Imperial College London

Uterine Transplantation

The move from the animal model into the human setting – surgical, reproductive & clinical aspects

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PhD Thesis

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…Dedicated To My Family…

No words can describe the extent of my gratitude

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…'The only difference between monkeys and us is the attention to detail'…

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'If you can trust yourself when all men doubt you,

But make allowance for their doubting too'.

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"…It may be that the gulfs will wash us down;

It may be we shall touch the Happy Isles,

And see the great Achilles, whom we knew.

Though much is taken, much abides; and though

We are not now that strength which in old days

Moved earth and heaven, that which we are, we are,

One equal temper of heroic hearts,

Made weak by time and fate, but strong in will

To strive, to seek, to find, and not to yield…"

- ***Ulysses*, Lord Alfred Tennyson**

Declaration of Originality

Submitted in fulfilment of the requirements for the degree of
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Work presented in this thesis is my own work except for where indicated.

Srdjan Saso

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Publications

1. **Saso S**, Petts G, Thum MY, Corless D, Boyd M, Noakes D, Del Priore G, Ghaem-Maghami S, Smith JR. Achieving uterine auto-transplantation in a sheep model using iliac vessel anastomosis: a short-term viability study. *Acta Obstet Gynecol Scand* 2014 Nov 24. [Epub ahead of print].

2. **Saso S**, Sawyer R, O'Neill NM, Tzafetas M, Sayasneh A, Hassan Hamed A, Elliott F, Thum MY, Ghaem-Maghami S, Lee MJ, Smith JR, Del Priore G. Trachelectomy during pregnancy: What has experience taught us? *J Obstet Gynaecol Res* 2014 Nov 25. [Epub ahead of print]

3. **Saso S**, Petts G, Chatterjee J, Thum MY, David AL, Corless D, Boyd M, Noakes D, Lindsay I, Del Priore G, Ghaem-Maghami S, Smith JR. Uterine allotransplantation in a rabbit model using aorto-caval anastomosis: a long-term viability study. *Eur J Obstet Gynecol Reprod Biol* 2014; 182C:185-193.

4. **Saso S,** Bracewell-Milnes T, Ismail L, Hamed AH, Thum MY, Ghaem-Maghami S, Del Priore G, Smith JR. Psychological assessment tool for patients diagnosed with absolute uterine factor infertility and planning to undergo uterine transplantation. J Obstet Gynaecol, 2014; 15:1-4.

5. Lintner B, **Saso S,** Tarnai L, Novak Z, Palfalvi L, Del Priore G, Smith JR, Ungar L. Use of Abdominal Radical Trachelectomy to Treat Cervical Cancer Greater Than 2 cm in Diameter. *Int J Gynecol Cancer* 2013; 23:1065-70.

6. **Saso S,** Ghaem-Maghami S, Louis LS, Ungar L, Del Priore G, Smith JR. Uterine transplantation: What else needs to be done before it can become a reality? *J Obstet Gynaecol* 2013; 33:232-8.

7. Del Priore G, **Saso S,** Meslin EM, Tzakis A, Brännström M, Clarke A, et al. Uterine transplantation-a real possibility? The Indianapolis consensus. *Hum Reprod* 2013; 28:288-91.

8. **Saso S,** Hamed AH, Doctor C, Thum MY, Naji O, Smith JR, et al. Is there a role for transplantation in gynecologic oncology? Autotransplantation and other lessons. *Int J Gynecol Cancer* 2013; 23:413-6.

9. **Saso S,** Ghaem-Maghami S, Chatterjee J, Naji O, Farthing A, Mason P, et al. Abdominal radical trachelectomy in West London. *BJOG* 2012; 119:187-193.

10. **Saso S,** Ghaem-Maghami S, Chatterjee J, Brewig N, Ungar L, Smith JR, et al. Immunology of Uterine Transplantation: A Review. *Reprod Sci* 2011; 19:123-34.

11. **Saso S,** Logan K, Abdallah Y, Louis LS, Ghaem-Maghami S, Smith JR, et al. Use of cyclosporine in uterine transplantation. *J Transplant* 2012:134936.

12. **Saso S,** Chatterjee J, Smith JR. Radical trachelectomy: need for a randomized controlled trial? *Acta Obstet Gynecol Scand* 2011; 91:758.

13. **Saso S,** Del Priore G, Smith JR. Uterine transplantation: future directions. *Hum Reprod* 2011; 26:500-1.

Oral Presentations

1. Smith JR, **Saso S,** Sieunarine K, Corless DJ, Boyd M, Noakes D, Clarke A, David A, Thum MY, Clancy N, Lindsay I, Petts G, Hakim N, Lawson A, Ungar L, Del Priore G. Uterine Transplantation after Cancer. Oral Presentation at British Gynaecological Cancer Society, Annual Meeting, Imperial College London, July 2014.

2. **Saso S,** Lintner B, Tarnai L, Novak Z, Palfalvi L, Smith JR, Del Priore G, Ungar L. Abdominal Radical Trachelectomy in Cervical Cancers >2cm. 11th RCOG World Congress, Liverpool, UK. June 2013.

3. **Saso S,** Chatterjee J, Thum MY, Clancy N, Ghaem-Maghami S, Smith JR. Uterine Viability following Allogeneic UTx in a Rabbit Model. 11th RCOG World Congress, Liverpool, UK. June 2013.

4. **Saso S,** Thum Y, Ghaem-Maghami S, David A, Del Priore G, Smith JR. Pregnancy Following Allogeneic Uterine Transplantation in a Rabbit Model. The Victor Bonney Society, 24th April 2013. London, UK.

5. **Saso S,** Smith JR. Uterine Transplantation: The UK Experience 2010-2012. 3rd International Uterine Transplantation Conference: 'Human Uterine Transplantation: Have We Finally Arrived?', 30th November 2012. London, UK.

6. **Saso S,** Smith JR. Pushing the Frontiers of Transplantation Surgery: The Enigma Of Uterine Transplantation. 1st Lebanese Conference on Hand and Reconstructive Surgery, 17th-18th November 2012. Beirut, Lebanon.

7. Smith JR, **Saso S.** Ethics of Uterine Transplantation. RCOG Ethics Committee Meeting. 19th January 2012. Royal College of Obstetricians and Gynaecologists, London, UK.

8. Attitudes to Uterine Transplantation. Fertility Preservation Symposium: A Road Map for Human Uterus Transplantation', 4th-6th December 2011. Indianapolis, USA.

9. Smith JR, **Saso S.** Uterine Transplantation: Past, Present and Future. Fertility Preservation Symposium: A Road Map for Human Uterus Transplantation, 4th-6th December 2011. Indianapolis, USA

10. Smith JR, **Saso S.** Role of Uterine Transplantation in Adolescent Gynaecology. 4th World Congress of the International Society for Hypospadias and DSD, 17th-18th September 2011. Royal College of Surgeons, London, UK.

11. Smith JR, **Saso S,** Sieunarine K, David A, Corless D, Lindsay I. Epidemiology of Fertility Preservation, Ethical Issues and Uterus Transplantation. Society of Gynecologic Oncologists, 6th - 9th March 2011. Florida, USA.

Poster presentations

1. **Saso S,** Clarke A, Bracewell-Milnes T, Ismail L, Abdallah Y, Doctor C, Del Priore G, Smith JR. Absolute Uterine Factor Infertility Patients: How Suitable a Cure is Uterine Transplantation? 11th RCOG World Congress, Liverpool, UK. June 2013.

2. **Saso S,** Chatterjee J, Thum MY, Ghaem-Maghami S, Del Priore G, Smith JR. Is Pregnancy possible in Animal Uterine Transplant Models? 11th RCOG World Congress, Liverpool, UK. June 2013.

3. **Saso S,** Bracewell-Milnes T, Al-Memar M, Abdallah Y, Ismail L, Clarke A, Smith JR. Attitudes of Health Care Professionals towards Uterine Transplantation. 11th RCOG World Congress, Liverpool, UK. June 2013.

4. **Saso S,** Chatterjee J, Thum Y, Ghaem-Maghami S, Del Priore G, Smith JR. Pregnancy following Allogeneic Uterine Transplantation in a Rabbit Model. American Society of Reproductive Medicine, 20th-24th October 2012. San Diego, USA.

5. Hamed AH, **Saso S,** Lynch C, Shah A, Vianna R, Del Priore G. The Role of Transplantation in Gynecologic Oncology at a University Tertiary Referral Center. International Gynecologic Cancer Society, 12th-16th October 2012. Vancouver, Canada.

6. Clancy NT, Sauvage V, **Saso S,** Stoyanov D, Corless DJ, Boyd M, et al. Registration and analysis of multispectral images acquired during uterine transplantation surgery. Biomedical Optics, 29th-2nd May 2012. Optical Society of America, Miami, USA.

7. **Saso S,** Al-Memar M, Saso A, Jalmbrant M, Del Priore G, Clarke A, et al. Attitudes to Uterine Transplantation. Fertility Preservation Symposium: A Road Map for Human Uterus Transplantation', 4th-6th December 2011. Indianapolis, USA.

8. **Saso S,** Al-Memar M, Jalmbrant M, Del Priore G, Clarke A, Smith JR. Motivations and Opinions of Potential Patients towards Uterine Transplantation. Fertility Preservation Symposium: A Road Map for Human Uterus Transplantation, 4th-6th December 2011. Indianapolis, USA.

9. **Saso S,** Chatterjee J, Sousa C, Ghaem-Maghami S, Del Priore G, Smith JR. Immunology of Uterine Transplantation. A Road Map for Human Uterus Transplantation, 4th-6th December 2011. Indianapolis, USA

10. **Saso S,** Chatterjee J, Naji O, Abdallah Y, Ghaem-Maghami S, Farthing A, et al. Radical Abdominal Trachelectomy: Current Outcomes and Future Directions. 9th International Scientific RCOG Meeting, 28th-30th September 2011. Athens, Greece.

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Abbreviations

AA	Abdominal Aorta
ART	Abdominal Radical Trachelectomy
a.u.	Arbitrary Unit
AUFI	Absolute Uterine Factor Infertility
BMI	Body Mass Index
bpm	Beats per Minute
BSA	Bovine Serum Albumin
CCD	Charged Couple Device
CD	Clusters of Differentiation
CNI	Calcineurin Inhibitor
CO_2	Carbon Dioxide
CRL	Crown-Rump Length
DMSO	Dimethyl Sulphoxide
DPBS	Dulbecco´s Phosphate Buffered Saline
eCG	Equine Chorionic Gonadotrophin
EG	Ethylene Glycol
eLASCA	Endoscopic Laser Speckle Contrast Analysis
ET	Embryo Transfer
FACS	Fluorescence-activated cell sorting
FSH	Follicle-Stimulating Profile
GW	Gestational Week
H&E	Haematoxylin & Eosin
Hb	Haemoglobin
βhCG	(beta) human Chorionic Gonadotrophin
HTK	Histidine-Tryptophan-Ketoglutarate
IFNγ	Interferon gamma

IL	Interleukin
IUGR	Intra-Uterine Growth Retardation
IVC	Inferior Vena Cava
IVF	In Vitro Fertilization
IRB	Institutional Review Board
LCTF	Liquid Crystal Tuneable Filter
LFIG	Leached Fibre Image Guide
LH	Luteinising Hormone
MRKH	Mayer Rokitansky Kuster Hauser
MSD	Mean-Sac Diameter
MSP	Modified Strassman Procedure
NSAID	Non-Steroidal Anti-Inflammatory Drugs
O_2Sat	Oxygen Saturation
PBMC	Peripheral Blood Mononuclear Cell
PBS	Phosphate Buffer Saline
PI	Perfusion Index
PIH	Pregnancy-Induced Hypertension
PV	Per Vaginam
RBC	Red Blood Cells
RCOG	Royal College of Obstetrics and Gynaecology
TGFβ	Tumour Growth Factor beta
TNFα	Tumour Necrosis Factor alpha
Treg	regulatory T cell
USS	Ultrasound Scan
UTx	Uterine Transplantation
VRT	Vaginal Radical Trachelectomy

Overview

Women with absolute uterine factor infertility (AUFI) are considered as being 'unconditionally infertile'. Uterine transplantation (UTx) may be a possible treatment option in the future for such women. This thesis describes a number of key areas of research that are important in order to move closer to a successful and crucially, safe transplant in the human setting.

Nine allogeneic transplants were carried out in a rabbit model to investigate anatomical and surgical aspects necessary for a successful UTx. An attempt to characterise and quantify the immunological mechanisms involved in allogeneic UTx (rejection patterns) was made. Out of the nine recipients, one was a long-term survivor. Embryo transfer was performed in this one doe with the aim of establishing pregnancy.

Performing UTx in a large-animal model is necessary as the pelvis resembles a woman's reproductive system more closely. In addition, the anastomotic technique is similar. Five sheep autotransplants were performed to further define surgical techniques. The anastomotic model was internal to external iliac vessel. Out of the five transplants, three sheep demonstrated adequate perfusion in the immediate post-operative period.

Furthermore, the suitability of two different imaging modalities, pulse oximetry and multispectral imaging, for assessing uterine perfusion and extent of ischaemia were been studied in both the rabbit and sheep models. Biophotonics was also applied in the form of Endoscopic Laser Speckle Contrast Analysis to characterize blood flow in the two models. Both Multispectral Imaging and Endoscopic Laser Speckle Contrast Analysis have never been assessed before in a gynecological context.

In order to transfer the concept of UTx to the human, we carried out a retrospective study of abdominal radical trachelectomy (ART) as a potential replacement for radical hysterectomy in patients with early stage cervical cancer desiring a fertility-sparing procedure. ART forms the

foundation of the original work into aspects of UTx. This original body of research revolved around the potential blood supply to a uterus.

Furthermore, an attempt has been made to analyse the motivations, aims and feelings of patients diagnosed with AUFI towards UTx. Forty patients were interviewed.

The final study involved the evaluation of the perceptions of health care professionals towards UTx, with 528 participants surveyed.

Aims

The aims of this thesis were to explore a set of important topics thought to be crucial in the process of moving UTx a step closer from the animal to the human setting.

Animal Studies

In order to achieve the aims below, both small (rabbit) and large animal (sheep) models were used.

1) To investigate anatomical, surgical and physiological aspects necessary for a successful UTx.
2) To establish pregnancy (rabbit model only) following allogeneic UTx and if successful to evaluate fetal growth and well-being using Doppler ultrasound.
3) To explore and compare the suitability of three different imaging modalities, pulse oximetry, optical spectroscopy, and enhanced laser speckle contrast analysis, for assessing uterine perfusion and extent of ischaemia and blood flow analysis.

Human Studies

1) To report the experience of ART as a potential replacement for radical hysterectomy in patients with early stage cervical cancer and who desire a fertility-sparing procedure.
2) To analyse the motivations, aims and feelings of patients diagnosed with AUFI and the perceptions of health care professionals towards UTx.

History and Evolution of Uterine Transplantation

Solid-organ Transplantation

Organ transplantation and the underlying processes have fascinated mankind throughout history. The idea of removing a part of an individual's body and grafting it onto a completely different entity transgresses beyond the scientific realm and into the philosophical and religious spheres. In the BC era, the Sushruta Samhita, a Sanskrit redaction text which contains all major concepts of ayurvedic medicine, had an innovative passage describing methods used by Sushruta to transplant a skin autograft during a nasal reconstruction. In the Far East, ca. 500BC, a legend describes how the first official Chinese physician**,** Bian Que used anaesthesia to perform a double heart transplantation in order to achieve balance between spirit and will in two men.[1]

In the modern era, the first successful human transplant was performed by Dr Eduard Zirm in Moravia, a province of Czech Republic, at the beginning of the 20th century, who transplanted a cornea. In the early 1900s, the French surgeon Alexis Carrel was awarded the Nobel Prize in Medicine for furthering the field of transplantation by developing successful and creative vascular anastomotic techniques.

Kidney and heart transplants represent two important milestones in solid-organ transplantation. On 23rd December 1954, Dr Joseph Murray, a plastic surgeon, led the team (including John Merrill (nephrologist), J. Hartwell Harrison (urologist), and Gustave Dammin (pathologist)) which performed the first successful solid-organ transplant. A kidney was transferred between genetically identical twins (Richard who suffered from chronic nephritis and Ronald Herrick), a process that inevitably removed the threat of rejection. Thirteen years later, on 3rd December 1967, in Groote Schuur Hospital, Cape Town, Professor Christiaan Barnard transplanted a heart from a brain-dead 24 year old donor into Mr Louis Washkansky who was suffering from chronic heart failure. He died 18 days later,

having been diagnosed with pneumonia. This possibly gave an early insight into the unique immune suppression requirements of each type of organ transplantation.

The idea of using organs to solve quality-of life issues was first introduced to the general public in 1998 following a controversial hand transplant. This case highlighted the important issue of psychologically assessing the recipient as would be required in uterine transplantation (UTx). The patient, a convicted con-man, failed to follow the post-operative drug and physiotherapy program and as a result the donated hand was rejected. In 2010, the first facial transplant was successfully performed confirming that quality of life issues can be successfully treated through organ transplantation (**Table A**).

Year	Organ	Surgeon (Place of Transplantation)
1905	**Cornea**	**Eduard Zirm** (Czech Republic)
1954	**Kidney**	**Joseph Murray** (USA)
1966	**Pancreas**	**Richard Lillehei** (USA) **William Kelly** (USA)
1967	**Heart**	**Christiaan Barnard** (South Africa)
1967	**Liver**	**Thomas Starzl** (USA)
1981	**Heart-Lung**	**Bruce Reitz** (USA)
1998	**Hand**	**Jean-Michael Dubernard** **Nadey Hakim** (France)
2010	**Face (full)**	**Joan Pere Barret** (Spain)
2011	**Uterus**	**Omer Ozkan** (Turkey)

Table A Timeline of successful solid-organ transplants[2]

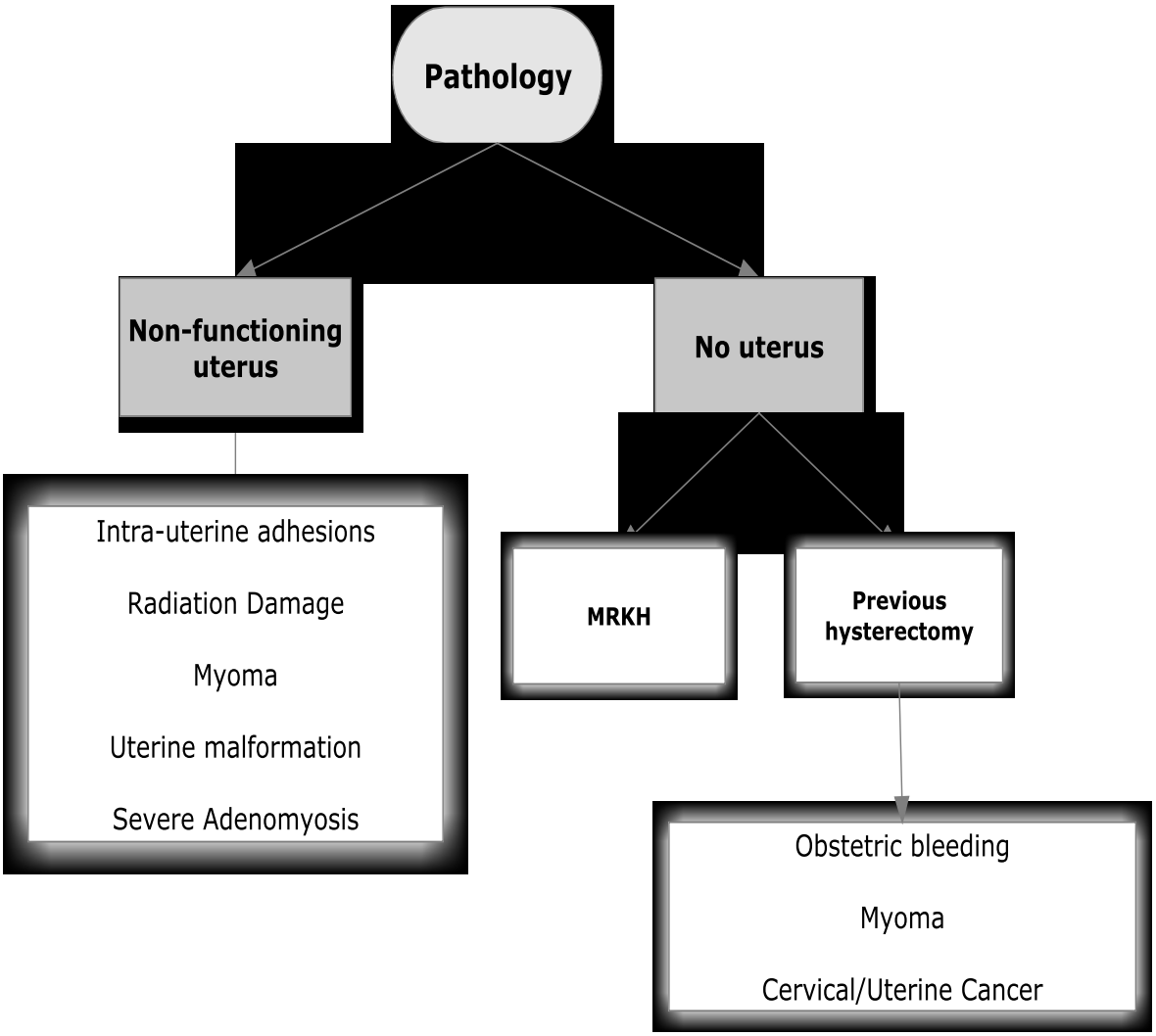
Background to Uterine Transplantation

Women with absolute uterine factor infertility (AUFI) are considered as being 'unconditionally infertile'. Currently, the only two options that women diagnosed with AUFI have with regards to managing their infertility are surrogacy or adoption. Potentially, these women may also benefit from a possible future third option: UTx.[3] Causes of AUFI are congenital, mainly Mullerian duct anomalies, or acquired (for example, a hysterectomy performed secondary to obstetric haemorrhage, myoma, endometrial or cervical malignancy).[4] Other uterine related causes albeit not absolute are secondary to pathology such as fibroids and severe endometriosis and adenomyosis (**Figure A**). Currently, it is estimated that even when restricted to women aged 15-34 years, there are around 7 million women in USA who have lost their uteri to benign causes or obstetric complications. Based on known fecundity rates, thousands of these women therefore require a resolution for their infertility. UTx has been proposed as a solution and thus, treatment for AUFI.[5,6]

Causes of AUFI - Mayer-Rokitansky-Kuster-Hauser

Women who present without a uterus are the most obvious group that may benefit from UTx in the future. These are women with either diagnosed uterine agenesis or who underwent hysterectomy. Uterine agenesis is the least common uterine anomaly and constitutes 3% of all Müllerian variances.[7] The syndrome is today referred to as Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome based on the work of four people who at various times over the 19th and 20th centuries described in the syndrome.[4,8] MRKH is a congenital condition, with an incidence of 1 in 5000, whereby the Müllerian duct fails to develop.[7,9,10] Therefore, as it is a congenital condition, the woman is born without a fully formed uterus (usually a rudimentary, solid bipartite uterus is seen) and Fallopian tubes. The morphology of the vagina is variable, from non-existent to only the upper third missing. Ovaries are usually present, and do ovulate normally. After pregnancy and Turner syndrome, it is the third most common cause of primary amenorrhoea.[7,11,12] The karyotype (46, XX) and hormone profile are normal, with no transgender issues and a usual development of a MRKH teenager into a female adult

following telarche and adrenarche. In those cases where vaginal agenesis is also present, normal sexual function is resumed after the creation of a neovagina.[11,13] Inheritance tends to be sporadic, with the syndrome appearing most likely to be inherited as a polygenic, multifactorial trait. Three MRKH-subtypes have been defined to date: (a) typical: described above, an isolated uterovaginal aplasia or hypoplasia - prevalence is around 50-60%; (b) atypical: uterovaginal aplasia or hypoplasia with associated renal and/or ovarian malformation – prevalence is around 20-25%; and (c) MURCS (Müllerian duct aplasia, Renal aplasia and Cervicothoracic Somite dysplasia) syndrome - prevalence is around 10-15%.



Key:

MRKH, Mayer Rokitansky Kuster Hauser Syndrome

Figure A Causes of Uterine Factor Infertility (adapted from Brannstrom et al) [4]

Petrozza et al carried out a study on whether MRKH is inherited in an autosomal dominant fashion. As currently the only way for MRKH women to have their own genetic child is via surrogacy, questionnaires were sent to all infertility treatment centres performing surrogacy in-vitro fertilization (IVF) in the US.[14] 32 programmes responded (60%). 58 MRKH women underwent a total of 162 IVF cycles, and 34 live-born children were delivered (female: n=17). No congenital malformations were found in the female neonates. One male child had a middle ear defect and hearing loss.[14]

Causes of AUFI - Hysterectomy

The other group of women who present with an absence of the uterus - women who underwent hysterectomy - represent the largest group of AUFI patients.[4,15] These women also tend to be young and therefore seek surrogacy services to find a solution to their infertility. *Goldfarb et al* reviewed and evaluated retrospectively the experience of an IVF surrogate gestational programme in a tertiary care and academic centre during a 15 year period.[16] 50% of the women enrolled underwent a hysterectomy previously. The overall pregnancy rate per cycle after IVF surrogacy was 24% (38 of 158), with a live birth rate of 15.8% (25 of 158). Interestingly, their conclusion that ovulation induction had a better effect in patients with congenital absence of the uterus rather than in patients who had a hysterectomy, most likely secondary to ovarian compromise brought on by the actual hysterectomy, is of value to future UTx patients.[16] Hysterectomy is carried out either as an emergency or an elective procedure. An emergency hysterectomy is a last-line, life-saving treatment for uncontrolled, severe post-partum haemorrhage where the patient is haemodynamically unstable. PPH that warrants a hysterectomy is usually caused by a Caesarean section, uterine atony or rupture, or placental adherence to the uterine wall. As the rate of Caesarean section rate continues to steadily increase, so one would expect the PPH hysterectomy rate to also increase from the present 1:2000.[4,17-19]

Elective hysterectomies are performed for either benign or malignant uterine pathologies. The most obvious malignant pathology is gynaecological cancer. The surgical treatment for endometrial cancer and uterine sarcoma is a radical hysterectomy. However, the incidence of these cancers (uterine

cancers not involving the cervix) is 2.5% in women under the age of 40.[20,21] Worldwide, cervical cancer remains the most common malignancy, with hysterectomy continuing as the most widespread surgical treatment method. This leads to good results with respect to survival and minimal morbidity but importantly a loss of a future fertility potential. This has led gynaecologic oncology surgeons to consider more carefully possible fertility sparing options in order to improve quality of life outcomes. Early stage (IA2-IB) cervical cancer, measuring less than 2cm in diameter can be treated with a fertility preserving operation, known as trachelectomy. However large tumours and those more serious than stage IB still require a radical hysterectomy.[4] *Sonoda et al* found that 89/186 (48%) patients under the age of 40 who underwent a radical hysterectomy for early-stage cervical cancer, were eligible for a fertility preserving surgery i.e. trachelectomy.[22] It is precisely this group of patients, women under the age of 40 who underwent a hysterectomy for cancer treatment who may benefit the most from UTx.[4]

Causes of AUFI – Asherman's Syndrome

Another cause of AUFI that may lead to a uterus which is not compatible with pregnancy is Asherman's Syndrome or intrauterine adhesion. Prevalence is around 1-2%, with a higher prevalence in the developing world secondary to genital tuberculosis.[23,24] Genital tuberculosis can have quite deleterious effects - it may obliterate 50% of the uterine cavity.[25] Asherman's Syndrome may also be caused by an inflammation of the endometrium secondary to viral or bacterial exposure or even surgical trauma; for example aggressive uterine curettage when evacuating retained products of conception.[26] Infertility rates are around 50% of affected women, with also a high rate of miscarriage, ~40%, in those patients that do fall pregnant.[27] A solution may be hysteroscopic adhesiolysis which can cure infertility in 90%, 70%, and 30% of cases of mild, moderate and severe Asherman's Syndrome.[4,28] However the treatment is not absolute. If infertility is not resolved, hysterectomy followed by UTx may be an option for this group of women.[4]

Causes of Uterine-Factor Infertility

Below are described causes of infertility that are uterine-related. They are however not absolute i.e. treatment may be possible for each one of them. Described above was the most severe congenital malformation which leads to AUFI, that of uterine agenesis. More common however are congenital uterine malformations where the uterus is present. Apart from infertility, this type of pathology also leads to a multitude of other gynaecological and obstetric complications such as pregnancy loss, pre-term delivery and difficulty during labour.[29] Uterine malformations are found in 6-8% of the general as well as infertile female population and around 16-18% of those women that suffer from recurrent miscarriage.[4,30] It is important to note that despite a relatively high prevalence, majority of women with congenital uterine malformations are in fact fertile. The most common type of uterine anomaly (35% of cases) in women considered infertile is a septate uterus, with the second most common type (25% of cases) being a bicornuate uterus.[4,7,30] Spontaneous miscarriage occurs in 80% and 35% of pregnancies respectively, following conception in the above two types.[31] Hysteroscopic metroplasty can decrease the rate considerably but many still cannot carry a pregnancy to 24 weeks.[4,32,33] Other common uterine anomalies are unicornuate and didelphic uteri, which have a live birth rate following conception of 50%.[7] The solution in future for those women permanently infertile may be a hysterectomy followed by UTx.[4]

Treatment of non-gynaecological cancers during childhood, puberty or early adulthood (under the age of 40) may lead to a non-functioning uterus. Survival into adulthood from a childhood cancer is now at 70-80% so cancer treatment is important with respect to future fertility.[34] Chemotherapy does not seem to affect the uterus with respect to morphology or size. *Larsen et al* assessed uterine volume using a transvaginal ultrasound in 100 childhood female cancer survivors.[35] Their results showed that cytotoxic therapy did not affect uterine volume, endometrial thickness or uterine artery blood flow. Radiotherapy however has an irreversible and damaging effect on both the uterus and the ovaries.[4] Uterine size is reduced in size as demonstrated by a number of studies.[35,36] *Holm et al* assessed the internal genitalia and uterine blood flow by ultrasound in 12 females 4-10.9 years after total body

irradiation and allogeneic bone marrow transplantation for childhood leukaemia or lymphoma.[37] Median uterine (around 60%) and ovarian volumes were significantly reduced compared with normal controls. Follicle growth and uterine blood flow were impaired. Significantly hormone replacement therapy could not generate normal uterine growth despite inducing bleeding and suppressing other signs of premature menopause.[37] *Wallace et al* presented the first model to reliably predict the age of ovarian failure after treatment with a known dose of radiotherapy. Gonadotoxicity is brought on by radiation doses as low as 5-20Gy which leads to immediate menopause in 90% of post-pubertal females.[38]

Benign pathologies that may may render a uterus incapable of carrying a healthy pregnancy to term include leiomyomas. The incidence of uterine leiomyomas varies anywhere between 8-16%, with the incidence increasing in older and Afro-Caribbean women.[4,39-41] *Marshall et al* quantified the incidence of uterine leiomyoma according to age and race among 95,061 premenopausal nurses aged 25-44 with intact uteri and no history of uterine leiomyoma.[39] 3.5% of those surveyed aged 30-39 were found to have undergone a hysterectomy. Submucosal and intramural leiomyomas are most commonly associated with infertility, especially if above 4cm in diameter.[42,43] Hysteroscopic resection and laparoscopic or open myomectomy are commonly used procedures to increase the probability of a successful pregnancy.[4,44,45]

History of Uterine Transplantation

In-vitro fertilization

It is worth summarising in brief the arrival of IVF, because of its crossover with UTx research with respect to the ethical arguments of whether it should be permitted. The first major piece of work that proved that the transfer of a normal embryo into a mammalian uterus could lead to a birth of a healthy offspring was performed in 1959 by Dr Min Chueh Chang at the Worcester Foundation for Experimental Biology based in Massachusetts, USA.[46] With regards to human IVF, the majority of

the ground-breaking work and a series of 'world's firsts' in the 1970s and 1980s had been done in Australia by Professor Carl Wood's team at the Monash Medical Centre in Melbourne. Their work included the world's first IVF pregnancy, albeit only confirmed biochemically and not via an ultrasound scan, the world's first birth of a healthy IVF baby conceived via frozen embryos (1978), the world's first IVF baby conceived using a donor egg (1983), and the world's first IVF baby following the use of novel sperm retrieval surgical techniques (1986).

What changed IVF from an experimental technique to a successful international practice was their introduction of hormonal medication (clomiphene citrate and human chorionic gonadotrophin) to stimulate ovarian follicle production in order to achieve better control of oocyte maturation and collection. The first team to achieve a 'real', non-biochemical pregnancy as well as live birth was headed by Professors Patrick Steptoe and Robert Edwards, based in the UK. In 1976, they reported a re-introduction of a human embryo in transition between a morula and blastocyst after culture *in vitro* into the mother's uterus via the cervix. The resulting pregnancy was found to be located in the Fallopian tube. The ectopic embryo was removed at 13 weeks gestation. However, on 25th July 1978, the world's first baby to be conceived by IVF, Louise Brown, was born in Oldham General Hospital, Greater Manchester, UK.[47,48] The second such baby, also their work, Alastair MacDonald, was born on 14th January 1979 in Glasgow. Professor Carl Wood's team in Australia then achieved a total of nine births from 14 pregnancies, although theirs was not the first IVF baby in that country. That honour went to a team led by Ian Johnston and Alex Lopata whose work led to the birth of the world's third live IVF baby, Candice Reed, born on 23rd June 1980 in Melbourne.[49] The pioneers of IVF in USA were Drs Georgeanna Seegar Jones and her husband, Howard Wilbur Jones II, based in Eastern Virginia Medical School in Norfolk, Virginia.[50] On 28th December 1981, their work on IVF gave birth to Elizabeth Jordan Carr, the first American test tube baby.

Uterine transplantation research - The beginning

Below is a review of UTx research before 1985, a cut-off chosen as it came in the middle of a decade when the current immunosuppressant medication as well as IVF had reached the clinical stage in many parts of the world. The post-1985 research is of a much greater relevance to this thesis and is described separately in the coming chapters. The majority of UTx research published in the 1960s through to the 1980s, involved transplantation of the entire female genital tract rather than the uterus specifically. Aims were to cure infertility but also offer a novel treatment option for various pathological conditions of the female genital tract. Furthermore, the transplantation process was autogeneic, rather than allogeneic. The first study to report a UTx attempt was by *Zhordania et al*.[51] Both small and large animals were used: 20 dogs, 10 rabbits and 18 ewes. The uterus, Fallopian tubes and ovaries were all excised and placed within the omentum, thus resulting in a heterotopic autotransplant. This was an original idea, which introduced the concept of omentopexy, whereby the omentum is wrapped and sutured around the graft in order to bring about revascularization of the chosen graft. The follow-up data for the ewes was reported in detail. Revascularisation had been achieved with a week, demonstrated neatly by methylene blue dye coursing through the descending aorta, then into the vessels of the omentum and finally into the uterus, Fallopian tubes and ovaries. The morphology of the grafted genital organs was completely normal by two weeks. Histology at one month showed normal and viable tissue. The authors did not report any complications with any of the animals, although it is unclear whether this is an omission or an actual fact. The most impressive result was that 10 out of 16 ewes achieved pregnancies after mating, all of which resulted in normal deliveries. The study used a significant number of animals, introduced a novel technique and reported surprisingly impressive results, with respect to preservation of both morphology and function. Their conclusion supporting the concept of revascularization of autotransplanted internal reproductive organs via omentopexy is appropriate given the results. However, it is worth noting that the omentopexy technique did not demonstrate such a high success rate in subsequent studies (viability: 16/18=89%; functionality: 10/16=62.5%).[51]

O'Leary et al performed a similar experiment in a dog model, whereby an omental pedicle graft was used as a source of uterine revascularization.[52] Specifically, the omentum was divided, with one pedicle sutured into the back of the uterus and Fallopian tubes, and the other across the top of the uterus, thus encasing the uterus and tube within itself. The number of animals used was appropriate (n=32). 23 out of 32 dogs were adequately re-vascularised at 4-6 weeks, with the appearance of the graft resembling its original morphology. *De novo* anastomotic channels were established even after two weeks post-operatively. However conception and pregnancy were not attempted so one cannot make a conclusion related to graft functionality. Even though the myometrium and cervix appeared normal histologically, the endometrium is not commented upon, most likely as the ovaries were removed so hormonally there would have been no effect upon the endometrium.[52]

Scott et al also applied the principle of omentopexy in a dog model but this time specifically for auto-UTx in order to see whether women with AUFI could be helped in the future.[53] This was a study looking at both auto-UTx and allo-UTx. Eight auto-UTx involved the application of an omentopexy whereas 10 allo-UTx involved the use of an omental pedicle. Two autotransplants were performed without the use of an omentopexy. The uterine graft was obtained via a subtotal hysterectomy, with the rest of the genital tract left untouched. Both the auto-transplants and homotransplants were performed immediately. Immunosuppression, azathioprine and prednisolone, was provided to five of the allotransplants. By 12 weeks, the two non-omentopexy transplants fibrosed completely, whereas viability was normal in seven out of the eight omentopexy transplants. Normal uterine functionality involving conception or pregnancy was not demonstrated. Acute rejection was grossly visible in all grafts that underwent allo-UTx. Progressive oedema, necrosis and atrophy were seen within the first two weeks. This continued until at first the endometrial layer was completely necrosed, and at four weeks, complete non-viability of all uterine layers was seen. Interestingly, administration of immunosuppressive drugs did not alter the rate of rejection or appearance of the graft, suggesting that mostly likely that the drugs were poorly administered or at a too low a dose. The study demonstrates the potential efficacy of the omentopexy technique with a high percentage viability of the auto-transplanted grafts. However, a similar conclusion regarding allo-UTx, which has a direct bearing on

human UTx, could not be made. The early demise of the grafts was most likely because of rejection but no attempt was made to differentiate those signs caused by rejection and those signs that may have been caused by a poor blood supply. Furthermore, acute rejection was simply noted and described using only macroscopic and histopathological features, but no attempt was made to characterise the response itself using imaging or biochemical markers. Their statement that histocompatibility testing between the donor and recipient, as well as an appropriate immunosuppressive regimen are required to ensure long-term allo-graft survival was correct, albeit obvious.[53]

Both *Barzilai et al* and *Paldi et al* also performed an omentopexy-autoUTx type of study in a dog model.[54,55] However, the results contradicted the favourable conclusions towards omentopexy of the above studies. The studies offered an original methodology of comparing two different perfusion techniques; one group underwent utero-ovarian autotransplantation and omentopexy and the other group underwent the same type of transplant but with a vascular re-anastomotic technique. In order to avoid problems related to rejection which would have made comparison more difficult, an autotransplant model was selected on purpose. In the first study, 12 dogs were used in each group.[54] In the omentopexy group, all 12 grafts showed massive necrotic degeneration, with no viable tissue present. The re-anastomosis group (common iliacs), however, revealed autografts (10/10; two dogs died immediately post-operation) to be viable, with normal uterine and ovarian tissue. Imaging using a hystero-salpingogram demonstrated patent tubes and cervico-vaginal anastomosis. Uterine function was normal in one dog; pregnancy was achieved with delivery of three healthy pups. The conclusions here changed somewhat the perception of the omentopexy technique being a viable perfusion technique for a transplanted graft. Follow-up was appropriate, as was the number of dogs in each arm of the two groups. The authors also demonstrated that they understood which variables would be key in future human attempts by reporting related data: warm ischaemic time, maintenance of a patent vascular tree and sound re-anastomosis. The vascular re-anastomotic technique demonstrated encouraging results and added to results from number of other studies employing the same technique which together highlight the way forward with regards to uterine graft perfusion.[54] *Paldi et al* also reported a 100% uterine viability rate three months post-transplant in the group which underwent

uterine auto-UTx with vascular re-anastomosis. The graft proved their functionality with subsequent implantation of embryos, pregnancy and delivery of healthy pups.[55]

The above two studies pointed out the inferiority of the omentopexy method when directly compared to the vascular re-anastomotic technique.[54,55] This vascular model had already been tried by various teams, with initial studies published in the 1960s. The first such study was by *Eraslan et al* in 1966, again performed using a dog model.[56] 18 virgin female dogs underwent uterine and ovarian dissection. It is worth highlighting that unfortunately the authors chose not to disconnect the organs from the body throughout the procedure, which impacts negatively on the study. With regards to the pelvic vascular supply, the common part of the internal iliac artery was dissected from its origin and freed inferiorly with ligations of all branches except the uterine and superior vesicle arteries. Division and re-anastomosis were performed immediately with the vagina still intact. The uterine veins on the other hand were dissected superiorly from the uterus to the fusion of the internal and external iliacs. The ovarian vessels were divided but not re-anastomosed. The vagina was then divided and re-anastomosed. The author understood the importance of warm and cold ischaemia and flushed the graft with heparinised saline through its common iliac artery after 30 minutes of warm ischaemia. Ten dogs out of 18 survived the one year follow-up. All had viable uteri based on their gross appearance and microscopic analyses. Uterine artery patency was normal in all animals and was appropriately studied using retrograde aortography. Following mating, three pregnancies were confirmed with two healthy litters delivered. Importantly, the physiological increase of the diameter of pelvic arteries during pregnancy did not affect the re-anastomotic sites. The study itself was the first such study in the medical literature to prove a useful concept - that vascular re-anastomosis does lead to perfectly viable and well perfused uteri, compatible with carrying a healthy pregnancy to term.[56]

Mattingly et al furthered the above concept by performing both auto- and allo-utero-ovarian transplants (again dog model) in a study designed to assess ovarian function.[57] However data related to the uterus is provided and the study is summarised below. The anastomoses in the two different models were different. In the autotransplants, end-to-end common iliac arterial and inferio-veno-caval

anastomoses were applied. However, in the allotransplants, the anastomoses were aorto-common iliac and cavo-common iliac. The autografts revealed very similar rates to the *Eraslan* study above with six out of seven viable autografts post-operatively. Two dogs carried intrauterine pregnancies with one full term and one premature delivery. The autotransplant group almost served as a preliminary project prior to the commencement of the more numerous allotransplant cohort. 50 were performed in total. 29 were administered azathioprine as an immunosuppressant three days pre-operatively whilst 21 also underwent a splenectomy. In the first cohort, only there dogs survived long-term, with 21 cases of early rejection. In the second cohort, eleven dogs did survive between 6 and 21 days and demonstrated graft viability. Assessment techniques were appropriate. Viability was evaluated on gross appearance via laparotomies and via imaging using arteriograms. Rejection was assessed using an alkaline phosphatase, haematoxylin-eosin and histochemical stains. The study again emphasises the need to study the rejection response and the specific role of immunosuppressants in preventing acute rejection in UTx specifically. Histocompatibility matching among donor and recipient is also required and was not carried out by the authors. The autotransplantation aspect of the study further reconfirms the technical advantage of a vascular re-anastomotic model in UTx. It also demonstrated that the re-connection of the nerve supply had no bearing on future successful pregnancy.[57]

Wingate et al focused specifically on an allogeneic UTx experimental model.[58] The graft was utero-tubal-ovarian and was transplanted with its vascular pedicle, thus continuing the development of the vascular anastomotic model. 17 dogs were used in total for a set of cross-transplantations. Ten did not receive any immunosupression, whereas seven did (six - azathioprine and corticosteroid and one-antithymocyte serum). The numbers used are appropriate, although it is not apparent why one dog received a completely different immunosuppressant to the other six. The graft was a development on previous examples and resembles vascular grafts used for both small and large animals by UTx surgeons today. It consisted of the uterus, Fallopian tubes, and ovaries, with its vascular pedicle of the uterine, internal and common iliacs to include two inches of the aorta and vena cava. Anastomoses in the recipient were end-to-side to the aorta and IVC. Those dogs that did not receive an immunosuppressant (eight out of ten survived to the tenth post-operative day) followed the usual

acute rejection course: adhesions, oedema, and necrosis. Those that received immunosuppressants also experienced necrosis as well as thrombosis of both the medium and large-sized vessels within the grafts. This means that the immunosuppressant doses were not adequate enough or that the combination of immunosuppressants was not appropriate. Mixed leukocyte culture test demonstrated an inadequate suppression of leukocyte transformation, thus suggesting an insufficient dose of immunosuppressant. It is a criticism of the study that it would have been more useful to have a small number of dogs not receiving immunosuppression (n=5 for example) and therefore have a larger number of dogs receiving immunosuppression but split into groups where a dog in each group received a different immunosuppressant dose. This was acknowledged in the conclusion. The authors also suggested that the donor and recipient should be tested for histocompatibility. They also recommended the setting up of a recognized monitoring tool for the efficacy of the immunosuppressants. The conclusions are relevant and appropriate and will be evaluated again in the 2000s.[58]

The final study of relevance to UTx research carried out pre-1985 was by *Yonemoto et al.*[59] Again a dog model was used, involving both auto- and allo-(cross) transplantation of the uterus and ovaries, and applying vascular anastomotic techniques. In total, 14 recipients received a utero-ovarian graft, of which five recipients were male. The immunosuppressant chosen was azathioprine, which was started pre-operatively and continued post-operatively. With respect to the vessels supplying the graft, the arterial vasculature included the lower aorta whereas the veins reached up to the common iliac veins. The anastomosis was a little different to the above study, with the aorta anastomosed to the internal iliac arteries and the common iliac veins to the respective veins in the recipient, in both cases in an end-to-side manner. 8 out of 14 recipients survived the operation, six female and two male. The authors highlighted the surgical complexity of the procedure in the conclusion. Out of the six females, four had viable uterine grafts, and the other two revealed complete necrosis upon euthanasia. The two male dogs revealed partial necrosis at day 21 and complete necrosis with rejection at day 37 post-UTx following euthanasia. The conclusions themselves do not reveal anything new to what has been describe above. The data added to the existing body of knowledge whereby the uterus is confirmed to

be a non-privileged site, acute rejection does follow a routine course seen with other organs and the myometrium is rejected later than the endometrium, with its architectural structure maintained for the longest. The authors explained those conclusions clearly, thus setting a clear path for future work. They also usefully reiterated the need for continued experiments focusing on the ideal combination and dose of immunosuppressant required and that all future experiments should use the vascular re-anastomotic model. The latter point has been confirmed by recent studies as will be discussed later in the thesis.[59]

One major criticism of all the studies above is that the studies do not provide in their reports any data concerning durations of utero-tubal-ovarian retrieval and whether the surgical technique with regards to accuracy and duration altered with the number of transplants performed.

First Human Uterine Transplant

Despite the promising research described above, UTx research had reached a 'dead-end' by the end of the 1970s. In the 1980s, the situation did not change greatly. Fertility research was dominated by developments in IVF, following the birth of the first 'test-tube baby' Louise Brown in 1978 in Oldham. The rest of the field of transplantation, following a much needed impetus based on recent scientific breakthroughs into the specific mechanisms behind the immunological response to 'foreign' cells (infection, cancer, autoimmunity and transplantation), concentrated on life saving transplantation, for example, heart, kidney and liver transplantation. This was more so from the 1980s onwards following the discovery of cyclosporine (CsA), a calcineurin inhibitor, which at the time represented a major breakthrough in the field of transplantation. The medication demonstrated that organ rejection after allogeneic transplantation could be prevented.

However this all changed from the mid-1990s as a result of three important factors. First, gynaecologic oncologists began to look at techniques whereby a gynaecologic cancer could be surgically removed, yet the fertility of the patient would also be preserved. An example is abdominal

radical trachelectomy, applied to treat early stage cervical cancer in those women wishing to preserve their fertility.[60] Based on the surgical techniques established at the time, some of these surgeons developed ideas as to how one may help those women whose uteri are rendered incompatible with carrying a healthy pregnancy or are simply not present.

Second, and most crucially, on 6th April 2000, UTx was attempted for the first and until 2011, only time in the human setting.[61] This was a compelling, courageous and historic attempt by a team in Saudi Arabia which should be commended. They transplanted a donor uterus into a 26 year old female who had previously had a hysterectomy for post-partum haemorrhage. The donor, a 46 year old woman, had completed her family and agreed to a hysterectomy and bilateral salpingo-opherectomy to treat her bilateral multi-loculated ovarian cysts. The consenting process was queried in the international literature as a result of the fact that the standard treatment for such a pathology is a laparoscopic salpingo-oophorectomy.[62] However following clarification, this was later retracted.[63] HLA tissue matching, ABO compatibility, and negative cytotoxic antibodies in the recipient were confirmed prior to transplant procedure. Briefly, the surgical technique is described. As with a hysterectomy, the uterine vessels were separated from the ureters, a process aided by the transaction of the round ligaments and opening of paracervical/pararectal spaces. Heparin (20,000iu) and prednisolone were given after vaginal transaction intravenously (iv) to prevent clotting and inflammation. The authors did not mention or describe the uterine veins or their division levels. The uterine arteries were divided 25mm away from the uterus. Also not reported is the duration of uterus retrieval surgery or the warm and cold ischaemia times. The first warm ischemia probably extended over several minutes. The organ was subsequently immersed *ex vivo* in cold saline and flushed with Euro-Collins solution. The Fallopian tubes were included in the transplanted graft which is a step that no team would perform today. Thus, only the uterus with its blood supply would be transplanted minus the ovaries and oviducts. This would be to avoid the risk of ectopic pregnancy following natural conception, which would be particularly high following radical pelvic surgery. The hysterectomy was modified so as to maintain the length of the vascular pedicle as long as possible (uterine arteries and veins were extended bilaterally by 6-8cm using long segments of the saphenous

vein), thus ensuring adequate tissue perfusion and tissue integrity. The uterus was subsequently grafted into the recipient's pelvis. The position was orthotopic with additional fixations to the pelvic side walls and ligament remnants as well as the cervical anastomosis to the vaginal vault. To ensure uterine perfusion, the uterine artery and three uterine veins were first connected to sections of the great saphenous vein, and then joined onto the external iliac arteries and veins, respectively.

As this was an allogeneic UTx, immunosuppression was administered in the form of the following drugs: oral CsA, azathioprine and prednisolone. Graft rejection was monitored by Echo Doppler studies which indicated uterine perfusion for two months, magnetic resonance imaging and measurement of the CD4/CD8+ cell ratio in the peripheral blood. There was only one episode of acute rejection on the ninth post-operative day, treated and controlled promptly with anti-thymocytic globulin and increased steroid and azathioprine doses. Following that episode, the patient did not experience any further issues. Apart from normal Doppler studies, the uterus showed adequate function with endometrial proliferation reaching a maximum of 18mm following the administration of combined oestrogen-progesterone therapy and two withdrawal bleeds once the medication had been stopped. The authors did not explain why the hormonal treatment was administered and the recipient had her ovaries preserved. The graft was removed after 99 days due to thrombosis of the uterine vasculature, which caused subsequent necrosis of the transplanted uterus, leading to a rescue hysterectomy, following the visualisation of a 'dusky-coloured' and prolapsing uterus. The authors reported '*acute vascular occlusion appeared to be due to inadequate uterine structure support, which led to probable tension, torsion, or kinking of connected vascular grafts.*'

Histopathology revealed acute thrombosis in the vessels of the uterine body, with resulting infarction. Interestingly both Fallopian tubes appeared healthy and viable, with no evidence of rejection. Therefore, the thrombosis of the uterine vessels was either because of the double anastomosis for each vessel or inadequate uterine structural support. This would have meant that the uterus, instead of being properly supported, instead 'sagged' under the influence of gravity leading to tension, torsion, or kinking of the connected vascular uterine grafts.[61] However, the authors did not attempt to explain

why the Fallopian tubes were of normal histology, as they are also supplied via the uterine arteries. It is likely that future attempts would have a greater chance of succeeding provided longer vascular pedicles were obtained with increased uterine fixation. Despite this, the actual event provided much needed impetus for research into UTx (**Figure B**).

The third and final factor was the area of ovarian transplantation, involving ovarian cortex transplantation and whole ovary transplantation, fresh and frozen in both cases. To date, more than 20 pregnancies have been reported after ovarian cortex transplantation.[64]

Conclusion

Currently, gestational surrogacy, adoption, or lifetime infertility are the only options available to affected women with AUFI. However, these options may not always be appropriate for every woman because of emotional, ethical, religious, social and legal issues.[3] Therefore, UTx can be justified as another potential solution to the afore-mentioned quality-of-life pursuits. With continued advances in transplantation surgery, improved methods of controlling tissue rejection, and advances in IVF technology, UTx can bring an end to AUFI by allowing a select group of women to become mothers.[65] It is worthwhile to recall and note the parallels in the controversy surrounding now 'standard' assisted reproductive techniques when they were first introduced and UTx today.

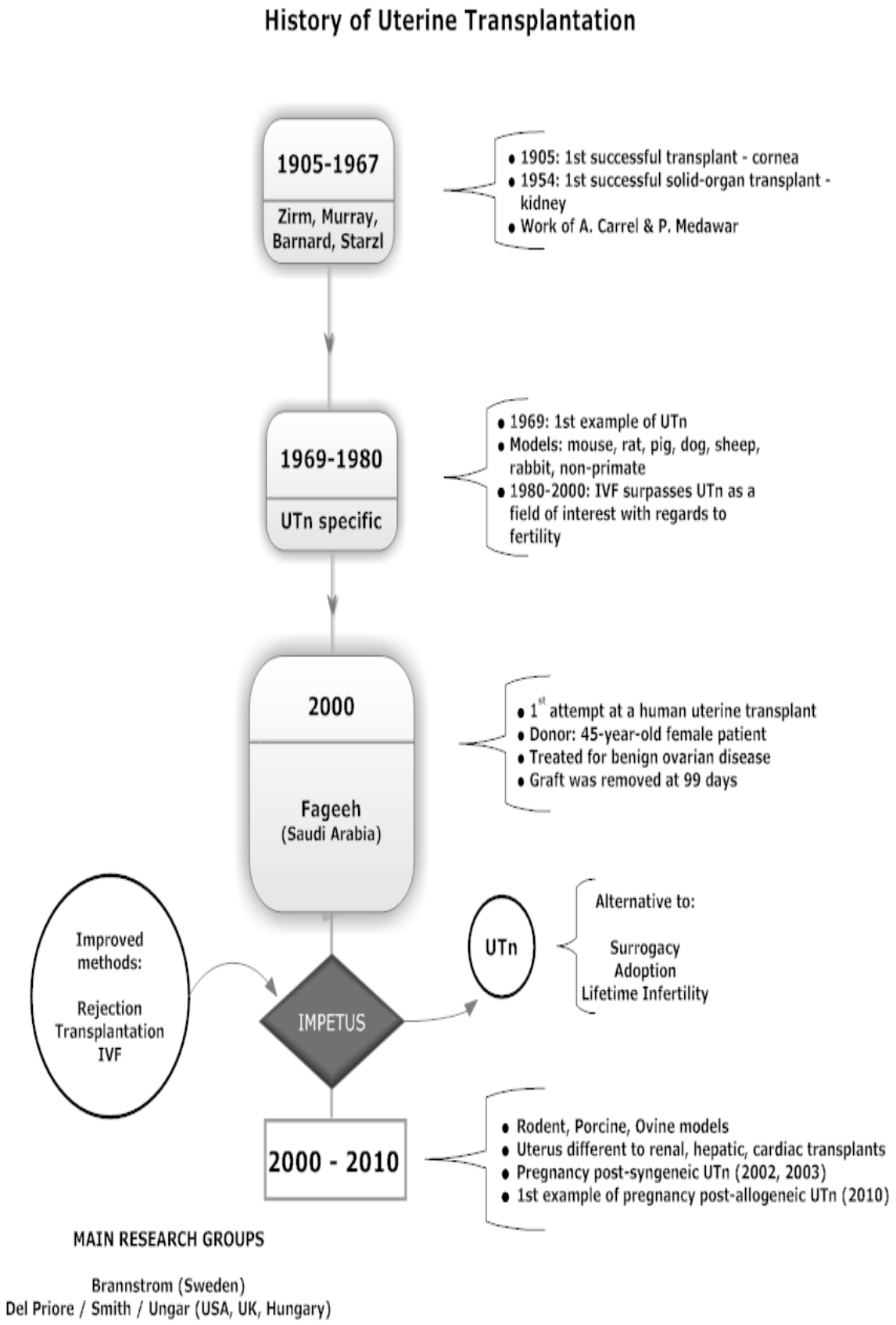


Figure B History of Uterine Transplantation.[2] Depiction of the orgins of modern organ transplantation leading up to current research into uterine transplantation.

Key areas of UTx research

The publication of the world's first human UTx was a catalyst which sparked a new wave of research specifically focusing on AUFI and UTx. Three international teams have carried out the vast majority of the research: Professor Mats Brannstrom's team in Sweden, Professor Giuseppe Del Priore's team in USA and Mr J. Richard Smith's team her in the UK. The key areas of interest, which all feature in the thesis, are listed below. Each is explained in detail in the coming chapters.

a) Surgical techniques, retrieval and grafting

How to achieve an adequate blood supply for the transplanted uterus still presents the biggest challenge in UTx. A number of animal models have been tried, including non-human primate [66,67], sheep [68,69], rat [68,70,71], and mouse.[72] The UK team's (*Smith JR et al*) previous experience regarding this question initially focused on uterine artery anastomosis, then a microvascular anastomotic model and finally 'the large-vessel patch technique'.

Small and large animal models have demonstrated that current surgical techniques used during UTx do not seem to interfere with normal uterine function. This is important as both uterine size and blood flow undergo substantial changes during pregnancy.[73] However, because of high rates of vascular thrombosis in transplant models, it is important to ensure that the vascular anastomotic sites and uterine structural support are improved for better adequacy.

b) Ischaemia-reperfusion injury

A transplanted graft is at risk of injury to its parenchyma, both short- and long-term, from ischaemia and reperfusion. Actual injury, known as ischaemia-reperfusion injury,[74,75] is strongly linked to: (a) delayed graft function[76] and post-transplantation perfusion leading to post-transplant capillary no-reflow, leukocytic inflammation, and persistent tissue hypoxia;[4,77] (b) increased frequency of

rejection, and (c) enhanced acute and chronic rejection.[78-81] These mechanisms eventually lead to graft failure.[82-84] During the ischaemic period, the uterus lacks circulation, and at reperfusion, it is exposed to a 'blood flow rush' after extended ischaemia. Two ischaemic periods (organ retrieval and vascular anastomosis) and one cold ischaemic period (organ storage) have been defined. The first warm ischaemic period occurs during organ retrieval. It commences at a point when the vessels supplying the graft are clamped and lasts until the graft is flushed with a cold preservation solution. At that point, the cold ischaemic period begins. The second warm ischaemic period starts when the graft is retrieved form the preservation solution and ends when the vascular anastomoses are established. It is thought to be less harmful, because of a gradual rise in organ temperature and also partial protection offered by a preservation solution in which the graft is placed.[80,84,85] Pathophysiology of the ischaemic-reperfusion injury involves a number of different mechanisms, already described in literature. They include vasoconstriction,[75] oxidative stress,[86,87] cellular damage (complement, cytokines, leukocytes)[88,89] and eventual cell death.[4,90,91] In addition, the normal immunological process invoked by antigen presenting cells, brings about acute and chronic rejection.[4,84]

The main strategy of reducing ischaemia-preservation injury involves the use of a cold preservation solution, made up of intra- or extracellular-like electrolyte compositions. The vascular system of the graft as well as the graft itself is flushed with the solution, which besides electrolytes also contains additives such as colloids, antioxidants, metabolic precursors, chelators of calcium and iron, as well as vasodilators.[84,92,93] The cold preservation solution decreases tissue damage by simply slowing down the metabolic rate of the graft. With every 10ºC that are lowered, metabolism declines by about 50%. The period during which a graft may be kept in a cold reservation solution varies with different organs. Maximum recommended times are around six hours for the heart and 36 hours for the kidney.

The method of separating the potentially harmful effects of ischaemia-reperfusion and surgical trauma from the degenerative process of rejection needs further developing. One of the solutions is to use auto-/syn-geneic transplant models to exclude rejection.

c) Uterine perfusion

The possible injury that may be brought on by ischaemia and reperfusion requires a method of assessing uterine perfusion, both intra and post-operatively and comparing those values to pre-transplant levels. Various methods exist, both invasive and non-invasive, ranging in their degree of accuracy. A need exists for a technique which will be able to assess the level of ischaemia and reperfusion injury and provide related diagnostic information during the intra-operative and immediate post-operative periods, as well as prior to possible pregnancy. No such technique exists.

d) Immunology of UTx

The immune response of a host towards a uterine graft can be just as deleterious as the host response towards other transplanted organs. The mechanisms are revised here. Rejection can be defined as destruction of the donor graft by the host's immune response, activated against the graft's alloantigens because of a difference in the genetic make-up of the two individuals. As outlined in an extensive review article by *Nankivell and Alexander* in the *New England Journal of Medicine*, rejection is classified according to: a) time (post-transplant) when rejection occurs: hyperacute (minutes), acute (days to weeks), late acute (after 3 months), or chronic (months to years); b) histological changes (cellular-interstitial, vascular, antibody-endothelial); c) physiological function; d) adaptive or innate immune system response.[94] Pregnancy in solid organ transplant recipients has not changed this generally observed pattern and timing of rejection.

Hyperacute rejection occurs almost immediately after the donor graft is perfused within the recipient's body. It is a peri-operative complication, where the diagnosis is clinical with macroscopic evidence revealing the extent and speed of rejection. Microscopically, host antibodies, activated by the complement cascade, attack the HLA antigens expressed on the graft endothelium. UTx hyperacute rejection would be expected to have the same natural history and diagnosis as seen in other transplanted organs.

Acute rejection usually begins one week after transplantation but can occur months to years after transplantation, with the risk highest in the first three months. It is caused by a Human Leukocyte Antigen (HLA) mismatch, a risk factor which is impossible to completely remove as HLA genes are found in all cells of the body. The HLA system is present in the human uterus, endometrium and fetal trophoblast. Its role has been described throughout human pregnancy. Acute rejection episodes are either antibody-mediated or, more commonly, due to T-cell activation. If the response to the episode is prompt, graft failure will rarely occur. Antibody-mediated acute rejection is triggered by the membrane-attack complex (C5b–C9), which causes localized endothelial necrosis and apoptosis and therefore, a rapid graft dysfunction due to inflammation. In a kidney allograft, histopathology usually reveals microthrombi, with haemorrhage and arterial-wall necrosis and infarction.[95] T cell–mediated rejection, as described by *Issa et al* in their review is initiated when foreign donor HLA alloantigens found on the surface of cells in the graft, are presented to the recipient T cells by both the recipient (indirect) and donor (direct) APCs (dendritic cells, macrophages, B cells)[96] which bind to those antigens and subsequently migrate to the lymph organs in order to locate the T cells.[97]

Three T cell subtypes have been extensively described in literature: CD4+ T cells (T helper cells, Th1, Th2, Th17), CD8+ T cells (T killer cells) and regulatory T (Treg) cells.[98] With regard to their roles, CD4+ cells initiate both a humoral response via cytokines interleukin-4, interleukin-5, and interleukin-13, as well as a CD8+-led cellular response, via interferon-γ and interleukin-2.[99] The latter T cell subset is responsible for the cytotoxic graft lysis. The former subset is also a prime 'mover' in a delayed hypersensitivity reaction which causes increased vascular permeability, local accumulation of lymphocytes and macrophages and therefore microvascular injury. Together these three effects represent an important mechanism in graft rejection and eventual destruction. T cell function has also been described in everything from early implantation to inflammatory pre-term labour.

Chronic rejection occurs months to years after transplantation because of an accumulation of various assaults by the host immune system over time. These assaults are mediated by host antibodies and

lymphocytes which manage to eventually wear down the immunosuppression administered to the patient. Of course long term chronic rejection will not be an issue for UTx recipients. UTx is envisaged as a temporary graft that will be removed after Caesarean birth or a period of infertility despite UTx.

Tremendous advances have been made in the field of solid-organ transplantation, which has led to greater benefit and increased survival in patients with pathologies that were once deemed terminal. A key discovery was the introduction of CsA, a calcineurin (protein phosphatise) inhibitor, in the 1980s. Along with tacrolimus, also a calcineurin inhibitor, it exerts its action by blocking the phosphatase activity of calcineurin, which then leads to an inhibition of lymphocytic interleukin-2 production. Therefore, interleukin-2-dependent T-cell activation is depressed.[100,101] Quality of life issues depend greatly on the magnitude of the immunosuppressant 'burden', mainly the type, dose, frequency of intake, time period during which they must be taken and the potential side-effects. Relevant to this research and to obstetrics and gynaecology as a whole, effects of immunosuppressants on fertility and fetal outcomes are also of great interest. However these issues have had to be balanced against the risk of graft rejection, which can occur at any time if the immunosuppressant regime is altered. Therefore, research has attempted to decrease the immunosuppressant 'burden' to a level which will not result in rejection, yet improve the patient's quality of life. Common approaches have included avoidance of steroids and reduced exposure to calcineurin inhibitors.

e) Pregnancy following UTx

Pregnancy has now been achieved in auto-, syn-, and allogeneic models, both small and large animals, and after both embryo transfer as well as natural conception. To date, there have been no reports of pregnancies in primate models, both human and non-human. It is also important to establish whether pregnancy, once achieved, and subsequent offspring are affected negatively in any way by a combination of immunosuppression, adequate uterine perfusion or uterine graft position.[4]

Literature Review

(a) Small-Animal Model

Surgical Techniques: Uterine retrieval and harvest

Techniques for uterine retrieval have been developed in several small-animal models to ensure a safe procedure for a human living donor. Initial experiments and operations were therefore performed in small animal models, with results and observations subsequently applied on large-animal models, where the anatomy, vasculature and size of the organs closely resemble those of humans. Uterine retrieval is a technically demanding procedure involving complicated retroperitoneal dissection of the pelvic vasculature and ureters. The technique varies depending on size/anatomy of the uterus and vasculature. Donors are sacrificed in small animal models since the uterine vessels are too small to be dissected and therefore, the vascular segments of the graft contain either the common iliac vessels (**Figure C**) [68,69] or the abdominal aorta (AA) and vena cava (IVC).[102]

Initial UTx endeavours in the small animal model involved rodents. Various ways of ensuring adequate uterine perfusion post-UTx were attempted (for example, including ovarian vessels in the graft) but the idea remained the same: the vascular anastomosis which would lead to adequate perfusion would be AA-AA and IVC-IVC or common iliac to common iliac. The uterus in rodents consists of a left and right horn. Retrieval involved the either one or both horns, and a macrovascular patch. The 'patch' is made up of the infra-renal aorta, IVC, common and internal iliac vessels and the uterine arterial and venous tree. If only one horn is retrieved, the patch would only include the aforementioned vessels from that side i.e. left or right. On occasion, the ligation may occur at the commencement of the common iliacs and therefore would not involve the AA and IVC. Together with one or both horns, the patch *en bloc* is successfully harvested intact as the large vessel patch and graft.

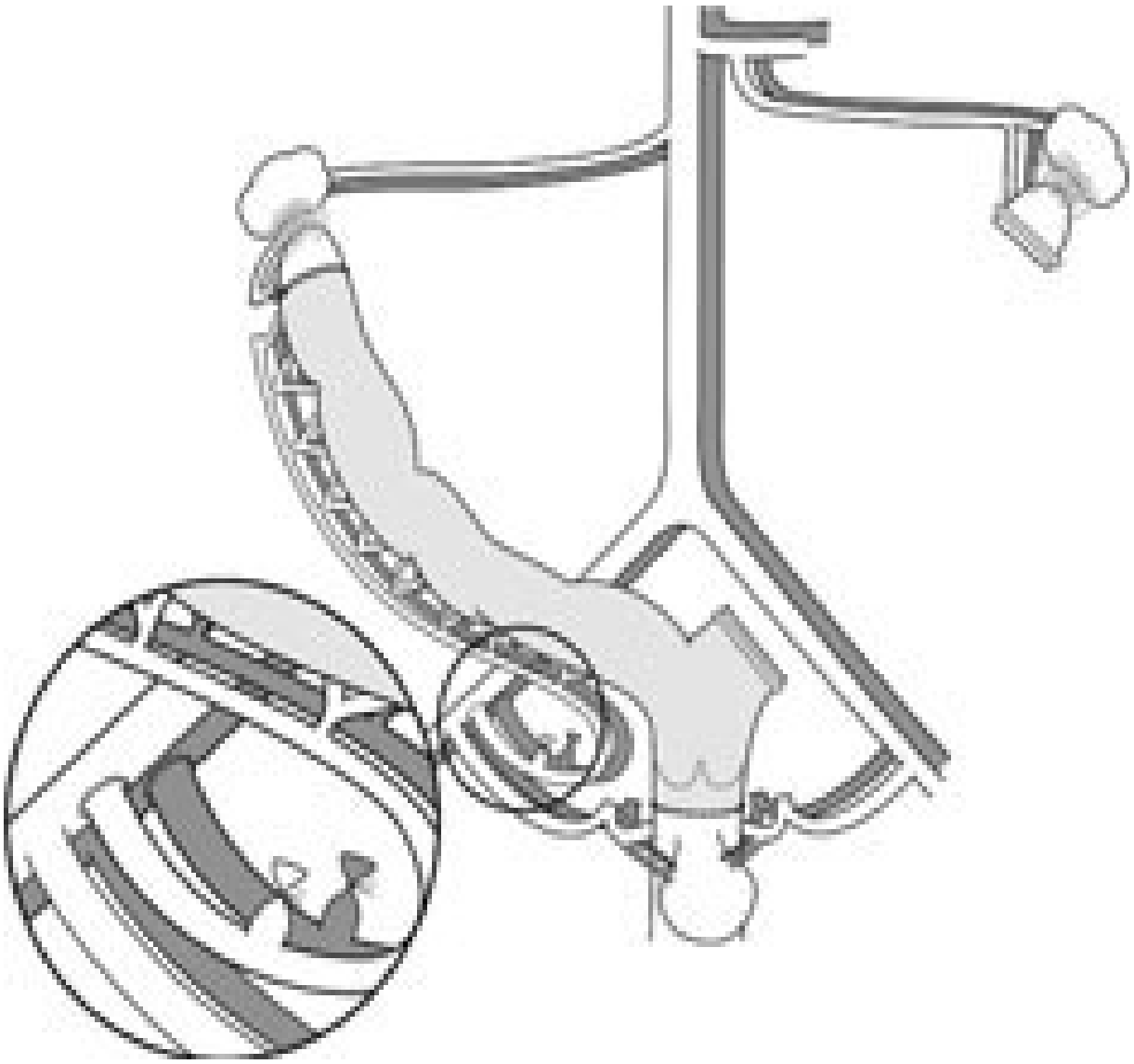


Figure C End-to-side vascular anastomosis between the common iliac vessels of the recipient and the donor graft in a rat UTx model. The vagina of the recipient and the vaginal rim of the graft are connected and the horn is attached to the utero-tubal junction (courtesy of *Hanafy et al*).[80]

The idea of using a patch had evolved as a result of difficulties in ensuring adequate perfusion of the graft in question when using smaller vessels for anastomosis. The uterine vessels in the mouse and rat are too small for dissection and anastomosis; therefore, the parametria, containing these vessels, are preserved up to the internal iliac vessels. *Lee et al*, in their review of replantation and transplantation of reproductive organs in rodents, were the first authors to use a macrovascular patch in an animal model of UTx. More importantly, they correctly hypothesized that a full understanding and

acquisition of microsurgical techniques and anatomy of the reproductive system would eventually lead to successful allogeneic transplantation of genital organs in both males (penis/testis) and females (uterus/ovary).[70] Their attempts in female rats focused on *en bloc* (vagino-)utero-tubal-ovarian allo- and syn-transplantation with an aorto-vena cava patch. Total number of transplants was satisfactory (n=44); 24 were syngeneic and 20 were allogeneic. All lumbar branches of the aorta, iliolumbar vessels, branches of the uterine arteries and veins at the level of the vagina, and iliac branches below the uterine vessels were ligated and cut. The AA and IVC were clamped about 1cm above the ovarian artery origin. They were therefore prepared to a level superior to the origins of the ovarian arteries and left renal vein and inferior to the origin of the internal iliac vessels. The vagina was cut at the level of the upper two-thirds. Following clamping of the recipient IVC and AA, routine end-to-side AA-AA, IVC-IVC anastomoses were performed using 9-0 microsutures. No apparent differences were seen between the syngeneic and allogeneic transplants on day one. By day five, moderate haemorrhagic oedema was noticed with some loss of mesosalpingeal vascular pattern, and by day 10, it was present in all allo-recipients suggesting rejection. However, the adequacy of the surgical model was supported by the syn-recipients which demonstrated reperfusion and revascularization as well as normal mesosalpingeal vascular arrangements. This persisted until sacrifice at day 20 or three months (whichever came first) with the only abnormality on histopathology being abundant suture granulomas at the vaginal anastomotic sites but normal vaginal mucosa. The ovarian follicles and stroma were normal. Furthermore, recipient survival was 100%.[70] As on original concept regarding uterine perfusion, the *en bloc* graft which includes a macrovascular patch proved promising and effective. It would have been ideal if the functionality of the graft was assessed (i.e. pregnancy attempts), and an attempt to suppress the acute rejection response.

Since the above publication, the aorto-vena cava patch has been used by other teams. *Jiga et al* describe a series of 15 whole-uterus-and-ovaries rat allotransplants.[71] However, in this case, the transplantation was heterotopic with the AA and IVC anastomosed to the femoral vessels. The smaller diameter of the femoral vessels in comparison to the AA and IVC, as well as the allogeneic nature of the transplant most likely led to failure in 74% of the grafts at 72 hours. Early thrombosis, starting

from the capillary bed and progressing towards the main feeding pedicles, was demonstrated.[71] This model is not advisable as in addition, to the high likelihood of thrombosis of the femoral vessels, it also creates an obvious risk of ischaemia to the hind limbs.

The next set of experiments to advance the techniques relevant to UTx were designed and performed by Professor Brannstrom's team in Sweden. They were all methodical, appropriately planned, with each experiment an extension of the previous. Importantly all the experiments were novel in what they were trying to achieve. Also, they all had in common a single aspect: to further evaluate the principles of a macrovascular patch. *Wranning et al* developed and evaluated the model with the aim

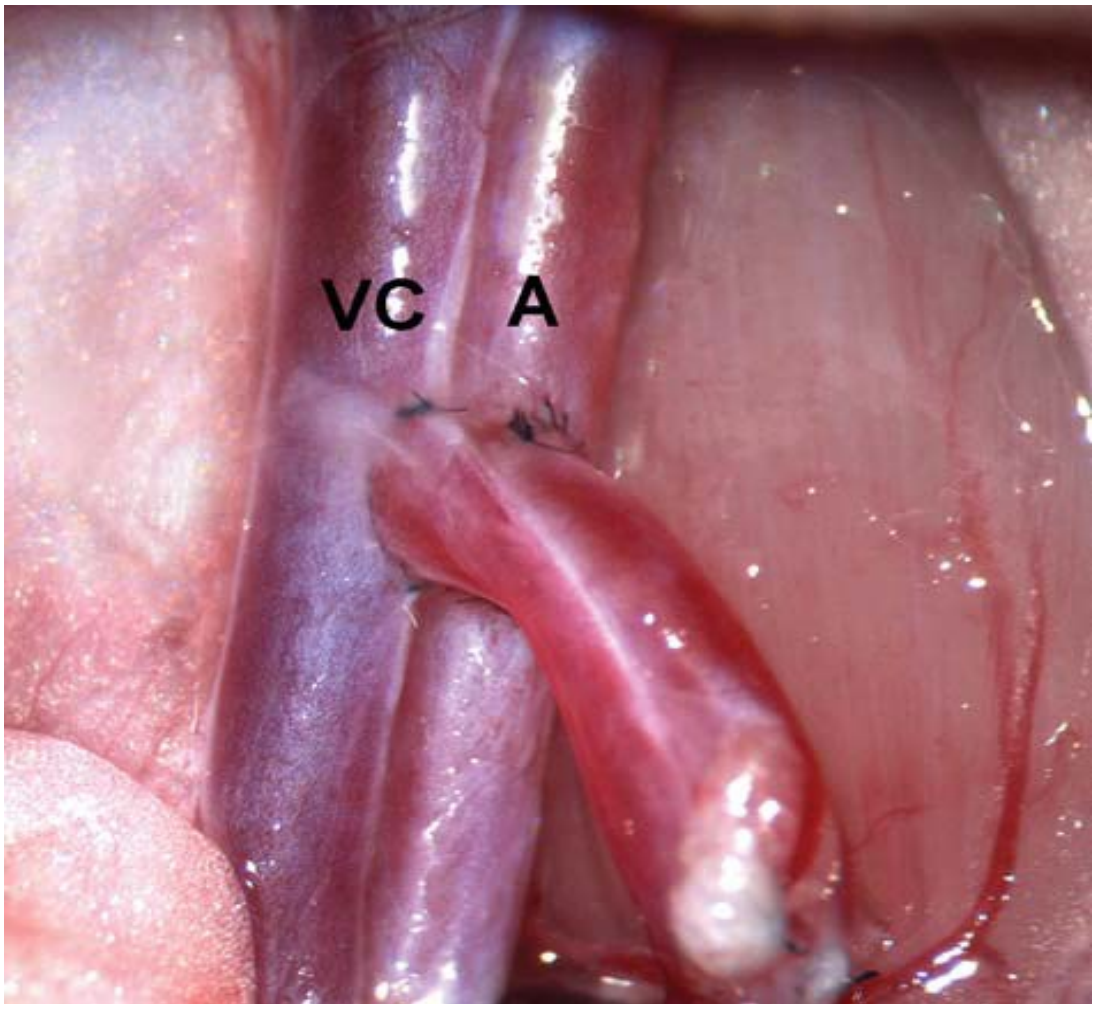


Figure D Close-up view of end-to-side vascular anastomoses between the common iliac artery and vein of a rat to the vena cava (VC) and the aorta (A) of the recipient rat.[68]

of reproducing and using it in future UTx-related studies. Adult, virgin female rats of inbred Lewis strain were used as donors and recipients. Therefore, transplants were performed between syngeneic animals where rejection is absent and immunosuppressive treatment is not required. Hence, any damage is directly attributable to the surgical process and post-operative inflammation, and not secondary to a rejection process. The UTx graft involved only one uterine horn, the common uterine part, the cervix and a vaginal rim.[68] The right uterine artery and vein were dissected cranially to the common iliac vessel, with ligation of all other branches. The vascular graft was transplanted by anastomosing the right common iliac artery and vein of the graft end-to-side (**Figure D**) to the AA and IVC.[68] The transplants were performed by two individuals with no previous microsurgical training with a recording of their learning curves. There was no removal of the native uterus, with it being left *in situ*. The transplanted uterus was then placed in a heterotopic position with the vaginal rim of the graft externalised and connected to a cutaneous stoma. When transplant survival exceeded 70% for both surgeons, 15 animals were transplanted and grafted uteri were evaluated at day(s) 1, 7 and 21 by assessment of morphology and recording of infiltrating neutrophilic granulocytes. Promisingly for UTx in general, both surgeons gained the necessary microsurgical skills needed to achieve above 70% transplant survival at a similar rate. The duration of uterus retrieval decreased from 90 to 60 minutes after 30 operations. The signs of post-operative inflammation on day one after transplantation were minor and further reduced at later time points. Survival transplant was high (70-80%), which together with the healing of the grafted horn demonstrated a fitting functionality of the surgical method. Thrombosis was most likely caused by a sub-optimal technique for the venous anastomosis, since the team found narrowing of the vein at the anastomosis site.[68]

This model has now been validated and modified by subsequent transplantations. The main modification was at the anastomotic level with subsequent anastomosis occurring between common iliac vessels (**Figure C**). This has led to pregnancy in both syngeneic [103] and allogeneic models.[104] The team's initial rodent model for UTx research was in the mouse.[69] The mice chosen were F1-hybrids of inbred C57BL/6xCBA/ca (B6 CBAF1) mice of 6–8 weeks of age (n=42). The right uterine horn, common uterine cavity, cervix and a vaginal rim were first isolated using microsurgical techniques

which preserved the vascular supply from the aorta through the right uterine artery, as well as the venous drainage back to the IVC. So that the recipient animal could be prepared, the organ was put on ice. Xylocaine and heparinised saline were used to expand the vessels and prevent thrombosis. The recipient's uterus was left intact and remained *in situ.* This was followed by transplantation to the left side of the abdomen, with vascular anastomosis to the donor mouse. Here, as a result of the small diameter of the iliac vessels, the AA and IVC of the graft were anastomosed end-to-side to the AA and IVC of the recipient animal with 11-0 sutures. It is worth noting the diameter of the vessels: only 0.7 and 1.5mm respectively. The duration of uterus retrieval decreased with time from 60 to 45 min with increased experience. Despite the difficulty of dealing with anastomosis of vessels with such small diameters, the overall survival rate of the recipient animals increased from 38% for the first 21 animals to 71% for the second 21 animals. The respective percentages for the proportion of viable grafts, as confirmed by normal blood flow (or at least similar to that of the native uterus) and histology showing normal morphology was 25% and 87%. 90% of the grafts exhibited normal blood flow on post-operative days 15-30. Light and electron microscopy confirmed normal structure in both native and graft uteri. Obviously, the gold standard test for the functionality of the transplanted uterus is its ability to implant transferred embryos and subsequently carry a pregnancy to term. In one of the transplanted mice (eight weeks old), six blastocysts were transferred to the uterine-transplanted mouse one week post-UTx. Three blastocysts were placed in the native uterus and three blastocysts were placed in the graft, with needle piercing the uterine wall in both cases. Three and one normal fetuses were seen in the native and transplanted uterus respectively. In the transplanted uterus, one absorbed pregnancy was seen. Thus, this is first report of a successful UTx with proven functionality in the mouse.[69] Prior to that, no record in literature exists whereby the function of a cross-transplanted uterus had been assessed.

An extension of the above experiment involved a further 19 syngeneic uterine transplants. The transplants were heterotopic; therefore, the native uteri were not removed. The vascular anastomosis was end-to-side aorto-aortic and cavo-caval. This time however the cervix was externalised with a formation of cervico-cutaneous stoma. This was done to ensure adequate mucus and fluid drainage

from the transplanted graft and thus, ensure implantation. The study was well designed, with controls used (no transplant, normal mice). A considerable improvement was seen with regards to complication/failure rate during the surgeries. Out of 19 transplanted uterus recipients, 12 had viable grafts (a satisfactory number) and were used as embryo recipients, with eight becoming pregnant (study 5 deals in greater detail with fertility aspects following UTx in a small animal model). The set of live births reported are a first set of such cases following cross-UTx.[105]

Both experiments demonstrated the need to attempt the same type of transplant in an allogeneic model. Also, they do not hide from the technical difficulties with regards to microsurgical techniques in achieving adequate anastomosis. A steep learning curve for the team in question is almost a necessary step towards successful UTx.

The small animal model applied in this thesis was the rabbit model. There is very limited literature on UTx in rabbits. *Confino et al* described the first experiment involving a rabbit UTx model. It is an original experiment outlining the surgical retrieval of the rabbit uterus and its non-vascular transplantation to the surface of the broad ligament with sutures.[106] A number of different study groups were formed: a) total uterine horn autotransplants (n=4); b) supracervical uterine horn autotransplants (n=4); supracervical unilateral uterine horn cross-transplants (n=10) with the last six cases subjected to immunosuppression with CsA. The first group had comprised results secondary to direct spread from a non-sterile vagina. All cases revealed tissue necrosis following a month post-operatively. In the second group, only two cases were followed up for at least month. One horn was well preserved whereas the other had necrosed. In the final group, the recipients which did not receive immunosuppression showed total rejection. Pelvic abscesses were seen in 75% of recipients that did. Despite the lack of big numbers within each group, the experiment is well thought-out with a reason behind the design of each group. It also highlights the disadvantages brought on by the use of immunosuppression. However, their conclusion is that non-vascular transplantation is an appropriate method is not supported by the data.[106]

Sieunarine et al used a rabbit cadaver to study the feasibility of the macrovascular patch including the AA and IVC, discussed in length above.[107] The infra-renal aorta, IVC, common and internal iliac vessels and the uterine arterial and venous tree together with the uterus en bloc were successfully harvested intact as a large vessel patch and graft in a single New Zealand white rabbit. The ovaries and its vascular pedicle were not included as part of the *en bloc* harvest because the diameter of the ovarian vessels (less than 1mm) is too small for feasible anastomosis. Also, this would not be attempted in a human. The exercise proved useful to the team in testing the feasibility of the aortic/vena caval macrovascular patch, with respect to the anatomy and surgical vascular dissection. Ideally, more than one rabbit cadaver would have been used but it was suitable as a preliminary experiment. The vascular tree in a rabbit is much more vascular than in the other rodent models. Therefore, the key feature of a rabbit macrovascular patch dissection is the care that must be taken to ensure the inclusion of the entire uterine vascular tree, especially the lateral and inferior pelvic branches, without accidental transection which would compromise the whole transplantation process. The option of using the IVC as a valid venous patch is adequate, yet it will remain a challenge to both dissect and anastomose to the recipient IVC.[107]

The next experiment by the same team attempted to test the use of this macrovascular patch in a series of six uterine allotransplants.[102] Again, the uterus and its blood supply *en bloc* were successfully harvested with an aortic-caval macrovascular patch from six rabbit donors. The rabbits used were standard New Zealand whites weighing between 3 and 4.5kg and of age 6-12 months. After one hour of cold ischemic storage, the uterine allograft was transplanted to six recipients using an aortic-aortal cavo-caval end-to-side anastomosis as practised in the experiments described above. An attempt was made to prevent both venous thrombo-embolism with administration of subcutaneous heparin. Dose used was 250IU and was given to the donor and recipient pre-operatively to prevent thrombosis. The same dose was given twice daily post-operatively. There was no mention however as to why those particular doses were chosen. All six rabbit recipients surgically survived the procedure. Immediate post-operative recovery was satisfactory in all six recipients. Demise was for the following reasons: three recipients developed non-resolving post-operative paraplegia and were killed on days two, three

and four post-operatively. Limb paraplegia was as a result of poor positioning of the hind legs and thus insufficient support to the spinal curvature in the supine position for a prolonged operative time. It was independent of surgical technique. Two recipients died unexpectedly on days two and three post-operatively. Post-mortem demonstrated a pulmonary embolus in both case. The final rabbit died of an intra-peritoneal haemorrhage, as a result of poor haemostasis over the left horn anastomosis. Importantly for the macrovascular patch concept, all recipient uteri (horns and cavity) appeared viable with no evidence of graft vessel thromboses and thus patency of all graft vessels. The aorto-aortic and cavo-caval anastomotic sites showed no suggestion of any bleeding or leakage, even in the final recipient. The authors believed that the experiment proved the feasibility of allo-UTx using a macrovascular patch, in both anatomical and surgical terms. However, this may be perhaps a little pre-emptive for two reasons. First longest survival was four days post-operatively - therefore, no long term survival. Second, the vascular anastomosis part of the surgery has a tremendously steep learning curve, with success dependent on the skill and experience of the surgeon. In this case, the IVC and aorta anastomotic times varied considerably.[102] Despite this the potential of a macrovascular model has been further enhanced - it has been attempted in a third small-animal model and it does work in the immediate post-operative period.

The above experiment was repeated by the team, involving five allo-transplants in a rabbit model.[108] The aim was to see if: (i) a large vessel aortocaval vascular patch technique may bring about long-term graft survival after allogeneic uterine transplantation (UTx) in a rabbit model; and (ii) fertility can be achieved following natural mating post-allogeneic UTx. The technique was the same as described above. Natural mating was attempted if long-term survival had been achieved. The main outcome measures were: (i) long-term recipient survival; (ii) long-term adequate uterine perfusion; and (iii) successful pregnancy post-UTx. All five recipient animals survived the surgery with satisfactory immediate postoperative recovery. Recipients 1, 2 and 4 died within the first 4 postoperative days. Both long-term survivors failed to conceive following introduction of a proven male breeder despite evidence of mating. Necropsy at 9 and 11 months showed a lack of patency of uterine cornua at the point of anastomosis, albeit a small uterus in recipient 3 and a reddish brown

amorphous material at the site of the transplanted uterus in recipient 5. This further demonstrated the feasibility of uterine allotransplantation using a macrovascular patch technique, but could not demonstrate conception because of blocked cornua. To address this, the authors suitably proposed using embryo transfer techniques in order to achieve conception.[108]

Ischaemia-reperfusion injury

A number of different preservation solutions have been used in the three small-animal models; murine, rat and rabbit. A small number of studies has looked at the length of cold ischaemia that will still lead to a functionally and morphologically adequate uterine graft. *Lee et al*, as mentioned above, first discussed a microsurgical model involving a macrovascular patch to ensure adequate uterine graft perfusion. However, they do not report on the type of preservation solution used or on any ischaemic conditions. Despite this, it is reassuring to read that all their syngeneic grafts (n=24) demonstrated revascularisation for the first three months.[70] It is worth reiterating that in a syngeneic model, thrombosis secondary to an immunological rejection reaction is not possible. Any damage is therefore as a result of surgical performance, ischaemia-reperfusion injury or post-operative inflammation. In the other rat syngeneic model, the uterus was flushed *in situ* with 2ml of cold heparinised Ringer Acetate supplemented with xylocaine and then 1ml of Perfadex.[68] Cold ischemia and second warm ischemia lasted 60 and 90 minutes, respectively. The graft survival was 80% (n=12), with good healing shown as assessed by gross appearance and histology at different post-operative time points. Only mild oedema and neutrophil influx were noted.

A syngeneic murine model was subjected to two different solutions by *Racho El-Akouri et al*.[69,84] In the first example, the uterus was gently flushed to gently flush the specimen with 0.4-0.6ml of ice-cold saline supplemented with heparin sulfate and xylocaine to expand vessels and prevent thrombosis. As is customary, one could see whether the donor uterus was properly perfused by evaluating that the uterus blanched and that clear liquid flowed through the incision of the IVC. The donor uterus was then stored in the ice-cold saline. The second half of the recipient cohort

demonstrated survival results of 71% and graft preservation of 87%, with average cold ischaemic times of about 35 minutes and second warm ischaemic time of 50 minutes. However, the study was not systematic in that it did not assess cold ischaemic preservation strategically. No time intervals were used to assess what may be the maximum amount of time that a graft should be preserved in cold solution between donor retrieval and a recipient transplant. The second example did try to answer this question. It also involved flushing with ice-cold saline supplemented with heparin sulphate and xylocaine to empty the organ from blood. However this time a proper preservation solution (UW solution) was used for further flushing and subsequent graft immersion in the solution at 4°C for 24-72 hours prior to transplantation.[84] Per usual, graft viability was assessed by evaluation of morphology and contractility *in vitro*, which after 24, 48 and 72 hours of storage in UW solution revealed a relatively high tolerance of the murine uterus to cold ischaemic preservation. Normal morphology was seen at 24 hours, with minimal degenerative changes seen after 48 hours. Both spontaneous and stimulated contractions using prostaglandin were witnessed after 24 and 48 hours. At two weeks following the transplant, blood flow and morphology were normal, with no necrosis in only those uteri that were preserved for only 24 hours. This two week 'rest' period is advisable; it allows for graft and recipient recovery from the effects of inflammatory trauma brought on by the surgery. The functionality of these 24 hour preserved uteri was further demonstrated when five out of the six mice developed pregnancies after embryo transfer, with offspring showing normal weight and growth trajectory.[84] This study was the first to demonstrate that a transplanted uterus may carry a pregnancy despite undergoing 24 hours of cold ischaemia when preserved in UW solution.

In the rat utero-tubal-ovarian allotransplant model described by *Jiga et al*, the chosen flushing fluid was Ringer solution again.[71] Cold ischaemia time did not differ greatly to previous attempts (~30 minutes), whereas the second warm ischaemia period lasted around 60 minutes. As this was an allotransplant, 76% of the grafts were thrombosed at 72 hours as a result of acute rejection. Again this study did not try to answer the question as to what is the maximum cold ischaemic time possible, and the allo-nature of the transplant gives us an obvious cause of the thrombosis.

It was the Brannstrom team that again attempted to quantify specifically the relationship between ischaemia and uterine graft damage.[109] This time their focus was on the second warm ischaemia period. This period is likely to be time consuming, involving pelvic vascular anastomosis. The predicted number of such anastomoses is four, two on each side. Therefore how the uterine graft responds during a warm ischaemic period that may be lengthy if the duration of achieving adequate anastomosis is prolonged is of interest. The aim of the study was to assess the viability of the transplanted rat uterus after exposure to the second warm ischaemic period, in order to mimic a time frame likely to occur in a human situation following UTx.[109] There is no record in literature of an experimental UTx study trying to analyse the consequences of warm ischaemia on graft survival. The surgery was performed as described previously.[69,84,103-105] Pseudopregnant rats were randomly allocated into two intervention groups: a standardized syngeneic UTx procedure (control; $n = 10$) with a minimized warm ischaemic period and a modified UTx protocol with a four hour extended period of warm ischaemia ($n = 10$).The graft was flushed through the common iliac artery with 2ml of cold (4°C) Perfadex preservation solution, supplemented with xylocaine and heparin, followed by storage in the Perfadex solution. After flushing, the graft was initially kept in cold (4◦C) Perfadex during preparation of the recipient. Cold ischaemia time was 120 minutes in both groups. As predicted, the group undergoing four hours of warm ischaemia demonstrated obvious signs of necrosis in five of 10 animals compared with only one of 10 in the control group (examinations were performed on days 3 and 6). The same pattern was demonstrated when evaluating gross morphology scores, with the control group faring much better. Surgical findings (colour, consistency, texture, oedema, patency, myometrial bleeding, adhesions, and laparotomy scar) correlated with the above histopathological findings at inspections three and six days after surgery. Both the gross morphology and histopathological variables (the presence of neutrophils, oedema, vasoconstriction, thrombosis, loss of endometrial/glandular integrity, haemorrhage and necrosis) were measured using two different validated scales. One can see how the avoidance of long warm ischaemic periods above five hours is a requirement as otherwise it can have a detrimental effect on the survival of the transplanted uteri. An interesting fact is that once you excluded the grafts that had necrosed by day three, the remaining 'warm ischaemia' had a similar total score on day six to the control group. This may be an important

finding, whereby those grafts which survive the initial post-ischaemic period should normalize morphologically, with functionality still to be tested.[109]

The two UTx studies in a rabbit model did not focus on the relationship between the level of ischaemia-reperfusion injury and graft functionality. However the preservation solution is described in both. *Confino et al* performed a non-vascular autoUTx in the rabbit where the graft was washed *ex vivo* in 37°C lactated Ringers solution.[106] Cold ischaemic and warm ischaemic times were not reported. Around 75% of the autotransplanted organs were preserved. No scale was used to differentiate between the deleterious effects of ischaemia and reperfusion, and acute rejection.[106] *Sieunarine et al* stored the graft for 1 hour in the transplant storage solution, Celsior, between 2°C and 8°C while the recipient doe was being prepared.[102] In all six recipients, histological analyses showed viable transplanted uterine graft tissue throughout with patency of the graft vessels. However, the rabbits survived between 1-4 days only and any extrapolation beyond would not be accurate. Furthermore, no validated scale was used to try and assess the level of viability of the graft. In addition, this was an allogeneic transplant, and again, there is no attempt to measure whether any graft tissue has been rejected and whether any damage is as a result of ischaemia or rejection.[102]

The major limitation of all the above studies is the modest sample sizes. Also only two studies looked at the area of ischaemia-reperfusion specifically. However it must be acknowledged that UTx is a time-consuming and exhausting procedure both with regards to planning and performing. Also, differences do exist between the human uterus and that of the mouse, rat, or rabbit, a fact which should be highlighted prior to extrapolating any of these results to possible human attempts. Later chapters will discuss the effects of ischaemia and reperfusion in large animal and human models.

Immunology of UTx

The process of rejection in allogeneic UTx was first described by two studies in 1969.[59,110] UTx was performed in dogs in both instances. Two further sets of experiments carried out a year later looked at

the use of azathioprine and cortisone to mediate graft rejection (in dogs).[53,58] Rejection occurred in all four cases, both with vascular [53,59,110,111] and avascular UTx.[58] Endometrium was the primary layer to fail. Classic rejection signs were described i.e. haemorrhage, patchy necrosis, intravascular thrombosis and features of inflammation: oedema, erythema, and increased temperature reported on the fourth post-transplant day. The therapeutic potential of azathioprine and steroid was also demonstrated with the level of rejection appearing non-uniform and seen even on the 49th post-operative day.[59] In 1971, *Scott et al* also reported allogeneic UTx in a non-human primate. The grafts were received following sub-total hysterectomy and full rejection was reported on day 14 post-transplant.[67]

The first UTx study to be published after the implementation of CsA in practice was by *Confino et al* in 1986.[106] As described above, this was the first example of allogeneic UTx in a rabbit model. The originality of the experiment comes from its attempt to transplant the uterus using a non-vascular method. Exploration of the rejection response to the uterine graft was not the aim. However the results were reported and are useful. Ten allogeneic transplants were performed, of which only six recipients were treated with CsA. Four were not given any immunosuppression and all revealed rejection, supporting the already established hypothesis that a uterine graft behaves in a similar manner to the kidney, liver and cardiac grafts. The uterus was transplanted in an avascular manner, with both the endometrial and myometrial layers surviving for 4 weeks on CsA (the only immunosuppressant administered). However, out of the four recipients that survived a month of follow-up, 75% cases developed pelvic abscesses. This negative aspect of using immunosuppression with UTx was rightly reported by *Confino et al* as a concept that will need addressing in future allogeneic models.[106,112]

Jiga et al were the first team to actively attempt to study graft rejection following allogeneic UTx.[71] Allogeneic utero-tubal-ovarian transplantation was performed in 15 rats and the time course of graft rejection was studied. After 72 h, the anastomosis sites were thrombosed in 76% of the grafts and the uteri were necrotic. Early thrombosis was seen in the capillary bed, which then progressed to occlude the larger vessels as well as the anastomotic sties. Such a pattern of rapid failure may be a result of

both a poor anastomotic site and an uncontrolled rejection response, a conclusion elaborated on by the authors. The other two examples of allogeneic *en bloc* uterus-oviduct-ovarian transplantation also stated that severe rejection occurred within 72 hours.[70,113] The importance of studying the early rejection response mechanism, which leads to early endothelial destruction and thrombosis of capillary vessels, was also made.

Brannstrom's group has performed a set of highly useful experiments using the murine model in attempt to characterise the rejection response. The mouse is a suitable research model for immunological studies because of the ease of creating inbred and gene deleted strains. In 2006, the team published the results of a study aiming to characterize the time course of rejection within the first 28 post-operative days in a fully allogeneic mouse UTx model. Mice of the BALB/c strain were used as donors and mice of the C57BL/6 strain as recipients. C57BL/6 mice were the recipients because this strain is the background strain used in the development of most knockout and transgenic mice. The MHC complex of the mouse, H-2, has its genetic loci on chromosome 17 and is homologous to HLA. The H-2 haplotypes of BALB/c and C57BL/6 mice are termed H-2d and H-2b respectively and demonstrate dissimilarity in all alleles except one. Therefore, the equivalent human model for the allogeneicity of this model would be a MHC mismatch at both major and minor loci between the human donor and recipient. The surgical methodology has already been described above.[69,84] The cervix of the graft was exteriorized through the abdomen and sutured to the skin to create a stoma, thus allowing for a non-invasive assessment of the viability of the graft and level of rejection using biopsies. The rejection response was monitored using the following parameters: uterine blood flow using laser Doppler flowmetry, macroscopic and histopathological appearance of the graft, and density of T lymphocytes during the post-operative period using immunohistochemistry. 32 out of 63 recipients had to be excluded as a result of post-operative complications, thus further supporting the conclusions from previous studies relating to the technical difficulties of the macrovascular patch model faced by the surgeons. Hyperacute rejection was not the cause of immediate death since a similar portion of mice with unsuccessful grafts was seen in the previous syngeneic study.[69] Early signs of rejection were noticed from day 2 to day 5. Minimal inflammatory

changes were seen from day 5 and massive inflammation and thus severe rejection were seen from day 10 to day 15. By day 28, the graft was fully rejected with massive necrosis and fibrosis visualised. Microscopically, the density of CD3+ T-cells was increased in the grafted uterus from day 2 in the myometrium and from day 5 in the endometrium. Blood flow in the grafted uteri was reduced from day 15. The study on the whole is the first such attempt at characterising the rejection response post-UTx. This has been done rigorously, using an appropriate number of mice (n=29). A murine study model for the characterisation of rejection post-UTx has also been defined. The conclusions are appropriate.

In addition, Brannstrom's work demonstrated that exploring immunological issues specific to UTx is more valuable than simply relying on evidence from renal, liver and cardiac transplants. This is for two reasons. First, each specific types of transplanted organ will affect the rejection process differently. Results reported by *El-Akouri et al* that correspond to severe acute rejection are similar to those of cardiac allografts at days 8-15, suggesting an immunological similarity between the heart and uterus.[53,114,115] Therefore, rejection mechanisms as shown by markers in the recipients' blood recipients are different depending on the type of tissue/organ involved. In addition to the similar timing of the rejection phases as with the murine cardiac transplants, no spontaneous acceptance of the uterus was seen. With transplanted murine liver and kidneys, the opposite occurs - spontaneous acceptance is the case.[114] Second, from the histological examination, endometrial and myometrial layers differ significantly during the rejection process. Endometrial rejection appears to be more rapid than myometrial, with earlier lymphocyte infiltration seen in the endometrial glands. Furthermore, the myometrium had the greatest resistance to rejection and retained its tissue structure for the longest time.[116] This pattern was already seen in the canine allo-UTx experiments described previously.[53,58] This can be explained by the 'Dr Jekyll/Mr Hyde' role played by the endometrium: it defends the uterus from various pathogens while simultaneously, offering immunological tolerance to semen and the early embryo/fetus. Therefore, the uterus is a very different organ immunologically when compared to other transplant organs.

The same group followed up the above study by evaluating the effect of CsA in a mouse UTx model, the first time such a study had been designed. The effect of CsA varies with different species despite the conservation of the CsA signalling pathway as a result of evolution.[117,118] The dose required varies as does the immunosuppressive duration period based on the type of allograft. For example, immunosuppression can be completely halted in certain liver transplants which is not the case with kidney or heart transplants.[117] Therefore the aim of this original study was to evaluate the effect of the immunosuppressant CsA on the rejection of the allotransplanted uterus in the mouse. CsA was neatly administered to three groups of uterine graft recipient mice: (a) positive control - no CsA (n=5); (b) dose: 10mg/kg (n=5); and (c) dose: 20mg/kg (n=5). Three recipients were syngeneic and acted as negative controls. The numbers in each group may be a low, but two different doses are compared. Also, two control groups were used; one to ensure a rejection response does occur between the donor and recipient strains and the other group to ensure that no rejection occurs if the mice are syngeneic. Both actions (dose and controls) are to be commended. All mice were culled on day 10 post-UTx. Grafted uteri were examined for gross appearance and biopsies were taken for histology and quantification of T cells using immunohistochemistry. Necrosis was seen in all recipient mice, but to a lesser extent in the CsA mice as expected. Those mice administered the higher dose (20mg/kg) showed the least rejection, with suppression of apoptosis and inflammation. The authors' conclusion that CsA, at does given, delayed the progress of rejection of grafted uteri but suppressed T cell infiltration insufficiently is suitable. Furthermore, the morphological and histological signs of graft rejection were not reduced suggesting that if long-term graft survival is to be achieved, either CsA should be administered at higher doses or it should be used in combination with other immunosuppressive agents. This point was correctly highlighted by the authors. With respect to the cellular response, CD4+ count was increased in all groups without a significant difference between them. However, the CD8+ count was higher in the mice receiving CsA as opposed to the non-CsA recipient mice. This observation is important and the authors' do provide an appropriate explanation. They hypothesised that the CD8+ increase was secondary to a CsA-dependent depression of activation induced cell death (AICD) of the activated and alloreactive CD8+ T cells. *Cebecauer et al* supported this theory in their study whereby soluble major histocompatibility complex–peptide

complexes failed to do induce rapid AICD in the presence of CsA, even though the opposite was seen *in vitro*.[117,119] The authors also suggested that those CD8+ cells that avoided cell death need to be investigated in order to see whether they have retained their cytotoxic activity or instead produce cytokines that promote rejection by modes other than CD8+ cytotoxicity.[117]

The next experiment by the Brannstrom group continued this pattern of original and highly enlightening work. Following on from the above two studies that assessed the time course of acute rejection as well as the correct CsA dose, this study aimed to explore exactly which leucocyte populations were responsible for bringing about the rejection response to an allogeneic uterus transplant.[120] By characterising the rejection response in this manner, the immunosuppression regimen could then be specifically tailored to control rejection. Again, as the two studies above, this was an original study, answering a question that had not been looked at before: 'What is the character of the acute rejection response?'. The surgery has been described before.[69] The graft was placed in a heterotopic position, with the native uterus left *in situ* to act as a control. The plan to remove both uteri on post-UTx day 2 (n=5), day 5 (n=5) and day 10 (n=6) is fitting. Immunohistochemistry was carried out for neutrophilic granulocytes, macrophages, cytotoxic CD8+ T-cells, CD4+ T-helper cells and B-cells. The authors explained how this allogeneic model corresponds to a human situation. First, the MHC of the mouse H-2 is homologous to HLA in the human.[121] Second, the two strains used here showed dissimilarity in all alleles except that of the q locus, which is the human equivalent of difference at both major and minor MHC loci.[120] The decision to evaluate cellular density in both myometrium and endometrium is useful, because of the immunological differences of the two layers. The endometrium is more susceptible to infection as it has external contact and also needs to accept a semi-allogeneic embryo. The main conclusion that predominately neutrophils, macrophages, and CD^+4/CD^+8 T cells were responsible for acute rejection of the allogeneic murine uterine transplant between days two and day five appears correct. Primarily, the main leucocytes that invade the graft are macrophages and neutrophils. The former are found in the myometrium at day 2 and in the endometrium at day five, and the latter at day five in the myometrium. CD8+ cells are also present at day two and increase in density by day five; this day two to five increase is not reproduced with CD4+

cells. T cells are the most abundant of the leucocytes. CD19+ B-cell density was low throughout, with no time-dependent changes in the myometrial or endometrial layers.[120] Therefore, as with the previous study, the results here suggest that a potential immunosuppressive regimen for UTx should include both a calcineurin inhibitor and a steroid. The authors definitely offered both a time- and site-specific description of the infiltration of particular leukocyte subtypes during acute rejection of a mouse uterine allograft.

Two very recent studies by the Brannstrom team focused specifically and in more detail on the effects of calcineurin inhibitors on the acute rejection response.[122,123] This was an extension on the above study focusing on CsA,[122] with an additional experiment broadening the knowledge of UTx-related research to include other types of calcineurin inhibitors (tacrolimus).[123] Both studies were enlightening, as they reported on the cytokine effect during acute rejection, a previously unknown area of uterine graft rejection. Furthermore, the chosen model was a rat model. In the CsA study, a total of 23 rats received a uterine graft, transplanted in an orthotopic position. In 16 recipients, the graft appeared healthy at inspection on the second post-operative day. The recipients were given CsA (10 mg/kg) once daily or no CsA until they were sacrificed on day seven post-UTx. As usual with the Brannstrom group, controls were used - syngeneic transplanted Lewis rats. In addition to immunohistochemistry and light microscopy, the following additional markers were measured: mRNA levels of the implantation/inflammation-related markers leukaemia inhibitory factor (LIF), galectin-1, CD200, interleukin (IL)-1a, and IL-15. To date, no such study has been attempted. As with the previous CsA study, CsA decreased the level of inflammation initially as well as the CD8+ count and mRNA levels of IL-1. The mRNA levels of galectin-1 and IL-15, two agents responsible for immunosuppression and immunotolerance respectively were lower in the non-CsA-treated mice. There was no difference between the groups concerning mRNA levels of CD200, or LIF, the latter result of importance as the LIF is essential for maintaining pregnancy. The authors' conclusion that CsA administration suppresses rejection-associated graft inflammation is apt. The real benefit of this study is however the results and therefore conclusions relevant to other immunological mediators. CsA appeared to normalise the expression of some mediators that may have further influence on

allograft survival but also on pregnancy potential which is of particular value.[122] The other study had the same aims (investigate whether tacrolimus therapy may prevent rejection and gage the effects on the uterine expression of markers related to inflammation and implantation) and also used a rat model.[123] The only difference was in the calcineurin inhibit used - tacrolimus. The rats were culled on day 14. The tacrolimus effect was synonymous with CsA, with rejection and T-lymphocyte infiltration suppressed and expression of IL-1 and IP-10 normalized. The author also correctly recognised the need for future tests to focus on the effect of calcineurin inhibitors on pregnancy in an allogeneic uterine graft.[123]

Deciding on the correct type of immunosuppressant also depends on the effect of that immunosuppressant on the fertility hormones (oestrogen and progesterone) which are required to sustain and 'nourish' a pregnancy. CsA may be a powerful and effective drug to inhibit T cell mediated immunity and thus the rejection response, but may be deleterious to both the mother (renal toxicity) and the fetus. Furthermore, immunosuppression in general has a potent role in ensuring graft survival yet it can cause greater morbidity for the recipient in question. The down-regulation of the immune system renders that individual at risk of Epstein-Barr Virus-induced lymphoid tumours and opportunistic fungal, viral and bacterial infections.

The next study by the Brannstrom group was again 'a literature- first' study assessing reproductive health in animals exposed to immunosuppressive drugs (CsA) *in utero*.[124] This is because little is known about the effects of intrauterine exposure to CsA in the growing and adult offspring, and in particular how CsA may affect reproduction. The study itself was well thought out with careful analysis of blood CsA concentrations, pregnancy variables (implantation and resorption rates, and fetal weights) and fertility potential of the offspring. To assess the latter, both female and male offspring were mated to partners that did not encounter CsA *in utero*, with subsequent analysis of pregnancy outcomes. Female mice were exposed to different doses of CsA from one week before mating and throughout pregnancy [0 mg/kg/day - n=10; 10 mg/kg/day (n=10), 20mg/kg/day - n=10) and 30mg/kg/day - n=9). The choice of CsA dose is sound, with a good variation and follows on from

previous studies. Number of mice per group is also suitable. The animals were euthanized on day 18 (gestational age for a mouse is around 21 days). CsA blood concentrations (before and during pregnancy), mating frequency, implantation rate, intrauterine deaths of fetuses and fetal weights were recorded on the day of euthanasia. Their results demonstrated that direct maternal and *in utero* exposure to high doses of CsA reduced implantation rates, fetal survival and adolescent growth but importantly did not affect offspring fertility. Offspring birthweight was also negatively affected. This negative correlation was dose-dependent, with higher doses exhibiting a more pronounced negative effect. These results are of particular relevance to UTx research as immunosuppression will be the key factor to implantation; however one criticism of the study is that it did not involve a UTx group in addition to the other groups.

The reduced implantation rates have been reported in previous similar experiments involving murine models,[125,126] suggesting that CsA can affect the endometrium or decidua in a negative way. In fact there is evidence which reveals a possible link between CsA and suboptimal ovarian function, as a result of hindrance to sex steroid secretion at the time of ovulation.[127,128] This in turn leads to issues with conception which tends to occur at the time of ovulation. The endometrial layer depends on the sex steroids for its maintenance and if their secretion is impaired, the lining is also dysfunctional. Conception, as a result, is impaired, an effect even more pronounced as a result of the negative role CsA plays in both T cell and NK cell activation.[129]

Interestingly, when organ transplantation is performed after long-term organ failure and is deemed successful, it results in the restoration of normal endocrine function.[127] Ovarian and menstrual function is usually restored in both renal and liver transplanted women within an average of six to ten months.[130-132] The hypothalamus-pituitary-ovary axis in a transplant patient is thought to be functioning as normally as it would in a non-transplant female, with serum gonadotrophin and prolactin levels very similar in the two groups.[129] Higher levels of oestradiol are reported in renal graft patients in comparison to non-transplant women.[133] This bodes well for a potential future uterine graft recipient who would need an unaltered and healthy reproductive function to sustain the pregnancy.

There are reports of the decreased levels of libido secondary to a fall in testosterone secondary to steroid use, but this would be inconsequential to an established *in utero* pregnancy.[129]

Pregnancy

The holy grail of UTx is a live birth of a healthy neonate following allogeneic UTx (**Figure E**). Embryo transfer techniques should be used to transport the already formed embryos into the uterine graft after transplantation in order to exclude any events which might compromise the graft. For example, it is likely that natural conception would not occur as the Fallopian tubes would not be functional and if it did occur, it could lead to intra-/extra-uterine ectopic and tubal miscarriages. Some propose that the Fallopian tubes should not be transplanted as part of an UTx.[65,104]

The Brannstrom group has described five examples (three murine and two rat models) of successful pregnancy post-UTx. The first example in 2002 consisted of a syngeneic transplantation of the right uterine horn and cervix, with the native uterus remaining in situ and subsequent successful fertility following blastocyst transfer and implantation in a single mouse model. The mouse was 8 weeks old, with the embryo transfer occurring one week post-UTx. The protocol for blastocyst production and retrieval was sound, with six blastocysts retrieved from a naturally mated B6 CBAF1 female. This was done only following the visualisation of a vaginal plug. Three blastocysts were transferred to the native uterus and three were placed into the transplanted graft, using the protocol described by *Hogan et al*.[134] The technique was a standard embryo transfer technique involving the insertion of a glass pipette through a hole in the uterine wall made by a 30 gauge needle. This was done following the 'preparation' of the female i.e. three days after the mouse was mated with a male. Number of fetuses present were counted ten days following embryo transfer when the mouse was killed. The native uterus had three fetuses present whereas the transplanted uterus had one fetus present, and one absorbed pregnancy. Fetal weights were similar to that of fetuses in the native uterus and controls. This proved, for the first time, the concept of pregnancy following a uterine transplant in a non-autotransplant model. The only other pregnancy recorded following UTx was in an autotransplant

model, where three out of 18 pregnancies were achieved in female dogs.[56] An attempt in only onemouse means that it is difficult to make any conclusions relevant to the actual fetuses, but it does allow one to base further experiments specifically focusing on the pregnancy aspect of UTx. The aim

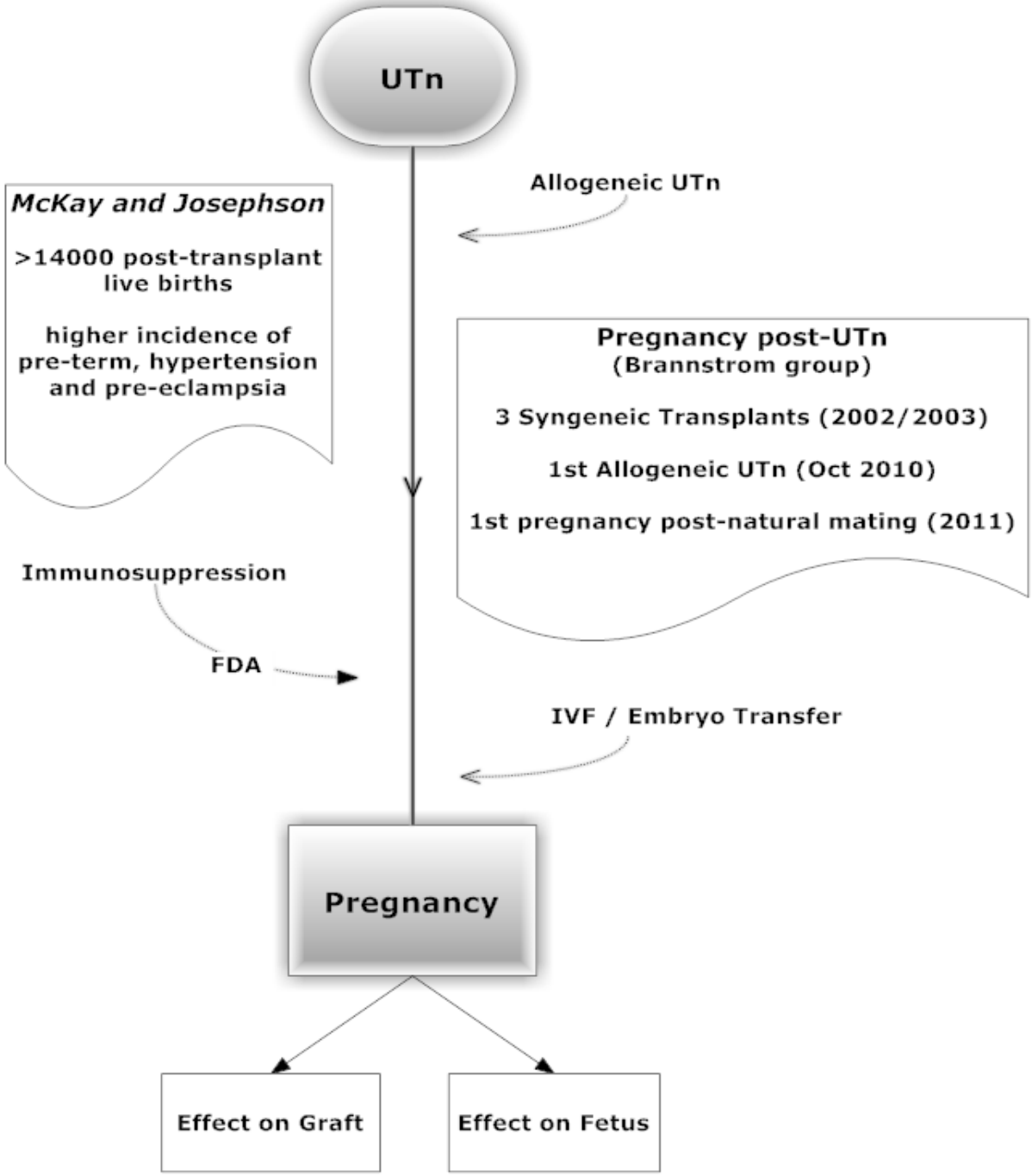


Figure E Pregnancy and Uterine Transplantation.[2] The goal of UTx is to achieve pregnancy and the birth of a healthy term infant. With regards to pregnancy following organ transplantation, evidence has slowly been gathered (McKay and Josephson). Pregnancy following allogeneic UTx exists only in small animal models. The effect of pregnancy on both the fetus and the mother following UTx is an unknown quantity.

of the authors was to assess the macrovascular anastomotic model previously described; the pregnancy experiment was a secondary aim.[69]

The second example of pregnancy post-UTx was in fact a pregnancy-related study.[105] It was an extension of the above study with the focus on pregnancy-related factors. UTx was performed in a syngeneic mouse model that used a macrovascular anastomosis concept to ensure uterine perfusion. Embryos were transferred 1-3 weeks post-UTx in both the transplanted and native uteri. The embryo preparation was no different to standard mouse protocols.[134] Embryos were also transferred to a control group of non-transplanted 8- to 10-week-old B6CBAF1 female mice. Three to six blastocysts were transferred to the lumen of each of the grafted and native uteri, and non-transplanted uteri on days 3-4 of the pseudo-pregnancy. The method was the same as described in the above study.[69,105] Three sets of experiments were then performed. In the first set, pregnancies were followed up to day 18 of pregnancy. In the second set, pregnancy was continued until term as the aim was to assess post-natal development of the offspring. The offspring were divided into three groups, offspring from: (a) non-transplanted mice (control group); (b) native uteri of UTx-mice; and (c) grafted uteri of UTx-mice. The weight of the offspring was then continuously measured until eight weeks of age. The third and final set of experiments looked at the fertility of the offspring. 19 mice received a transplanted uterus. Out of those, 12 had viable grafts and thus received an embryo. Pregnancies were found in nine of 12 native uteri of transplanted mice and in those nine, eight also had fetuses within the transplanted uterus. With respect to he controls, eight out of 13 mice were pregnant. The numbers for resorbed pregnancies were respectively, one, two and four. The lengths and weights of the fetuses, as well as weights of the placentae, were similar in pregnancies in native, grafted and the 'control' uteri. The placental aspect should be highlighted as the data may be extrapolated to the human model. Major similarities exist between mice and humans (and primates in general) with regard to placentation.[105,135] For example, the terminal blood space is not lined with endothelial cells, but with trophoblast cells. Also, retrograde intra-arterial trophoblast migration occurs in both species. In this experiment, UTx did not compromise placental development. Offspring from all three groups followed a similar post-natal growth pattern until eight weeks of age. They were then tested to see if

they could continue the progeny. Both female and male offspring proved to be fertile and the pups reported birth weights within the normal range. Therefore, the experiments demonstrated similar implantation and pregnancy rates, as well as birthweight and growth curves (8 weeks) between the native and transplanted (syngeneic) uteri. The second-generation offspring from transplanted animals were all fertile with normal birth weights.[105] The study was an extension of their previous study [69] which further confirmed the concept that a UTx model which is not an autotransplant, in this case a syngeneic model, can result in pregnancy. It is also the first study in which the ability of a transplanted uterus to implant inserted blastocysts and to carry a pregnancy has been truly evaluated. It was reassuring to have a control group with results that demonstrated no significant difference to both the 'native uteri' and 'grafted uteri'. The offspring exhibited normal patterns of development and weight increase and future fertility was not compromised.[105]

In the same year, *Racho El-Akouri et al* performed studies assessing the effect of cold ischaemic storage on post-UTx pregnancy.[84] Using the same methodology as described in the experiments above, embryos were transferred into six mice (two weeks post-UTx) where the transplanted uteri (right uterine horn and cervix) had been preserved for 24 hours in University of Wisconsin solution. Five recipient mice fell pregnant and all five gave rise to offspring from the transplanted uterus. In total 25 pups were live born; 20 survived through the first day. Eight pups born from native and seven from graft uteri were weighed at regular intervals up to 8 weeks of age. The two groups of animals followed normal weights and growth trajectories.[84] Again, this study did not differ from the two above. The model was murine and syngeneic, the graft was made up of a right uterine horn and cervix and anastomosis was macrovascular involving an AA- IVC end-to-side anastomosis. The numbers were smaller than the previous study (six recipients received an embryo transfer only)[105] but here we had the novel factor of a pregnancy attempt post-storage in a preservation solution for 24 hours. Therefore, achieving pregnancy in this case was a further proof of functionality of a grafted uterus, following both a transplant operation and subsequent 24 hour storage in cold ischaemic conditions. Both experiments mimic what would happen in a human attempt.

However, the above murine experiments still differ somewhat from a human model. Three areas in particular are immediately obvious: gestational lengths, no immunological rejection mechanisms to consider and anatomical differences. The following experiments tried to counter some of these differences. In October of 2010, the Brannstrom group described the first recorded pregnancy in an allogeneic uterine transplant (rat model). Three groups underwent a UTx-related procedure. The anastomosis was at the common iliac level. With regards to fertility, all groups were exposed to fertile males during one oestrous cycle between 35 and 38 days after surgery. The first group consisted of eight female rats that had undergone UTx and were immunosuppressed by tacrolimus. The second group underwent removal of only one uterine horn, with the five rats treated with an identical tacrolimus protocol, whereas the third group did not receive tacrolimus (n=6). Caesarean section was performed 17 days after initiation of the mating period. The pregnancy rates in groups I-III were 5/8, 3/5, and 5/6 respectively. Median number of fetuses for a pregnant rat in each group were 1 [0–3], 1 [1–5], and 3 [1–4] respectively. Four of the five pregnant mice that underwent UTx (group I) demonstrated resorption. Again like with the previous experiments, it would be easy to criticise certain aspects of the study. No control group is mentioned (i.e. non-transplanted rats with and without tacrolimus), the rejection response is not evaluated, with no assessment of the level of acute rejection of the graft, and there is no mention of whether the level of mismatch between donor and recipient was carried out. However, these were not the aims. The authors wanted to advance the concept of pregnancy post-UTx from a syngeneic model onto an allogeneic model and therefore bring the field of UTx closer to what may happen in a human experiment. In this they succeeded. Even though, no live pregnancies were recorded, this experiment is a first time record of a pregnancy after allogeneic UTx. It is therefore a central proof of a concept that a uterus can function following its transplantation from a donor to a recipient and that it can do so under immunosuppression.[104]

Their next experiment was also an advancement on the existing work - again a rat model, but with an attempt to bring about pregnancy following natural mating.[103] Furthermore, the actual transplant was orthotopic for the first time i.e. the native uterus was removed and the graft was placed *in lieu*. Female Lewis rats underwent hysterectomy and received syngeneic uterine transplants (with one horn

removed). The uterine graft was placed in an orthotopic position i.e. in the pelvis with two tissue anastomoses: to the vagina and the upper part of the native uterine horn. The anastomosis was end-to-side between the common iliac vessels of the recipient and the graft. Control rats had only one uterine horn removed. The aim was to compare a number of outcomes: mating and pregnancy frequencies, successful deliveries and offspring weight and development trajectories. 19 out of 27 recipients survived the surgery or the immediate post-operative period. The overall pregnancy rates after introduction to male rats were comparable in UTx (11/19; 58%) and control (12/19; 63%) rats. From the UTx rat, only one rat delivered two healthy male pups. Two other UTx rats gave birth to at least two pups each but committed infanticide prior to the offspring count. The remaining four UTx rats experienced stillbirth, with signs of distress at birth. Dead pups of full-term size were found in both the recipient vagina and in the common cavity and right uterine horn. The weight and development outcomes post-birth for the two surviving male rat pups from the one UTx rat were similar to the control rat offspring (32 live born pups). The main differences between the UTx and control groups were the increased number of resorptions and decreased number of successful deliveries in the former group. Thus, some unknown factor is compromising fetal wellbeing post-UTx, most likely blood flow or denervation. In the control group, no nerve manipulation occurred and there were no issues with labour. Also vaginal scarring at the anastomosis may cause a synthetic obstruction to the labour pathway. Obviously in future models, both animal and human, elective Caesarean section would remove these obstacles. In conclusion, this model may be reproduced to assess fertility outcomes following allogeneic UTx and ensuing natural mating. The study demonstrates that conception by natural mating, and subsequent pregnancy, and birth of live and healthy offspring is possible with orthotopic, syngeneic UTx.[103]

Similarly to other solid-organ transplants, the most pronounced effect of the graft on the fetus will be indirect, i.e. via the immunosuppressants that must be administered to the recipient. The idea of using immunosuppressive drugs to curb rejection was first suggested by Sir Peter Brian Medawar while he was working for the National Institute for Medical Research. He made significant in-roads in the 1940s in understanding the mechanism behind rejection. Today, immunosuppressants are routinely

used after transplantation to minimize the risk of rejection. However, long-term use has its drawbacks, mainly the risk of recurrent infections and neoplastic disease, as well as necessary close and meticulous medical care.[136] UTx would be short term i.e. 1-2 years thus avoiding this risk. Once the uterus and immunosuppressants are removed, any associated neoplasm risk should theoretically return to baseline. Many transplant related lymphomas are known to regress after removal of the graft.

Nevertheless, care is taken with the type, dose and monitoring of immunosuppressants prescribed as they can cross the human placental barrier and enter the fetal circulation, possibly affecting the immune system of the fetus.[137] Medications used to suppress immunologic activity include glucocorticoids, nonsteroidal anti-inflammatory drugs (NSAIDs), and the antifolate medication (methotrexate). In addition, cytokine modulators (CsA: CsA), purine-related medication (analogues: azathioprine and 6-mercaptopurine; purine synthesis inhibitor: mycophenolate mofetil), alkylating agents (cyclophosphamide and chlorambucil), biologic response modifiers (TNF antagonists) and intravenous immunoglobulin are used.[138] With pregnant patients, a selected number of available immunosuppressants have been stratified into categories, depending on the level of risk that the immunosuppressant poses on the fetus (**Table B**).

McKay and Josephson have summarised the outcomes of approximately 14000 post-transplantation live births since the first birth to a transplant recipient occurred in 1963.[139] Therefore, the stratification of immunosuppressants is necessary as observations on post-transplant pregnancies indicate a higher incidence of preterm deliveries, low birth weights, and maternal hypertension but no increased rate of structural malformations.[140-143]

As succinctly reported by *Framarino dei Malatesta et al*, hypertension and pre-eclampsia with premature membrane rupture are the main source of preterm delivery and low birth weight.[140,144,145] The pregnancy incidence of these three complications is related to the type of immunosuppressive agents prescribed: hypertension incidence varies between 22% and 29% with corticosteroids,[146] 68%

and 73% with CsA, and 47% and 54% with tacrolimus,[147] pre-eclampsia on the other hand develops in approximately 30% of pregnant kidney or kidney-pancreas recipients, and 25% of liver, heart, or lung recipients.[140,144,148,149]

With respect to the recipient of UTx, immunosuppression would only be a potential risk to her general health as long as the graft is within her pelvis. The plan is that the transplanted uterus will be surgically removed after the patient has completed one pregnancy, thus removing the need for immunosuppressants.

Category	Drug	Animal / Human studies
A	**Paracetamol**	No risk in human studies
B	**Corticosteroids**	No risk in animal studies OR Risk in animal studies but that risk not demonstrated in human studies
C	**Tacrolimus** **Rapamycin** **Cyclosporine A** **Mycophenolate Mofetil**	Fetal risk demonstrated in animal studies BUT no adequate and well-controlled studies in humans. Drugs can be used if potential benefits outweigh risks.
D	**Azathioprine**	Fetal risk demonstrated in human studies. In exceptional circumstances, drugs can be used if potential benefits outweigh risks.

Table B Immunosuppressant risk categories according to US Food and Drug Administration [150]

Nevertheless, we must acknowledge that long-term data on allograft loss, maternal survival after pregnancy (due to an increased risk of viral diseases and neoplasm secondary to immunosuppressants)[151] and offspring outcomes of transplant recipients is rather limited. Despite several reports indicating no increase of congenital anomalies among the offspring of recipients,[140,152] long-term outcomes focusing on physical and mental development, and immunological and oncological pathologies later in life in these offspring is necessary.[150] Unfortunately, currently, only one study has compared pregnancy outcomes in a single group of women before and after transplantation. It concluded that despite an initial increased risk for pre-eclampsia, growth restriction, pre-term birth, and the risk of miscarriage, the odds of these four outcomes were all statistically similar pre- and post-transplantation.[153]

Theoretically, UTx in animal models could be used to further characterize immune related diseases such as recurrent pregnancy loss or immunologic PIH. In these models, an intact gravid uterus would be transplanted into a recipient. Or more likely, a pregnancy would be established after UTx into a recipient with a known disease propensity and studied for induced changes. In this way UTx could isolate 'maternal versus fetal' factors in a particular disease state.

It is important to recognise that unfortunately pregnant transplant recipients cannot wait for long term results to clearly demonstrate superiority of one regimen over the other. This is especially a problem regarding pregnancy outcomes among transplant patients. Regardless if the pregnant transplant recipient has a kidney, heart, lung or uterus, it will be impossible to ensure safety to mother and fetus if the goal is a healthy child that matures to a healthy adult.

(b) Large-Animal Model

Below is a description of the research performed using a porcine model and a sheep model, two of the most commonly used large animals.

Porcine model

As already explained in the chapter on trachelectomy, the development of ART served as a prelude to research into UTx. Initial concerns were about how adequate uterine perfusion may be achieved, whether the answer lay in uterine artery anastomosis and the subsequent function of these vessels in pregnancy. The first UTx related study in a porcine model was performed by Dr J. Richard Smith's (head of UK Uterine Transplantation) team in the UK.[154] It was an original, preliminary study to the possibility of UTx. The aim was not to actually perform a transplant but to see whether division and subsequent re-anastomosis of uterine vessels still allowed for normal uterine perfusion. The decision to attempt conception and pregnancy was suitable. Thus, the uterine arteries in two sows were divided and re-anastomosed. At six weeks, the sows including the control were inseminated. Pregnancy occurred and proceeded uneventfully, and all three sows farrowed normally with average litter sizes. Three months after delivery, the sows were killed, and post-mortem studies were undertaken. Histopathology of the uterine arteries revealed minimal intimal fibrosis across all anastomotic sites, with successful re-anastomoses of the uterine arteries accomplished in both study sows. Thus, uterine artery anastomosis in the porcine model was shown to be feasible with subsequent normal vascular function in pregnancy of the anastomosed vessels. The expected 'hour-glass effect' at the anastomotic site did not compromise uterine function. Obviously, the number of sows used was particularly small (n=2) and this needs to be taken into account when discussing the conclusions.

JR Smith et al next attempted to assess the feasibility of UTx in a porcine model by attempting autotransplantation of the uterine graft.[155] Despite pre-1985 canine studies, as well as post-1985 small animal studies demonstrating that adequate perfusion of the graft could only be achieved via large

vessel anastomoses, the team wished to see whether microvascular anastomoses could work in a porcine and thus large animal model. The vessels in question were uterine. A supracervical hysterectomy was performed on eight sows of proven fertility. After one hour of cold storage in a transplant solution (*ex vivo* for one hour in University of Wisconsin/Celsior solution), the specimen was reintroduced, followed by stepwise vascular re-anastomosis. The use of objective perfusion index measurements to assess uterine perfusion added to the quality of the study. The results suggested adequate uterine perfusion after transplantation. Post-operatively, sow 1 survived to 3 months with no signs of oestrus, and sows 2, 6, and 8 were killed on days 6, 33, and 54, respectively, for pelvic infection. Histopathology of the uterine grafts revealed gradual vessel thromboses suggesting that microvascular re-anastomosis is unsuccessful in auto-UTx (and most likely in allo-UTx) because of gradual vessel thromboses. The advantage of using an autogeneic model was that the effects of immunological rejection could be removed, and therefore any conclusion was specifically related to the surgical techniques and the anastomotic model chosen. In this case, the anastomotic model that should be first choice is a large vessel model. In addition, the porcine model proved to be problematic for the authors as it is highly susceptible to post-operative infection.[155]

The Brannstrom group also carried out a study, which further looked to develop the auto-transplant model in a pig.[156] They added to existing literature by also evaluating the early reperfusion events after short-term cold ischaemia. The bicornuate uterus was dissected and removed from the pelvis, put in a sterile isolation bag and continuously flushed through the arterial cannulas with heparinised ice-cold Ringer Acetate for a time period of between one and two hours. The uterus was then placed in its orthotopic pelvic position and the uterine arteries and veins were anastomosed end-to-end to their origin. Following re-anastomosis, the graft was reperfused for 100 minutes. Blood samples and tissue biopsies were taken in order to monitor reperfusion events and detect of ischaemia-reperfusion injuries. Number of auto-transplants performed was commendable (n=19). A novel criterion was introduced to judge how successful reperfusion of the auto-transplanted uteri was. The following parameters were applied: (i) blanching of the uterus during perfusion for cold ischemia; (ii) the colour-shift during reperfusion from the initial bluish appearance towards a more reddish colour,

typical for well-perfused and oxygenated tissue; (iii) adequate bleeding from the bed of uterine tissue when cut through the myometrium at the end of the experiment; and (iv) blood flow past the venous and arterial anastomosis sites at the end of the experiment by bisection of the vessels. However, only seven grafts could be appropriately flushed and those were kept for cold ischaemia. From these seven, only four grafts were felt to be well reperfused according to the above variables. At least two of these transplants were well re-perfused, with no severe ischaemia-reperfusion injuries following analysis of blood-gas and metabolite parameters and histology. Helpfully for future attempts, the authors described a useful technique alteration whereby the suture placement for the uterine vein anastomosis was changed from continuous to interrupted as the use of the former led to constriction and thrombosis.[156]

The conclusions were well explained and important for future UTx research. First, the authors demonstrated that acceptable reperfusion can be achieved following uterine vessel re-anastomosis. Despite this, the success rate was low (~20%). The primary reasons were the large total size of the pig uterus with long uterine horns (~one metre) and the small size of the vessels available for re-anastomosis, causing most likely thrombotic events within the uterus or markedly reduced flow through the sites of anastomosis. Also hindering the process was the large number of ligations of the internal iliac branches which would have inevitably led to microthromboses. The authors understood that the small calibre of the uterine vessels, and the resulting inadequate blood flow meant that technically, small-vessel anastomosis is not the way forward with regards to graft perfusion and UTx research in general. The answer lay in developing a large-vessel anastomotic model as in the small-animals experiments described in the previous chapter, but not involving the great vessels. Finally, the authors did explain their reasons for choosing a porcine model. First it parallels well human anatomy and physiology. Second, the animal itself is relatively available and there are fewer ethical and economical considerations involved in its use in research compared to the use of rabbits, dogs, sheep or primates. Their final conclusion that the porcine model is a fairly difficult model for further studies on UTx is in total agreement with the Smith team.[156] However, the statement that this was the ‘first

report ever on auto-transplantation of the pig uterus' is false as the actual first study was *Sieunarine et al*, described above.[155]

Avison et al completed an original work and to date the only allogeneic UTx in a porcine model. UTx was heterotopic, using genetically defined mini-pigs.[157] The choice of immunosuppression was appropriate. Tacrolimus acted as an induction agent and was administered intravenously for the first 12 days post-UTx. This was followed by oral CsA and methylprednisolone, which were used as maintenance immunosuppressants, and in higher doses to deal with rejection episodes. The authors detailed how they would monitor the rejection response. First, drug levels were measured by the application of tandem mass spectroscopy on human peripheral blood. Second, the actual graft was assessed by both endoscopy and biopsy. Finally, direct visualisation of the cervix and vaginal vault was made possible via exteriorization as a stoma in the lower right abdominal wall. In total ten transplants were performed, with five animals surviving for 6-12 months post-UTx. The anastomotic model was large-vessel, with an AA-AA and IVC-IVC end-to-side anastomosis. The authors correctly explained why they decided upon this, citing previous thrombosis when using small vessels. However, their decision not to perform a vaginal anastomosis is to be queried as this step should be part of the operation because it provides additional structural support to the graft within the pelvis. A cold preservation solution was used to minimise ischaemia-reperfusion injury. The aorta of the donor was cannulated above the level of the renal arteries for infusion of cold University of Wisconsin solution with the uterus preserved in it until completion of vascular anastomosis in the recipient. The team also defend their decision to perform a heterotopic transplant, citing clinical advantages relevant to avoidance of the pelvis which is frequently the site of pathology, previous surgeries, and radiation. However, an orthotopic transplant should still be the primary choice as the lower pelvis allows for natural growth of the uterus during pregnancy without interference with other organs such as bowel.[157]

The five deaths were caused by pneumonia (n=1), intussusception of the graft (n=1), cardiorespiratory arrest during anaesthesia (n=1), and complications of the stoma (n=2). It is evident that the use of stoma in a porcine model was less than ideal with complications including stoma prolapse and

infection. The authors' conclusion that the immunosuppression regimen proved to be effective in preventing rejection in the uterine transplants for a few weeks after the procedure is suitable. Although it is noted that induction immunosuppression was not sufficient and therefore, additional maintenance immunosuppression was required to maintain a rejection-free state in the transplanted graft. Despite the 50% early mortality rate as well as stoma-related complications, the actual model proved that UTx may be successful when using macrovascular anastomoses as long-term survival was 50%. The conclusion that further studies are required to determine whether pregnancy is possible in allogeneic UTx model is appropriate. Finally, it is worth highlighting that the suitability of the porcine model was again (similarly to *Sieunarine et al* described above)[155] brought into question. The main issue was the high susceptibility of the animal to ascending pelvic infection via the vagina or stomas because of its general state of cleanliness.[157]

Ovine model

A sheep model is particularly appropriate for UTx research and more suitable than a porcine model. Both the body and pelvic size is more equivalent to that of a woman, as well as the size and anatomy of the pelvic vasculature. UTx in a sheep model really commenced together with the main body of UTx-related research following the first human attempt in 2000. There are however reports of auto-UTx in the 1970s involving a heterotopic grafting technique with vascular anastomosis to the carotid artery and jugular vein.[158,159]

The first two studies involving a sheep model was by *Wranning et al* (Brannstrom group).[68] The former involved an autogeneic model, whereby the aim was to study the effects of cold ischaemia and reperfusion after transplantation of the sheep uterus and to compare the preservation solution Perfadex with Ringer's acetate. This was an important study carried out a time when UTx research in large animal models was at its early stage and there was limited data on the effects of cold ischemia and reperfusion injury on the transplanted graft. It is essential to ascertain the time limit of cold ischaemia and the degree of reperfusion injury that takes place after initiating circulation in the recipient. One

uterine horn with the common uterine cavity and cervix of sexually mature ewes was auto-transplanted following one hour of cold ischaemic storage in either Perfadex (n=5) or Ringer's acetate (n=5). Anastomosis was as follows - the utero-ovarian vein and the anterior branch of the iliac artery of the graft were attached end to side to the external iliac vessels with continuous 6-0 polypropylene sutures. Two ewes in each group were excluded as a result of inadequate reperfusion of the uterus after anastomosis. Uterine venous blood was collected and analyzed for several parameters during three hours of reperfusion. The choice of outcomes to be studied was valid, all-encompassing and of diagnostic value. They included: pH, pCO_2, pO_2, and lactate, as well as indicators of oxidative stress, such as semi-dehydroascorbate radicals (ascorbyl radicals), thiobarbituric acid–reactive species (TBARS), and total antioxidant capacity, analyzed in uterine venous blood and plasma. In addition, light microscopy was used to detect histological signs of ischaemia-reperfusion injuries and quantify neutrophils in tissue biopsies. Results showed that storage in Ringer's acetate was more detrimental to the uterine tissue, with a decline in pH and a rise in lactate and pCO_2-pO_2, as well as an elevation of antioxidant capacity, lipid peroxidation, and intensity of ascorbyl radical electron spin resonance signal. Storage with both preservation solutions revealed mild inflammation. The study itself was useful in providing a time limit to cold ischaemic exposure as well as differentiating between two preservation solutions. The authors' conclusion that a cold-ischaemia time of between one and two hours does not induce any immediate severe reperfusion injury in the sheep uterus is appropriate. Also, the use of the more complex and protective buffer Perfadex improved the status of the analyzed parameters, by decreasing oxidative stress and inflammation when compared with the case of Ringer's acetate.[160]

Two further studies by the Brannstrom group were performed on an auto-UTx sheep model. *Dalm-Kahler et al* carried out a study which was an extension of the study describe above by *Wranning et al*,[160] studying the effects of cold ischemia and reperfusion after sheep auto-UTx.[161] The aim of this study was to develop and evaluate auto-UTx in a sheep model, but to an orthotopic position in the pelvis. Thus, the study is not simply a repeat of *Wranning et al* but is in fact the next step. Seven ewes were subjected to laparotomy with the uterus and its vascular supply and drainage being surgically

isolated. The excised uterus was kept *ex vivo* at +4°C in a preservation solution for 60 minutes and then auto-transplanted, single horn, uterine cavity and cervix, with vascular end-to-side anastomoses to the external iliac vessels (**Figure F**). A question arises as to why the authors used Ringers-Acetate when in their previous study, they demonstrated the superiority of Perfadex solution. The assessment of uterine blood reperfusion efficacy had already been evaluated.[160] The same methodology was therefore applied: measurements of pCO_2, pO_2, lactate and pH in uterine venous blood. Uterine contractility and histology were assessed after three hours of reperfusion. The percentage of ewes demonstrating reperfusion was identical to the previous study (71%; 5/7),[160] a statistic which supports the anastomotic model chosen (anastomosis of utero-ovarian vein and the anterior branch of iliac artery in an end-to-side fashion to external iliacs). The authors provided a satisfactory explanation as to why the first uterine grafts did not demonstrate a re-established blood flow after vascular anastomosis. The reason was that heparin was only given at one time-point, about three to four hours before re-initiation of flow. This was too early as the half-life of heparin in the circulation is only about one-two hours and thus the effect would have been sub-optimal at this crucial stage. The pCO_2/pO_2-ratio and the lactate level were initially elevated but decreased and became normal after 60 minutes. Promisingly, visible tissue blood flow and spontaneous uterine contractions were seen after three hours of reperfusion. The latter measurement was novel and had not been attempted in UTx research previously. This is an important finding as the confirmation of uterine contractions points to a non-disrupted inherent neurological function as well as appropriate general signs of functionality. The adequacy of uterine perfusion was further supported by histological analysis which revealed a mild inflammation, but no oedema or stasis. In conclusion, the study further confirms that auto-transplantation of a uterine graft in a sheep model can be successful. Placing the graft in an orthotopic position was a novel attempt in a large animal model which worked well as did the novel vascular connections. Both techniques would be used in a future human model, and the model itself is suitable for future experiments studying long-term uterine viability, contractility and even pregnancy. However, once again the transplantation of just one horn as opposed to both is highlighted. Perhaps, the operation should have mimicked a human attempt as much as possible and therefore both horns should have been transplanted. The authors did not offer an explanation.[161]

Leading on form the last study, the following Brannstrom experiment carried out the next logical step.[162] It investigated the long-term function of a transplanted uterine graft by seeing whether a sheep recipient is able to harbour normal pregnancies after auto-UTx achieved using the above vascular anastomotic techniques. The study differed slightly to the previous in that together with the single uterine horn, both the Fallopian tube and ovary were also retrieved and subsequently auto-transplanted. 14 ewe recipients were used. The anastomotic model involved end-to-side anastomosis of the vessels of the graft to the external iliac vessels. Of the14 autotransplanted ewes, seven were excluded from further experiments due to post-operative complications. Only one recipient suffered from an anastomotic bleed. The seven surviving recipients all had intact ovarian activity and grafts demonstrating normal appearance. Two ewes were examined in more detail two months after surgery,

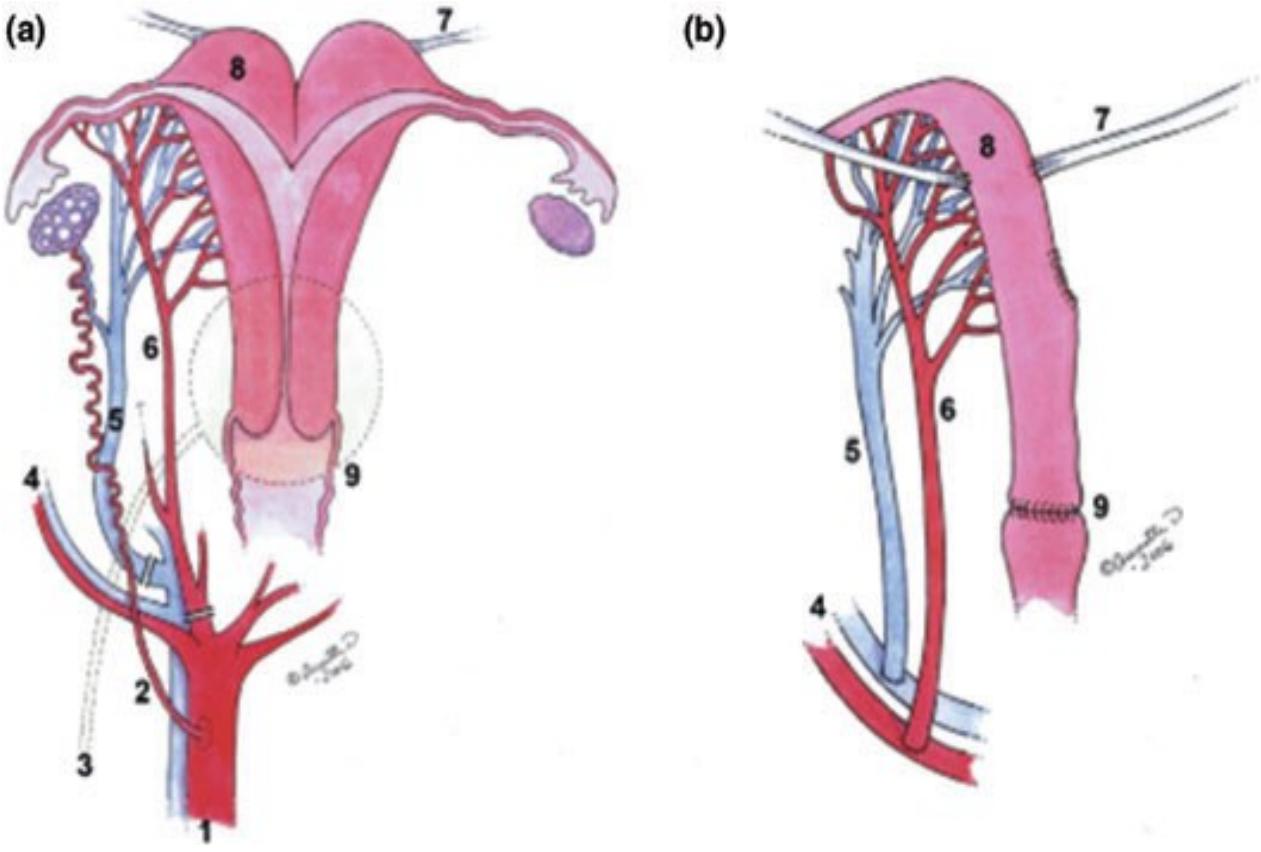


Figure F Schematic drawings of the sheep uterus, adnexae and their vasculature before transplantation (a) and after transplantation (b). (courtesy of *Dahm-Kahler et al*) [161] Sites for division of the arterial supply and venous drainage are indicated by straight double lines in panel (a); 1, aorta; 2, ovarian artery; 3, ureter; 4, external iliac vein and artery; 5, utero-ovarian vein; 6, uterine artery; 7, round ligament; 8, left uterine horn; 9, vagina.

to establish that the procedure had not caused changes that would risk the animals' well-being during the fertility experiments. The remaining five ewes underwent surgical examination and serum progesterone measurements to determine appropriate healing and ovarian activity. Afterwards, five autotransplanted and five control ewes were placed with a ram for mating. The use of control ewes further strengthened the study. All offspring were delivered via Caesarean sections performed before the estimated delivery date and fetal-relevant data were compared. Mating occurred in four of five transplanted ewes and in all five controls with three transplanted and five control ewes conceiving. Torsion of the uterus was observed after spontaneous initiation of labour in one transplanted ewe. All fetuses from transplanted mothers were comparable in size to those of controls. The authors achieved their primary aim which was to investigate whether orthotopic graft transplantation and a new vascular supply affects the fertility potential of the transplanted uterus. They also cleverly avoided problems which may arise from immunological reactions and immunosuppressant drugs by using an autotransplantation model. A number of techniques have now been firmly established: vascular flushing, cold ischaemic preservation of the transplant and the technique and site used for vascular anastomosis. The authors draw attention to the 50% survival rate but rightly point out that five out of the seven euthanized ewes were put down as a result of species-related problems. Also, fertility rate was 60% in the transplanted ewes as opposed to 100% in the controls. One of the reasons may have been that the Fallopian tube and ovary were also transplanted together with the uterus. Therefore, post-UTx inflammation may have led to obstruction and prevention of conception. The current proposed human model does not include the transplantation of a Fallopian tube and ovary so the risk of ectopic pregnancy and obstruction within the Fallopian tube would be avoided. The study is first report to demonstrate fertility and pregnancies going to term after removal of the graft from the abdominal cavity (*Eraslan et al* did not remove the graft)[56] and auto-transplantation of the uterus using a macrovascular anastomotic technique as opposed to an avascular process (dog [53] and rhesus monkey [67]), in a large animal with a pelvis that is comparable in size to a woman's.[162] The plan to use external iliac vessels as recipients of the graft vessels received further support here.

Ramirez et al have published data on allogeneic UTx in a large-animal model.[72] UTx was carried out in a sheep model, with the second 2011 study being to date the only study to validate the purpose of UTx in a large animal model.[163] It demonstrated pregnancy following the transplantation of a uterine graft between two non-related ewes. The initial pilot study was performed in 2008, with the aim of developing a uterine transplant procedure in the sheep model that may be suitable in women suffering from AUFI. The reason for this is that a ewe's pelvis resembles a woman's pelvis with regards to anatomical landmarks and vascular anatomy, with the exception of a bicornuate uterus. UTx was performed through a minilaparotomy incision with the application of a 900-500 modified Mobius retractor device. Ten sheep received allografts. Immunological graft rejection was managed with CsA therapy. Uterine vessels were re-anastomosed in this case series using a continuous end-to-end non-interlocking approach to ensure uterine perfusion. This choice of using uterine vessels which failed in previous porcine models was a risk, and would not have been recommended by the other international teams. It is worth noting that could only be applied in a clinical situation where hysterectomy is performed as part of the procedure in the recipient. Therefore, total abdominal hysterectomy without oophorectomy was performed. The authors conveniently quantified warm and cold ischaemic times. A cold ischaemic time of 45 minutes was recorded. Despite small-vessel anastomoses, complete tissue reperfusion of the graft was surprisingly achieved in all ten animals within 30 seconds after vascular re-anastomosis without evidence of arterial or venous thrombosis. All ten animals were followed-up for six months, which is a suitable period of time to make conclusions related to long-term graft viability. At the end of the follow-up period, hysterectomies were performed and histological analysis revealed viable uterine tissue and vascular patency in six out of the ten uterine allotransplants. Interestingly, the site of uterine vessel re-anastomosis was patent. Neo-vascularization with presence of smooth muscle and glandular endometrial tissue was also demonstrated. The conclusions are valid - the authors have indeed developed a modified procedure that has allowed them to perform successful uterine transplants in the sheep model. The model itself has proved useful especially with its correlation to a woman's pelvis. This is despite using a re-anastomotic model not deemed suitable by other UTx teams. It is also important to recognise that this is the first reported case in the literature

documenting a successful procedure of allo-UTx in the ewe. Finally, the authors do state their desire to demonstrate that a pregnancy can be achieved following allogeneic UTx in a sheep model.[72]

The second study in 2011 was precisely this attempt.[163] It is therefore of particular significance, as it reported the first case of pregnancy following large-animal allogeneic UTx. The number of sheep used was appropriate; allogeneic UTx was performed in 12 sexually mature African sheep. Usefully, a control group was present, consisting of pregnant Romney Marsh sheep with non-transplanted uteri. Outcomes that were measured and compared with the control group were all focused on pregnancy and the fetus: fetal development, uterine and placental histological findings, and blood samples of progeny of the uterine transplant recipient sheep. All animals underwent UTx via a mini-laparotomy incision using a Mobius retractor device. The vascular model was again a bilateral end-to-end uterine artery re-anastomosis, with the same immunosuppression regimen applied but a higher dose of CsA. Four months after the initial UTx, fresh and frozen blastocyst donors were transferred accordingly to the surviving five uterine allografts via a mini-laparotomy incision. Three of these resulted in pregnancies. One was an ectopic gestation, one sheep carried the pregnancy to 105 days, and one delivered a fully developed lamb from the transplanted uterus that was delivered via caesarean section. Neonatal lamb blood gas values and chemistry, gross organ examination, and ventilation and respiratory compliance studies yielded results normal for gestational age. Although a 60% pregnancy rate seems high, the actual live birth rate is 20%, a much lower figure and one which calls for cautious optimism. A criticism of the two studies was that the rejection response was not adequately studied, as well as the level of graft rejection and to what extent the immunosuppressants prevented rejection. However, the knowledge that pregnancy can be carried in an allotransplanted uterus in a large-animal model which resembles the human pelvis closely, with the end result a successful delivery, is of huge value to future research.[163]

The last study to describe uterine allotransplantation in a sheep model was by *Gauthier et al*.[164] Like the previous studies, this was also an original experiment as an aorto-caval patch was applied to ensure uterine perfusion. A standard and appropriate number of recipients (n=10) received a uterine

allograft in an orthotopic position. As mentioned, the anastomotic model was macrovascular with the application of an aorto-caval donor patch on the left external iliac vessel recipient (**Figure G**). The advantage of the model is that it minimises the risk of thrombosis, yet the procedure is only applicable when the organ is recovered from a deceased donor i.e. it is not applicable for all types of donors. The authors explained that in their view a brain-stem dead heart-beating donor would be the better choice, as opposed to a living donor, as the risk of ureteric and vascular injury would be non-existent with the former. This is a significant point and one with which the UK Team is in total agreement.[164]

The other highlight of this study was its secondary aims. They were to assess the practicability of prolonged immunosuppression in ewes and to assess graft integrity by vaginoscopy, magnetic resonance imaging, and second look laparotomy at 6, 8 and 10 weeks. The immunosuppressive protocol was a combination of CsA (10mg/kg/day) and mycophenolic acid (3g/day). The exposure of the graft to the immunosuppressant medication was assessed by measuring the area under the curve of the immunosuppressive plasma concentrations. The graft was flushed through with 1000ml of chilled protective Celsior solution at 100mmHg pressure. Flushing was continued until clear fluid drained from the vena cava. The median times for cold and warm ischemia were acceptable: 95 and 91 minutes respectively. All the vascular anastomoses were patent at the end of the surgery. Two recipients died from graft haemorrhage within six hours of the operation and one died from intestinal obstruction. One recipient expelled the graft from the vagina two months after the operation, with no clinical signs or consequences with regards to the well-being of the graft. Thus, only six recipients were assessed. Cervical biopsies showed signs of necrosis in all six ewes, with thrombosis also seen in all six aortocaval vascular pedicles. Most likely reason for the thrombosis is the pedicle length and non-prevention of graft mobility via pelvic side wall fixation. This probably gave rise to mechanical stress on the vessels, which in turn could have caused thrombosis and subsequent necrosis. The authors acknowledged the major weakness in their study which was the lack of early graft assessment to distinguish whether necrosis was caused by mechanical or rejection-related reasons. is a major weakness. Their follow-up plan to monitor rejection in a future study is apposite; it would include regular biopsy monitoring of the uterine cervix, immunochemistry for neutrophilic granulocytes,

macrophages, cytotoxic CD8+ T cells and CD4+ T-helper cells and an early MRI scan immediately post-UTx.[164]

The authors discussed at length the impact of potential rejection on the graft and how it may have brought about rejection. The immunosuppression doses used were higher than those used in humans which have decreased the impact of acute rejection on renal, hepatic and cardiac transplantation. However, the area under the curve was clearly inferior to those recommended for humans. Usefully, the authors recommend that future studies should look at setting up an optimum UTx sheep model. The studies should assess the relationship between dose and plasma concentration for immunosuppressive drugs in sheep for a particular route of administration. Two ewes had grafts which demonstrated viability, with graft histology revealing endometrial tissue in the two. Peripheral neovascularisation was the explanation as to why perfusion was possible in two grafts despite aorto-caval thrombosis. This is a valid justification and the knowledge that neovascularisation occurs post-UTx is surely promising for future attempts. The authors deemed that ischaemia and reperfusion injury was not the cause of the necrosis as the times for both cold and warm ischaemia were relatively low and the standard preservation solution was applied. The use of MRI to estimate graft viability was a novel technique introduced by the authors that had not been recorded before in a sheep model. The MRI data correlated with the macroscopic observations of the 'second look' laparotomy and appears to be a good easy-to-perform, non-invasive examination for graft estimation. Pelvic MRI could thus serve as a reference examination in post-UTx follow-up, as well as act as a functional reference examination to check vascularisation patency. It is worth highlighting that a lack of control group (syngeneic ewe which underwent the same surgical procedure without any immunosuppressant treatment) was noticeable (acknowledged by the authors). To conclude, this was a welcome addition to the relatively small pool of data on allogeneic UTx in a sheep model, with particular obstacles that need further work highlighted: mobility of the transplant within the pelvis, the length of the vascular pedicle and difficulty in administering an optimal immunosuppressive treatment in ruminants. The potential of MRI to evaluate graft morphology and vascular patency and thus distinguish ischaemic risk from the risk of rejection when used in parallel to biopsy is a novel and important idea.[164]

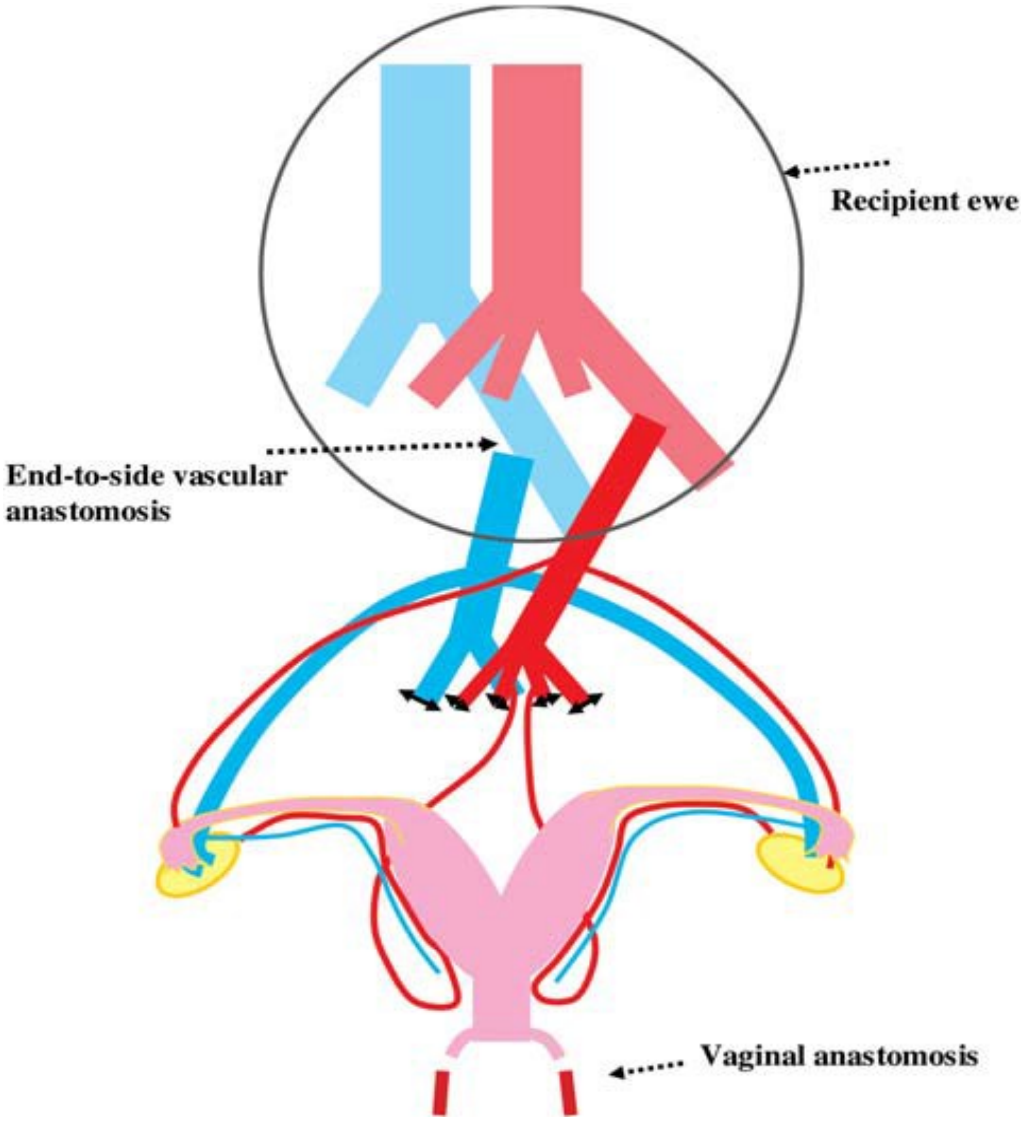


Figure G Orthotopic uterine alloUTx in the ewe. The lower donor aorta and vena cava were anastomosed end-to-side with the recipient's external iliac vessels (courtesy of *Gauthier et al*) [164]

(c) Uterine Perfusion

In current medical practice, tissue pathology is diagnosed by the application of two different processes: volumetric visualisation of tissue and biopsy. The former is a macroscopic process. It includes imaging modalities such as X-ray, γ-ray and computed tomography and magnetic resonance imaging which estimate the extent of a disease. The latter is a microscopic process and involves the resection of tissue and its subsequent analysis under a microscope. This process is opposite to the above as it supplies a restricted but detailed picture on tissue biochemistry and biostructure.

Existing blood imaging systems as well as the current methods of ischaemia diagnosis, such as angiography, CT and MRI, reveal that none of them can be used intra-operatively. They also provide information on anatomic modifications resulting from ischaemia but that could have been caused by other pathologies.[165] Finally, the processing time of these techniques is long, whereas early diagnosis is crucial for graft survival.

Both processes have brought enormous benefit to patient health care and in particular disease diagnosis. However the information they provide does not overlap, and a missing link becomes apparent. This is the absence of real-time functional and molecular data, pre-, intra- and post-operatively. It may lead to missed or wrongly diagnosed lesions, resulting in needless biopsies and operations. Furthermore, no data at a molecular level means that serious pathologies (e.g. cancer) cannot be diagnosed early when 'abnormal' change is occurring at a molecular level, which first precedes and subsequently triggers anatomic alterations. Additional problems include cost, logistical constraints and inadequate technology for use with infants and elderly patients. The exception is X-ray fluoroscopy which may be used intra-operatively.

Biomedical photonics is a field with potential to bridge the gap between the macroscopic and microscopic methodologies described above. It studies the interaction between light and tissue and can provide information on processes occurring at a molecular level, both at microscopic spatial resolution and nanometre spectral resolution.[166,167] The manner in which the light interacts with tissue (absorption, transmission, diffuse reflection, fluorescence) may vary, but it carries the common goal of supplying more complete tissue diagnostic information to the surgeon with minimal trauma and procedure duration. In particular, the technique has shown to be advantageous over medical imaging and biopsy in a number of cases. First, it is atraumatic to the patient, whereas ionising radiation is used in medical imaging and biopsy involves tissue cutting and therefore damage. Second, biomedical photonics can generate immediate data at the time of an operation which therefore speeds up diagnosis and subsequent management. With medical imaging and biopsy one is forced to wait for a

period of time until the data is processed. Third, the resolution of biomedical photonics is highly detailed, with the ability to characterize tissue in the micrometric range and thus, probe biochemical alterations that pre-stage pathology. This is definitely not the case in medical imaging and biopsy, where a clear demarcation line between diseased and healthy tissue is often unclear.[168] Today, biomedical photonics are well-established and commonly used tools in hospitals. For example, they are applied in pulse oximetry, endoscopes and colposcopes, laser therapy, and microscopes used to examine histological sections. Diffuse reflectance spectroscopy in the visible range has been successfully used to diagnose chronic mesenteric ischaemia during an endoscopic procedure.[165,169]

We have already discussed the problems posed by potential ischaemia and reperfusion injury during the transplant process. The critical stage of this operation is the uterus re-perfusion in the recipient during which ischaemic injury can cause parenchyma and microcirculatory function impairment.[4] A need exists for a technique which will be able to assess the level of ischaemia and reperfusion injury and provide related diagnostic information during the intra-operative and immediate post-operative periods, as well as prior to possible pregnancy. No such technique exists.[170]

Biomedical photonics have the potential to do just that by processing data related to haemoglobin concentration and saturation of oxygen directly and intra-operatively. Importantly, the data will be non-invasive, real-time, localized and high-resolution. A type of photonics known as reflectance spectroscopy has been successfully used to diagnose chronic mesenteric ischaemia during an endoscopic procedure.[165,169] The physics element behind the use of biomedical photonics is beyond the scope of this PhD. However, the background is outlined briefly below. The principle of biomedical photonics is based on the complex phenomenon that light is modified by tissue, with the spectral range of interest lying in the non-ionizing and richly diagnostic visible domain (400nm - 700 nm). When striking the surface of a tissue, light can either be reflected or refracted. Reflected light is of no particular interest as it does not penetrate the tissue and therefore does not contain significant diagnostic information. It is referred to as a specular reflection. The amount of refracted and reflected light depends on the refractive index at the air/tissue interface and the angle between the in-coming

light beam and the tissue normal. The photons forming the refracted light can be absorbed or scattered by the tissue chromophores and scatterers. The fraction of the photons which emerge from the tissue after multiple scattering events is called diffuse reflectance as the scattering induces the spreading and the loss of directionality of the incident beam.[170]

Two photonic applications which are both real time and can be used intra-operatively are described below.

Application of Pulse Oximetry in Uterine Transplantation

Pulse oximetry is a non-invasive method of measuring the arterial oxygen saturation (O_2Sat) of haemoglobin. A probe is placed around the cornua in our experiments which is linked to a microprocessor unit displaying a waveform, O_2Sat, and pulse rate. Two light emitting diodes are contained within the probe. The light they emit passes through the cornua to a photodetector. Some of the light is absorbed by blood and soft tissues during its passage through a select tissue. The amount of absorption in general and at each light frequency is proportional to the degree of oxygenation of haemoglobin within the tissues. The microprocessor can then calculate the proportion of oxygenated haemoglobin by computing the absorption at the two wavelengths. The pulse oximeter measures O_2Sat and perfusion index (PI). PI is independent of O_2Sat and acts as an indicator of total blood volume. The oximeter produces a graph related to the amount of light absorbed by the tissue over time. The microprocessor can select out the absorbance of the pulsatile fraction of blood (arterial flow) from absorbance of non-pulsatile venous or capillary blood and other tissue pigments.

The first experiment to assess whether pulse oximetry could be a suitable tool for confirming adequacy of uterine perfusion was performed by the Smith group in the UK (as advised by Dr Andrew Lawson, Consultant Anaesthetist). *Sieunarine et al* looked at the uterine vascular supply during a set of abdominal radical trachelectomies, a novel surgical procedure for conservative cervical cancer management.[171] Pelvic vessels are selectively ligated during ART. Thus, the question of how many

vessels the uterus requires to ensure its viability arose. Originally, the group believed falsely that four of the following six vessels (two uterine, two ovarian and also a collateral supply from two vaginal vessels) were necessary to maintain a viable uterus. The aims of this highly original study were to investigate the vasculature of the infundibulopelvic and broad ligaments, to assess the contribution of the ovarian and uterine vessels to overall uterine perfusion, and to consider the clinical applications of selective pelvic vessel ligation. The number of dissections performed was adequate. Ten fresh dissections of the infundibulopelvic vessels, broad ligaments of benign total abdominal hysterectomy, and bilateral salpingo-oophorectomy specimens were performed. PI and O_2Sat measurements using a modified probe were taken at specified intervals at the uterine cornua during ten routine benign abdominal hysterectomies to assess the contribution of the ovarian and uterine vessels to overall uterine perfusion. Conclusions drawn from PI and O_2Sat measurements implied that uterine and ovarian vessels contribute almost equally to uterine perfusion. Of the six supplying vessels (ovarian, uterine, and vaginal) to the uterus only two (ovarian or uterine or a combination thereof) are required for uterine viability. Such a uterus is indeed capable of pregnancy and delivery [172] except if ART is performed in pregnancy when uterine artery preservation is required to allow the pregnancy to run to term.[173] This fact is particular importance to uterine transplantation where according to the above, only two arteries and two veins would be required to ensure adequate perfusion of the uterine graft. Clinically, selective ligation of the pelvic vasculature (uterine and ovarian vessels) has been utilized in certain gynaecological procedures at particular risk of torrential life-threatening uterine haemorrhage; for example, surgical management of chemoresistant gestational trophoblastic disease (Strassman procedure),[174] and fertility-sparing surgery in ruptured cornual ectopic pregnancies.[175]

A similar study by the same team was performed to validate the above results. The aim was to further confirm that the measurement of PI and O_2Sat using pulse oximetry could be used to assess the contribution of uterine and ovarian vessels to the overall uterine perfusion.[176] During routine hysterectomies, PI and O_2Sat were measured over the right and left uterine cornua. These measurements were taken before any vessels were ligated (baseline), after only the ovarian vessels were clamped and then after the uterine vessels were clamped. Clamping of the ovarian and

subsequently uterine vessels produced statistically significant decreases in PI and O_2Sat. Both pairs of vessels therefore contribute almost equally to uterine perfusion and a definite role for O_2Sat and PI variables exists in determining the success of uterine and ovarian vessel re-anastomosis in UTx.

Application of Multispectral Imaging in Uterine Transplantation

Multispectral imaging requires a multispectral laparoscope to acquire high resolution images of changes in tissue oxygen saturation. This technique involves using a white light source and tuneable filter to acquire images at many wavelengths in the visible range in order to build up a reflectance spectrum at each pixel.[177] Here, like with pulse oximetry, the haemoglobin concentration and oxygenation, as well as scattering decide the shape of the visible light spectrum reflected from tissue. These spectra are then fitted using a linear regression model which uses prior knowledge of the optical absorption spectra of oxy- and deoxy-haemoglobin, from which their relative contributions are calculated. Previous implementations of this technique have also used hyperspectral data with numerical techniques to aid tissue visualisation and ischaemia detection.[177,178] Multispectral imaging refers to techniques that use a relatively low number of wavelengths (up to 40-50) while hyperspectral imaging refers to techniques that can apply hundreds of wavelengths. A strict threshold between the two types does not exist but 'hyper' usually means hundred or more.

The technique has been applied in current medical practice in a number of ways: gingival inflammation quantification,[179] brain tumour demarcation,[180] fundus analysis,[181] imaging of Hirschsprung's disease,[182] and analysis of facial skin lesions.[183]

Endoscopic Laser Speckle Contrast Analysis

The application of biophotonics and its potential benefit to the field of medicine, with regards to diagnosis and treatment is outlined above. In particular, its application to assessing uterine perfusion. The technique utilizes the interaction between light and tissue, and thus the processes of light

refraction, absorption and scattering, to detect, measure and image the vasculature and circulatory function. Three main variables are assessed: blood function, vascular structure and flow velocity, using both invasive and non-invasive methodology.[184]

Apart from assessing uterine perfusion, an additional problem that one may face in a human UTx model is how to ensure an adequate blood flow of those vessels supplying the uterus: immediately post-anastomosis, in the early and late post-operative periods, and during pregnancy when those anastomotic sites will be severely tested because of an increase in the size of the diameter. Therefore, it is of crucial interest to be able to image the uterine circulatory system that maintains the flow of oxygen and nutrients to the relevant tissue. By investigating tissue circulation, one can make conclusions relevant to tissue oxygenation. The level of oxygenation of an organ is directly proportional to the health of that organ.

Parameters of interest with regards to blood flow specifically are blood pressure, blood volume and blood speed. Blood pressure can be easily assessed using a sphygmomanometer; however, the measurement is systemic and not a reading of the blood flow speed in a specific area. Blood volume can be measured using electrical-impedance plethysmography and chamber plethysmography. The former has poor accuracy as the method is based on three assumptions which are often not met: the vessels expand uniformly; the resistivity of the blood remains constant; and the direction of the current is parallel to the flow. The latter depends on the blockage of the venous flow and measurement of the volume, and it is therefore only applicable to the limbs, and not the internal organs. Finally, blood flow speed can be assessed by a number of different methods: electromagnetic flowmeters, ultrasound-Doppler flowmeters, and optical methods such as photoplethysmography, laser Doppler flowmetry and imaging, photoacoustic methods and optical coherence and Doppler tomography. However, none of the methods above are able to assess in combination blood flow velocity, blood function, vascular anatomy and tissue perfusion. Thus, the measurement of three variables in particular: blood flow speed, haemoglobin concentration ([Hb]) and oxygen saturation (O_2Sat).

An imaging technique which could offer a solution, known as Endoscopic Laser Speckle Contrast Analysis (eLASCA), is an imaging system which monitors vessel blood flow by measuring changes in the light reflected from tissue under laser illumination because of the presence of moving 'scatterers' (red blood cells).[184-186] A laser speckle pattern is produced when a rough area, surface or pattern is illuminated by coherent laser light and is imaged onto a camera (**Figure H**). Randomly distributed bright and dark areas on an observation plane are created by the interference of the reflected and backscattered light. It is precisely this image which is called a speckle pattern. When imaging the surface or the whole particle(s) whilst in motion, the speckle pattern that is generated at each pixel varies with time and space. These variations contain flow information about the motion of the scattering particles which can be calculated by measuring either the temporal intensity fluctuation of a speckle (laser-Doppler flowmetry) or the spatial intensity fluctuation (LASCA). The latter fluctuations, assessed by LASCA, appear as blurs which are evaluated by the contrast. Contrast decreases with increases in the speed of the scatterers. Therefore the speed can be calculated by examining the change of the contrast value which is the basic principle of LASCA.[186,187]

Laser speckle is a random phenomenon and can only be described statistically which is beyond the scope of this thesis. Speckle contrast analysis is based on the statistics of the intensity of the speckles. Speckle Contrast is generally described as the ratio of the standard deviation over the mean intensity of a small area in the speckle image:

$$C = \frac{\sigma}{\langle I \rangle}$$

where C is the contrast, σ is the standard deviation of the signal and $< I >$ is the mean intensity value. In experiments the contrast is calculated based on the values recorded by the CCD pixels. Usually in an area defined by an 'n' number of pixels, the mean intensity and the standard deviation are calculated and the speckle contrast number is allocated for this area.[186]

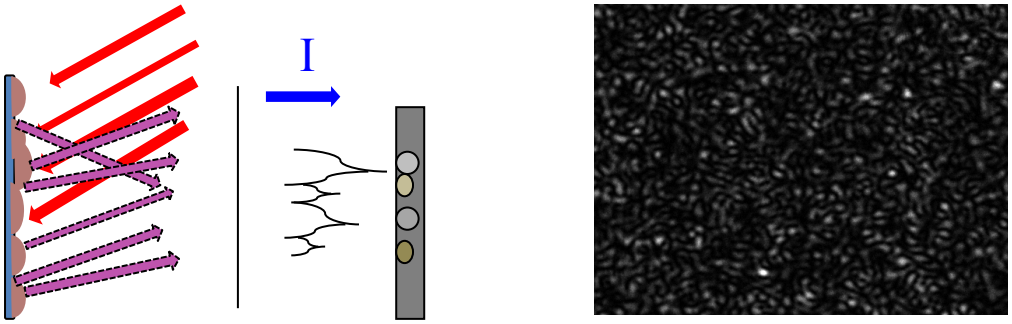


Figure H (a) Generation of the speckle pattern. The red arrows represent the laser illumination beam and the purple arrows the scattered beam which is the same wavelength as the laser. The scattered beams converge to an interference pattern with random intensities and phases. (b) This is the speckle image as it appears from the laser illumination of a paper card.

When an object moves, the speckle pattern it produces changes. For small movements of a solid object the speckle pattern moves as a whole, i.e the speckle pattern remains correlated. For faster motions, the speckles 'decorrelates' and the speckle pattern changes completely. Decorrelation also occurs when the light is scattered from a large number of individual moving scatterers, such as particles in a fluid. An individual speckle appears as if it is 'twinkling' like a star. This phenomenon is known as a 'time-varying' speckle.[186] *Stern at al* first recognised that the biggest potential for the application of speckle pattern fluctuations could be in assessing the flow of red blood cells.[188] In the 1980s, this effect was used in a photographic technique known as single-exposure speckle photography which was developed to image the blood flow at the retina.[189] A camera was used to obtain a quick snapshot image of the time integrated speckle pattern from which blood flow in the retina was estimated from the spatial statistics of the speckle pattern. The term LASCA was then introduced to describe this analysis.[186]

Speckle images of tissues *in vivo* could be acquired by fast cameras as series of snapshot images with acquisition occurring at 1/1000 sec (1ms) with a total duration of several seconds or minutes. The contrast calculation is based on a single frame image processing, which is further applied to the whole stack of images with image processing algorithms. For a single digital frame the computer program

selects a window of an area of 5x5 pixels or 7x7 pixels known as 'kernel' and calculates the mean intensity and the standard deviation over the mean intensity. Speckle contrast C is automatically calculated as the division of the standard deviation over the mean intensity, and the value of C is being assigned over the selected pixel area. The "kernel' window then moves by a pixel step across the digital image and the process is repeated. The result is the contrast image of the Area Of Interest. The diagram of the calculation of contrast is shown in **Figure I**.

Theoretically the speckle contrast has values between 0 and 1. A completely stable object maximizes the speckle contrast to 1 indicating that there is no blurring and therefore no motion, while moving particles with certain speed results in blurred speckle images with contrast values close to 0. The faster the motion, which in case of red blood cells is the blood flow, the darker the speckle contrast. Pseudo-colour may be added to the contrast map according to the contrast value so that the different speeds can be distinguished by different colours.

The application of LASCA is limited however because laser light can be absorbed and then scattered whilst passing through tissue. Therefore, the maximum penetration depth is several millimetres. A solution is the combination of LASCA with endoscopy (eLASCA) which would allow imaging of internal organs for clinical diagnosis and then surgery if deemed necessary, assessment of the recovery from disease or injury, as well as the acquisition of real-time information of the *in vivo* organ blood supply. Variations in cardiac and respiratory rates can be extrapolated from the analysis of the signal over time as well as the blood oxygenation status based on the changes of concentration of oxygenated and deoxygenated haemoglobin. Such information will improve upon current imaging and non-imaging techniques for post-operative tissue monitoring, and will therefore analyse uterine 'well-being' both in its non-pregnant and pregnant state, assess recovery and understand haemodynamics and oxygenation levels. It is therefore potentially a system which may be capable of detecting tissue perfusion, oxygenation level, pulsation and respiration at the same time.[185,186,190]

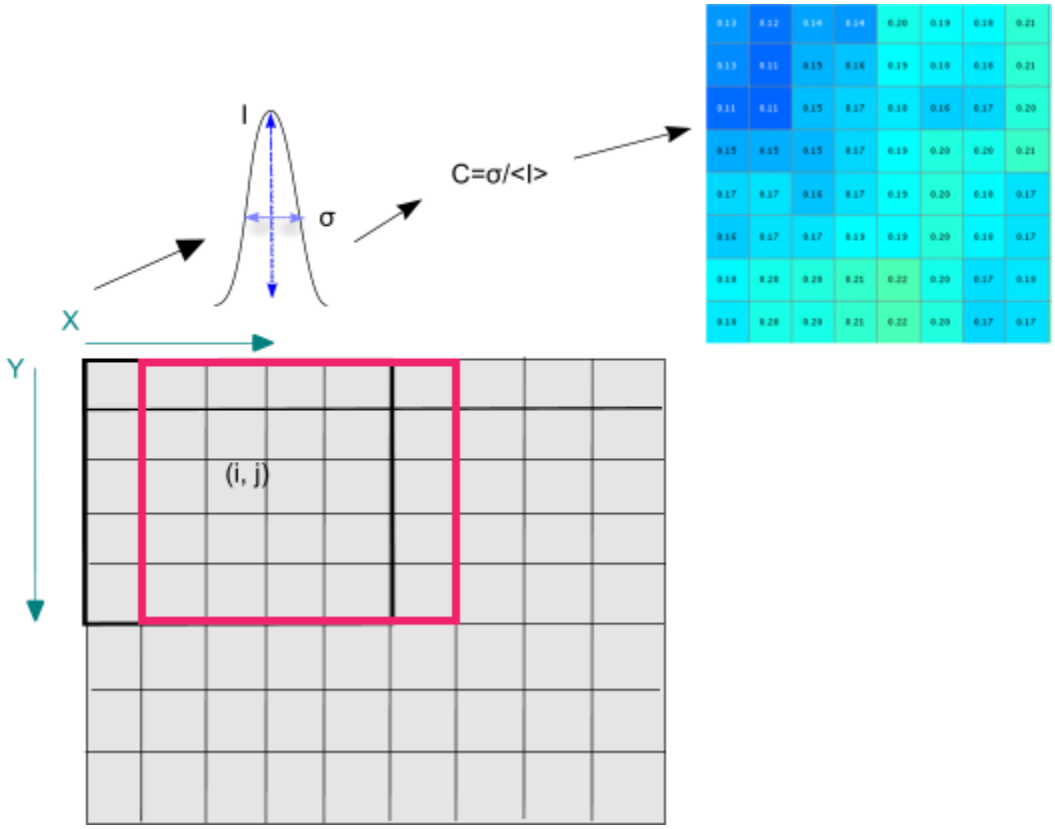


Figure I Diagram of the Speckle Contrast calculation. The table represents the detector chip and each small square represents one pixel. The black square represents the kernel window with the size of 5x5 pixels. The contrast value C of the pixels is assigned to the pixel (I,j) and the kernel is moved across X axis and then along Y axis to perform the spatial contrast calculation of the whole image. The red rectangle shows the position of the kernel for calculating the contrast. For every kernel position a number indicating the contrast is assigned to the whole kernel area. A speckle contrast image is generated by the derived contrast values.

Currently, LASCA has been applied in practice to quantify and assess blood circulation in the skin, retina and cortical tissue.[185,191,192] All these areas are easily accessible and that is because LASCA is limited by the penetration depth of the light the system emits, as explained above. The combination of endoscopy and LASCA has been tried to date but not in gynaecology. The ultimate aim was to see whether disease diagnosis and intra- and post-operative supervision could be carried out simultaneously. The first application of eLASCA was by *Bray et al*, who used it to image tissue perfusion and blood flow in the medial compartment of the knee of five patients requiring arthroscopic knee surgery.[193] Changes in tissue perfusion were brought about by tourniquet application and intra-articular adrenaline injection, and were then displayed as real-time video perfusion images of tissue blood flow in the knee joint. Only a high resolution CCD and a standard

arthroscopic sheath with which to cover the rigid Hopkins lens type endoscope were needed for this study.[193] The next study advanced on the previous. *Zimnyakov et al* applied a fibre-optic bundle and a CCD camera instead of the above rigid endoscope to monitor a speckle-contrast pattern. However, it was a point measurement and could therefore not provide images of the scatter speeds.[194]

(d) Patients and Uterine Transplantation

With respect to AUFI and fertility in general, it is well established in the literature that infertility is strongly associated with depression and a reduced (Quality of Life) QoL.[195,196] Being able to conceive and subsequently bear a child can have a huge influence as to how women perceive their own femininity and QoL.[196] Unfortunately, the society and culture of the woman in question is an important influence. One of the most important groups of AUFI patients are women diagnosed with MRKH Syndrome. The above pressures are further pronounced in MRKH women. This group of women had to undergo the afore-described introspection at a much earlier age, in particular as teenagers. It is around this time that a woman awaits menarche, which, if normal and occurring within the expected time period, is indicative of the woman's physiological normality.[196] For the woman in question, the onset of menarche is a symbol of reproductive ability.

MRKH patients therefore are forced to face up to a rather distressing diagnosis from a relatively young age, with implications related to their body image, psychosexual development, identity and femininity.[197] All attributes of womanhood are in doubt; absence of menarche equates to loss of physiological value, no uterus means no capacity to child bear and in the sub-group of MRKH women with vaginal agenesis, failure to engage in sexual intercourse. Ultimately, the malformation itself does lead to narcissistic damage.[198] The evidence however has remained largely anecdotal.[199] *Heller-Boersma et al* presented one of the few investigations on the psychological impact of MRKH.[200] A group of 66 women with MRKH were compared with 31 control-group women on a range of self-rating scales assessing psychological distress and self-esteem. Women with MRKH had significantly more pathological scores on some of the scales and subscales, such as phobic anxiety and

psychoticism (interpersonal alienation), with a similar trend for subscales measuring depression and anxiety. The authors concluded that MRKH has a lasting negative impact on affected women's level of psychological distress and self-esteem.[200]

MRKH Syndrome is primarily characterised by uterine agenesis, although vaginal agenesis is also common. Genetically, the patient is XX and the external phenotype is a normal female. Therefore, the management of this condition has mainly focused on the creation of a neovagina for sexual function using both surgical and non-surgical approaches.[11] Our aim is to widen management options by enabling UTx to become accepted in human practice as an infertility treatment. The experience of units offering the creation of a neovagina has demonstrated the importance of a holistic approach when managing this group of patients. As succinctly described by *Edmonds et al*, a patient who will undergo an anatomic correction or creation of a vagina will first partake in routine psychological assessments at the UK National Centre for Adolescent and Adult Females with Congenital Abnormalities of the Genital Tract, based at Queen Charlotte's & Chelsea Hospital, London. The aim of the treatment as is the aim of UTx is not simply the creation of a new organ i.e. vagina or uterus, and thus an improved sexual relationship between a couple and end to infertility but also, an improved quality of life and psychological well-being of the patients.[11] *Edmonds et al* demonstrated that attempting and then managing to find a 'cure' to one of the pathologies associated with MRKH (in this case, a non-existent vagina) did lead to improved long-term QoL outcomes. Their retrospective study (largest ever reported on the non-surgical management of vaginal agenesis) examined the efficacy of vaginal dilators in the management of MRKH Syndrome. The main outcome measure was achieving functional vaginal length (defined as greater than 6 cm in length and maximum width throughout the vagina and especially at the apex) and sexual satisfaction. Out of 245 patients, 232 (94.9%) achieved a successful vaginal length and sexual function. When the program was completed by all patients, 100% of patients were successful, suggesting that a) surgery is rarely, if ever, required; and b) adoption of a holistic approach to management of MRKH patients with a strong psychological input can result in long-term QoL outcomes.[11]

Additionally, a number of studies and reviews have confirmed the presence of psychological problems, such as anxiety and depression, in women suffering from infertility and in particular MRKH syndrome.[11,196,201,202] *Laggari et al* was the first study to examine psychological difficulties in adolescents with MRKH. They used the Beck Depression Inventory (BDI) and the State-Trait Anxiety Inventory (STAI-Gr) to measure depression and anxiety respectively in 49 adolescent girls, of whom 27 were patients with confirmed menstrual disorder, 22 with PCOS and 5 with MRKH. 22 girls were healthy adolescents with a normal menstrual cycle (control group) matched by age and school grade. The results demonstrated that the PCOS group was 1.08 times more likely to have higher scores than healthy adolescents on the anxiety scale and for the MRKH group it was 1.12 times more likely. Furthermore, the MRKH group was 1.40 times more likely to have a higher number of depressive symptoms than the control group. Therefore, the authors findings, although based on a small sample, suggest a relationship between PCOS and MRKH and anxiety and depressive symptoms in adolescents.[196]

A number of various psychological treatments have been used with MRKH patients. *Heller-Boersma et al* developed a cognitive-behavioural group treatment (CBT) for MRKH patients.[203] 39 women with MRKH were randomized to group CBT (n=19) or waiting list (n=20). Participants allocated to group CBT showed significantly reduced psychological symptoms on the SCL-90-R, whereas those on the waiting list remained unchanged.[203] Using the Medical Research Council's (2002) framework for the development and evaluation of complex health interventions, the same team then went on to develop a specific model of the core negative psychological impact of the MRKH diagnosis and medical treatment as well as CBT for MRKH.[199] This approach may also be applicable to a number of other congenital or acquired gynaecological conditions such as premature ovarian failure, female genital mutilation, Turner's Syndrome, ovarian dys-/agenesis or, Complete Androgen Insensitivity Syndrome have a psychological impact not dissimilar to MRKH in terms of these women's sense of self-esteem and femininity.

For MRKH, and AUFI women in general, UTx may offer a way for these women to re-discover their own femininity through the restoration of fertility. Thus, when faced with a patient who may undergo UTx, the afore-mentioned phrase 'holistic approach' takes on an extra meaning. This is because the psychological element is two sided for these patients. On one side lies the psychology of infertility, and on the other side, equally important is the substantially higher prevalence of psychiatric disorders in transplant candidates and recipients than in the general population. Additionally, a psychiatric issue may have an impact on a medical outcome for a non-transplant-related disease. It is for this reason that the pre-transplant evaluation phase routinely includes an assessment of the mental health of transplant candidates. This helps to highlight both psychological and psychiatric issues that may be affecting a potential candidate's pre-operative preparation and post-operative care. The patient's overall QoL can therefore be improved as identifying and treating mental health issues before or in the early aftermath of a transplant could reduce symptoms and distress. Frequent monitoring of psychiatric and psychological symptoms during the first year after transplantation is in place in some institutions.[204]

To accomplish this, a validated assessment tool for patients who have registered a wish to undergo UTx and which addresses the above points is necessary. No such tool exists in the current medical literature. The face transplantation (also a QoL improving procedure) effort faced a similar problem approximately ten years ago. This was highlighted by the report drafted by the Royal College of Surgeons working party set up to investigate and provide recommendations related to face transplantation.[205] The report stated that despite the adequately developed surgical techniques required to carry out face transplantation, '*the risks of failure and long-term consequences of immunosuppression presented very considerable psychological and ethical problems.*'[205] Their revised report in 2006, produced following further research into face transplantation, provide good practice guidelines - 15 core requirements that had to be fulfilled before a research ethics committee/institutional review board could approve a proposal to carry out face transplantation.[206] A number of these requirements focused on factors necessary for adequate selection, preparation, evaluation and monitoring of those patients selected to undergo face transplantation.[207] Similarly,

these issues are also of importance in UTx. The afore-mentioned tool must be designed in such a manner that it addresses these points as well as any other similar psychological and social concerns (**Box A**).[206,207]

Box A Psychological and Societal Issues identified by the Royal College of Surgeons Working Party Report on Facial Transplantation 2007 that may be of importance to UTx (courtesy of Clarke & Butler, 2009) [207]

- **Psychological responses to transplantation in general**
  - Fears related to viability of transplanted organ
  - Fear of aftermath of rejection
  - Anxiety related to potential side effects of immunosuppressive medication, including risk of infection and malignancy
  - Feelings of personal responsibility for the success of the graft, linked to the need for adherence to a drug routine, regular monitoring of symptoms, regular attendance at outpatient appointments
  - Integration of the transplant into existing body image and sense of identity
  - Emotional responses to the experience of receiving a transplanted organ

- **Potential additional factors**
  - Effectiveness of surrogacy and adoption and prior engagement with these alternative interventions
  - Psychological support for UTx patients (pre-, peri-, post-transplant and for those not suitable for the procedure)
  - Motivation to seek treatment and expectations of outcome
  - Psychological stability and adjustment
  - Level of cognitive functioning
  - Likelihood of adherence to treatment regimens
  - Social support
  - Implications of chronic rejection
  - Impact of transplant failure/fetal & neonatal abnormalities
  - Recipient and donor family support

- **Societal issues**
  - Negative impact on national organ donation programs
  - Press intrusion for recipient and donor family
  - Pressure on AUFI [patients to seek surgical solutions
  - Raised expectations for many, solution for few

Psychological Domain	Measure
Cognitive Function	Wechsler Adult Intelligence Scale III
Mood	Hospital Anxiety and Depression Scale Beck Depression Inventory
Post-traumatic Stress Disorder	Brief Post-traumatic Stress Disorder Screen
Quality of Life	Short Form-36
Appearance Anxiety and Social Avoidance	Fear of Negative Evaluation Scale
Beliefs Regarding Medicines	Beliefs Regarding Medicines Questionnaire

Table C Standardised psychological measures used in assessment (courtesy of Clarke & Butler) [207]

STUDY 1

1. Study 1: Abdominal Radical Trachelectomy in West London

This study has now been peer-reviewed and published as per following reference: **Saso S,** Ghaem-Maghami S, Chatterjee J, Naji O, Farthing A, Mason P, McIndoe A, Hird V, Ungar L, Del Priore G, Smith JR. Abdominal radical trachelectomy in West London. *BJOG* 2012; 119:187-93.

Abstract

Objective: Traditionally, the surgical management of invasive cervical carcinoma that has progressed beyond microinvasion has been a radical abdominal hysterectomy. However, this results in the loss of fertility, with significant consequences for the young patient. This report describes abdominal radical trachelectomy (ART) as a potential replacement for radical hysterectomy in patients with stage IA2-IIA cervical cancer who desire a fertility-sparing procedure without decreasing the curative rates.

Design: Observational, retrospective study.

Setting: Teaching hospital and regional cancer centre in London, UK.

Population: Patients undergoing ART. Methods: Patients presenting during the period 2000-2009 with cervical cancer stage IA2-IIA were offered a trachelectomy, if they expressed a desire to preserve fertility. The type of trachelectomy (vaginal/abdominal) was chosen based on patient anatomy and neoplastic and magnetic resonance imaging characteristics. Each patient was counselled as to the experimental nature of the procedure.

Main Outcome Features: Survival, recurrence and fertility issues among ART patients.

Results: A total of 30 patients underwent ART (open and laparoscopic) between 2001 and 2009. Three patients presented with a recurrence, two of which have died (median follow-up: 24 months). Only three patients required further surgical re-intervention because of operative complications. Ten patients attempted to conceive, resulting in three conceptions (30%) and two live children.

Conclusions: Abdominal radical trachelectomy provides a feasible, cost-effective and safe treatment option for young women who have been diagnosed with early-stage cervical cancer and wish to preserve their fertility.

1.1. Background

The UK's efforts into developing the field of UTx began in the late 1990s as a continuation of a number of studies looking at the potential of Abdominal Radical Trachelectomy (ART) as a curative and fertility-preserving surgical procedure.[60] This work focused on finding out how many vessels were required to keep a uterus viable. It is supplied by two uterine, two ovarian (via collaterals) and two vaginal arteries (via collaterals) (**Figure 1.1**). At the Society of Gynecologic Oncologists Conference (USA) in 1996 during an interactive session between the panel and members, the membership believed that at least four of these arteries should be patent to maintain a viable uterus.[60] Subsequent experience with ART has shown this hypothesis to be false, with two ovarian arteries and veins proving to be enough. This is because ART results in the division of vaginal arteries to remove the cervix and upper vagina and the uterine arteries to complete adequate ureteric dissection. It was these lessons learned with regards to preserving adequate uterine blood supply post-operatively, as well as the technical aspects of ART that led a few gynaecological surgeons to consider UTx (also a fertility-preserving procedure) as a realistic rather than 'science fiction-esque' goal. A study of ART and its clinical and practical applications is therefore a necessary introduction into the field of UTx.

Traditionally, surgical management of invasive cervical carcinoma which has progressed micro-invasion has been a radical abdominal hysterectomy and pelvic lymphadenectomy. This treatment method has shown excellent results in terms of survival but results in the loss of fertility, which can cause added stress and reduced quality of life to a young female patient.

In current gynaecological oncology practice, fertility preservation has become a significant and meaningful issue when deciding on how to treat stage IA2-IB cervical cancer.[208,66,209] A fertility-preserving operation, known as 'radical trachelectomy' was coined in 1994 in a seminal paper by the late *Daniel Dargent* [210] who described a new technique suitable for exophytic tumours of Stages IA to IIA. This procedure preserves the uterine body while removing the cervix, parametrium and upper

one-third of the vagina.[211,212] Two types of radical trachelectomy are now recognised: vaginal (VRT) and abdominal (ART).

The benefits of VRT, in treating early-stage cervical cancer and successful fertility outcomes thereafter have been detailed.[213,214] However certain problems have arisen. Recurrence rates for example range from 12.5% to 29% for tumours that are greater than 2cm in diameter.[211,215]

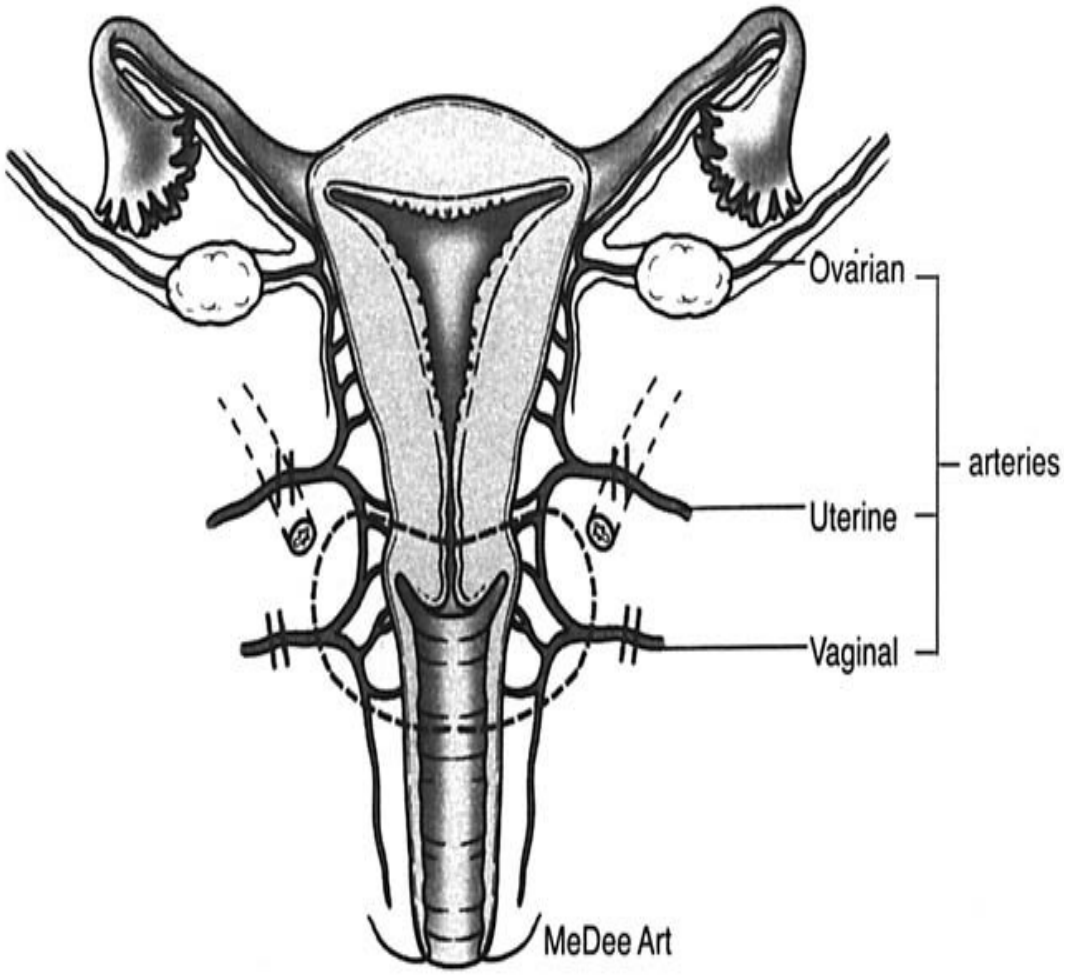


Figure 1.1 ART involves the removal of the cervix, upper third of the vagina and parametrium. This area is shown in the figure below enclosed by the dotted line. The vaginal and uterine arteries are divided (courtesy of *Smith et al*) [60]

The radicality of the ART procedure is identical to that of a traditional type II Wertheim hysterectomy. The similarity in the operative techniques between the two operations have allowed ART to become an acceptable alternative. It has also been used to treat pregnant women diagnosed

with cervical cancer.[216] *Mandic et al* [217] and *Abu-Rustum et al* [218] reported their experiences of performing ART on pregnant women in the 19th and 15th gestational week (GW) respectively. In both cases, the patients had a caesarean section in the 36th GW and 39th GW respectively and delivered a healthy newborn. No further relapse has been demonstrated post-ART.

1.2. Materials and Methods

This was a retrospective study performed at the beginning of the research programme. Project proposal was reviewed and approved as a clinical audit project by the Quality and Safety Co-ordinator for the Women's and Children's Clinical Programme Group at Queen Charlotte's Hospital. Case notes were retrieved and studied and the following data were extracted: mean age, mean length of operating time, pre- and post-ART tumour histology (tumour type, stage and grade), size and margin status of cervical tumour, number and disease status of lymph nodes removed, estimated blood loss and type of cervical suture used to anastomose the uterine stump with the upper vagina. We also recorded post-operative complications, follow-up time and outcome, recurrence of disease and fertility outcomes.

With respect to the ART procedure, approval to perform it was granted by the Institute Review Board of the Chelsea and Westminster Hospital Ethics Committee in 1997, which is the where JR Smith first performed an ART.[60] Since the publication of that initial study, the selection criteria employed in order to choose suitable patients has remained unaltered. At West London Gynaecological Cancer Centre, all patients presenting from 2000 to 2009 with cervical cancer stage IA2 to IIA expressing a desire to preserve fertility were given the option of undergoing an ART. Each patient was counseled as to the novel nature of the procedure and appropriate consent was obtained. The surgical technique has been described previously.[172,219] The operation was performed by the five gynaecologic oncologists at the West London Gynaecological Cancer Centre (Queen Charlotte's and Hammersmith Hospitals).

Follow-up

All patients were discharged from hospital once they demonstrated an adequate level of comfort and fluid and food intake, complained of minimal abdominal pain only and had voided a sufficient amount of urine. They were also instructed, prior to discharge, to seek medical help if a significant amount of blood was passed *per vaginam*, lower abdominal pain became intolerable or problems with micturition became apparent.

Routine follow-up was organised in the gynaecology oncology clinic every three months in the first year, four months in the second year, six months in the next three years and annually thereafter. Each appointment consisted of a focused history and examination, followed by a 'cervical' smear and pelvic ultrasonography if deemed necessary. It is also worth mentioning that our patients had direct access to our Cancer Nurse Specialists if they required specific support. With regards to attempts at pregnancy, our recommendations were for the patients to use contraception during the first year following ART to allow the tissue to heal.

Prior to surgery, all patients had preoperative magnetic resonance imaging of the pelvis to assess tumour location, tumour size and involvement of the parametria and upper endocervix. Computerized tomography of the chest, abdomen and pelvis was performed to investigate possible distant spread. Examination under anaesthetic and cystoscopy +/- sigmoidoscopy were performed in order to complete the clinical staging process. Pathologic parameters were gathered from the pre- and post-operative reports. At our institution, all cervical specimens were evaluated by specialist gynaecologic pathologists. Case notes were reviewed by two of the investigators to confirm the accuracy of the data collected.

Data Presentation and Analysis

Statistical analysis was not required for this study with the data described using descriptive tables.

1.3. Results

General outcomes

The patient characteristics and surgical outcomes are listed in **Table 1.1**. Following in-depth consultation between the patient and members of the gynaecology oncology team, 31 women chose to undergo an elective fertility-sparing ART. This operation was selected over a radical hysterectomy primarily in order to protect the patient's fertility while at the same time managing the chief diagnosis of cervical carcinoma.

At the time of diagnosis the mean age of the 31 patients was 32.5 years (range 23-41). The stage of cervical tumour that patients presented with was: stage IA2 (n=2; 6.7%), stage IB1 (n=25; 83.3%), stage IB2 (n=2; 6.7%) stage IIA (n=1; 3.3%). Five tumours (16.7%) were greater than 2cm in diameter. Notably, patients with non-squamous tumours were advised of the risk of ovarian and distant metastases.

ART was performed simultaneously with bilateral pelvic lymphadenectomy. Mean length of operating time was 2 hours 50 minutes (range: 1 hours 50 minutes to 5 hours). The tumour was excised completely in 27 patients, with the median number of lymph nodes removed from the pelvis equalling 24 (range 7-52). 27 patients (90%) had no lymph node involvement. In the 3 patients (10%) who did demonstrate such spread, the lymph nodes involved were left internal iliac (1/23), right and left internal and external iliac (3/22), and right obturator (2/28). Lymph nodes removed were from the following groups: external, internal and common iliac, sacral and obturator. Microscopic evaluation demonstrated the resection margins of more than 3mm to be tumour-free in all cases, with endo- and ecto-cervical and circumferential margins commented on most frequently. None of the tumours displayed perineural spread. During surgery, 11 patients (36.7%) had a frozen section of the margins sample sent for histology. All revealed tumour-free resection margins. Magnetic resonance imaging revealed the largest tumour to be of the following size, 5cm x 3.7cm x 6cm, with a volume of 48cc

(stage IIA) and in contact with the posterior wall of bladder. Following EUA and imaging, as well as a team discussion, it was felt that ART could safely remove the tumour. The patient was also keen to preserve her fertility. She did not suffer from recurrence during follow-up and is still alive.

Finally, with regards to the cervical suture, insertion depended on ease of application during surgery and surgeon's choice. 24 patients (80%) had a cervical cerclage to aid future pregnancies.

Pre- / Intra- / Post- operative characteristics	
Patient characteristics	
Age	32.5 years (mean)
Follow-up	24 months (median)
	Number of patients (%)
FIGO Classification	
Stage IA2	2 (6.7)
Stage IB1	25 (83)
Stage IB2	2 (6.7)
Stage IIA	1 (3.3)
Histological Type	
Squamous	15 (48)
Adenosquamous	4 (13)
Adenocarcinoma	10 (32)
Glassy cell carcinoma	1 (3.2)
Intra-operative / Post-operative characteristics	
Mean Operative Time (minutes)	170 (110-300)
Median number of lymph nodes retrieved	24 (7-52)
	Number of patients (%)
ART - open approach **ART - laparoscopic approach**	27 (90) 3 (10)

Post-operative Antibiotics	8 (27)
Estimated Blood Loss	813 ml (50-4500)
Transfusion	6 (20)
Lymphatic invasion	3 (10)
Post-operative complications	14 (47)
Re-operation	3 (10)
Recurrence	3 (10)
Survival Rate	28 (93)
Pregnancy Rate	3 (10)

Table 1.1 Pre- / Intra- / Post- operative characteristics [220]

Recurrence and Mortality

28 patients (93.3%) who underwent ART are alive at the time of writing this manuscript, with a median follow up of 24 months (range 7-113 months). Two patients have died following recurrence of disease. One patient (30 years of age) presented ten months after her ART for stage IB1 cervical adenocarcinoma (diagnosed in June 2006) with non-specific respiratory symptoms, breathlessness and intermittent pleuritic chest pain, as well as several lumps situated in the anterior abdominal wall. Imaging revealed a highly suspicious right lung and abdominal wall lesions with para-tracheal lymphadenopathy. CT guided biopsies demonstrated neoplastic lesions of identical histology as the original cervical cancer, mucinous adenocarcinoma, and therefore, confirmed a metastatic picture. The patient underwent surgical removal of the abdominal masses and several cycles of chemoradiotherapy but died in 2008, twenty-two months after her initial diagnosis.

The second patient also underwent ART for stage IB1 cervical adenocarcinoma. A follow-up MRI three months post-ART revealed extensive disease in the anterior portion of the uterus, invading

towards the bladder and posteriorly towards the rectum. She was treated with radical chemoradiotherapy and 2 months post-treatment, a new MRI scan showed residual disease still present and new lung nodules, thus confirming further metastatic disease. As her condition worsened, the patient underwent a defunctioning colostomy formation following admission for large bowel obstruction. She died 11 months after diagnosis.

One other patient has presented with disease recurrence (November 2010). She was diagnosed with a grade 2 squamous cervical carcinoma (stage IB) in November 2007 and underwent ART with bilateral pelvic lymphadenectomy (24 lymph nodes, all clear). Resection margins were tumour free. Her post-operative recovery period had been uneventful until the time of recurrence. It consisted of a pelvic mass, extending to pelvic side walls and pressing against rectum and bladder, with right ovarian involvement. She underwent chemoradiotherapy with no subsequent evidence of persistent disease.

Pregnancy

Prior to ART, four patients had a history of pregnancy, with only one patient out of the four reporting the birth of a healthy baby. The other three patients had suffered a miscarriage(s). Ten patients reported a desire to conceive during the follow-up period. Fertility treatment or oocyte cryopreservation was not sought by any of the patients prior to ART. The mean age of this particular group was 30 years (range 26-36 years), with a median follow up of 53 months (range 11-113 months). The pregnancy rate was 30%, with two of the patients delivering successfully.

Two patients conceived spontaneously four and five years after ART respectively and both experienced an uneventful pregnancy. Healthy term female infants were delivered at 37 weeks gestation via a Caesarean Section. Noting the sole reliance on ovarian vessels for uterine perfusion during pregnancy, we were reassured by the weight of the 'term' babies, which were within normal range. The third patient conceived six years post-ART via IVF but had to undergo a termination of pregnancy at 17 weeks gestation. This was a consequence of spontaneous rupture of membranes and

subsequent fulminating chorioamnioitis. Notably, all three patients had a cervical cerclage suture inserted at the time of ART, with the suture removed in the chorioamnioitis case.

1.4. Discussion

The drive behind UTx research was fuelled by initial work in ART, another fertility-preserving surgical procedure used to treat early stage cervical carcinoma. Although worldwide, most cervical cancer patients are treated with hysterectomy, fertility-preserving methods are becoming more accepted and available as the survival rates continue to mimic those of a radical hysterectomy.[221] This important treatment alteration followed the realisation that the uterine body can be preserved without apparent compromise of therapeutic efficacy.[221] The current accepted fertility-preserving procedure in Europe and Canada is VRT with laparoscopic pelvic lymphadenectomy, with over 600 cases reported in literature, and therefore, the largest experience to date as a method of fertility-sparing surgery in early cervical cancer. ART is becoming a favoured fertility-sparing procedure in the UK and is well-established in the USA, with extremely promising and improved oncological results (1.9% recurrence rate in tumours < 2cm following ART) but worse fertility outcomes in comparison to VRT (15.5% versus 30% of all pregnant women from the international series), for tumours < 2cm in size.[222]

When looking at the literature, ART seems to be a reasonable option for selected patients whose tumours are < 2cm in diameter. We are aware that the fertility rates currently favour VRT and this issue requires further investigation. Despite this, we believe that in future, gynaecological oncologists should consider both ART and VRT with every patient diagnosed with early stage cervical cancer (tumour size < 2cm) who wish to preserve their fertility. The choice should depend on the eligibility criteria (**Box 1.1**), treatment centre, availability of trained personnel and patient choice. The patient in particular should be made aware of recurrence, mortality and fertility outcomes of ART and VRT, as well as the experience of surgeons at that institution in both procedures. The techniques for an open ART and routine radical hysterectomy are extremely similar. Therefore, in theory, a learning curve should not exist as more trainee surgeons should be able to master the operative techniques of open

Box 1.1 Eligibility of patient for ART - What are the criteria? [220]
Cervical carcinoma: FIGO Stage IA1 with lymph-vascular space involvement (LVSI) or Stage IA2 / IB1
Histological sub-types: squamous, adenosquamous, adenocarcinoma
No evidence (clinical or radiological) to suspect extra-pelvic spread or lymph node metastases
Lesion size greater than 2cm
Desire to preserve fertility
No diagnosis or indication to suspect infertility
Favourable age (relating to fertility outcomes)

ART relatively easily because radical hysterectomy is readily taught and reproducible in any gynaecologic oncology unit. The overall incidence of intra- and post-operative complications is likely to be lower with ART than with VRT when performed in average-sized gynaecologic oncology centres. This cohort of patients supports this theory with no intra-operative injury demonstrated. Only three patients required further surgical re-intervention because of complications related to the operation: surgical treatment of haematocolpos, laparoscopic repair of an omental prolapse and low transverse laparotomy, ovarian cystectomy and adhesiolysis. With regards to urinary symptoms, only one patient experienced a single episode of urinary retention in the first month post-operatively.

ART is a safer option with regards to bulky, exophytic or > 2cm tumours as the extent and location of tumours this size and shape require increased radicality of parametrial, sacrouterine, vesico-cervical and pelvic lymphatic tissue resection, which is not possible with VRT, where the approach limits the parametrial resection to tissue in the medial half of the broad ligament. With regards to the two deaths from this cohort, one of the patients was advised that ART would not give her the greatest chance of cure because of her bulky tumour mass (>4cm). However, she still opted for ART for fertility reasons (patient was only 29 years of age at time of diagnosis), which unfortunately did not lead to recovery.

Fertility

Only ten of our cohort attempted to conceive, in part due to our earlier recommendation that each patient waits for two years following the procedure while undergoing close surveillance. This recommendation has now changed to one year. Our pregnancy rate (when focusing on the sub-group who attempted to conceive) was 30%, and delivery rate 20% (term pregnancies). This is comparable to the rates reported in literature; worldwide, 194 patients have undergone ART, 15% became pregnant and 10% delivered.[222] Furthermore, when compared to the fertility rate following hysterectomy (0%), one can immediately see that as a fertility-sparing procedure, ART is a definite success.

Ungar et al have reported previously the birth of two healthy term infants and the loss of 3 fetuses in the 7th, 8th and 15th gestational weeks of five pregnant patients who underwent ART (Hungarian patient cohort).[216] The patients were followed-up for 40 months with no record of relapse. However, on closer analysis of the 'lost' fetuses, we note that 2 of these 3 were in fact the first 2 cases performed. We believe this to be of significance because since then, the surgical technique has improved as both knowledge and experience of the procedure have advanced.

The potential use of ART in pregnant women during the second trimester is an added challenge that is currently being a debated. Management of cervical cancer during pregnancy depends on five factors: stage of the disease (and the tumour size), nodal status, histological subtype of the tumour, term of the pregnancy, and whether the patient wishes to continue her pregnancy.[223] The gynaecological team has three choices when deciding upon treatment during pregnancy. First is a radical hysterectomy with termination of pregnancy. Second is a planned delay in treatment until delivery. Finally, the third option is chemotherapy until lung maturation has occurred, both followed by a radical hysterectomy.[10] The trachelectomy approach in early gestation is feasible and may result in the most favourable oncologic and obstetrical outcomes when compared to the above three options. It also offers the added

bonus of preservation of future fertility. In addition, it is worth highlighting that the role of adjuvant therapy in node-positive post-ART patients deserves further investigation.[223]

We believe that trachelectomy should now be offered as a first line treatment in very specific cases of women diagnosed with early stage cervical cancer during pregnancy. It is a cost-effective, feasible and safe treatment option. Both ART and VRT should be considered prior to deciding which operation is more suitable for treating tumours <2cm in diameter. For tumours >2cm in diameter, ART seems to be the most appropriate choice.

Certain criteria must apply in order to arrive at a carefully chosen group: (a) Patient diagnosed with early stage cervical cancer during second trimester pregnancy; (b) Trachelectomy should be offered only in specialist gynaecologic oncology centres with long-standing experience of performing this procedure; (c) Loss of fertility and/or loss of fetus secondary to a standard hysterectomy will impact considerably on patient's quality of life; (d) The benefits of performing trachelectomy far outweigh the risks; and (e) The final decision should rest with a fully informed and consented patient in all cases. The discussion regarding the management of the patient should take place within a gynaecologic oncology multidisciplinary team meeting, with pre-operative counselling and post-operative support already in place.

In conclusion, radical trachelectomy may expand the management options for pregnant women diagnosed with stage IB1 cervical cancer who wish for both immediate cancer treatment as well as prevention of definitive pregnancy termination. We look forward to more publications on this topic, with further welcome news regarding successful pregnancies post-trachelectomy performed during a second trimester. This data should be accumulated but more importantly openly discussed in order to verify the strengths, weaknesses, efficiency and safety of the technique.[216] Only successful outcomes with regards to both cancer clearance and fertility preservation can resolve any arguments relevant to the moral, ethical and financial aspects of performing a trachelectomy procedure in a pregnant

woman. Furthermore, we strongly encourage surgeons to report in detail post-operative outcomes, including complications, length of follow-up, and future fertility outcomes.

Limitations

We acknowledge, however, that our conclusions have their limitations, mainly as a result of the size of our data set and duration of follow-up. Our data set includes only 31 patients and the median follow-up length of time is only 24 months.

The fact that the final decision rests with the principal surgeon was perfectly highlighted when you apply the ART eligibility criteria (**Box 1.1**) to our data. Our patient cohort 'disagreed' with the criteria on two accounts: size and stage. Imaging revealed only five patients with tumour size > 2cm and 3 patients were diagnosed with a stage IB2/IIA tumour. With respect to the latter 3 patients, all are still alive at the time of writing. Our conclusions below are partly based on these findings.

Finally, it is worth asking the question whether ART is an adequate fertility-preserving procedure when only a third of the cohort attempted to become pregnant post-ART and the mortality rate was 6.7%. This proposition can be rejected when looking closely at the results. One of the two patients that died was advised against ART. Second, all women in our cohort underwent ART as opposed to radical abdominal hysterectomy in order to preserve their fertility for the purpose of future pregnancy. A third of the cohort has tried to get pregnant post-ART but this is only to date (median follow-up: two years). From the remaining 20 patients who have not attempted pregnancy, some did not have a partner/were not married at the time of the operation, whereas others felt that they wanted to wait longer prior to starting a family. Hence, the low number of women attempting to conceive to date should not be confused with the fact that all of our ART patients were hoping to fall pregnant eventually.

Need for a RCT?

Ultimately, we believe that for patients wishing to preserve their fertility and diagnosed with early stage cervical cancer, the next stage of trachelectomy research should be a treatment comparison between abdominal and vaginal RT (open and laparoscopic) in a large-scale, multi-centre, prospective randomized trial of ART vs. VRT vs. cone biopsy and lymphadenectomy for lesion size 0–2cm, correlating recurrence rates and fertility outcomes as primary end-points. If fertility is no longer an issue, then vaginal RT vs. RH is also a valid trial. No such trials exist in the literature.[224]

We do not believe that it is ethically acceptable to do a trial of abdominal RT compared to vaginal RT for lesion size 2–4cm because of Dargent's high recurrence rates after VRT of tumours >2cm (6/27).[225] Secondary aims should try to define the incidence of specific post-operative complications within the three trial groups, arrive at a more suitable method of pre-operative patient selection for a specific procedure and investigate how to apply adjuvant therapy in node positive patients. A closer look at surgical techniques employed in the formation of a neocervix, application of a permanent cervical cerclage suture (and timing: during ART or pregnancy) and the process of re-anastomosis are also worthy of study. To provide quality assurance of surgical technique between hospitals, a limited number of centres with recognized surgical skills to perform this procedure should be included first. Another trial option may be to randomize patients between either 'a radical trachelectomy and lymphadenectomy (any approaches)' and 'simple trachelectomy and lymphadenectomy' for small volume lesions, as recent literature is slowly suggesting that radical surgery is probably not necessary in most cases. We hope that information gained from the research proposed above would greatly improve our understanding of fertility-sparing surgery and ultimately demonstrate its appropriateness in treating the patient group highlighted in this study, with respect to oncological safety and pregnancy outcomes.

Relevance to UTx

The ART patient series described in this report represents the largest data set from a single institution in the UK. Fertility outcomes and the role of adjuvant therapy in node-positive, post-ART patients deserve further investigation. We also believe that ART should qualify as a 'required skill', and therefore, feature on the Gynaecologic Oncology surgical curriculum.

There is an ultimate benefit with respect to UTx research. First, as a fertility-preserving procedure, it can raise awareness of other such procedures such as UTx and lesser known operations, for example Modified Strassman Procedure (MSP). The standard management of placental site trophoblastic tumours is a radical hysterectomy with pelvic lymph node sampling. MSP is an alternative fertility-sparing technique. *Saso et al* presented five cases who underwent MSP to treat a presumed solitary uterine placental site trophoblastic tumour.[174] Following surgery, all patients remained in remission. Only one patient has remained in remission with her fertility intact. The other four underwent a completion hysterectomy because of possible incomplete excision of the disease. No residual disease was later found in two of these four uteri.[4] Since then, the sole patient who had her fertility preserved has delivered a healthy, term baby. Therefore, an MSP can be added to an ever-growing list of gynaecological surgical procedures which are both curative and fertility preserving. Apart from radical trachelectomy (vaginal and abdominal), other cases described involve the first case of fertility sparing surgery for a giant adenomatoid tumour of the uterus (a tumour previously managed by hysterectomy),[226] a successful cessation of haemorrhage in a patient with a ruptured cornual ectopic pregnancy without recourse to hysterectomy,[175] and the surgical management of uterine arterio-venous malformation where selective temporary ligation of the uterine and ovarian vessels was applied.[227]

Second, the surgical techniques required to carry out ART overlap significantly with the graft retrieval and anastomotic aspect of the UTx procedure. Finally, lessons learned from ART with regards to uterine perfusion can be applied to UTx. Namely, the fact that the uterus remains viable if supplied by

two vessels out of the six that supply it (three pairs of ovarian, uterine and vaginal arteries). Post-ART, the uterus is most commonly supplied and drained by the left and right ovarian vessels only.

STUDY 2a

2. Study 2a: Uterine allotransplantation in a rabbit model using aorto-caval anastomosis: a long-term viability study

This study has now been peer-reviewed and published as per following reference: **Saso S,** Petts G, Chatterjee J, Thum MY, David AL, Corless D, Boyd M, Noakes D, Lindsay I, Del Priore G, Ghaem-Maghami S, Smith JR. Uterine allotransplantation in a rabbit model using aorto-caval anastomosis: a long-term viability study. *Eur J Obstet Gynecol Reprod Biol* 2014; 182C:185-193.

Abstract

Objective: Uterine transplantation (UTx) has been proposed as a treatment option for women diagnosed with absolute uterine factor infertility. Allogeneic UTx has been attempted in a number of animal models, but achieving an adequate blood supply for the transplanted uterus still presents the biggest challenge. Microvascular re-anastomosis was unsuccessful in a number of animal models. The aim was to assess whether a large vessel aortic-caval vascular patch technique can bring about long-term graft survival after allogeneic UTx in a rabbit model.

Study Design: A longitudinal study involving uterine cross transplantations (n=9 donors, n=9 recipients) was performed in New Zealand white rabbits using an aortic-caval macrovascular patch harvested as part of the uterine allograft. All rabbits were allogeneic and of proven fertility, with at least one previous litter each. The end result of the donor graft harvest was a total hysterectomy transecting across the vagina and the most lateral aspects of the uterine horns together with an aortic-caval macrovascular patch (aorta, inferior vena cava, common and internal iliacs, and uterine arterial and venous tree). Tacrolimus (500µg twice daily) was administered for immunosuppression post-transplant. The recipients were closely monitored until death or euthanasia.

Results: In this case series, long-term rabbit survival was 11% (n=1). Surgical survival was 56% (n=5). Three rabbits (UTx #3, #4 and #8) died intra-operatively as a result of blood aspiration, ventricular hematoma, and massive haemorrhage. Three does (#1, #2, #7 and #9) died within the first 24h as a result of the veno-vena and anastomosis breakdown. Does #6 and #9 died secondary to pre-

operative pneumonia and a pulmonary embolus, respectively. Only one rabbit survived longer than a month.

Conclusion: Our method used a macrovascular patch technique to ensure adequate blood supply to the donor uterine graft. We have demonstrated the feasibility of uterine allotransplantation using this technique in the rabbit, but were unable to demonstrate a higher long-term survival percentage because of issues related to using a rabbit model.

2a.1. Background

Various models, both small-animal (mouse, rat, dog, and rabbit) and large-animal (sheep, pig, and primate) have been used to study different aspects of UTx.[4] In this chapter, efforts to perform UTx in a rabbit model are described. Thus, the focus with respect to surgical techniques will be on small animal models. The literature review has already been covered in an earlier chapter titled 'Literature Review'. The next chapter deals with a large animal model.

Aims

The primary aim was to investigate anatomical and surgical aspects necessary for a successful UTx. In those recipients that survived the surgery, the (secondary) aim was to characterise the immunological mechanisms involved in allogeneic UTx (rejection patterns) and suppress acute rejection with an adequate immunosuppression regimen. Finally, the tertiary aim was to assess whether fertility (conception, pregnancy and fetal well-being) was possible following orthotopic, allogeneic UTx, whereby the recipient had demonstrated long-term survival and had been administered immunosuppression. Presented also is a comparison of pre- and post-operative reproductive and multi-organ function.

2a.2. Materials and Methods

Rabbit model

The feasibility of the uterine allograft dissection together with its vascular supply that includes the internal and common iliacs, the AA and the IVC was first ensured by a series of uterine transplantations in the rabbit model performed by this team.[3] Here, we performed two surgically explorative procedures and nine allogeneic uterine cross transplantations in the rabbit model using a pre-determined protocol.

18 New Zealand white rabbits were used as donor and recipient animals (nine donors, nine recipients). None of the transplants were syngeneic; all were allogeneic. The animals were acquired a few days before the operation to ensure appropriate acclimatization to their surroundings. The rabbit uterus is similar in shape to the rat/murine uterus. It is composed of two long horns, approximately 7-9cm in length and 1cm in width, which join in the midline to form a single uterine cavity-like structure **(Figure 2a.1a-b)**.

Surgical protocol

This particular surgical procedure has already been described in detail.[3] A brief outline is provided below. The donor rabbit was anesthetized using a combination of intramuscular ketamine (35mg/kg) and xylazine (7mg/kg). After intubation, anaesthesia was maintained with 1.5–2.0% isoflurane. The anterior abdominal wall was shaved and cleaned, and the abdomen was opened through a midline incision. The end result of the donor graft harvest was a simple hysterectomy transecting across the vagina and the most lateral aspects of the uterine horns together with an aortic-caval macrovascularpatch. Following graft harvesting, the AA was cannulated and the uterine specimen with its vascular patch was generously flushed with heparinised normal saline and transplant storage solution, *Histidin-Tryptophan-Ketoglutarat (HTK)*.

The uterine allograft along with the AA, IVC, common and internal iliacs, and uterine arterial and venous tree all intact were harvested *en bloc*. The uterus was flushed and subsequently stored for 90 minutes in the *Celsior* solution, between 2°C and 8°C while the recipient doe was being prepared. The recipient animal was anesthetized and prepared in a similar manner as the organ donor and injected with 250 IU subcutaneous heparin. A midline laparotomy was performed, and the loose connective tissue overlying the infra-renal aorta and IVC were removed to expose this area of the major vessels. Soft tissue vascular clamps were placed first around the recipient's AA. A longitudinal cut (4mm

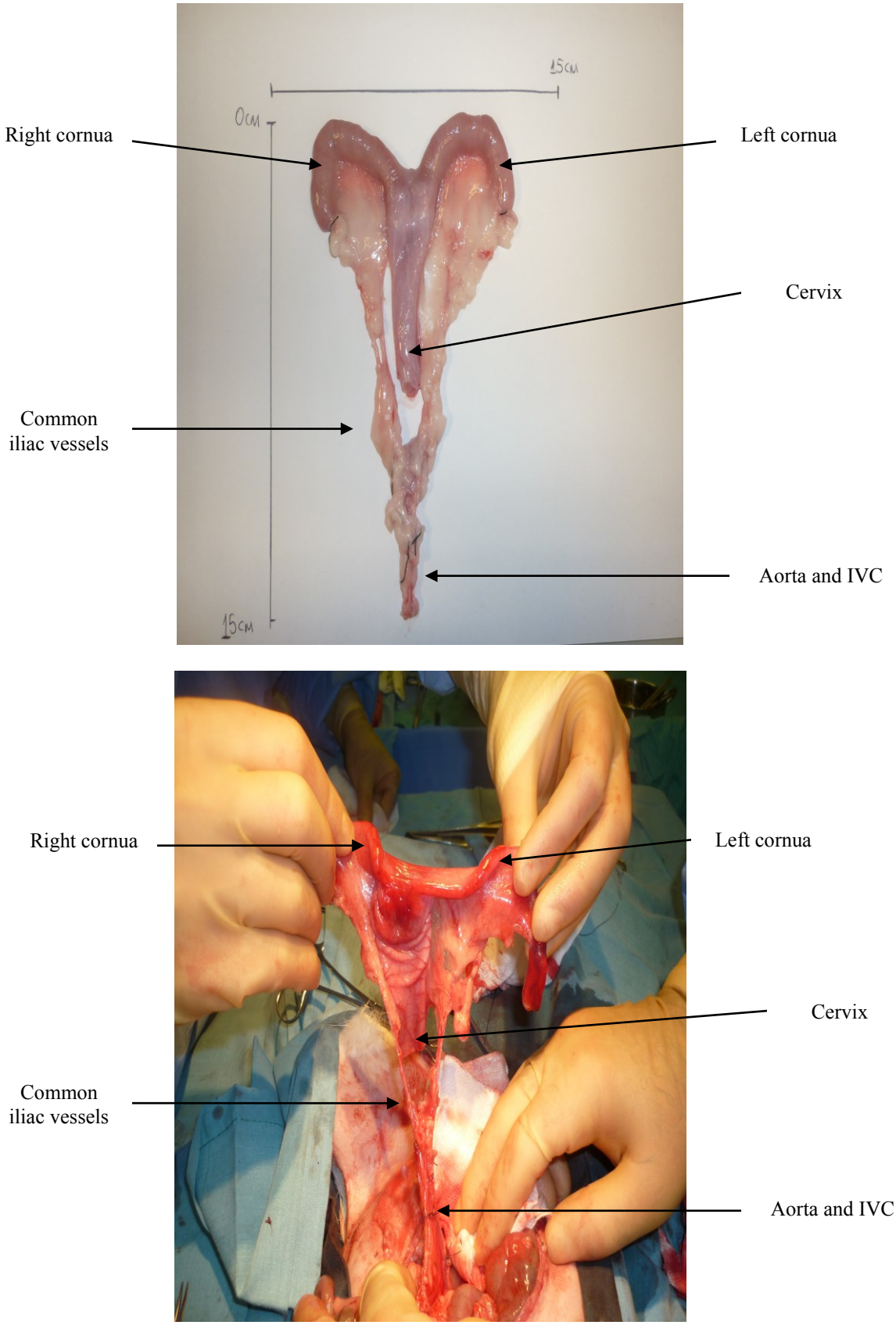


Figure 2a.1a and Figure 2a.1b. Rabbit uterus with 'macrovascular patch'. The first figure is a photograph of the recipient uterus following hysterectomy. The macrovascular path showing the AA and IVC is clearly shown. The second figure shows the graft uterus immediately after the end-to-side AA and IVC anastomosis.

length) was made in the anterior aortic wall to create an opening. An end-to-side anastomosis was performed to the AA of the graft using 6-0 Prolene sutures. A total of six to eight stitches were used for the aortic connection. A similar procedure was carried out for the cava-cava end-to-side anastomosis. The vagina was anastomosed initially to ensure proper orientation and positioning of the specimen followed by the aorto-caval patch, end-to-side vascular re-anastomosis to the recipient's AA/IVC, ensuring vascular integrity, haemostasis, and patency. The uterine horns were finally rejoined. Successful reperfusion of the graft was judged on the colour shift of the uterus during reperfusion from its blanched appearance after flushing to a more reddish colour. Blood flow past the venous and arterial anastomotic sites was also ensured.

The graft retrieval and transplant was performed by Mr J. Richard Smith and Dr S Saso. The vascular anastomosis was carried out by Mr DJ Corless (Consultant Upper GI Surgeon, Mid Cheshire Hospitals Trust), with Mr JR Smith and Dr S Saso assisting. Dr M Boyd (Veterinary Anaesthetist, Royal Veterinary College) was the anaesthetist for all nine transplants. Blood samples from the does were taken by either Dr M Boyd or Dr S Saso. Advice on technical aspects and rabbit physiology during a major surgical procedure was given throughout by Professor D Noakes.

Medication

Subcutaneous heparin 250 IU was given to the donor before organ procurement and to the recipient pre- and intra-operatively to prevent thrombosis. Empirical immunosuppression was administered in allotransplants #4-#9 using CsA and hydrocortisone (intravenous intra-operative doses of 10µg/kg and 40mg respectively) commenced during the vascular patch anastomosis, followed by oral tacrolimus (500µg twice daily postoperatively) and prednisolone (10mg post-operative days 1-4 and 5mg post-operative days 5-7). Quinolone enrofloxacin (Baytril) was used as antibiotic prophylaxis at an intra-operative dose of 5 mg/kg through a slow intravenous infusion.

Post-operative course

The rabbits were closely monitored post-operatively within a specialist enclosure at The Royal Veterinary College, London. Multiple siring of transplanted rabbits occurred during this period. Heart rate and oxygen saturation were recorded daily in the first two weeks with twice weekly measurements taken subsequently until their demise. They were allowed to eat *ad libitum* and were fed with autoclaved hay as well as with a proprietary pellet called GD99 that contains a coccidiostat.

The room temperature varied between 19ºC and 21ºC, with a relative humidity of 47-62%. Their lighting schedule was 12 hours on and 12 hours off. Close collaboration with veterinary specialists was extremely important, and all work undertaken was strictly under the Animals (Scientific Procedures) Act 1986.

Post-mortem & Histopathology

All post-mortems were performed within two hours of the rabbit dying. The same laparotomy incision as used in the actual surgery was opened and extended to become a thoraco-laparotomy. The thoracic and abdominal cavities were inspected, including the macrovascular patch, the uterine graft and each organ individually (heart, lungs, liver, kidneys, spleen). These organs were then placed in formaldehyde to fix the tissue and prepare it for histopathology. An incision was made on the uterine cornua to allow for the formaldehyde to access the inside of the cornua.

The gross morphology findings were recorded according to a scoring system previously validated by the Brannstrom team [117] and also used by *Diaz-Garcia et al* in UTx.[109] The following characteristics were evaluated by the scoring system: (a) abnormal appearance of the uterus (colour, texture and hardness); (b) presence of adhesions; and (c) thrombosis of the graft vessels (both artery and vein).

Prior to haematoxylin & eosin (H&E's) staining, tissue was cut and placed on slides, with the assistance of Dr Iain Lindsay (Consultant Pathologist, St Mary's Hospital, London). All routine H&E's were stained on the Tissue-Tek staining machine. The haematoxylin dye is a poor dyeing agent. To obtain purposeful results it needs to be converted to its oxidation product haematin. This is achieved by the use of an oxidising agent such as mercuric II oxide (HgO). Before this conversion is brought about, haematoxylin is added to an aqueous solution of aluminium potassium sulphate. This is the mediating step, where aluminium ions combine with the haematoxylin dye. The resultant aluminium-haematin complex then stains via the metal ion - Al^{++}. Haematoxylin possesses acid-base indicator properties (red in acid - blue in alkali solutions) so the stained tissue components must be 'blued' after differentiation using an alkaline solution (London tap water or a blueing agent). This then allows the rest of the tissue components to be counterstained as desired (normally eosin). The sections are first immersed in water. Visible nuclei are then stained in Harris haematoxylin for five minutes. This is then followed by a wash in water and differentiation in 1% acid alcohol. The well is washed in water and 'blue' in Scott's water. Subsequently the sections are all washed in water again, followed by staining in 1% eosin Y for five minutes. Washing in water implies de-waxing in xylene in 1-2 minutes, then in 99% alcohol for 10 seconds which is repeated three times and finally repeated in running tap water for 10 seconds. A repeat wash in water, followed by dehydration, clearing and mounting. This implies the following method: dehydration in 99% alcohol for 10 seconds repeated three times, clearance in xylene for 20 seconds and mounting on cover-slipping machine.

Immunological, reproductive and organ profiles

Blood samples and donor grafts were used for the experiments. We have performed nine allogeneic UTx in the rabbit model:

3 transplants (no immunosuppression): (3 donors, 3 recipients)

6 transplants (immunosuppression): (6 donors, 6 recipients)

Peripheral blood samples (6ml - 4ml for immunological profile and 2ml for reproductive and organ profiles) were obtained from the marginal auricular vein or artery and placed into sterile heparin-containing anticoagulant tubes on the following post-transplant days: D0 (prior to transplant - control group), D5, D12, and from then onwards at weekly intervals until (depending on which occurred first) either the rabbit died or the time of the Caesarean section providing pregnancy had been achieved.

Markers of rejection consisted of all lymphocytes, circulating T lymphocytes - CD4+ T helper cells and CD8+ cytotoxic cells - B lymphocytes and monocytes/macrophages.

Hormones levels measured with regards to presenting a reproductive profile were as follows: Follicle-Stimulating Profile (FSH), Luteinising Hormone (LH), Testosterone, 17β-oestradiol and progesterone.

Standard biochemistry markers were used to assess organ function: urea and electrolytes (U&Es), creatinine for kidneys, liver function tests (LFTs) for the liver, albumin for general metabolic function and C-reactive protein to monitor the inflammatory process.

Tacrolimus levels were also measured to therapeutic levels.

With regards to the isolation of peripheral blood mononuclear cells (PBMCs), blood obtained from the transplanted rabbit was first placed into a Falcon tube and subsequently centrifuged at 1500rpm for 5 minutes to separate the plasma and supernatant. The separated plasma was collected and stored in cryovials at -20^{o}C to -80^{o}C for future functional studies (five cryovials containing the following volumes: one cryovial of 1ml and four vials of 250µl). The remaining plasma supernatant was discarded. The plasma free blood was then layered over Histopaque/Ficoll (solution containing polysucrose and sodium diatrizoate, adjusted to a density of 1.077 g/mL, Sigma-Aldrich Biotechnology LP and Sigma-Aldrich Co.-10771, Axis Shield, Cambridge, UK) in a 1:2 ratio (blood pellet is first diluted with Phosphate Buffer Saline, PBS in a 1:1 ratio) and centrifuged at 2500 rpm for 25 minutes at room temperature with no brake. Composition of PBS was as follows: 8g NaCl, 0.2g

KCl, 1.44g Na_2HPO_4, 0.24g KH_2PO_4. The mononuclear cells formed a white layer ('buffy coat') which was removed using a pipette and transferred into a clean tube. Cells were then washed in PBS three times. Any red blood cell (RBC) contaminants were removed by re-suspending the pelleted cells in a RBC lysis buffer for 10 minutes at room temperature. Lysed cells were subsequently removed by washing in PBS. The number of PBMCs extracted from 4ml of rabbit blood ranged from 6-60 million cells.

Flow cytometry was the next step. PBMCs were washed and counted. They were then re-suspended in PBS in eight FACS tubes. The number of PBMCs pipetted into each FACS tube ranged from 600,000-1.3 million cells. Antibodies were added at the appropriate dilution (**Table 2a.1**), and the cells were incubated in the dark on ice for 15 minutes. Optimal antibody concentration was based on titration experiments. Following washing with 1ml of PBS and subsequent centrifuging at 1500rpm for 5 minutes, cells were incubated with a secondary antibody (goat anti-mouse IgG:FITC), or if the primary antibody was directly conjugated, cells were re-suspended in around 100µl of PBS. PBMCs which required a secondary staining (in bold in **Table 2a.1**) were re-suspended in PBS containing the secondary antibody for a further 15 minutes in the dark on ice. Cells were then washed a further time in 1ml of PBS. The antibody stained cell suspension was then transferred to FACS tubes containing 300µl of PBS for FACS analysis using the FACScalibur (Becton Dickinson) and analysed using the FlowJo software (Tree Star, Inc.). The machine was set to analyse exactly 10,000 cells/FACS tube.
Both forward (cell size/volume) and side scatter (cell granularity) detectors were aimed at the column of cells passed through the FACScalibur. The unstained and mouse IgG1 antibody stained samples were used as negative controls for this experiment. All cells located to the right of the latter group on the scatter plot were defined as being positive for the marker in question.

Marker Antibody	Target	Fluorochrome	Concentration	Source
Mouse IgG1	Negative control	FITC	10µl/10^6 cells	AbD Serotec
Mouse Anti-Rabbit CD4	'Helper' T lymphocytes	FITC	3µl/10^6 cells	AbD Serotec
Mouse Anti-Rabbit CD8	'Cytotoxic Killer' T Lymphocytes	FITC	3µl/10^6 cells	AbD Serotec
Mouse Anti-Rabbit CD25	Activated T cells	FITC	3µl/10^6 cells	AbD Serotec
Mouse Anti-Rabbit CD11b	Monocytes/Macrophages	FITC	3µl/10^6 cells	AbD Serotec
Mouse Anti-Rabbit CD45	All lymphocytes	FITC	3µl/10^6 cells	AbD Serotec
Goat Anti-Rabbit IgM:FITC	B cells	FITC	2µl/10^6 cells	AbD Serotec
	Secondary Antibody			
Goat Anti-mouse IgG:FITC	IgM (B cells)	FITC	1µl/10^6 cells	AbD Serotec

Table 2a.1 List of antibodies used for FACS analysis

Pregnancy

The protocol was prepared by the rabbit fertility team of the Polytechnic Institute, Valencia, Spain, led by Professor Jose Vicente. It has already been described in Dr Gamal Mehaisen's thesis, supervised by Professor J Vicente. Sixty hours before insemination time, superovulation was induced in nine donor does following subcutaneous injection of 20IU/kg of eCG (Gonaser, Hipra, S.A.). Ejaculates from five proven males were collected using an artificial vagina. A 10µL aliquot samples from ejaculates were diluted 1:10 with Tris-citrate-glucose extender (250mM tris-hydroxymethylaminomethane, 83mM citric acid, 50mM glucose, pH 6.8-7.0, 300 mOsmkg-1) for a previous motility rate evaluation. Ejaculates with an estimated motility higher than 70% were pooled. From ejaculates, two aliquot sample of 10µL were taken. The first sample was diluted 1:10 with Tris-

citrate-glucose extender for motility rate evaluation in a computer assisted sperm analysis (CASA) system. The second sample was diluted 1:10 with 1% of glutaraldehyde solution in phosphate buffered saline to calculate the concentration in a Thoma chamber and to evaluate both the percentages of normal intact acrosome and abnormal sperm by phase contrast at a magnification of x400. Only ejaculates with more than 70% of motility rate, 85% of normal intact acrosome, and less than 15% of abnormal sperm were pooled and used to inseminate donor does. Pooled semen was diluted to 40 million per milliliter adding Tris-citrate-glucose extender in order to elaborate the seminal doses. Donors were inseminated with a seminal dose of 0.5 ml.[228,229]

Ovulation was induced in two does immediately following insemination by an intramuscular injection of 2μg of Buserelin Acetate (Hoechst, S.A.). The embryos were recovered *in vivo* by ventral midline laparoscopy 76-78 hours after the insemination, applying a method described by *Besenfelder et al.*[230] First, anaesthesia was induced by an intramuscular injection of 16mg xylacine (0.8ml of Rompun; Bayer AG, Leverkusen, Germany), followed by an intravenous reinjection of ketamine chlorohydrate (Imalgene; Merial, S.A., Lyon, France) to maintain does under anaesthesia during laparoscopy. Rabbit donor does were placed in a head-down position at a 45ºC angle. A Verres needle was inserted in the lower right abdominal quadrant and attached to CO_2 automatic insufflator. After CO_2 abdominal distension, a trocar-cannula unit of 5mm diameter was inserted into the abdominal cavity at 10cm caudal to the sternum. Another accessory (3mm diameter) trocar-cannula unit was inserted 4-5cm laterally to right of the former. After both were removed, they were respectively replaced by the laparoscope (Wolf paediatric 0º) and grasping forceps (length: 28.5cm). Subsequently, an epidural needle (inner diameter: 1mm, Vigor Epidural G17) was inserted near the ovary and a sterile polyethylene tube (inner diameter: 0.3 mm) attached to a 5ml sterile syringe was introduced for 2-3cm through the epidural needle into the oviduct. After fixation with the grasping forceps, ova and embryos were flushed from the oviducts to uterine horns with 5ml of Dulbecco´s phosphate buffered saline (DPBS, Sigma) containing 0.2 % (w/v) of Bovine Serum Albumin (BSA, Sigma) and supplemented with antibiotics (200 IU/mL penicillin G and 0.25mg/mL dihydrostreptomycin; Penivet 1). This was followed by flushing of the uterine horns, elevated with the grasping forceps placed near

the oviduct-tube junction, with 50ml of the same flushing solution. It was achieved via insertion of a sterile epidural needle directly through abdominal wall into each uterine horn. Finally, the recovery medium was aspirated from the vagina by a Foley Catheter (Minitube, Tiefenbach, Germany) connected with a vacuum pump of 20-40mmHg. After the recovery of does, the reproductive tract was washed with 0.1% ethylenediaminetetraacetic acid solution in PBS in order to prevent any abdominal adhesions post-laparoscopy.[228]

In both groups, recovered embryos were scored by morphological criteria. Only embryos in the morula stage with homogeneous blastomeres as well as a visible regular mucin coat and zona pellucida were catalogued as normal embryos.[67,71,228,231]

Vitrification and thawing process

All normal embryos (morulae) were vitrified by the methodology described by *Vicente et al*.[232] Briefly, the vitrification procedure was carried out in two steps at 20ºC. In the first step, embryos were placed for 2 minutes in a vitrification solution consisting of 12.5% (v/v) dimethyl sulphoxide (DMSO, Sigma) and 12.5% (v/v) ethylene glycol (EG, Sigma) in DPBS supplemented with 0.2% (w/v) of BSA. In the second step, embryos were suspended for 1 minute in a solution of 20% (v/v) DMSO and 20% (v/v) EG in DPBS supplemented with 0.2% (w/v) of BSA. Then, embryos suspended in vitrification medium were loaded into 0.125ml plastic ministraws (IMV, L´Aigle, France) between two drops of DPBS separated by air bubbles. Finally, the straws were sealed and plunged directly into liquid nitrogen. De-vitrification was performed in two steps, first ministraws were placed to 10 cm from vapour nitrogen until vitrified fraction begin to cristalize (20-30 sec) and, then, submerged into a water bath at 20 °C for 10 sec. To remove the vitrification, warmed embryos were introduced into a culture dish containing 0.33 M sucrose in DPBS supplemented with 0.2% BSA, after 5 min embryos were washed in DPBS for another 5 minutes. Thereafter, 30 de-vitrified embryos were cultured for 48 hours in Medium 199+20% FBS (Sigma) at 38.5ºC, 5% CO_2 and saturated humidity to assess post-vitrification viability. Twenty-four embryos reached a state of 'hatchable' blastocyst (80%).[67,171,228]

Embryo transfer

The recipient doe was injected intramuscularly with 25IU of eCG 60 hours prior to induce the ovulation with an intramuscular injection of 2μg of Buserelin Acetate (Hoechst, S.A.). The transfer was performed by mini-midline laparotomy, using previous scar to enter the abdomen. The uterus was externalised to allow for ease of access. Seventeen intact de-vitrified embryos were placed in cornua using an epidural catheter (G17, Baxter, Deerfield, Illinois, USA).[228]

Pregnancy monitoring

Experiments were carried out in the first four weeks following the above ET. 3ml of blood was taken at appropriate intervals during the gestational period to obtain a reproductive profile (βhCG, FSH, LH, Testosterone, 17β-Oestradiol and Progesterone). Furthermore, a colour Doppler ultrasound with a two-dimensional 7.5MHz (range: 7-10MHz) probe was used to monitor fetal development and growth. The protocol has been described already by *Chavatte-Palmer et al*.[233] The ultrasound examination was performed from right to left with the probe in the sagittal orientation, following localisation of the urinary bladder. The initial scan was performed on Day 5 post-ET with the aim to judge whether any fetal sacks were present. Complete fetal sack examination is performed on a maximum of six fetuses (three first on the right and three first on the left) in order to try to avoid repeated measurements on one fetus. Subsequent scans are done at 3-4 day intervals until the day of the elective Caesarean section. With respect to measurements, for the fetal sack, they are made when the largest surface area appears on the screen. Important for growth, crown-rump length (CRL) is determined as the maximum distance from crown to tail basis with the fetus on a sagittal plane.[154]

Data Presentation and Analysis

Statistical analysis was not required for this study. Data has been presented using 2D graphs, histopathology slides, figures and descriptive tables.

2a.3. Results

Three recipient does died intra-operatively. Five died within 24 hours. One doe survived long-term and was culled in the fourth post-operative month.

The mean weight of the donor rabbits was 3.93kg (3.4-4.4kg) and of the recipient rabbits 437kg (3.8-5.1kg). All rabbits were New Zealand White from an outbred strain. The first two UTx procedures were carried out with 'virgin' does. Recipient rabbits #3-#9 were all of proven fertility, having carried at least one litter in the past. Immunosuppression was given to recipients #4-9 as the initial aim was to characterise the acute rejection response using recipients #1-3. Operative details and hepatic, renal and reproductive profiles are outlined in **Tables 2a.2 and 2a.3**. Morphological findings are summarised in **Table 2a.4.**

Intra- and Post-operative course

Recipient #1 was euthanized Day 1 post-operatively (15 hours). Clinically, the rabbit appeared ischaemic and pale inferiorly from mid-abdomen and the hind legs were paralysed. On opening of the abdomen at post-mortem, a significant intra-abdominal collection of blood was revealed. Post-mortem examination and histology of fixed organs revealed a healthy looking and well perfused uterine graft. A haematoma at the right cornu-cornual anastomosis was visualised. The impression as to the cause of the rabbit's demise was most likely an exsanguination due to bleeding from an anastomotic breakdown. It was not possible to confirm whether an aorto-aorta/veno-vena anastomotic breakdown had occurred histologically. Therefore an alternative explanation could be a slow bleed from a peripheral vessel (haematoma noted at the cornua), causing a large haematoma that put pressure on the iliac (or uterine vessels) causing the ischaemia of the lower limbs. The well perfused uterus supports this as it suggests that the major inflow aortic and major outflow veno-vena anatamoses were intact thus ensuring perfusion and prevention of congestion and stasis related ischaemia respectively. However, ultimately the cause of death is still exsanguination due to the operation.

Recipient #2 died Day 1 post-operatively (12 hours). The rabbit was initially well post-operatively but found dead between regular checks with early rigor mortis. At post-mortem there was significant intra-abdominal bleeding. Again, the impression as to the cause of the rabbit's demise was exsanguination due to bleeding from an anastomotic breakdown, probably relating to intra-operative donor IVC injury. Major anastomotic breakdown was further supported following post-mortem examination and histology of fixed organs which revealed a dusky and veno-congested looking uterine graft suggesting poor perfusion. In this case the recipient uterine horns and utero-vaginal anastomoses appeared healthy suggesting that the recipient blood supply to these areas had remained largely intact.

Recipient #3 died intra-operatively. The rabbit was anaesthetised and prepped. However during intubation, she bit her tongue and aspirated ~5-10ml of blood. As a result her respiratory rate and heart rate both increased and it took 30 minutes to stabilise the rabbit and proceed with surgery. Between the re-commencement of the hysterectomy and the start of recipient engraftment (32 minutes), the recipient doe deteriorated and arrested four times requiring intravenous dose of adrenaline. Following the fifth arrest the decision was made to stop the surgery. Four anastomoses were completed before the rabbit died (vagina, left and right cornua, veno-vena). The cause of death was attributed to aspiration of blood resulting in cardiorespiratory arrest.

Recipient #4 also died intra-operatively. As with the previous attempt, the donor graft, together with the macrovascular patch was harvested *en bloc*. Four anastomoses were completed before the rabbit died (vagina, left and right cornua, veno-vena). However this time the anaesthetic and surgical preparation had been uneventful but the doe arrested five times during the course of the surgery (adrenaline given for all four arrests) with the decision being made to stop the surgery after the fifth arrest. The cause of death was unknown at the time of the post-mortem.

Recipient #5 is the only long-term survivor at the time of writing (post-operative D74). Intra-operatively she arrested twice during the uterine engraftment part of the surgery. However she was successfully rescued with adrenaline both times and experienced no further intra or post-operative problems. The abdominal wound had healed by the tenth post-operative day and normal patterns of behaviour such as self - grooming were re-established around the same time. She was mobilising well by the end of the second post-operative week. Her weight had returned to the pre-operative level by the end of the third week. She did not experience any further complications in the period up to D74.

Recipient #6 was the third rabbit to die intra-operatively. This time the doe had arrested twice and was revived successfully both times. The actual transplantation was completed with the donor graft, together with the macrovascular patch harvested and grafted *en bloc*. All anastomoses were completed (vagina, left and right cornua, veno-vena, aorto-aorta) and the abdomen was closed before the rabbit was pronounced dead exactly two minutes post-abdominal closure. The cause of death was unknown at the time of the post-mortem.

Recipient #7 followed a similar course to recipients #1 and #2. The team attempted a novel strategy where the cornua were not anastomosed in an attempt to decrease the time during which the doe anaesthetised. The cornua were left free-floating in the lower abdomen. Excellent vascular control was achieved with under-running of the IVC with fine ties and use of bulldog clamps. Shortening the length of prolene suture made for a faster and easier vena cava and aorta anastomoses. The aorta-cava was skeletonised with extensive skeletonisation (top 1.5cm aorta-IVC well separated), over an area of 4cm. The uterine allograft along with the aorta, inferior vena cava, common and internal iliacs, and uterine arterial and venous tree all intact were harvested en bloc. Blood loss was minimal, four sutures were required for the two vascular anastomoses and following clamp removal the uterus appeared adequately perfused. The time under anaesthesia was decreased by 20 minutes compared to the six previous cases. The doe recovered well and was placed back into the cage where she showed immediate signs of alertness. Surgically the team felt that this novel surgical strategy had gone well. However, within 12 hours of the operation ending, the recipient was found dead. At post-mortem a

large haematoma was found in the abdomen and the vagina. However the uterus appeared well perfused. The source of the post-operative haemorrhage was not identifiable macroscopically and it is felt that it could have been bleeding from the un-anastomosed recipient cornua and/or tearing of the aorta-vena anastomoses secondary to twisting of the unanchored free-floating donor uterus cornua.

Recipient #8 died intra-operatively. Donor and recipient hysterectomy were performed without issues. Ligaclips were used during graft retrieval during this operation and therefore, no difficulties arose with bleeding from perforators as seen on occasion with previous transplants. As with recipient 7 the practice of not placing a suture into the IVC prior to dividing the IVC and under-running of both the aorta and IVC with fine ties was continued. The aorta-cava was skeletonised with the same extensive skeletonisation as last time. However, unlike Recipient 7, tubal cornua were anastomosed this time and were not left free-floating. Excellent vascular control similar to UTx #7 was achieved. Unfortunately, a stitch in the aorta to prevent a small bleed that started twenty minutes after the team had completed the surgery caused a sudden estimated blood loss of 30ml, leading to demise.

Recipient #9 received the final uterine graft in this series. Surgical technique was the same as with UTx #7 and #8. With regards to haemostasis, achieving adequate vascular control, and a successful IVC-IVC and AA-AA end-to-side anastomosis, the operation proceeded without concern. The doe was found dead in the first 24 hours of the operation finishing. No pooling of coagulated bloods was found in the organ, together with absence of any obvious pathology of the organs. The cause of death was unknown at the time of the post-mortem.

UTx	Graft Retrieval (min)	1st Warm Ischaemia (min)	Cold Ischaemia (min)	2nd Warm Ischaemia (min)	Clamp on (IVC/AA) (min)	Reperfusion (min)	Recipient Hysterectomy (min)	Grafting (min)	EBL (ml)
1	120	6	90	125	35 / 17	15	40	125	35
2	120	6	90	135	55 / 27	20	50	135	50
3*	165	5	90	78*	37 / NA	NA	45	78*	30
4*	98	5	90	107*	27 / NA	NA	40	107*	40
5	110	8	90	153	39/ 25	17	45	153	20
6	90	8	90	150	33 / 28	20	40	150	20
7	80	8	90	122	34 / 20	15	30	122	20
8*	90	7	90	118	38 / 24	20	35	118	20
9	85	6	90	115	35 / 65	10	25	115	15
Mean	**106**	**6.6**	**90**	**131**	**40 / 29**	**16**	**39**	**131**	**28**

Table 2a.2 Operative details from the nine transplant procedures in the rabbit model showing retrieval, ischaemic, clamping, reperfusion and recipient hysterectomy times in minutes

Marker	UTx 1		UTx 2		UTx 3	UTx 4	UTx 5										UTx 6	UTx 7	UTx 8	UTx 9
	D0	D1	D0	D1	D0	D0	D0	D2	D6	D9	D13	D20	D36	D46	D60	D74	D0	D0	D0	D0
Sodium	142	NM	140	NM	142	136	**151**	141	142	143	142	143	144	144	144	141	146	145	148	142
Potassium	5.1	NM	4.5	NM	4.3	5.6	3.9	4.7	NM	4.3	4.5	3.8	3.8	4.0	NM	4.2	3.8	3.7	3.8	3.8
Urea	7.2	NM	6.3	NM	6.2	6.8	8.9	**21.8**	**12.2**	7.6	6.2	6.8	5.8	6.7	7.6	7.2	10.7	6	7	5.9
Creatinine	65	NM	82	NM	69	72	131	115	91	89	82	79	82	91	106	95	130	96	96	112
AST	15	NM	22	NM	24	53	40	63	21	14	23	30	22	29	NM	25	25	21	17	18
ALT	27	NM	16	NM	19	68	28	71	44	26	17	22	21	27	31	23	26	47	46	35
ALP	38	NM	35	NM	34	31	41	**21**	NM	37	34	34	38	36	42	50	22	39	35	40
Albumin	40	NM	32	NM	30	43	**57**	42	**50**	**49**	45	**50**	**51**	**56**	**55**	**56**	**55**	**56**	**54**	**47**
CRP	NA	NM	NA	NM	NA	NA	NA	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6
Reproductive profile																				
17β-oestradiol	56	NM	46	NM	56	77	70	47	57	83	90	112	60	102	101	92	108	44	88	44
LH	0.1	NM	0.1	NM	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
FSH	0.1	NM	0.1	NM	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Testosterone	0.4	NM	0.4	NM	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Progesterone	0.5	NM	0.5	NM	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.6	0.5	0.7	0.5	0.5	1.3	0.8	0.5	0.5
Immunosuppressant																				
Tacrolimus level	**NA**		**NA**		**NA**	**NA**	**NA**	**NM**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**NA**	**NA**	**NA**	**NA**

Key: **NA.** Non-applicable, **NM,** Not Measured (lab error, insufficient sample received, haemolysed sample)

Table 2a.3 Hepatic, Renal and Reproductive Profiles (Non-Pregnant Rabbit)

Ranges [234]

SODIUM	mmol/L	138 - 148
POTASSIUM	mmol/L	3.4 - 5.1
UREA	mmol/L	3.5 - 10.7
CREATININE	umol/L	12 - 146
ALKALINE PHOSPHATASE	IU/L	10 - 140
ASPARTATE TRANSFERASE	IU/L	14 - 113
ALANINE TRANSFERASE	IU/L	14 - 80
ALBUMIN	g/L	24 - 46
FOLLICLE STIM. HORMONE	IU/L	
	Follicular	3.5 - 12.5
	Mid-cycle	4.7 - 21.5
	Luteal	1.7 - 7.7
LUTEINISING HORMONE	IU/L	
	Follicular	2.4 - 12.6
	Mid-cycle	14.0 - 95.6
	Luteal	1.0 - 11.4
PROGESTERONE	nmol/L	
	Follicular	0.6 - 4.7
	Periovulatory	2.4 - 9.4
	Luteal	5.3 - 86.0
TESTOSTERONE	nmol/L	
17-β OESTRADIOL	nmol/L	
	Follicular	46 - 607
	Mid-cycle	315 - 1828
	Luteal	161 - 774
FK506 (Tacrolimus)	ug/litre	

Post-mortem and Histopathology

Table 2a.4 and 2a.5 below and **Figure 2a.2a-i** summarise the findings following post-mortem and histopathology.

Table 2a.4 Summary of gross morphology findings at post-mortem, performed within 60 minutes of the death of the recipient doe (*Wranning et al*) [117]

UTx	Colour 0, pink 1, dusky 2, white	Texture 0, soft 1, firm 2, hard	Size 0, normal 1, oedematous 2, markedly bigger	Anastomosis (IVC-IVC) 0, intact 1, broken down	Anastomosis (aorta-aorta) 0, intact 1, broken down	Uterine bleeding 0, absent 1, present	Adhesions 0, absent 1, minor 2, severe signs of inflammation	Laparotomy scar 0, normal 1, swelling or inflammation 2, pus or hernia
1	1	1	1	1	1	1 (total blood volume within abdomen: 30ml)	0	0
2	1	1	1	1	1	1 (total blood volume within abdomen: 20ml)	0	0
3	0	0	0	0	N/A	0	N/A	N/A
4	0	0	0	0	N/A	0	N/A	N/A
5	0	1	2	0	0	0	1	0
6	0	0	0	0	0	0	N/A	N/A
7	1	1	1	1	1	1 (total blood volume within abdomen: 30ml)	0	0
8	0	0	0	0	0	0	N/A	N/A
9	1	2	1	0	0	0	0	0

Table 2a.5 Summary of histopathology findings (UTx #1-9)

UTx	Macroscopic examination of fixed tissue and Histopathology	Clinico-Pathological Correlation
1	**19 slides** **Heart & major vessels:** No infarction/thrombus/pericarditis **Lungs:** Small thrombi in branches of PA suggestive of DIC **Spleen:** No sepsis/neutrophilia **Graft w/ patch: Evidence of RBC extravasation at the anastomotic site;** no evidence of a thrombus or embolus	**Cavo-caval & aorto-aortic anastomosis breakdown leading to intraperitoneal haemorrhage**
2	**8 slides** **Heart & major vessels:** No infarction/thrombus/pericarditis **Lungs:** No evidence of PE **Spleen:** No sepsis/neutrophilia. Decreased number of RBCs **Graft w/ patch: Evidence of RBC extravasation at the anastomotic site;** no evidence of a thrombus or embolus; congestion of venous return, including IVC	**Cavo-caval & aorto-aortic anastomosis breakdown leading to intraperitoneal haemorrhage**
3	**5 slides** **Heart & major vessels:** No infarction/thrombus/pericarditis **Lungs: Upper, middle, and lower lobes of right lung all collapsed; RBC in trachea and right bronchus;** no evidence of PE **Spleen:** No sepsis/neutrophilia/pathology **Graft w/ patch:** Grafting process not complete	**Respiratory compromise following blood aspiration**
4	**5 slides** **Heart & major vessels: Haematoma in left ventricular papillary muscle** **Lungs:** No evidence of PE/thrombus/collapse/oedema **Spleen:** No sepsis/neutrophilia/pathology **Kidneys/Liver:** No pathology **Graft w/ patch:** Grafting process not complete	**Cardiac damage** (Left ventricular rupture)

5	**12 slides** **Heart & major vessels:** Acute venous congestion. No infarction/thrombus/pericarditis. **Lungs:** Mild oedema. No evidence of pulmonary embolus. **Kidneys/liver:** Overall no pathology. Mild focal interstitial inflammation in right kidney. **Spleen:** No sepsis/neutrophilia/pathology **Graft w/ patch:** Gravid uterus. Chorionic villi and syncytiotrophoblasts confirmed. Evidence of chronic inflammation, giant cell necrosis and muscle and fat necrosis witnessed.	**Rabbit culled electively**
6	**5 slides** **Heart & major vessels:** No infarction/thrombus/pericarditis **Lungs:** Evidence of cell destruction and fluid-filled alveoli **Spleen:** No sepsis/neutrophilia/pathology **Graft w/ patch:** No evidence of RBC extravasation at the anastomotic site; no evidence of a thrombus or embolus; congestion of venous return, including IVC	**Widespread pneumonia**
7	**10 slides** **Heart & major vessels:** Heart discoloured. No vascular abnormalities or thrombi identified. No evidence of ischaemia or infarction. Coronary arteries widely patent, no evidence of thrombosis. **Lungs:** No evidence of pneumonia, oedema or pulmonary embolism. **Spleen:** No sepsis/neutrophilia/pathology. **Graft w/ patch:** Vascular anastomosis showed intact sutures intact with no evidence of perivascular soft tissue haemorrhage. **Evidence of damage to vessel wall, likely because of surgical manipulation.** No evidence of thrombotic occlusion. Genital tract organs reveal intact sutures with early but mild ischaemic changes. Possibly some epithelial loss but very little associated inflammation so likely to be artefact.	**Shearing of the aorto-aortic and cavo-caval anastomosis leading to intraperitoneal haemorrhage**

8	**6 slides** **Heart & major vessels: Single small focus of acute inflammatory cell infiltration seen in the subendothelial myocardium of the septal wall of the left ventricle.** No other abnormalities seen. **Lungs:** Patchy alveolar capillary congestion is present throughout. Focal acute inflammation is seen and in one area an excess of alveolar macrophages is witnessed. **The features are consistent with early acute lung injury.** No evidence of pulmonary embolism. **Kidney:** An occasional acute inflammatory cell is seen within the wall of glomerular capillaries. **Red blood cells are seen in the renal tubules and collecting ducts – this may be artefact or secondary to acute kidney injury.** **Spleen:** No sepsis/neutrophilia/pathology. **Appeared congested.** **Graft w/ patch:** Tissue sutures intact. Vascular anastomosis reveal a single, non-occlusive thrombus in the lumen of a thin walled (vein) vessel. The reproductive organs reveal a mild chronic (lymphocytic) inflammatory cell infiltrate within the superficial endometrium. This may be related to normal endometrial turnover or may represent early ischaemic change. There is a patchy mild lymphocytic infiltrate in the outer muscle layer the lower genital tract. A single focus of coagulative necrosis is seen in the muscular wall of the vagina/cervix, consistent with ischaemic injury.	**Features in multiple organs of early ischaemia/hypoxia related injury, consistent with the history of hypovolaemic/haemorrhagic shock**
9	**Heart & major vessels:** Subendocardial fat deposition, consistent with age related changes. Possible thrombus in right ventricle. No myocardial rupture or infarction. **Lungs:** Parenchyma of both lungs congested with firm consistency. Large adherent thrombi seen in both pulmonary arteries, extending into parenchymal vessels. Appearance consistent with a bilateral pulmonary embolus. One large vessel (artery) contained possible thrombus - features may be consistent with pulmonary embolism. **Kidney:** No pathology **Spleen:** No sepsis/neutrophilia/pathology **Graft w/ patch:** Vaginal sutures intact. Vaginal anastomosis shows changes consistent with recent surgery. Both cornua/oviduct anastomoses show marked vascular congestion of the recipient portion of the cornua/oviduct but no evidence of ischaemia. Both distal oviducts haemorrhagic and friable. No perforation. The donor cornua/oviduct appear viable and are without significant congestion. Vascular anastomosis shows sutures intact. No evidence of perivascular soft tissue haemorrhage. One thick walled vessel (artery) demonstrating inflammation of the media associated with luminal fibrin deposition.	Macroscopic features of a **pulmonary embolism** Features of **early peritonitis** which may represent a systemic inflammatory response to recent surgery or early infection This, along with the recent surgery, is a risk factor for **venous thrombosis and pulmonary embolism**

Key: **DIC;** disseminated intravascular coagulation; **PA,** pulmonary artery; **PE,** pulmonary embolus

Figure 2a.2a-i Selected histopathology microscopic images demonstrating cause of demise (slides are H&E unless stated otherwise)

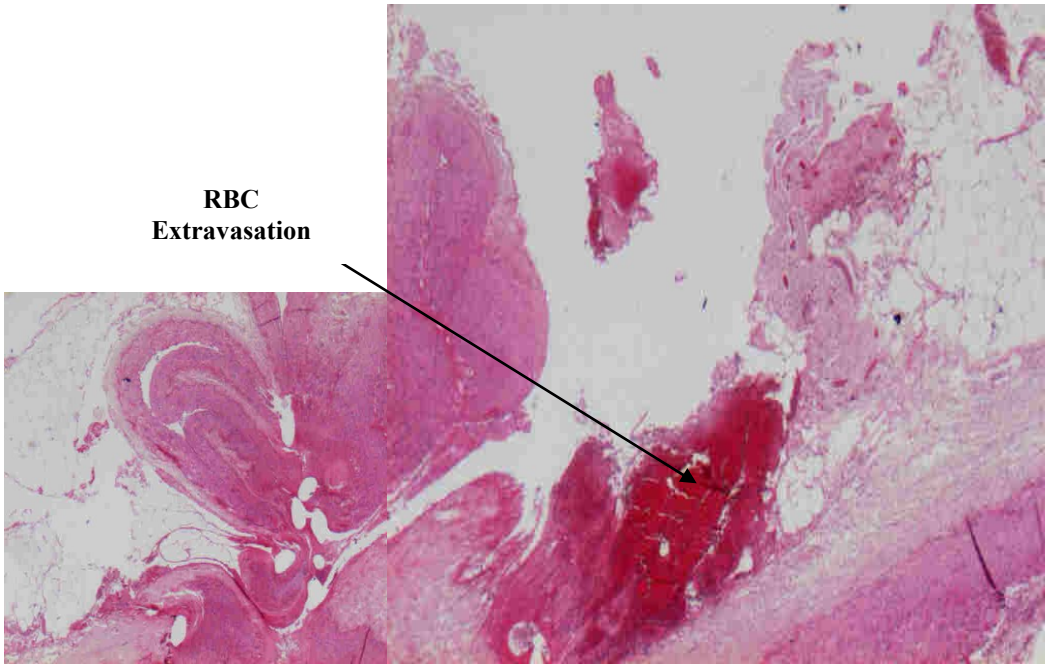


Figure 2a.2a UTx #1 - evidence of RBC extravasation at the IVC-IVC and AA-AA anastomosis

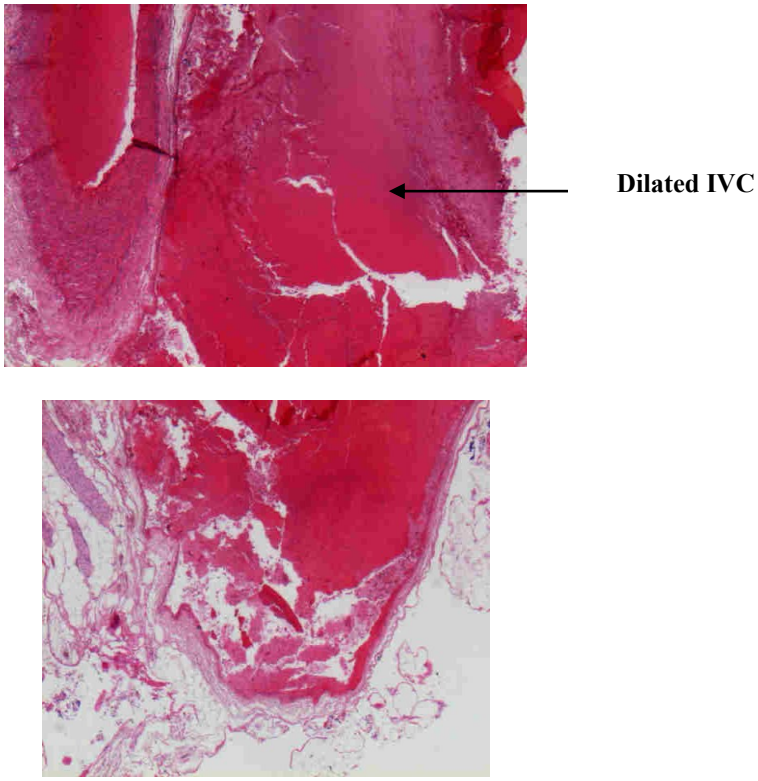


Figure 2a.2b UTx #2 - evidence of donor IVC dilatation secondary to anastomotic breakdown

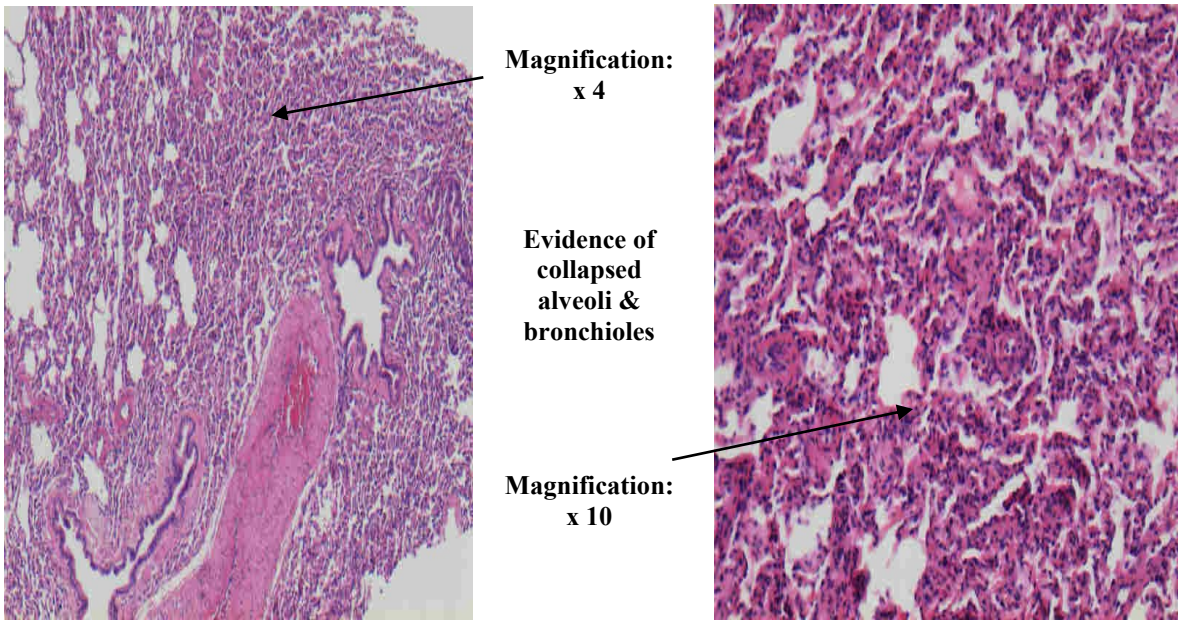


Figure 2a.3c UTx #3 - right lung collapse

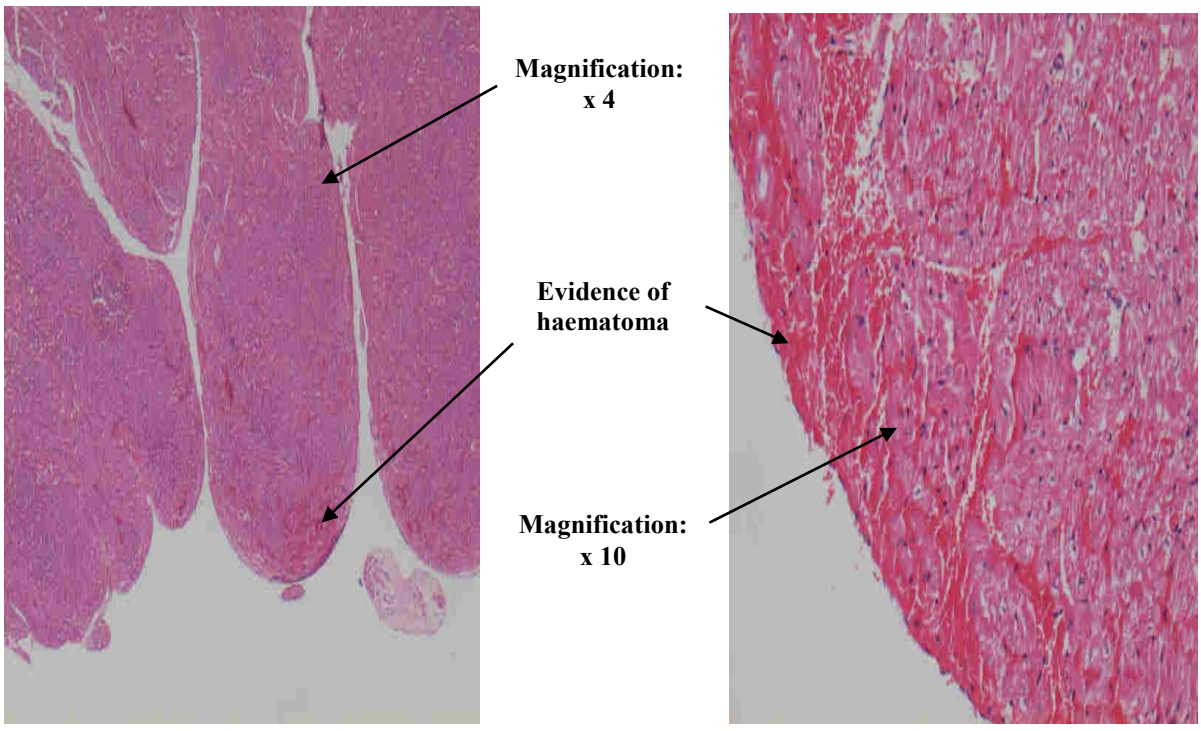


Figure 2a.4d UTx #4 - Left ventricular papillary muscle haematoma

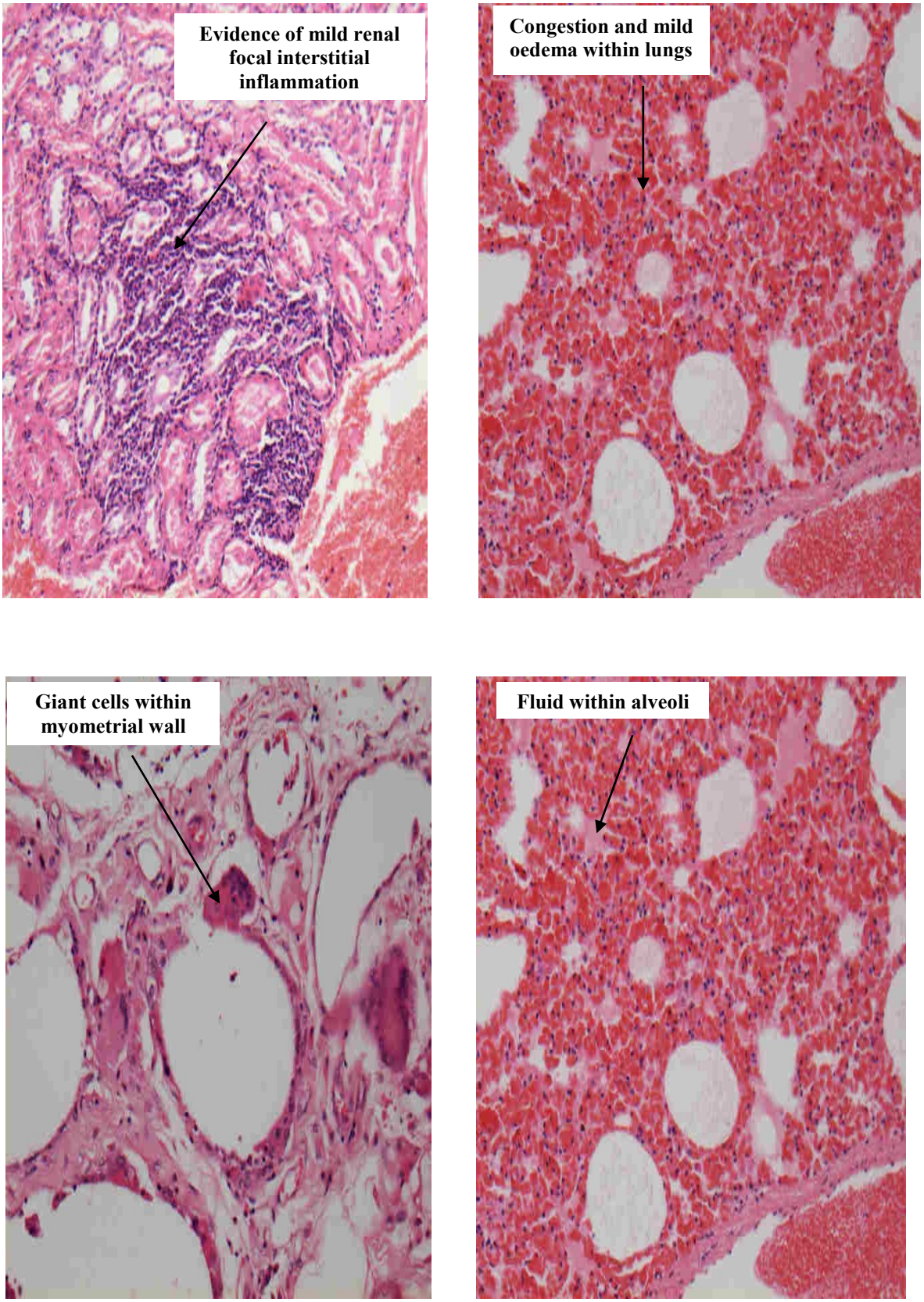


Figure 2a.4e UTx #5 - Evidence of necrosis and inflammation

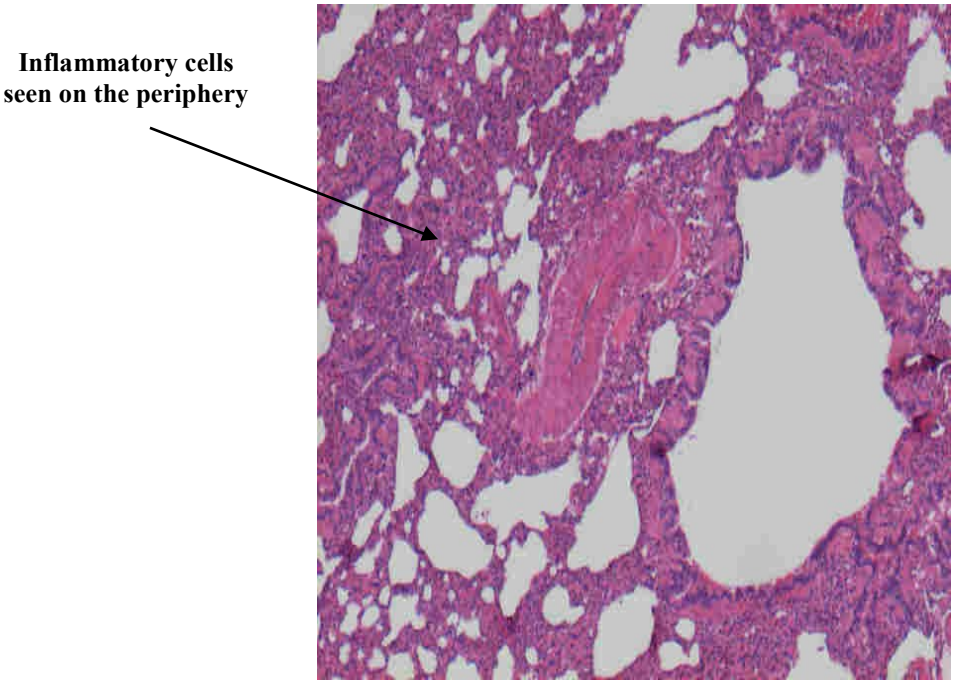


Figure 2a.2f UTx #6 - Evidence of pneumonia within the hilum of left lung

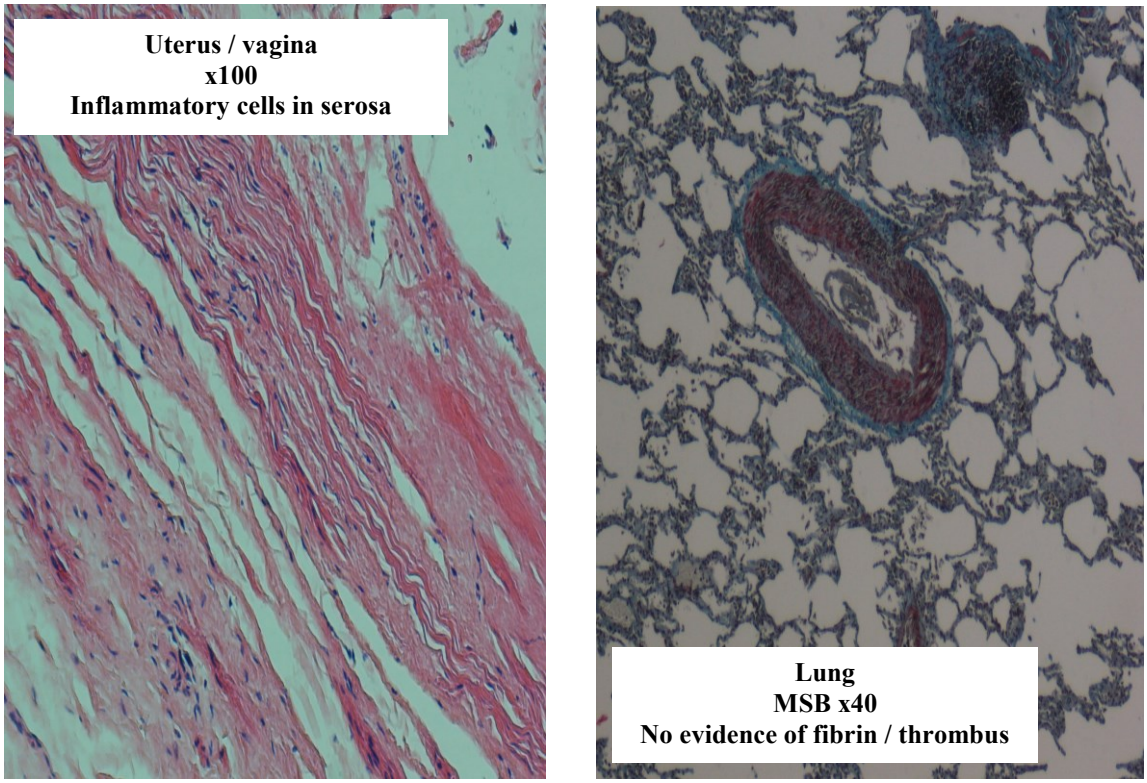
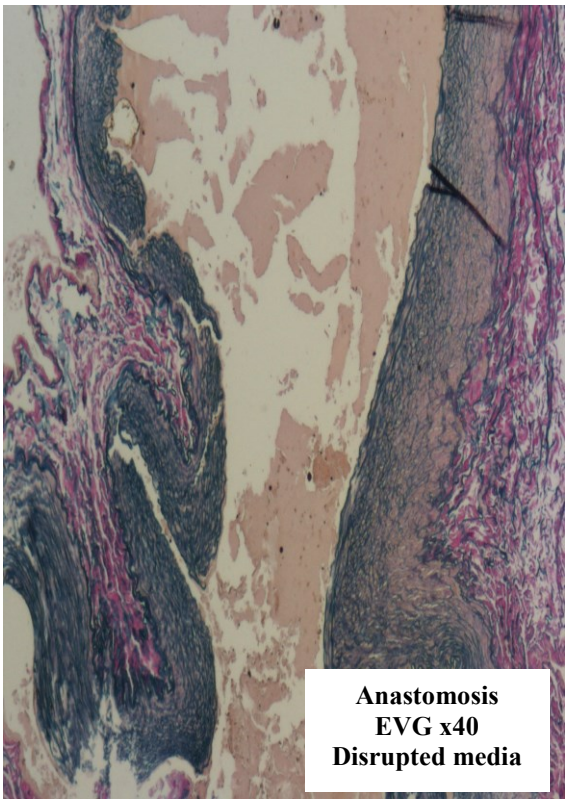
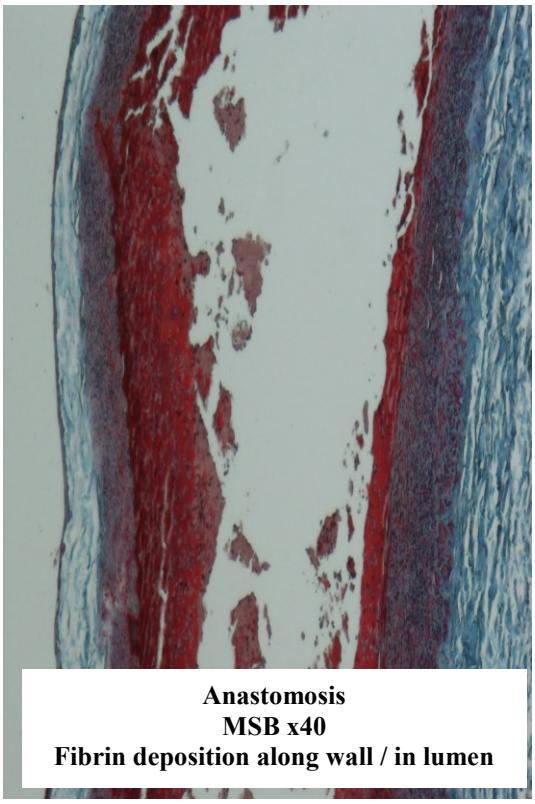


Figure 2a.2g UTx #7 - Anastomosis shearing and consequences

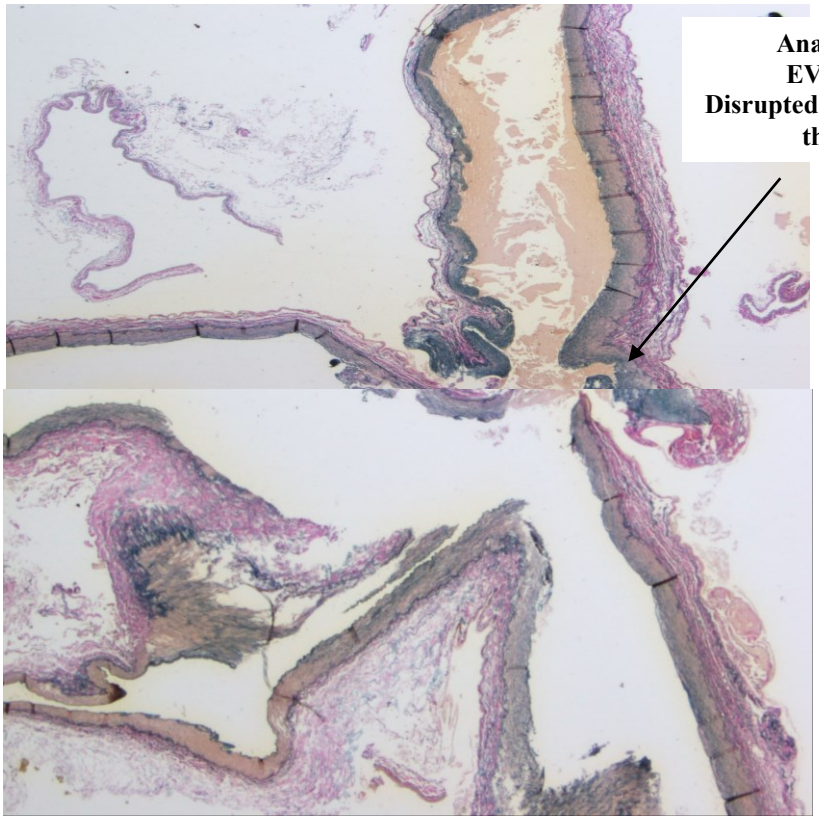
Figure 2a.2g (continued)



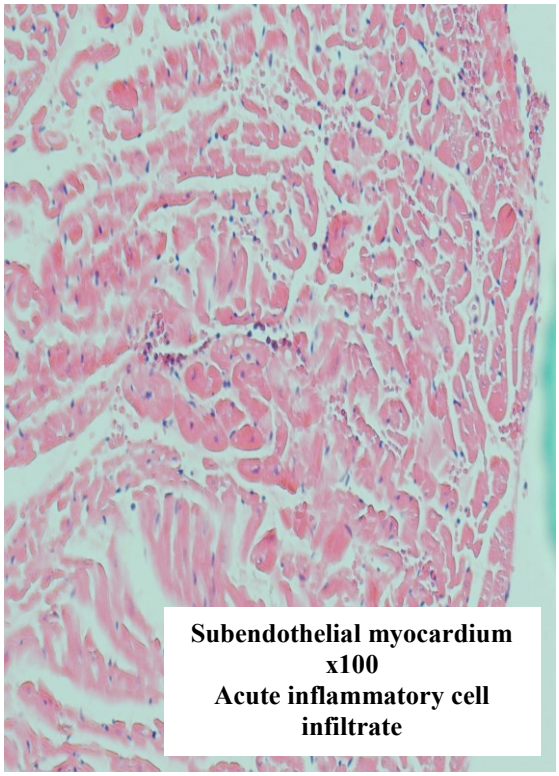
Anastomosis
EVG x40
Disrupted media

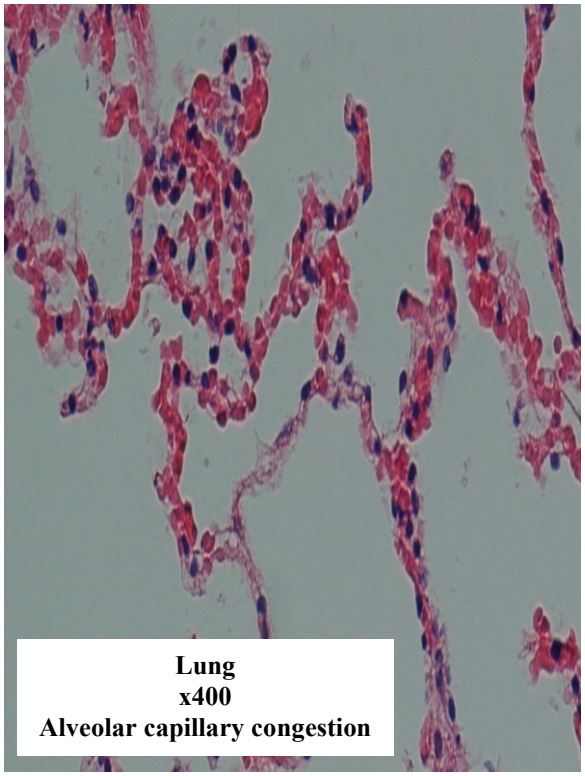


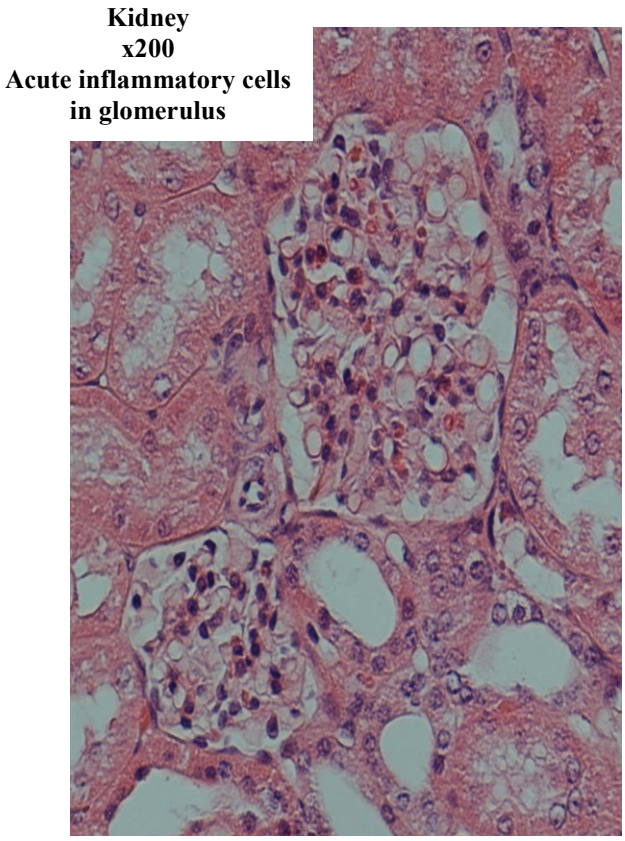
Anastomosis
MSB x40
Fibrin deposition along wall / in lumen



Anastomosis
EVG x12.5
Disrupted anastomosis at the base







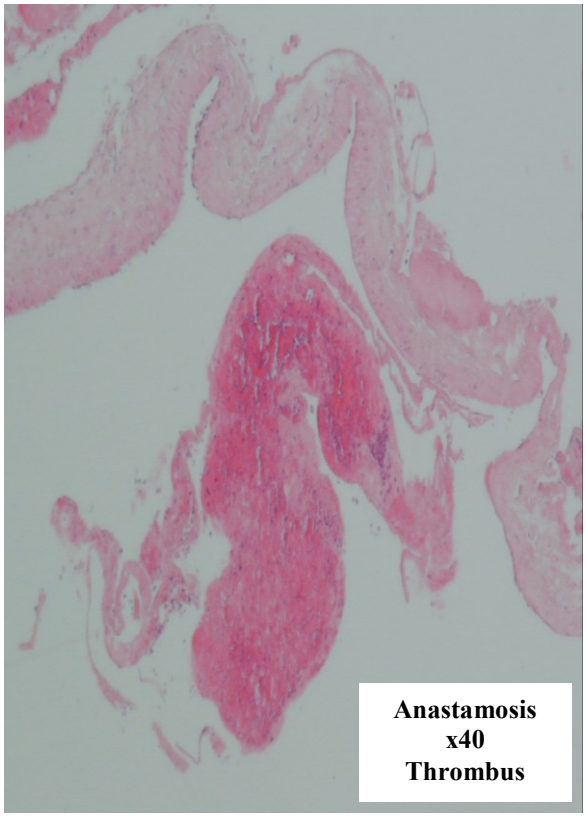
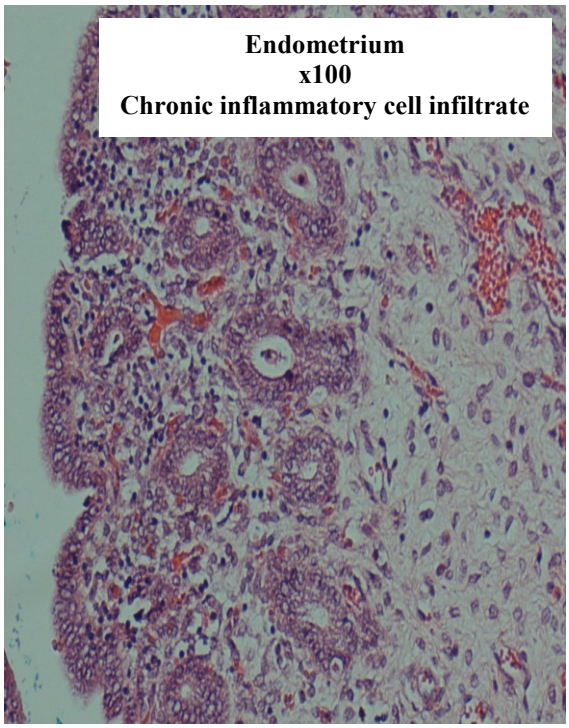
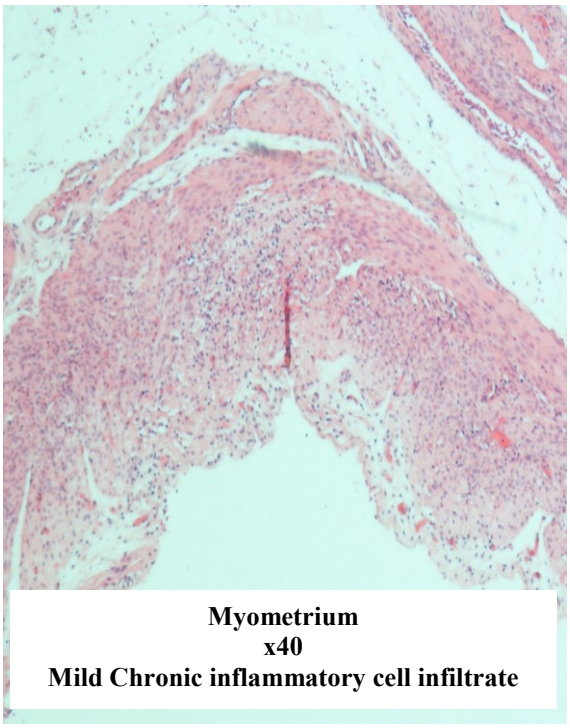
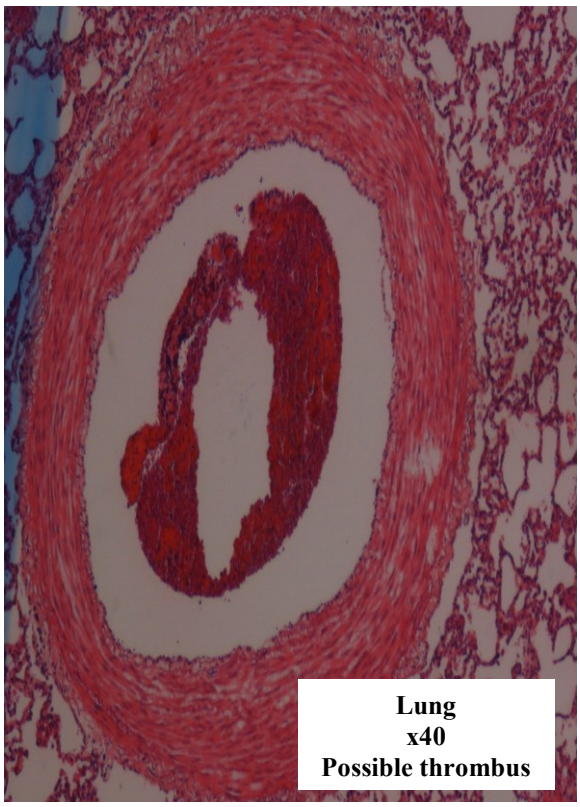


Figure 2a.2h UTx #8 - Evidence of early ischaemia/hypoxia related injury, consistent with the history of hypovolaemic/haemorrhagic shock secondary to aortic trauma







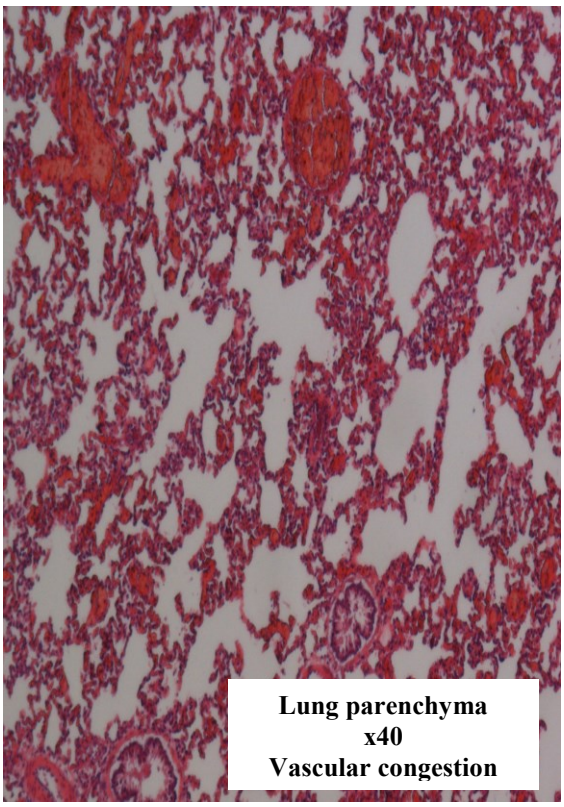


Figure 2a.2i UTx #9 - Evidence of Pulmonary embolus

Rejection response

The results (**Figures 2a.3 and 2a.4**) below describe the rejection response following UTx #5. As outlined above, rabbits #3, #4, #6 and #8 all died intra-operatively. Rabbit #1 died within the first post-operative 24 hours and was not given any immunosuppression. Rabbit #5 was the only long-term survivor of our cohort and received immunosuppression both intra- and post-operatively. Rabbits #7 and #9 also died within the first 24hours but were given immunosuppression. The distribution of the rejection markers in both UTx #1 and UTx #2 was not significant, most likely as we only had one sample group for each day. There was no difference between D0 and D1 (15 hours post-op) levels in UTx #1. In UTx #2, #7 and #9, the doe has had already died before blood could be extracted to study the rejection response.

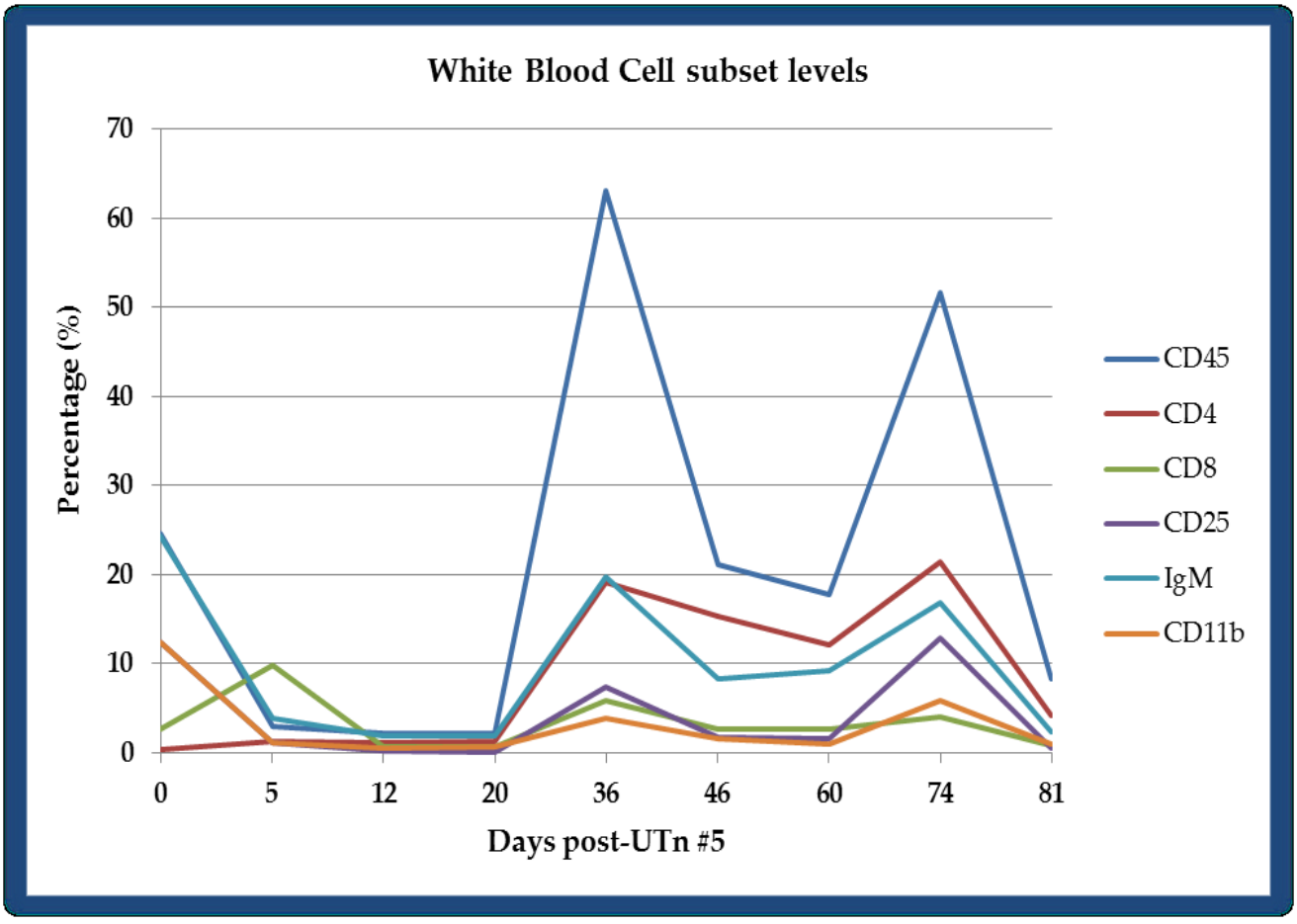


Figure 2a.3 White Blood Cell subset levels shown as a percentage of the total (y axis) following UTx #5 (day zero to day 81, x axis)

Figure 2a.4 Cellular levels depicting rejection response following UTx #5 (from left to right, 1st row: unstained FACS sample: negative control; IgG1: negative control; CD4: T Helper cells; CD8: cytotoxic T cells; 2nd row: Monocytes/macrophages; B cells; all lymphocytes, activated T cells). The machine was set to analyse exactly 10,000 cells/FACS tube. Both forward (cell size/volume; x axis) and side scatter (cell granularity; y axis) detectors were aimed at the column of cells passed through the FACScalibur. All cells located to the right of the IgG1/negative control group on the scatter plot were defined as being positive for the marker in question.

Figure 2a.4a Post-UTx #5 Day 0

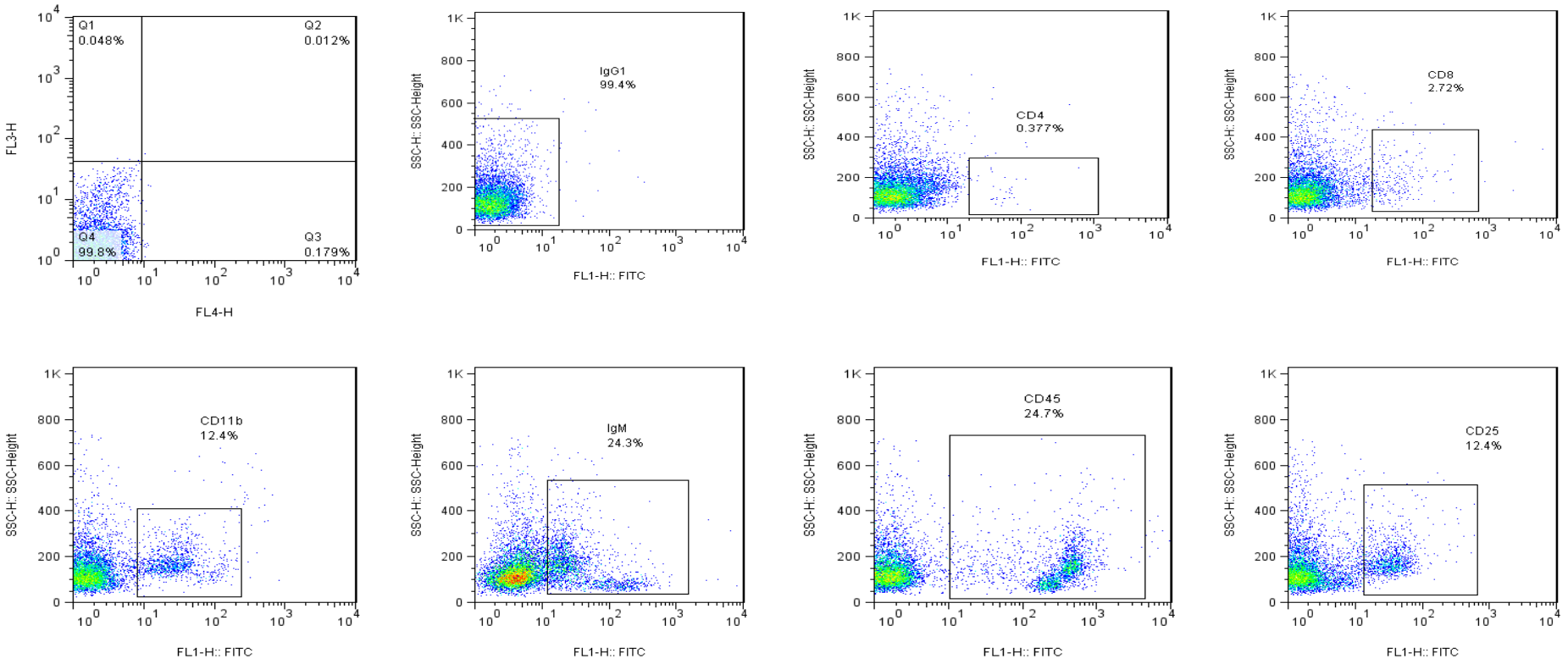
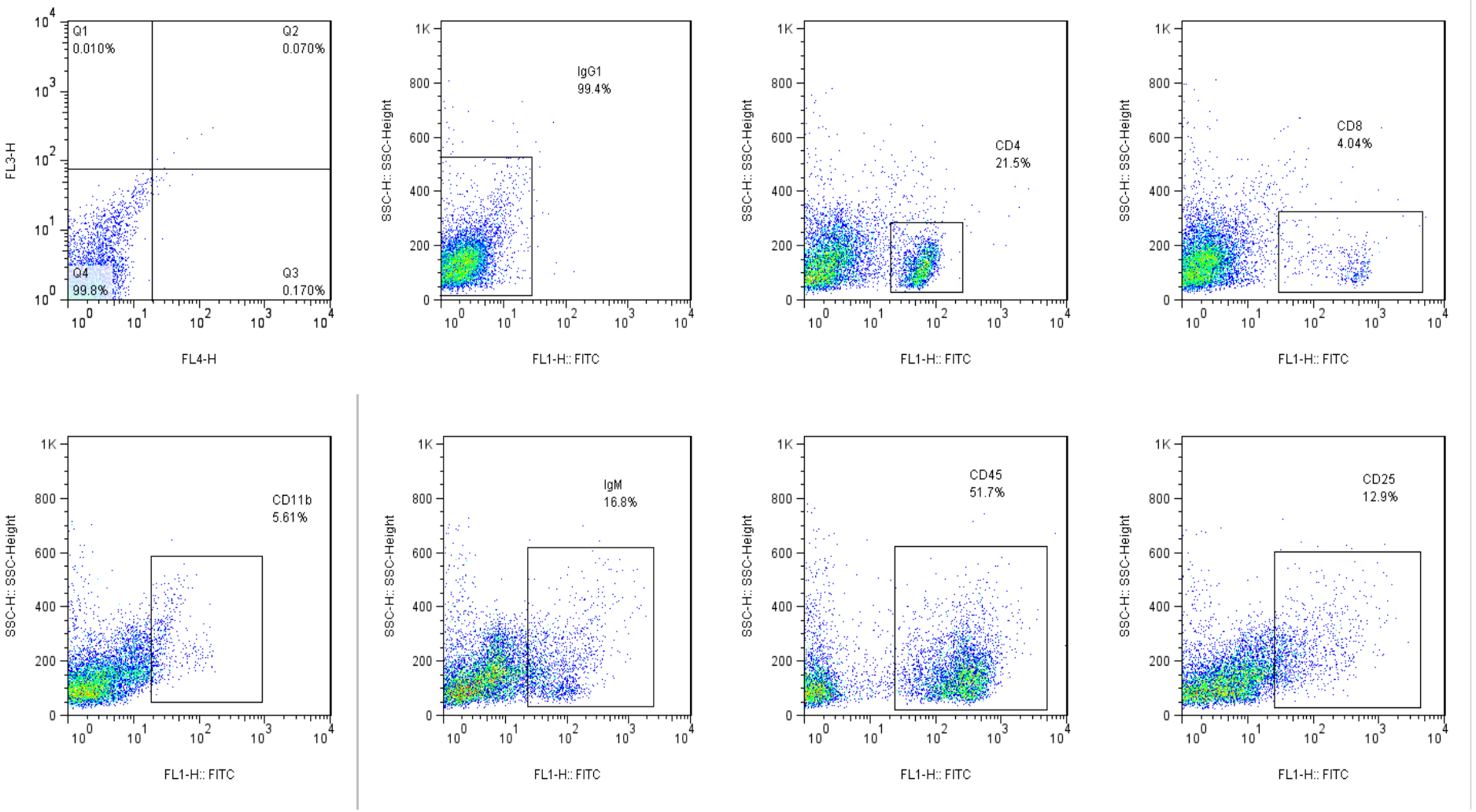


Figure 2a.4b Post-UTx #5 Day 74 (see legend above)



Long-term Survival and Pregnancy

Recipient #5 was the sole surviving doe out of the cohort of nine. The doe showed no adverse effects post-operatively and throughout the recovery period. She was feeding and drinking well, with appropriate movement and affect. Following visualization of certain signs suggesting a receptive doe, embryo transfer was performed on day 89 post-UTx. Vitrified embryos were prepared at the same time, with 17 transferred into the cornua of the sole surviving doe by open laparotomy and insertion of catheter transmurally (nine into the right and eight in the left cornua). The doe began to demonstrate signs of pregnancy, including nesting and an increase in abdominal size towards the end of the first gestational week.

D5 scan did not demonstrate a fetal sac. Fetal sac diameter on D9, D13, D16 and D18 was (mm): 17.3x13.1, 22.1x19.5, 25.2x22.7, and 10.2x6.3 (**Figure 2a.5**). CRL was only recordable on D9, D13 and D16 (mm): 10.2, 14.3, and 16.4 respectively. Subsequent scans on D22 and D25 did not demonstrate a fetal sac, which together with the fetal sac diameter was suggestive of resorption. No bleeding was witnessed around the cage. The doe's behaviour was noticed to have returned to pre-embryo transfer period four days before she was culled. The recipient was sacrificed on D27. Necropsy and histopathology confirmed evidence of a gravid uterus and the presence of a gestational sac (**Figures 2a.6 and 2a.7**).

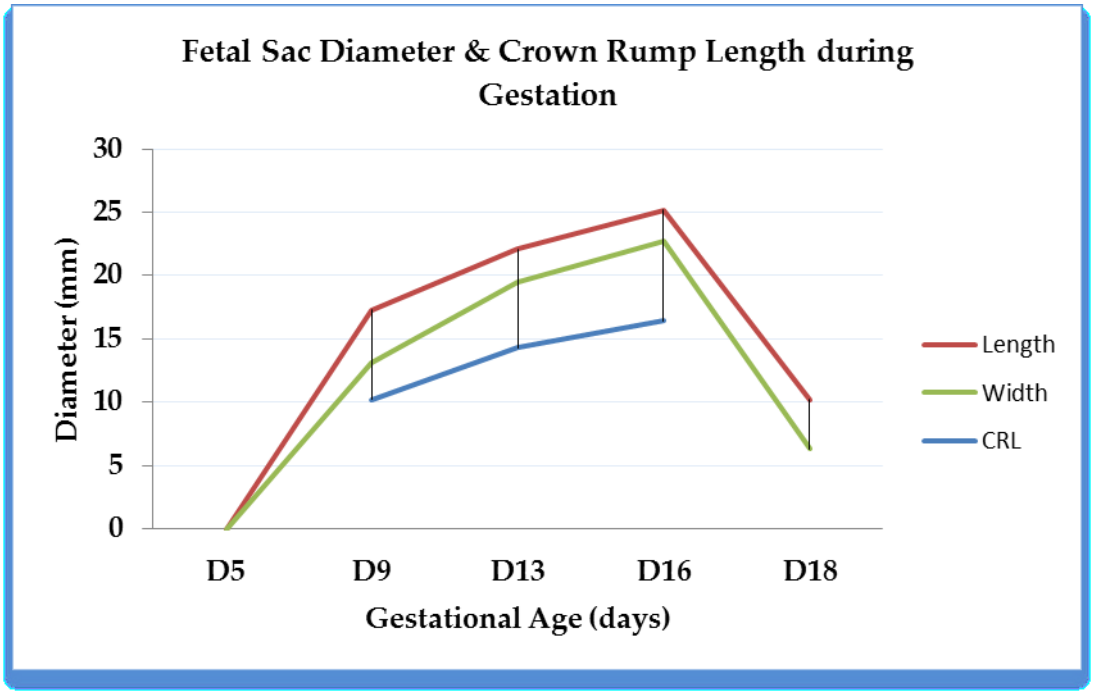
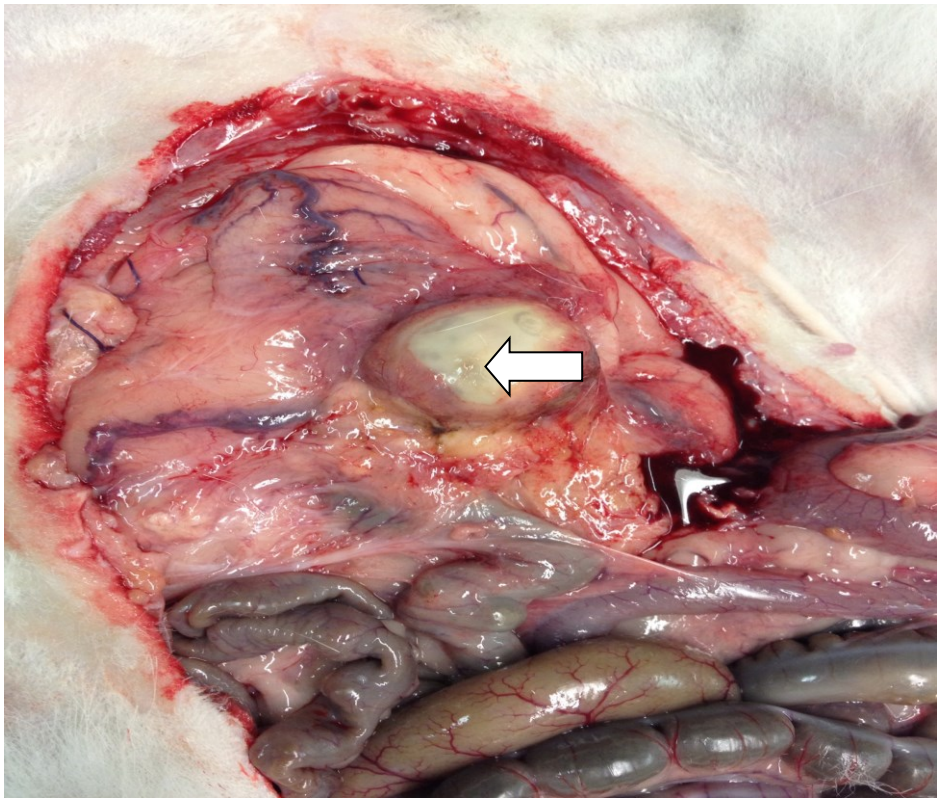


Figure 2a.5 Graph depicting the growth and subsequent decline in the size of the Fetal Sac Diameter and Crown Rump Length during Gestation



White Arrow-head Uterus containing fetal sac and now resorped fetus

Figure 2a.6 Photograph of the abdominal cavity on D113 following UTx taken prior to the commencement of the post-mortem

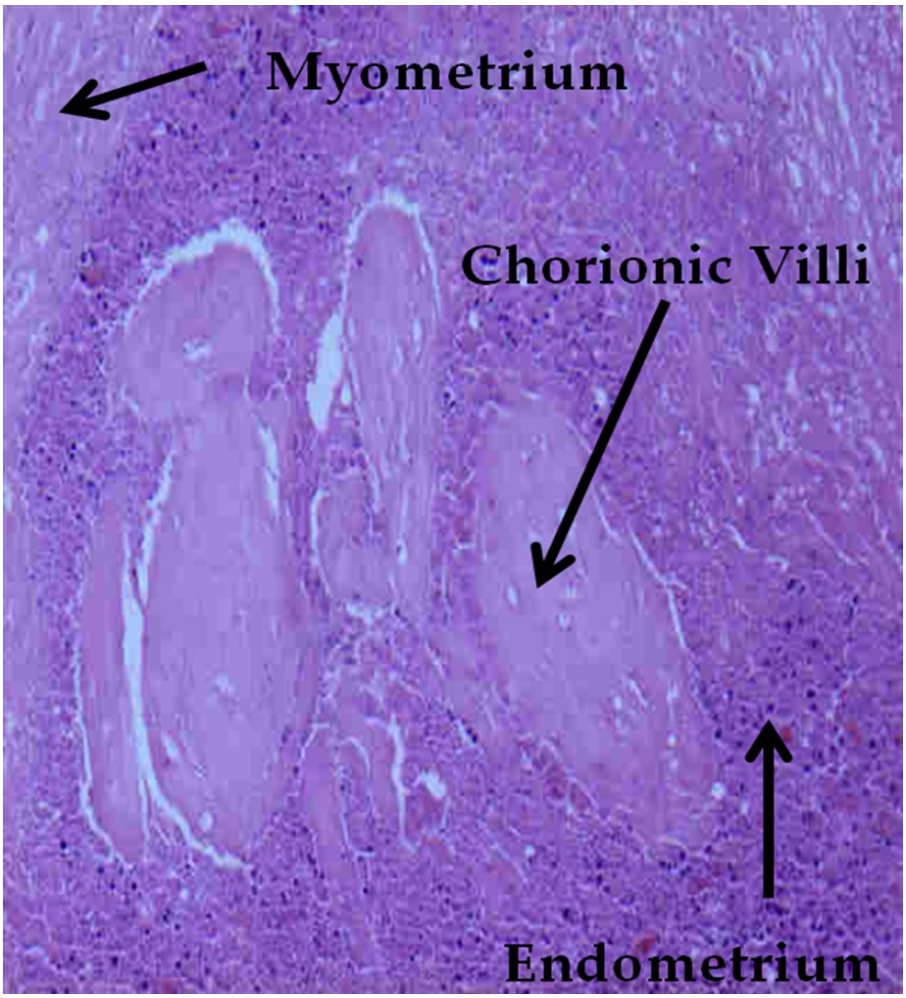


Figure 2a.7 A histopathology slide illustrating the chorionic villi embedded into the endometrium and therefore acting as proof for the presence of products of conception and thus pregnancy

Unfortunately as a result of resistance from the doe, only one blood sample was possible during the gestational period. The graph from **Figure 4.6** can be extended to include white cell subset levels from D102, which was D13 of the gestational period (**Figure 2a.8**). Similarly **Table 2a.6** demonstrates renal, hepatic and reproductive profiles from D0 to D102.

UTx 5											
Gestational Age	**D0**	**D2**	**D6**	**D9**	**D13**	**D20**	**D36**	**D46**	**D60**	**D74**	**D102**
Sodium	**151**	141	142	143	142	143	144	144	144	141	141
Potassium	3.9	4.7	NM	4.3	4.5	3.8	3.8	4.0	NM	4.2	4.4
Urea	8.9	**21.8**	**12.2**	7.6	6.2	6.8	5.8	6.7	7.6	7.2	7.6
Creatinine	131	115	91	89	82	79	82	91	106	95	95
AST	40	63	21	14	23	30	22	29	NM	25	36
ALT	28	71	44	26	17	22	21	27	31	23	19
ALP	41	**21**	NM	37	34	34	38	36	42	50	18
Albumin	**57**	42	**50**	**49**	45	**50**	**51**	**56**	**55**	**56**	48
CRP	NA	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	-
Reproductive profile											
17β-oestradiol	70	47	57	83	90	112	60	102	101	92	45
LH	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
FSH	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Testosterone	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Progesterone	0.5	0.5	0.5	0.5	0.5	0.6	0.5	0.7	0.5	0.5	15.5
Immunosuppressant											
Tacrolimus Levels	**NA**	**NM**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**-**

Table 2a.6 Renal, hepatic and reproductive profiles of recipient #5 from D0 to D102

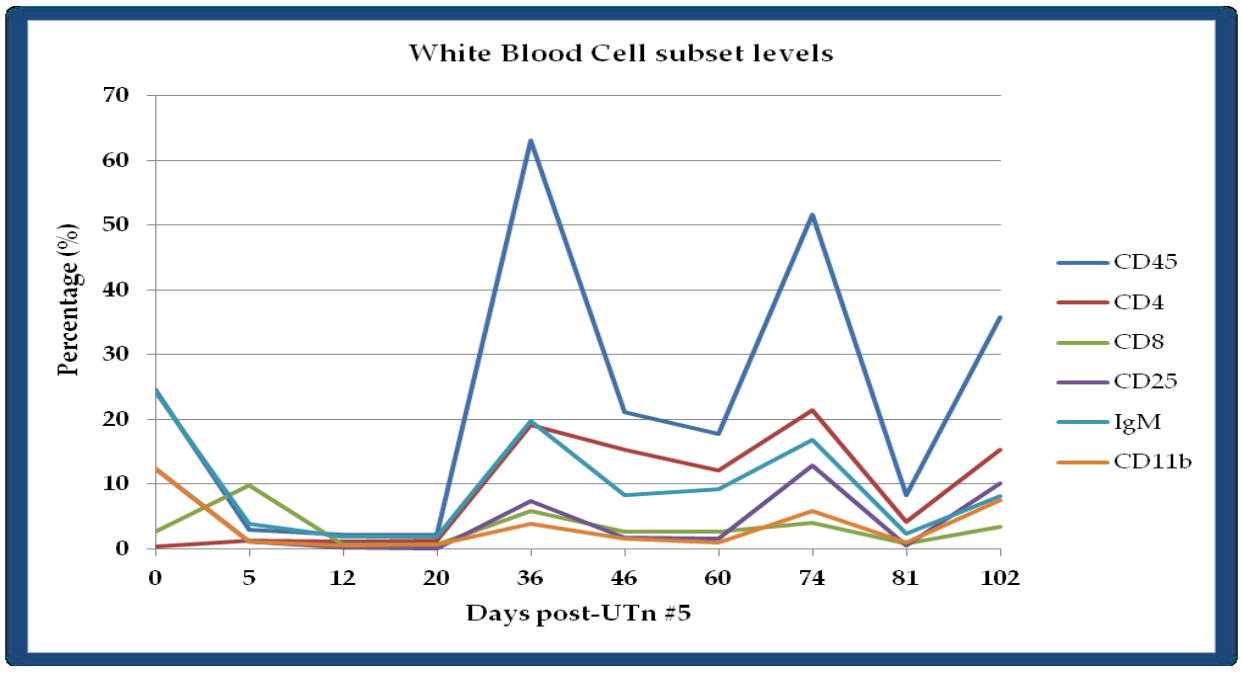


Figure 2a.8 White Blood Cell subset levels shown as a percentage of the total (y axis) following UTx #5, including those taken during pregnancy (day zero to day 102, x axis; D102=D13 post-embryo transfer)

2a.4. Discussion

This case series was a continuation of existing small animal models: murine, rat and rabbit, with the aim to further investigate the surgical model which may be applied in a human. Nine allogeneic UTx procedures in a rabbit model are presented here. They are an extension of work performed by *Sieunarine et al* and *Saso et al* described above.[108,235] In this case series, long-term rabbit survival was 11% (n=1). Surgical operative survival was 56% (n=5). Four rabbits (UTx #3, #4, #6 and #8) died intra-operatively or within one hour of the operation as a result of blood aspiration, ventricular haematoma, pre-operative pneumonia and massive haemorrhage. Three does (#1, #2 and #7) died within the first 24 hours as a result of the veno-vena and anastomosis breakdown and one died secondary to a pulmonary embolus. Part of the surgical procedure which took the longest time was grafting of the transplanted uterus, where great caution had to be taken to preserve the blood supply to the uterus throughout the operation and also to avoid leakage from the anastomotic sites. All rabbits demonstrated normal renal, hepatic and reproductive profiles throughout, both pre- and post-operatively (**Table 2a.3**). Our single long-term survivor revealed fluctuating 17β-oestradiol and no decrease in progesterone and testosterone levels despite the maintenance immunosuppression dose. FSH and LH remained constantly low throughout, even during fertility treatment suggesting an issue with the detection assay. This suggests a healthy and normal reproductive system. The sole long-term surviving doe underwent embryo transfer.

Surgical technique

Before commencing with this UTx series, we performed two hysterectomies in a rabbit model in order to re-acquaint ourselves with rabbit pelvic anatomy. Particular attention was paid to skeletonization of the infra-renal AA and IVC. From the outcomes above (long-term survival of 11%), one may conclude that the surgical learning curve has remained steep, and that it can only be surmounted with further attempts in the future. This was demonstrated by *Racho El Akouri et al* who reported a gradual learning curve for their murine UTx model.[69] The reported rate of successful uterine transplantation

was 25% for the first 1-21 animals, which increased to 87% for animals 22-42. However, on closer inspection, a number of issues challenge the above statement ('the surgical learning curve has remained steep'): (a) other factors that may have contributed to the recipients' demise; (b) decrease in duration of each stage of UTx procedure when comparing UTx #1-6 and UTx #7-#9; and (c) the actual rabbit model. Each is discussed individually below.

Failure secondary to the anastomotic technique was 43% (n=3/7); these were recipients #1, #2 and #8. Other deaths were independent of the macrovascular method proposed and would have most likely been avoided in the human setting. Recipient #3 died as a result of aspiration pneumonia, brought on by difficulties in intubation. In a woman, intubation is likely to be straight forward and any anatomical issues would have already been vetted during pre-operative pre-assessment. Recipient #4 died secondary to a ventricular haematoma, cause of which may be one of the following: intravenous CsA administered intra-operatively, an undiagnosed pathology already present pre-operatively which during stressed conditions brought on a decreased cardiac output and subsequent arrest or a pathology which developed intra-operatively. In human UTx, an intra-ventricular haematoma would have been reported during pre-operative assessment, in particular an echocardiogram. The human CsA dose is highly unlikely to cause an intra-cardiac bleed and a UTx procedure is equivalent to a hysterectomy with regards to patient risk. Thus an appropriately assessed patient should be able to cope with surgical stress. Recipient #6 died secondary to respiratory arrest brought on by a pre-operative pneumonia only diagnosed during histopathology. Again, this condition would have been diagnosed prior to the operation. Recipient #7 bled post-operatively from the anastomosis. However this bleed was secondary to anastomotic damage caused by non-fixation of the recipient cornual ends to the donor cornua. This was a decision made by the team to shorten the operative procedure and therefore decrease surgical stress on the rabbit. However the results were disastrous and it was not repeated again, with the previous method of cornual anastomosis re-applied. In a human UTx, the uterus would be fixed to the pelvic side walls and base and such a scenario would not arise. Finally, recipient #9 died secondary to a pulmonary embolus, despite the use of a dose of heparin that had worked in the previous 12 of 13 rabbit transplants. Pulmonary embolus in a woman following the use of an

appropriate heparin dose is extremely unlikely and if it occurs diagnosis should be imminent as the women will spend at least a week in hospital post-operatively.

It is therefore evident how factors independent of surgical technique and especially the vascular anastomotic technique contributed to reported early deaths. **Table 2a.2** clearly demonstrates the decrease in duration of various stages of the surgical procedure when comparing the last three operations to the first six: graft retrieval (UTx #1-#6: 114min; UTx #7-#9: 85min); cold ischaemia (UTx #1-#6: 118min; UTx #7-#9: 90min); grafting (UTx #1-#6: 128min; UTx #7-#9: 124min); recipient hysterectomy (UTx #1-#6: 43min; UTx #7-#9: 30min); and estimated blood loss (UTx #1-#6: 33ml; UTx #7-#9: 18ml). Subjectively, each transplant seemed an improvement with how the surgeons felt about each stage of the procedure with respect to anatomical awareness and technical expertise. Lessons learned were applied in each subsequent transplant (**Table 2a.7**). With regards to the vascular anastomotic model, the surgical survival rate of 54% (and 80% if you include previous rabbit transplants) as well as the macroscopic appearance of the transplanted uterus supports the use of a macrovascular model. This has been the accepted method for ensuring uterine perfusion with all animal experiments since the first human attempt in 2000. Omentopexy, uterine anastomosis and the use of venous grafts to extend the pedicle are now definitely unacceptable.

Despite this, the biggest difficulty remains the viability and length of time it takes to form the veno-vena and aorto-aorta anastomosis. The first two rabbits died directly as a result of this anastomotic breakdown, whereas #8 died because of uncontrolled blood loss during veno-caval anastomosis. Therefore, the anastomotic breakdowns described above did not occur in UTx #3-7 and #9. The number of stitches required to form the anastomosis may be a possible cause of breakdown but this number (three to five stitches for both IVC and AA anastomosis) was maintained from UTx #1 to UTx #9. Therefore, this number was perfectly adequate to allow for long-term survivor of recipient #5 as well as the two long-term survivors in the previous cohort. In addition, a suture used to be placed into the IVC at the time of graft harvesting to help distinguish it during veno-vena anastomosis. This practice was abolished for UTx #3-9 so as to minimise IVC damage prior to anastomosis. To improve

Table 2a.7 Modifications to Surgical Technique UTx #1-9

Transplantation Number	Learning Points
UTx #1	1) Smaller size Yankauer required with suction tube to go with Yankauer of this size - this should allow us to cope with unexpected blood loss. 2) Appropriate size Bulldog Clamps - clamps used here too large for AA/IVC of this diameter. A smaller size must be available. 3) Suction blood from abdomen for immunological studies to avoid potential difficulties with venesection or cardiac puncture. 4) Always remember to insert a suture into the posterior aspect of the uterus.
UTx #2	1) Blue cannula into IVC post-Celsior flushing and to remain there until the grafting stage of UTx. This should aid with IVC recognition. 2) To help with IVC recognition, only one stitch to be inserted into it during uterine retrieval (in-out, out-in, through the edge). Not 2 stitches. 3) Better skeletonization and separation of IVC from AA at the top 2 cm. 4) ~40 Ligaclips are required per operation. To be used predominantly instead of surgical ties. 5) Does from now on must be of proven fertility and no longer virgins.
UTx #3	1) Skeletonization of IVC and aorta should involve the top 4cm. 2) As blue cannula falls out from IVC, gray or green should be applied.
UTx #4	1) Try to minimize the time that the recipient is anaesthetized in any way possible. 2) When performing the all-important anastomosis, use vascular slings to underrun IVC and AA and ease the process. 3) No longer apply stitches into IVC. Instead, to locate it, run Celsior solution through IVC and watch for its exit through the lumen of the IVC. 4) To aid blood haemostasis, apply Flocil powder to the anastomosis.
UTx #5	Above lessons were all applied. Long-term survivor (> 90 days).
UTx #6	Above lessons were all applied BUT… should we consider a more robust animal model?
UTx #7	Uterine graft must be fixed to pelvic side walls and cornua must be anastomosed to the recipient's cornual ends. This is to prevent twisting at the cavo-caval and aorto-aortic anastamoses and subsequent shearing.
UTx #8	IVC anastomosis proved to be the most difficult again. 4 stitches are the maximum that should be attempted with the vascular anastomoses.
UTx #9	Prophylaxis of venous thromboembolic disease again an issue. Above lessons were all applied BUT… should we consider a more robust animal model?

the probability of long-term survival and a patent veno-vena and aorto-aorta anastomosis, we decided to change the rabbit used in UTx #1-2 from a virgin doe to a doe of proven fertility of a weight >4kg. This eased the process of uterine grafting as we were operating on a larger-size pelvis and therefore, an AA and IVC of a slightly bigger diameter.

Another particular problem of the anastomotic process is the IVC clamp placed just before the IVC is cut to allow for insertion of the first anastomotic suture. A delay in return of blood to the heart consequently ensues. The length of the delay does not seem to predict the level of compromise, but it was evident that the recipients did not react favourably once the clamp was placed. Heart rate and respiratory rate suddenly dropped in both cases, with each recipient experiencing at least four cardiac arrests until their demise. The solution to the problem is not a quick fix - the delay can only be minimised with an increase in speed of veno-vena anastomosis, which in turn can only happen with more practice.

With regards to a human UTx, the blood vessel diameter is longer, suggesting that achieving patient anastomosis should be more straightforward. If a graft is obtained from a multi-organ donor, it would include vessels such as the common iliacs or even IVC and AA. A graft harvested from a living donor would obviously not contain common, internal or external iliacs, or IVC and AA. Therefore, the vascular grafts will be shorter in length and diameter. Skeletonization of uterine vessels allows for free vascular pedicles of around 6cm. These can subsequently be utilised to form direct end-to-side anastomosis to both the internal and external iliacs. The hysterectomy was modified so as to maintain the length of the vascular pedicle as long as possible, thus ensuring adequate tissue perfusion and tissue integrity. This was achieved by transecting the round ligaments as laterally as possible. Also divided were the infundibulopelvic and uterosacral ligaments. The paravesical and pararectal spaces were dissected in order to ease identification and protection of the ureters, and avoid a potential confusion with uterine vessels. The uterine arteries were encircled with vessel loops, whereas the bladder was freed from the vagina and the cervix.

Finally one has to consider the genuine issue of using a rabbit model for such extensive surgery. A rabbit model was chosen because of its reproductive potential, its short gestational period and its large litters. However, as explained above, the small size of the rabbit pelvic vessels, the necessary development of surgical methodologies and achievement of adequate microsurgical skills to master vascular anastomosis require both dedication and long hours at the surgery table. This implies a steep surgical learning curve. When adding the previous rabbit UTx numbers to this cohort, surgical survival increased from 50% to 70% (14/20). However, long-term survival increased from 11% to only 15% (3/20). Despite 11 previous attempts, in the subsequent nine, only one recipient survived. This is not an acceptable result especially when compared to the Brannstrom murine and rat experiments described previously whereby the small-animal model is even smaller. At least 30 full transplantations are needed to achieve satisfactory graft survival rates (~70%) in a small-animal UTx model and in this experiment, the final result was considerably short of this target.[68,69] The rabbit model is therefore not recommended by the UK team for future UTx experiments.

Ischaemia-Reperfusion injury

Ischaemia-reperfusion injury occurs in different degrees during a transplantation procedure. Its effects are unavoidable, with the aim being to minimise them as much as possible. Ischaemia leads to deprivation of oxygen and blood nutrients to the tissue, which in turn brings about a steady loss of cellular homeostasis. Subsequently, reperfusion result is a sudden in-flow of blood, the constituents of which induce an inflammatory response that if severe can lead to loss of organ function.[160,236] The main way to minimize this damage is to store the graft in a storage solution, at a low temperature which decreases both the cellular metabolic and catabolic rates. Generally, preservation solutions are used to maintain the organ in optimal condition from the time of explanation until transplantation. Five major advantages to the transplantation programme are offered: time to transport the organ, time to allow tissue matching, time to prepare the recipient and surgical team, quality of organ function, and better post-operative recovery.

A number of different types exist: EuroCollins, University of Wisconsin, Celsior, Perfadex, and the solution used here HTK. HTK is not a colloid, with therefore a viscosity equal to that of pure water from 1 to 35^0C, and a mean flow rate three times that of University of Wisconsin solution at an equal perfusion pressure. Tryptophan acts as membrane stabilizer whereas ketoglutarate as an energy substrate. Interestingly, organs cool down to lower temperatures more rapidly than with other preservation solutions. According to the University of Pittsburgh criteria for Organ Preservation, HTK is now the gold standard solution for preservation of all organs. Its advantages are obvious - it has no additives, is ready to use immediately when retrieving from unstable donors, no solution waste if case is aborted, costs less, requires less packaging than other solutions, and transport/storage is at room temperature which further decreases the cost.

Different organs tolerate ischaemia for varying periods of time. Main reasons have been described extensively in the literature: energy demand, parenchymal cell function, and resident immune-cell populations. The kidney can tolerate cold ischaemia times of ≥24 hours,[237] whereas the heart and lungs can last for only <6 hours and intestinal mucosa of the small bowel degenerates after <12 hours of cold ischaemia.[238] Obviously the organs have completely different purposes which explains the above. The heart has a particularly high content of contractile protein, and more importantly, needs to be at its optimum with respect to function in a shorter period following the retrieval (life-saving organ). The uterus on the other hand would need to be functional at a much later point in time and can therefore take longer to repair damage induced during the transplantation process.

Furthermore, in comparison to the heart, the uterus is much more durable organ. The murine uterus for example produced healthy pups after embryo transfer, having been stored for 24 hours in cold ischaemia post-syngeneic UTx.[84] The Brannstrom team have also reported a similar time frame for a human uterus. Following its storage in a cold preservation solution for 24 hours, the human myometrium was able to contract spontaneously and to respond to prostaglandin. The preservation solutions are in most cases buffered saccharide solutions. They are designed to maintain a neutral pH, prevent cell swelling and loss of cellular homeostasis brought on by hypothermia by providing a high

concentration of an impermeant (mannitol, histidine, and phosphate), provide an antioxidant defence against reperfusion, and enable quick recovery from the ischemic depletion of energy stores. *Sieunarine et al* assessed the morphological changes in human uterine tissue after its preservation in a transplant solution under the influence of cold ischaemia.[235] Small tissue samples of human uteri were submerged in Celsior transplant solution which mimicked a cold ischaemic environment (2-8 degrees Celsius for up to 48 hours). To assess evidence of cold ischaemic injury, histological analysis by light and electron microscopy was applied. It did not demonstrate any major uterine tissue changes after 48-hour cold preservation. Electron microscopy after 24 hours confirmed unchanged structural integrity of the uterine myoendometrium. The authors concluded that human uterus is morphologically resistant toward cold ischaemia in Celsior preservation solution for up to 24 hours and may therefore be suitable for transplantation purposes.

Graft manipulation may also add to post-operative inflammation. Mechanical manipulation of intestines induces a local inflammatory response mainly mediated by the usual culprits, macrophages, followed by an infiltration of leukocytes peaking at 24 hours.[68,239,240] Similarly, mechanical manipulation during dissection of the uterus transplant could lead to an inflammatory response in the actual uterine graft. As per advice of *Wranning et al*,[68] the surgical team avoided overall extensive manipulation by creating an adequate operating field without touching the uterine graft unless absolutely necessary.

Perfusion of the uterine graft with a cold preservation solution functioned well in this cohort of recipients. Perfusion and storage of all nine uterine grafts was kept constant at 90 minutes, with the graft changing in colour from pale, dusky white to a pink or red. This allowed us to conclude that the vascular anatomy of the graft was intact and had not been damaged during the retrieval process, thus permitting flow of solution throughout the graft.

Recipients which exhibited any signs of cornual ischaemia were #5, #7 and #8 (3/9). The sole long-term survivor, recipient #5, demonstrated evidence of chronic inflammation, giant cell necrosis and

muscle and fat necrosis following its cull on Day 113 post-UTx. Microscopically, perfusion was evident and thus, the evidence of inflammation and necrosis would be most likely secondary to a failure to control the rejection process. Recipients #7 and #8 died as a result of a heavy haemorrhage which was gradual enough to bring about mild ischaemia of the graft. Recipients #3, #4 and #6 died intra-operatively and recipient #9 within 24 hours, secondary to reasons not related to significant haemorrhage and therefore, ischaemia was not expected. Recipients #1 and #2 did suffer from significant blood loss from the anastomotic sites. The lack of signs of ischaemia is most likely because of the sudden and total nature of the blood loss, leading to a quick death. Therefore, there was not enough time for ischaemia to be evident on the uterine graft.

It is difficult to draw definite conclusions as to whether 90 minutes of preservation in Celsior solution minimised ischaemia-reperfusion injury in this case series. The flushing process certainly allowed for assessment of uterine graft and especially the vascular integrity. The long-term survival rate of only 11%, coupled with a 44% intra-operative death rate means that judgment as to whether long-term protection is possible is not appropriate. However the lack of ischaemic damage in those that survived surgery (recipients #1, #2 and #9), the mild element of inflammation in recipients #7 and #8 as well as that macroscopically the graft of recipient #5 was judged ready for embryo transfer is promising. It is worth recognising that uterine tissue has a relatively high resistance to ischaemia and reperfusion injury despite moderately long ischaemic times during the grafting process and subjectively, minimal mechanical manipulation of the uterus itself.[68]

Venous Thromboembolic disease

It should be noted that pulmonary embolism, a major cause of post-operative mortality, has been addressed carefully in the previous cohort of five recipients as well as this case series. When addressing all rabbit recipients, 3/11 does died from a post-operative pulmonary embolus. In this present cohort, one out of nine recipients died from this pathology. The uterus was washed continuously with Celsior transplant solution which was run through under low flow pressure

produced by gravitational effect. High flow pressures can result in damage of the intimal layer of the arterial wall, thus increasing the risk of thromboembolic disease. Additionally, heparin was given pre-operatively to the recipient and intra-operatively to both the donor and the recipient. We did not administer any heparin post-operatively in order to prevent any intra-abdominal bleeding, especially form the vascular anastomotic sites. The type of heparin used is low molecular weight heparin as it has a longer duration of action and is easier to administer. In a human, the same principle would be applied, with the added benefit of post-operative thromboprophylaxis, which is administered in other types of transplantation surgery.

Immunology

The biggest milestone in UTx was the first human UTx in 2000, which we have detailed earlier. Because of limited use of animal model experiments prior to the human setting, important anatomical, technical and immunological factors were not tested, leading to the conclusion that the attempt may have been premature. With regards to immunosuppressants, they were administered pre-operatively: CsA A, 6 hours prior to surgery; intra-operatively: prednisolone (to maintain uterine perfusion); and post-operatively: conventional CsA A, azathioprine, prednisolone and a boost by antithymocyte globulin. CD^+4/CD^+8-ratio in blood and Doppler of uterine blood flow were the only techniques employed to monitor possible rejection patterns. Despite initial successful immunosuppression, the transplanted graft's vascular anastomosis failed and the UTx infarcted, surviving for only 99 days.[66]

This is the first attempt to characterise and suppress an allogeneic rejection response in a rabbit model following any type of organ transplant. Immunosuppressants were administered in UTx #4-9 as the initial aim was to characterise the acute rejection response when unaffected by immunosuppression. Therefore recipients #1-3 were not administered any immunosuppressants. However as none of the three were long-term survivors, it was not possible to achieve this. The study design was created so as to allow comparison of the rejection response when immunosuppressants are administered and when they are purposefully left out. Available data is only for recipients #1 and #5. Recipient #1 (died

within first 24 hours) was not provided with immunosuppression whereas recipient #5 (long-term survivor) was. Tacrolimus was the main immunosuppressant used. The other calcineurin inhibitor (CNI), CsA, was administered only intra-operatively. Tacrolimus was the post-operative CNI of choice, as it had been used extensively in the rabbit model, with a greater number of published results related to its effect and toxicity than CsA-related data.[241] In addition, tacrolimus had been used in our previous rabbit UTx cohort.[3] Two does were long-term survivors, with one uterus viable and well-perfused prior to the cull. Histopathology was also normal. The dose administered was 500µcg, twice per day. Finally, it has shown promising results when used in the UTx model. Three reports of its use related to UTx exist in the literature, with one involving pregnancy. *Brannstrom et al* in their description of a first time pregnancy after allogeneic UTx used a dose of 0.5mg/kg/day. The dose used was chosen according to an initial dose-study (0.2-0.5mg/kg/day), demonstrating the absence of morphological signs of uterus rejection by this dose.[115] The same team studied the effect of combining tacrolimus with CsA on long-term uterine survival in a sheep model.[242] Initially, tacrolimus was given for 12 days intravenously, followed by CsA only orally. Immunosuppression after allotransplantation consisted of administration of intravenous CsA (2-5 mg/kg) or oral tacrolimus (0.3-0.5 mg/kg) for 3 weeks and the animals were then examined and euthanized. In allotransplanted animals the ovary and uterus showed normal appearance in 33% (2 of 6) of CsA-treated and 66% (2 of 3) of tacrolimus-treated ewes at 3 weeks post-transfusion. Histology analysis of these confirmed normal appearance. These results indicate that immunosuppression by tacrolimus, rather than CsA is preferred in the sheep allo-UTx model. Finally, *Avison et al* also used tacrolimus in a porcine model. Ten transplants were performed, with five animals alive and healthy at the end of follow-up (0.5-12 months). Immunosupression given was as follows: intravenous tacrolimus administered for the first 12 days post-UTx, together with 10-20 mg/day of methylprednisolone for a month and oral CsA (10mg/kg/day) as maintenance. Rejection episodes were treated with steroids (10-20 mg/kg/day) and increased doses of oral CsA.[157]

With regard to other immunosuppressants, intravenous CsA and hydrocortisone (intravenous intra-operative doses of 10µg/kg and 40mg respectively) were administered only intra-operatively.

Prednisolone was also given orally throughout the first post-operative week (daily dose). The dose of CsA was chosen based on previous published reports describing CsA toxicity levels in rabbits.[243,244] In addition, results from *Wranning et al* demonstrated that CsA dose of 10-20μg/kg can delay the process of rejection.[120]

In our long-term survivor, acute rejection appears to have been adequately controlled, with IgM, CD11b and CD8 levels maintained below their pre-UTx levels. CD45 and CD25 counts spiked on Days 36 and 74, whereas CD4 was higher from Day 36 onwards. As CD45 is a marker for the white blood cells in general and CD25 for activated T cells, the spikes correlated to an increase in rejection activity. It was therefore important to suppress these two acute rejection episodes. 10mg prednisolone was given for three days following the results alongside an increase in tacrolimus dose from 500μg to 1g twice/day. The Doppler examination of the grafted internal iliac at the time was entirely normal and the rabbit did not appear distressed or unwell at any of point during this period. The rabbit maintained her weight and appetite, with no pus or offensive smell noticed from the vagina end. Decreased CD45 and CD25 levels following Day 36 and Day 74 and non-elevated CD8 levels throughout appear to demonstrate that the rejection episode was controlled. It would be interesting to see whether Tregs were responsible for this 'control' and therefore the overall CD4 T-cell increase post-Day 36. It has not possible to analyse Tregs as an appropriate marker for a rabbit model has not been found to date. The graft did not exhibit visible signs of rejection during embryo transfer. It appeared well-perfused, with no signs of ischaemia or necrosis. Results relevant to O_2Sat obtained with a multispectral laparoscope are awaited. The ultimate test is success with regards to embryo implantation and a subsequent pregnancy. This is discussed in the next chapter.

Theoretically, the risk of graft rejection is lowered during the pregnancy because pregnancy itself is thought to be inducing a transient 'physiological' state of immunosuppression within the host's body. *Chaouat et al* [150] described a variation in both the population and activity of immune cells during pregnancy, suggesting an existence of protective wall of effector T lymphocytes, Tregs and uterine-specific NK cells which all induce tolerance of the allogeneic fetus.[177,245,246] Nevertheless, the

rejection rate varies in comparison to the non-pregnant population. With regards to kidney allografts, pregnant to non-pregnant is similar,[247] whereas in cases of liver transplantation, reports state a higher rejection rate at the same interval after transplantation in the pregnant patient. For instance, an average of 10% of pregnant recipients suffered rejections at 39 months after transplant versus 3% in a comparable non-pregnant population approximately three years after transplantation.[140,150]

The biggest limitation of the study is the paucity of data. It was obtained on eight different post-operative days, ranging from day 0 to day 81. However, this was possible for only one recipient, as the other eight died within the first 24 hours. Also immunosuppressants were administered to the sole surviving doe. The data obtained from it has no control, i.e. a long-term survivor free of immunosuppression for which the rejection response could be monitored and used for comparison. We therefore recognise that it is difficult to make any conclusions regarding our immunosuppression regimen but the combination of a CNI and a steroid can clearly slow down the rejection process, if not completely inhibit it. This would correlate with other transplant programmes. If UTx were available in humans, one can envisage the addition of a monoclonal antibody for the induction part. Another limitation was that MHC matching was not carried out for the donor and recipient in the nine transplants and it was therefore not possible to comment on the adequacy of the donor and recipient parings. The breeders who provided us with the animals made sure that we received two does from the same family line (not twins) to ensure a degree of compatibility. This would mimic human UTx where a degree of MHC matching is expected.

The ability to characterise the rejection response in a rabbit allo-UTx model is of course dependent on long-term recipient survival. Ideally, data obtained would depict the rejection response in both a non-pregnant and, post-ET, pregnant doe. At least one model should characterise the rejection response in a doe not administered any immunosuppressants. Furthermore, all uterine grafts will undergo immunohistochemistry following post-mortem. Beyond the scope of this PhD, a need for future UTx animal models to clarify the type and dose of immunosuppressant ideal for prevention of uterine rejection exists. This choice must also prove to be of value from the safety aspect, with minimal harm

affecting the mother but in particular to the growth and well-being of the fetus. Small studies can also investigate whether combinations of different immunosuppressants (CsA, tacrolimus and steroids) carry greater clinical benefit. Finally, the results of such targeted research should be pooled together to create a protocol which outlines a clear methodological structure for the induction of allotolerance and more importantly, leads to long-term (i.e. 9 months) survival of the allotransplanted uterus.

Pregnancy

Our pregnancy did not result in the birth of a healthy term offspring. However the demonstration of pregnancy in this allogeneic model is a central proof of concept of UTx in a rabbit model and thereby an essential step in the research toward its clinical application in the human. The study represents only the third example of conception and pregnacy following an allogeneic UTx. To date, there has only been one live birth following allogeneic UTx. The animal model was large (sheep), and thus, no live births have been achieved in a small animal model.

The macrovascular patch model functioned in that the uterine graft received an adequate blood supply, allowing for implantation and fetal growth. The renal, hepatic and reproductive profiles were normal throughout the post-operative period, during which time the recipient doe was taking daily tacrolimus. Its daily routine was also normal, with no adverse characteristics demonstrated with respect to behaviour, eating and drinking patterns, movement and fertility induction. Embryo transfer was chosen as the optimum method to achieve conception following the conclusions from *Saso et al*.[108] In this study, the two rabbit long -term survivors following allogeneic UTx could not conceive via natural conception, most likely as a result of blocked anastomosed cornua.[108]

One of the key issues concerning UTx is finding a suitable immunosuppressive protocol which achieves graft protection from the body's rejection processes but also does not harm the embryo of the fetus. This resorbed pregnancy may therefore be explained by either the potential negative impact of tacrolimus on early fetal growth, or by a clinical rejected uterus because of inadequate

immunosuppression. With regards to the former point, tacrolimus was selected as it is now widely used in clinical organ transplantation. It has lower nephrotoxicity, with reports from large studies stating fewer episodes of acute rejection compared with the traditional calcineurin inhibitor CsA.[248] Its mode of action suppresses T-lymphocyte activation via the inhibition of calcineurin-dependent interleukin IL-2 production.[249] Importantly it can be used after initial induction therapy as a single immunosuppressant in liver transplantation,[250] and tacrolimus has been used extensively during pregnancies in organ transplant recipients (a significant consideration for UTx).[123,251] The latest study on the effects of tacrolimus on UTx, evaluated the effects of tacrolimus on the rejection of a transplanted rat uterus and on uterine expression of markers of inflammation and implantation.[123] Non-treated uterine grafts showed rejection with necrosis. Tacrolimus-treated transplanted group exhibited normal uterine morphology with low numbers of T-lymphocytes in all uteri except in two out of seven uteri of the tacrolimus-treated transplant group. Uteri of the non-treated transplanted group showed elevated mRNA expression of IL-1a and IP-10 and reduced galectin-1, compared with the tacrolimus-treated transplanted group. Therefore, tacrolimus monotherapy suppressed rejection of an allotransplanted uterus, normalized the expression of IL-1a and IP-10 and prevented T-lymphocyte infiltration. The tacrolimus in our experiment did overall decrease the rejection response (**Figure 2a.8**). However, three rejection episodes did occur, with the ultimate episode occurring during pregnancy. Despite managing to control the first two (occurring on days 36 and 74), some uterine structural damage must have occurred, thus rendering the graft less suitable for carrying a pregnancy until term. The third episode on day 102 could have been the final insult which led to miscarriage, most probably occurring three days later (**Figure 2a.6**). It is therefore unlikely that it was the effects of tacrolimus which led to intra-uterine death but rather that the rejection response itself was inadequate to protect the graft carrying the pregnancy. This could be either because the dose of tacrolimus was inadequate or that an additional immunosuppressant, in the form of a monoclonal antibody, should be added. Further such experiments are required but should be carried out in a larger-animal model, because of difficulties of using a rabbit model as described in the previous chapter.

Advances in immunosuppression as well as original modalities to induce tolerance of a transplanted uterus may reduce these problems in the future. The induction of immunological tolerance to a transplanted graft offers a novel potential solution to inhibiting immune-mediated graft rejection. Tolerance is defined as the specific and permanent absence of an attack or response by the immune system to a foreign antigen, which in our case is represented by the uterine graft antigen, without immunosuppression.[252] It can be classified as either natural which occurs via either a central (in the thymus and bone marrow) or peripheral (circulating mature T and B cells) pathway, or acquired. The ultimate clinical goal would be for the patient to 'acquire' tolerance, both in the non-pregnant and pregnant state, as natural tolerance cannot deal with foreign antigens. This would mean an almost complete allo-unresponsiveness to donor cells and therefore a diminished use of immunosuppressants and negligible damage to the uterine graft as a result of both the pre-clinical and clinical rejection process. Thus, it is important to differentiate between the role of immunosuppressants and tolerance modulation in the graft acceptance process. The level of an immunosuppressant administered to a patient can indeed portray the state of clinical tolerance, yet the drug does not provide tolerance induction in general. In certain cases, immunosuppressants can diminish the overall tolerance effect, for example, calcineurin inhibitors which downgrade the action of all T cells, including regulatory T cells (Tregs).[253]

Boosting the role of Tregs may offer a 'tolerance' pathway. Natural Tregs are a subset of CD4+ cells, known for their ability to induce peripheral tolerance and are also known as CD4+CD25+FOXP3 cells.[254] Tregs are developed either naturally from the thymus as a separate lineage or similarly to other suppressive T cell subsets like Th3 or CD8+ suppressor cells are induced peripherally under certain regulatory conditions.[253,255] It is the latter type of Treg which may be able to downregulate a post-transplant rejection response. The idea behind this process would involve *in vitro* isolation of Tregs specific to the uterine graft alloantigen prior to transplantation. Following Treg number expansion, these de novo cells would be transferred into the patient's peripheral circulation, with the aim to induce immunomodulatory graft tolerance, minimal rejection and a decrease (or even disappearance) of immunosuppressive medication. Protocols under good manufacturing practice

conditions have already been created,[253,256] with Phase I clinical trials in allogeneic bone marrow transplantation looking at this particular issue.[257] Treg expansion during pregnancy (they make up a third of all CD4+ cells in the pregnant uterus) has been demonstrated in both allogeneic and syngeneic pregnancies, suggesting that paternal alloantigens are not the primary cause for this rise.[245] *Aluvihare et al* confirmed the link between the establishment of a healthy fetus and normal Treg levels at the maternal-fetal interface by demonstrating a much smaller proportion of decidual Tregs following miscarriage, as opposed to elective abortions.[245,258] It is thought that this particular inherent model of localized (uterine) tolerance may be responsible for a decreased rejection pattern on the graft during pregnancy,[120] which necessitates further research into immunomodulatory techniques using the aforementioned agents, similar to the 'Treg' ideas described above.

The field of immunomodulation continues to develop, and could lead to the ultimate goal, a post-transplant world involving no regular immunosuppression medication. Aside from Tregs, immunosuppression may also be induced after a single treatment course. For example, T10B9, a monoclonal antibody directed against the alpha-beta heterodimer of the T-lymphocyte receptor complex, has been used successfully to treat acute cellular rejection in renal transplantation and as an immunosuppression induction agent in heart and simultaneous kidney-pancreas transplantation.[259] *Kawai et al* described combined bone marrow and kidney transplants from HLA single-haplotype mismatched living, related donors resulting in induced immunotolerance.[260]

Unfortunately, pregnant transplant recipients cannot wait for long term results to clearly demonstrate superiority of one regimen over the other. This is especially a problem regarding pregnancy outcomes among transplant patients. Regardless, if the pregnant transplant recipient has a kidney, heart, lung or uterus, it will be impossible to ensure safety to mother and fetus if the goal is a healthy child that matures to a healthy adult.[2]

We cautiously predict that from the immunological point of view, the risks to mother and fetus will be no different to those faced by renal, hepatic or cardiac transplant patients undergoing pregnancy. The

patient must be considered as high risk from the beginning of the pregnancy. She should be managed under a multi-disciplinary team, involving in particular a physician specializing in high-risk obstetrics and a transplant immunologist. The care pathway would be very similar to any pregnant patient with a transplanted organ.[150] Because of the effects of immunosuppressants on the maternal immune system, viral serology for cytomegalovirus as well as microbiological cultures of vaginal smears should be repeated monthly. Levels of immunosuppressive drugs in the blood should be monitored, thus permitting adjustments of drug dose relative to graft function and physiological changes of pregnancy. Doppler assessment of the vascular uterine supply as well as anastomosis may be helpful if not interesting. Visual inspection of the transplanted cervix would likely provide clinical clues of the graft's condition. Both vaginal delivery and a Caesarean Section would be possible for delivery. However, as the uterus will be removed following delivery, and in order to create a controlled environment during the labour process, a Caesarean section would be more advisable.[2]

Conclusion

Although this case series of allo-UTx described only nine cases, it highlighted some of the challenges with the choice of animal model and the complexity of the surgical procedure involved. The macrovascular patch technique involves aorto-aorta and veno-vena anastomoses. This type of anastomosis would not be attempted in a human because of its highly risky strategy involving the most important blood vessels. Damage to them could lead to catastrophic outcomes. Anastomosis at a lower level (common iliac, internal iliac or uterine arteries) would not be possible in a small animal model such as a rabbit as the diameter of these vessels is too small to allow for satisfactory anastomosis. Therefore, the macrovascular patch technique allows us to bypass this problem, and thus provide an adequate blood supply to the uterus - the most important prerequisite to long-term graft survival and subsequent pregnancy. In a human, internal iliac to internal iliac or even external iliac vessel anastomosis would be possible however as the diameter of these vessels is large enough for precise microsurgery.

With regards to the attempted pregnancy, despite the end result i.e. termination of pregnacy as a result of miscarriage, the study represents only the third example of conception and pregnacy following an animal allogeneic UTx. The surgical anatomical macrovascular model was successful, with an appropriate level of perfusion attained (see Chapter 3). Post-operative recovery was uneventful, with no adverse effects recorded. The cause of fetal demise is most likely secondary to inadequate prevention of the immunological rejection response.

In summary, when including all 20 rabbit UTx performed by the team, results were as follows: (a) surgical survival rate: 80% (16/20); (b) UTx success rate: 67% (11/18); and (c) anastomotic success rate: 83% (15/18). These results support the feasibility of uterine allo-UTx using a macrovascular patch technique in the rabbit with respect to anatomical terms and surgical vascular achievability. Immunosuppression would follow kidney transplantation, with definite use of a steroid and a CNI. With regards to the robustness of the rabbit to withstand the operation, a solution could be to transfer the UTx experience onto a larger-animal model. A porcine model has been attempted and proved also a less-than-ideal model.[84] A sheep model could be the next logical step, especially as the first birth post-allogeneic UTx was achieved using in this model.[163]

STUDY 2b

2. Study 2b: Achieving uterine auto-transplantation in a sheep model using iliac vessel anastomosis: a short-term viability study

This study has now been peer-reviewed and published as per following reference: **Saso S**, Petts G, Thum MY, Corless D, Boyd M, Noakes D, Del Priore G, Ghaem-Maghami S, Smith JR. Achieving uterine auto-transplantation in a sheep model using iliac vessel anastomosis: a short-term viability study. *Acta Obstet Gynecol Scand* 2014 Nov 24. [Epub ahead of print]

Abstract

Objective: To investigate, develop and evaluate anatomical, surgical and anastomotic aspects necessary for a successful uterine transplant in a large-animal model.

Design: Sheep model; longitudinal study involving five ewes.

Setting: Royal Veterinary College, London, UK.

Population: Five ewes of proven fertility.

Methods: The uterine allograft along with the internal iliacs, and uterine arterial and venous tree all intact were harvested en bloc. An end-to-side anastomosis was performed between the external iliac vessels and the internal iliac vessels of the graft using 6-0 Prolene sutures. Successful reperfusion of the graft was initially judged by the colour shift of the uterus during reperfusion. Blood flow past the venous and arterial anastomotic sites was also ensured by visual inspection, together with pulse oximetry and multispectral imaging.

Main Outcome Measures: Operative details (retrieval, ischaemic, clamping, reperfusion and recipient hysterectomy duration); physiological profiles; gross morphology and histopathology.

Results: Five autotransplants were performed. One procedure was abandoned because of the inappropriate size of sheep model. Another procedure was halted because the animal suffered from respiratory failure in the immediate intra-operative period. Three transplants were completed. In

those, at least two out of four possible anastomoses were finished and the grafted uteri demonstrated immediate perfusion and appropriate viability 45 minutes post-operatively.

Conclusions: Internal to external iliac vessel anastomoses is an acceptable surgical technique that should be applied in a human model to ensure adequate subsequent uterine perfusion.

2b.1. Introduction

The challenges that one faces when attempting uterine transplantation (UTx) in a small animal model have been described in length in the previous chapters. Additionally, the human UTx attempt in 2000 demonstrated the deleterious effects of vascular thrombosis, which in this case led to the demise of the graft and subsequent hysterectomy. Thrombosis was brought on by surgical techniques related to vascular anastomosis and the fixation of the transplant. Together, the above problems highlight the need for the development of a large animal model in order to further develop vascular anastomotic and other surgical techniques. Thus, the next step in achieving successful human UTx is carrying out the actual procedure in a large animal model. The large animal models resemble the human pelvis much more closely than small animals. Surgeons can therefore perform procedures that may be reproduced clinically in the future. The literature review has already been covered in an earlier chapter titled 'Literature Review'.

Aims

Five autogeneic UTx procedures in a sheep model are presented here. The aim was to investigate, develop and evaluate anatomical, surgical and anastomotic aspects necessary for a successful UTx.
UTx will be carried out in an orthotopic position in the pelvis and with vascular anastomosis to the external iliac vessels.

2b.2. Methods and Materials

Sheep model

The feasibility of the uterine autograft dissection together with its vascular supply that includes the internal iliacs up to the point of division, the AA and the IVC was assessed by a series of uterine autotransplantations in the sheep model performed by this team. Two surgically explorative

procedures and five autogeneic uterine cross transplantations were performed using a pre-determined protocol.

Five mature ewes (2–5 years of age) were purchased from an accredited breeder and used as both donor and recipient animals (auto-transplant). They were all of proven fertility with at least one previous litter each. The animals were acquired two to three weeks before the operation to ensure appropriate acclimatization to their surroundings. The animals had free access to hay and water and were fed pelleted concentrated fodder twice a day. The study followed the guidelines for the handling and care of experimental animals issued by the appropriate UK body and was approved by the Animal Ethics Committee in UK as well as the Institutional Review Board.

Surgical protocol

The aim of surgery was to isolate a specimen including the common uterine cavity and cervix, both uterine horns (without the oviducts and ovaries), the uterine arteries, leading to the anterior divisions of the internal iliac artery and accompanying vein, and ultimately the internal iliac vessels up to the point where they originate directly from the common iliac vessels.

The ewe was anesthetized using a combination of intramuscular ketamine (4mg/kg) and diazepam (2mg/kg). After intubation, anaesthesia was maintained with 1.5–2.0% isoflurane. The anterior abdominal wall was shaved and cleaned, and the abdomen was opened through a midline incision. The end result of the graft harvest was a radical hysterectomy transecting across the vagina and the most lateral aspects of the uterine horns together with an internal iliac macrovascular patch. Following graft harvesting, the internal iliac artery was cannulated and the uterine specimen with its vascular patch was generously flushed with heparinised normal saline and transplant storage solution, *Histidin-Tryptophan-Ketoglutarat (HTK)*.

The uterine allograft along with the internal iliacs, and uterine arterial and venous tree all intact were harvested *en bloc*. The utero-tubal-ovarian specimen was continuously flushed and subsequently stored for 60 minutes ex vivo in the *HTK* solution, between 2°C and 8°C while the recipient doe was being prepared.

The vagina was anastomosed initially to ensure proper orientation and positioning of the specimen followed by the uterine horns which were finally rejoined. Subsequently, soft tissue vascular clamps were placed first around the recipient's external iliac artery. A longitudinal cut (4mm length) was made in the anterior external iliac artery wall to create an opening. An end-to-side anastomosis was performed between the external iliac artery and the internal iliac artery of the graft using 6-0 Prolene sutures. A total of three to five continuous stitches were used for the aortic connection. A similar procedure was carried out for the venous end-to-side anastomosis. Successful reperfusion of the graft was judged on the colour shift of the uterus during reperfusion from its blanched appearance after flushing to a more reddish colour. Blood flow past the venous and arterial anastomotic sites was also ensured.

In all ewes, immediate surgical outcome was assessed 30-60 min after reflow by inspection of the patency of the vascular anastomosis sites, pulsation through the transplanted vessels, colour of the transplant and occurrence of spontaneous uterine contractions.

Blood samples (full blood count, kidney and liver function, reproductive hormone profile) were taken just before the commencement of surgery by Dr M Boyd and Dr S Saso and again just before the animal was culled.

The graft retrieval and transplant was performed by Mr J. Richard Smith and Dr S Saso. The vascular anastomosis was carried out by Mr DJ Corless (Consultant Upper GI Surgeon, Mid Cheshire Hospitals Trust), with Mr JR Smith and Dr S Saso assisting. Dr M Boyd (Veterinary Anaesthetist, Royal Veterinary College) was the anaesthetist for all nine transplants. Blood samples from the does

were taken by either Dr M Boyd or Dr S Saso. Advice on technical aspects and rabbit physiology during a major surgical procedure was given throughout by Professor D Noakes.

Medication

Subcutaneous heparin 1000IU was given both before and after organ procurement to prevent thrombosis. Quinolone enrofloxacin (Baytril) was used as antibiotic prophylaxis at an intra-operative dose of 5 mg/kg through a slow intravenous infusion.

Post-mortem & Histopathology

All post-mortems were performed immediately after the ewe had been euthanized. The same laparotomy incision as used in the actual surgery was opened and extended to become a thoraco-laparotomy. The thoracic and abdominal cavities were inspected, including the macrovascular patch, the uterine graft and each organ individually (heart, lungs, liver, kidneys, spleen). Certain organs were then placed in formaldehyde to fix the tissue and prepare it for histopathology. An incision was made on the uterine cornua to allow for the formaldehyde to access the inside of the cornua.

The gross morphology findings were recorded according to a scoring system previously validated by the Brannstrom team [117] and also used by *Diaz-Garcia et al* in UTx.[109] The following characteristics were evaluated by the scoring system: (a) abnormal appearance of the uterus (colour, texture and hardness); (b) presence of adhesions; and (c) thrombosis of the graft vessels (both artery and vein).

Prior to haematoxylin & eosin (H&E's) staining, tissue was cut and placed on slides, with the assistance of Dr Gemma Petts (Specialist Registrar, Pathology, St Mary's Hospital, London). All routine H&E's were stained on the Tissue-Tek staining machine. The haematoxylin dye is a poor dyeing agent. To obtain purposeful results it needs to be converted to its oxidation product haematin. This is achieved by the use of an oxidising agent such as mercuric II oxide (HgO). Before this

conversion is brought about, haematoxylin is added to an aqueous solution of aluminium potassium sulphate. This is the mordanting step, where aluminium ions combine with the haematoxylin dye. The resultant aluminium-haematin complex then stains via the metal ion - Al^{++}. Haematoxylin possesses acid-base indicator properties (red in acid - blue in alkali solutions) so the stained tissue components must be 'blued' after differentiation using an alkaline solution (London tap water or a blueing agent). This then allows the rest of the tissue components to be counterstained as desired (normally eosin). The sections are first immersed in water. Visible nuclei are then stained in Harris haematoxylin for five minutes. This is then followed by a wash in water and differentiation in 1% acid alcohol. The well is washed in water and 'blue' in Scott's water. Subsequently the sections are all washed in water again, followed by staining in 1% eosin Y for five minutes. Washing in water implies de-waxing in xylene in 1-2 minutes, then in 99% alcohol for 10 seconds which is repeated three times and finally repeated in running tap water for 10 seconds. A repeat wash in water, followed by dehydration, clearing and mounting. This implies the following method: dehydration in 99% alcohol for 10 seconds repeated three times, clearance in xylene for 20 seconds and mounting on cover-slipping machine.

Data Presentation and Analysis

Statistical analysis was not required for this study. Data has been presented using 2D graphs, histopathology slides, figures and descriptive tables.

2b.3. Results

Five uterine auto-transplants were carried out. The aim was to first remove the whole uterine graft (cervix, cavity, both cornua), including its blood supply (uterine vessels, anterior division of internal iliac and the internal iliac from their origin to the section where they divide into anterior and posterior divisions. Accompanying veins were also included. The anastomotic goal was to join the origin of the internal iliac vessels to the external iliac vessels bilaterally, perform both left and right cornua-cornual

anastomoses and finally carry out a cervico-vaginal anastomosis. Mastery of the microvascular techniques of iliac arterial and venous anastomosis was the primary focus.

The first cadaveric study focused on the vascular and pelvic surgical anatomy of the sheep model and its variations from the human pelvis. The dissections of the internal and external iliac vessels allowed us to believe that we could perform uterine auto-transplantation successfully. The morning of the first transplant commenced with the sheep being administered a general anaesthetic, and then washed and draped. The weight of the ewe was 80kg. The animal was not catheterised. A midline incision was made, which in retrospect should have been longer both in a caudal and cephalic direction. On opening the abdomen the uterus was easily identified. The uterine horns were then isolated, followed by identification of the middle uterine arteries and the utero-ovarian vessels which were clamped and ligated. The external iliac vessels were identified and skeletonised for the later attachment of vessels. A dissection was subsequently performed down to the vagina. An enormous number of vessels down the anterior aspect of the cervix were witnessed and the cervix in fact proved to be approximately four inches long. By the time the vagina was entered, the surgical team was deep into the abdomen in both an inferior and posterior direction. This depth was uncomfortable and would not have been encountered in a woman's pelvis. Having opened the vagina, the dissection of the uterine vessels was continued inferiorly and the uterine vessels were dissected free of the vagina. Unfortunately, it did not prove possible, having identified the internal iliac vessels, to allow for the dissection of the 'meeting' of the internal iliac with the base of the uterine arteries. They were too far under the pelvic joints to be visible. Therefore the uterine arteries were ligated with approximately 1.5-2.0 inches of spare artery to utilise, as the aim was now to attempt uterine artery to external iliac artery anastomosis. Unfortunately following the removal of the sample, it became clear that these arteries had grossly retracted and were the equivalent of a uterine artery in a woman. They were also impossible to separate from the cervix and furthermore, the uterus could not be anastomosed at the vagina as planned. Only one connection was possible, from the identified uterine artery and vein to the external iliac on the right side. This was duly cross clamped at the vein. Four sutures were put in. Bearing in mind the uterus was now in the wrong place, it proved impossible to complete the anastomosis and the procedure had to be

abandoned. The animal was subsequently culled. In order to see whether the technique could be improved, the incision was lengthened in the cephalic direction which delivered a better angle of approach and access. A Mercedes scar was subsequently utilised which did finally allow us to gain access to the distal end of the internal iliac. It did however involve cutting through a large body of muscle with potential for heavy haemorrhage secondary to incision of large-diameter vessels within the muscle. The decision was made that prior to the next transplant, a further cadaveric dissection was required to ensure that this procedure could be carried out in a sheep model. This was indeed performed, and the use of a lighter and smaller ewe solved the problem of the afore-mentioned abdominal depth which was greatly decreased. This allowed for definite visualisation of the point where the uterine arteries meet the anterior division of the internal iliac.

The second autotransplant commenced in the same manner as the transplant above. The sheep weighed approximately 50kg. Catheterisation proved difficult again and the bladder was emptied using a needle and syringe intra-abdominally. This was performed with the subsequent three autotransplants. A mid-line incision was made which proved difficult in the region of the mammary teats as a result of the venous supply within the subcutaneous fat. Having entered the abdomen, the bowel was packed posteriorly. Dissection then commenced by dividing the round ligament, opening the broad ligament and identifying the external and internal iliac vessels. The internal iliac vessel was completely skeletonised down to the insertion of the uterine artery and beyond. The ovarian ligament was divided separating the ovary and Fallopian tube from the uterus. The bladder was dissected down. Ureters were slung and dissected out in their entirety. The vagina was opened anteriorly and posteriorly and the uterine artery was then dissected free down a distance of approximately 8cm until it met the internal iliac. The ligation of the internal iliac beyond the uterine artery/venous junction proved difficult since a ligature could not be placed round it, since again it was well under the pelvic arch. Therefore, four large ligaclips were applied to good effect and this allowed separation of the uterus, cervix, upper vagina, along with attendant uterine arteries. At the end of it, the uterine arteries twisted rather easily which did not allow perfusion with the HTK solution in a satisfactory fashion. The external iliac vessels were completely skeletonised and rendered free to allow vascular clamps to

be put round them. The left external iliac vein was first incised. Appropriate haemostasis was difficult to achieve with the equipment present. This was however done and the internal iliac vein was anastomosed to the external iliac. Six sutures were required to create a seal. Identical steps were repeated for the arterial supply. There did not appear to be any arterial flow with the entire vessel seeming to have spasmed. After much discussion the decision was made to complete the experiments by joining the right internal iliac to the right external iliac artery. This was duly performed with the uterus in fact gradually appearing pink and perfused. The animal was kept alive for 45 minutes and then culled.

The third autotransplant proceeded in the same manner. The ewe weighed 48kg. It was again possible having identified the internal iliac vessels to make the dissection 'meet-up' i.e. internal iliac artery with the base of the uterine arteries. The skeletonization of the uterine vessels towards internal iliac was again a challenge but the uterus with the uterine vessels as well as the internal iliac vessels was dissected *en bloc*. All four vascular anastomoses were completed: left internal iliac to left external iliac artery, left internal iliac vein to left external iliac vein, right internal iliac to right external iliac artery, and finally right internal iliac vein to right external iliac vein. The uterus appeared well perfused at completion of surgery. The animal was kept alive for 45 minutes and then culled.

The fourth operation commenced in the same manner as the previous three autotransplants, with a midline laparotomy. The ewe weighed 55kg. The anaesthetics team had reported pre-operatively that intubation had been challenging, with the animal observed to be respiring at a higher rate than normal. Immediately on entering the abdomen it was clear that there was more intra-abdominal pressure than normal. The uterus was subsequently identified, with the bowel packed back. At that point unfortunately the animal arrested and died. Resuscitation was attempted without success. Full post-mortem was undertaken with retrieval of uterus, liver, lungs and heart. The lungs looked obviously diseased.

The fifth animal weighed 62kg. Following the mid-line laparotomy, the bowel was packed with good access obtained into the pelvis. The external iliac vessels were skeletonised and under-run in preparation for the anastomosis later. The internal iliac vessels were identified as far as the bifurcation where the uterine vessels branched off. This part of the operation was similar to auto-UTx #1 i.e. extremely deep in the pelvis. The uterus was isolated by dividing the ovarian ligaments. The uterine arteries were clearly identified running down either side of the vagina. The utero-vesical space was opened to reach the vagina down to 2.5-3 inches below the cervix. The recto-vaginal septum was also opened to achieve the same dissection. The ureters had slings applied. Unfortunately at the end of the dissection on the left side, accidental trans-section of the left uterine artery occurred, approximately 4cm from the cervix and 3cm from the internal iliac. On the right side it appeared that the uterine artery bifurcated twice and this resulted again in a short sample. This therefore led to us being unable to do an internal iliac to external iliac anastomosis. Thus, the anastomosis performed was uterine vessels to external iliac vessels. Hence, the uterine arteries were skeletonised clear of the uterus i.e. in the parametrium. The right uterine vein was anastomosed to the right external iliac vein and the left uterine artery was anastomosed to the left external iliac artery. The uterus immediately demonstrated good blood flow and was pink and functional. The animal was culled 45 minutes after completion of anastomosis.

Operative details for the above five auto-transplants are shown in **Table 2b.1** below. Blood results are shown in **Table 2b.2**.

UTx	Graft Retrieval (min)	1st Warm Ischaemia (min)	Cold Ischaemia (min)	2nd Warm Ischaemia (min)	Clamp on (LEIV/LEIA/ REIV/REIA) (min)	Grafting (min)	Reperfusion (min)	EBL (ml)
1*	478	5	60	30*	25 / - / - / -	30*	N/A	35*
2	375	5	60	120	35 / 26 / - / 22	120	45	200
3	300	5	60	125	42 / 23 / 22 / 15	125	45	800
4*	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
5	205	5	60	90	- / 24 / 27 / -	90	45	100
Mean	**340**	**5**	**60**	**115**	**34 / 24 / 25 / 19**	**115**	**45**	**367**

Key: * UTx not completed

Table 2b.1 Operative details from the five transplant procedures in the sheep model showing retrieval, ischaemic, clamping, reperfusion and recipient hysterectomy times in minutes

Marker	UTx 1		UTx 2		UTx 3		UTx 4		UTx 5	
	pre	**post**	**pre**	**post**	**pre**	**post**	**pre**	**post**	**pre**	**post**
Sodium	147	NA	145	145	144	142	143	NA	143	143
Potassium	4.1	NA	4.2	**6.5**	4.1	4.3	4.2	NA	4.5	**6.1**
Urea	6.3	NA	7.7	**11.7**	8.9	5.1	9.5	NA	8.5	**12.4**
Creatinine	82	NA	64	**186**	80	56	69	NA	73	**128**
AST	**72**	NA	**89**	**80**	**97**	**83**	**109**	NA	**239**	271
ALT	27	NA	25	21	21	60	34	NA	27	31
ALP	107	NA	146	**149**	138	125	**174**	NA	57	48
Albumin	36	NA	30	**23**	31	28	**33**	NA	**32**	**23**
CRP	<0.6	NA	<0.6	<0.6	<0.6	<0.6	<0.6	NA	<0.6	<0.6
Reproductive Profile										
17β-oestradiol	56	NA	73	88	56	91	133	NA	88	94
LH	0.1	NA	0.1	0.1	0.1	0.1	0.1	NA	0.1	0.1
FSH	0.1	NA	0.2	0.2	0.1	0.1	0.1	NA	0.1	0.1
Testosterone	0.4	NA	0.4	0.4	0.4	0.4	0.4	NA	0.4	0.4
Progesterone	0.5	NA	0.5	0.8	0.5	0.8	0.8	NA	0.5	0.8

Key: **NA.** Non-applicable, **NM,** Not Measured (lab error, insufficient sample received, haemolysed sample

Table 2b.2 Hepatic, Renal and Reproductive Profiles (Non-Pregnant Ewe)

Post-mortem and Histopathology

Tables 2b.3 and 2b.4 below and **Figures 2b.1-2b.3** summarise the findings following post-mortem and histopathology.

UTx	Colour 0, pink 1, dusky 2, white	Texture 0, soft 1, firm 2, hard	Size 0, normal 1, oedematous 2, markedly bigger	Anastomosis (vein-vein) 0, intact 1, broken down	Anastomosis (artery-artery) 0, intact 1, broken down	Uterine bleeding 0, absent 1, present
1	N/A	N/A	N/A	N/A	N/A	N/A
2	1	1	1	0+1	0+1	0
3	0	0	0	0	0	0
4	N/A	N/A	N/A	N/A	N/A	N/A
5	0	0	0	0+1	0+1	0

Table 2b.3 Summary of gross morphology findings at post-mortem, performed within 60 minutes of the death of the ewe (*Wranning et al*) [117]

Table 2b.4 Summary of histopathology findings (UTx #1-5)

UTx	Post-mortem	Macroscopic examination of fixed tissue and Histopathology	Clinico-Pathological Correlation
1	**N/A**	**N/A**	**N/A**
2	Left vascular anastomosis appears to have failed. Right vascular anastomosis appeared successful.	**10 slides** Vascular anastomosis: The left internal-external iliac anastomosis shows evidence of intravascular thrombosis and damage to the vessel wall (**Figure 6.3a-b**). The thrombosis appears extensive but it is difficult to comment on whether there is complete occlusion of the vessel due to suboptimal sectioning of the histology block. Vagina, uterus, and oviduct: The vaginal anastomosis shows diffuse mild to moderate acute inflammation of the squamous (**Figure 6.3c**) and glandular (**Figure 6.3d**) epithelium, probably representing mild ischaemic changes. The left and right cornua show evidence of early ischaemia (peri-glandular inflammation, **Figure 6.3e**). There are small polypoid stromal proliferations probably representing benign uterine polyps (**Figure 6.3f**). The right oviduct shows evidence of early ischaemic changes (**Figure 6.3g**). The left oviduct shows marked congestion and inflammation, suggesting more severe ischaemia (**Figure 6.3h-j**).	**Uterus and oviduct show evidence of mild to moderate ischaemia.** **Thrombus in the left internal-external iliac anastomosis but the extent of the vessel lumen occlusion cannot be assessed.**
3	No obvious pathology	Vascular anastomosis: No thrombus formation or significant abnormality identified. Extravascular connective tissue haemorrhage and fibrin deposition containing numerous polymorphs seen. Most likely representing a blood clot associated with handling and surgery (left vascular anastomosis, not photographed). Uterus, and oviduct: The uterine body shows small polypoid stromal proliferations possibly representing benign uterine polyps (similar to those seen in uterus 2). No other abnormalities noted. The left cornua appears congested. The right cornua appears within normal limits. There is no evidence of ischaemia.	**Uterus and oviduct showing no significant abnormalities.** **No evidence of thrombus in the vascular anastomoses.**
4	Heart: Small lesion in the lateral wall of the left ventricle Lungs: Congested with areas of ?haemorrhage/infarct.	**6 slides** Heart: The lesion in the wall of the left ventricle is a partially calcified nodule surrounded by a rim of dense connective tissue containing a chronic inflammatory cell infiltrate (**Figure 6.4a)**. The origin (infection, foreign body, infarction) of the nodule is unclear. In further sections taken from the wall of the left and right ventricles and septum there are occasional scattered aggregates of chronic inflammatory cells (**Figure 6.4b**). The overall appearance is of mild patchy myocarditis. Lungs: Cuffing of large airways by aggregates of	**Features of myocarditis and an allergen induced bronchial hypersensitivity reaction.** **Both of these, alone or in combination would have affected the animal's ability to withstand the physiological stress of anaesthesia.**

		eosinophils, consistent with an airway/bronchocentric allergic inflammatory reaction (in humans this would be associated with allergen induced bronchial hypersensitivity as seen in asthma)(**Figure 6.4c**). Focally there are aggregates of haemosiderin/degraded foreign material surrounded by giant cells, suggestive of previous aspiration. Mediastinal and paratracheal lymph nodes show reactive changes.	
5	No obvious pathology	**2 slides** Vascular anastomosis: There is a possible thrombus present in the right vascular anastamosis, however the plane of tissue sectioning is suboptimal and it is not possible to comment on the occlusive nature of the thrombus (**Figure 5.5a**). There are some inflammatory cells adherent to the luminal surface of the left vascular anastomosis but these are not associated with thrombus and probably represent early activation of the clotting system or early recruitment of inflammatory cells (**Figure 5.5b**). Uterus, and oviduct: The uterine body shows small polypoid stromal proliferations possibly representing benign uterine polyps (similar to those seen in UTx #2 and #3, therefore this may represent normal histology for a sheep uterus). No other abnormalities noted. The right and left cornua appears within normal limits. There is no evidence of ischaemia.	**Possible thrombus in the right vascular anastomosis.** **Uterus and oviduct showing no significant abnormalities.** **No evidence of ischaemia.**

Key: **DIC;** disseminated intravascular coagulation; **PA,** pulmonary artery; **PE,** pulmonary embolus

Figure 2b.1-2b.3 Selected histopathology images (slides are H&E unless stated otherwise)

UTx #1 - Histopathology was not performed here as the procedure was abandoned because of a poor choice of animal

Figure 2b.1 UTx #2 - Demonstration of anastomotic failure of left vascular field

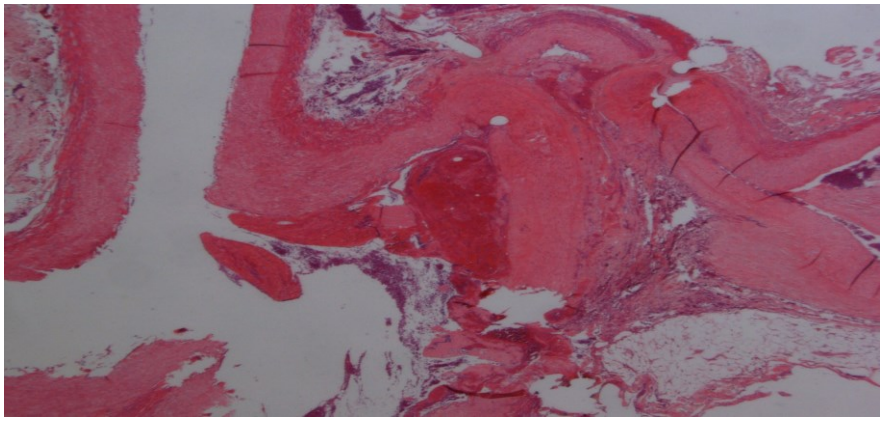
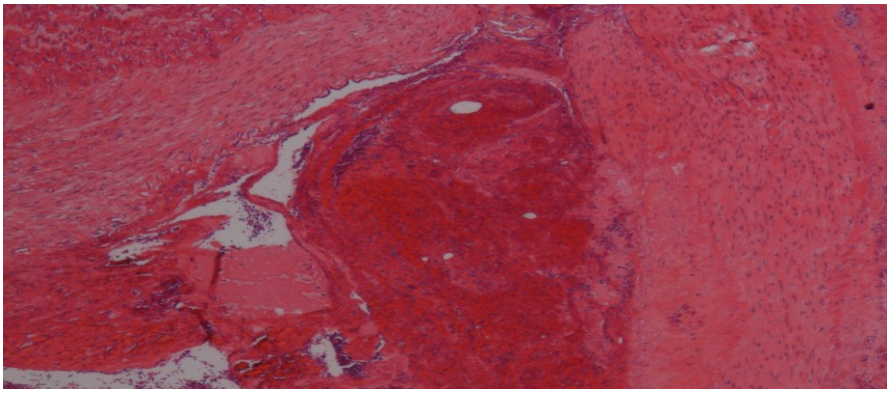
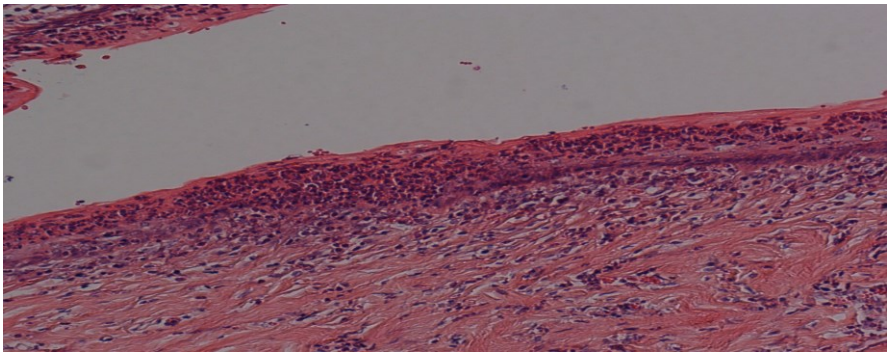
Figure 2b.1a Left internal-external iliac anastomosis. x 12.5, H&E. Intravascular thrombosis and damage to the vessel wall.	
Figure 2b.1b Left internal-external iliac anastomosis. x 40, H&E Intravascular thrombosis and damage to the vessel wall.	
Figure 2b.1c Vaginal anastomosis. x 100, H&E. Squamous epithelium, mild ischaemic changes.	

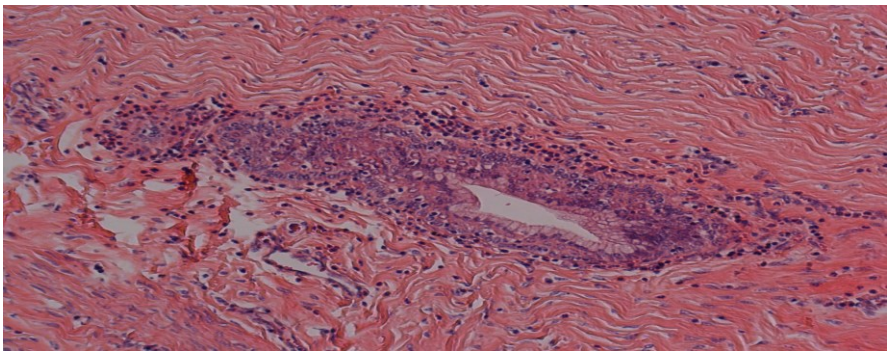
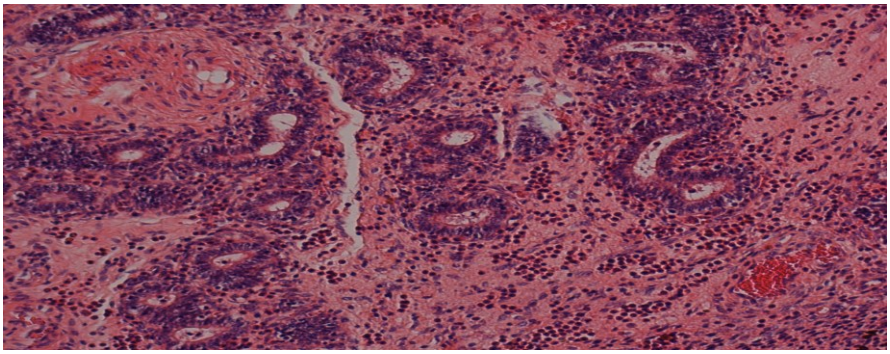
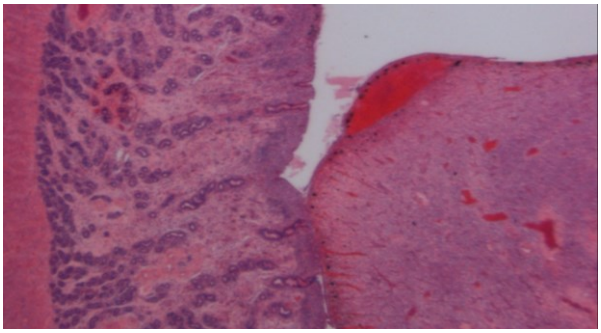
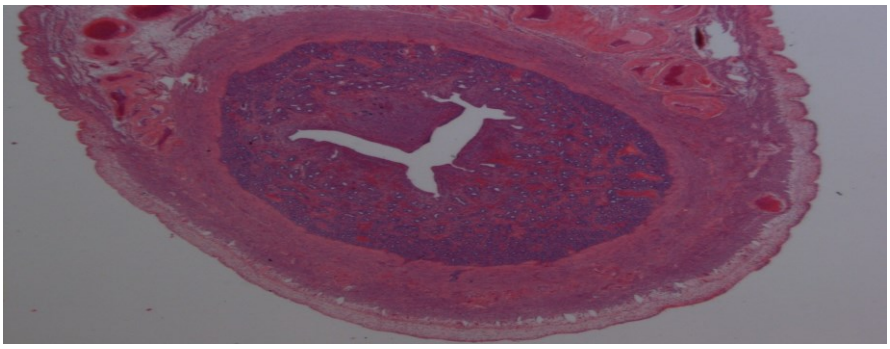
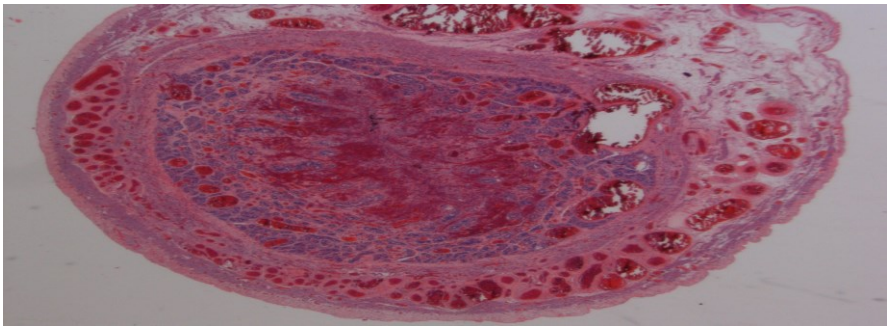
Figure 2b.1d Vaginal anastomosis. x 100, H&E. Glandular epithelium, mild ischaemic changes.	
Figure 2b.1e Left cornua. x100, H&E. Early ischaemia (peri-glandular inflammation)	
Figure 2b.1f Right cornua. x12.4, H&E. Benign uterine polyp.	
Figure 2b.1g Right oviduct. x12.5, H&E.	
Figure 2b.1h Left oviduct. x12.5, H&E. Marked congestion.	

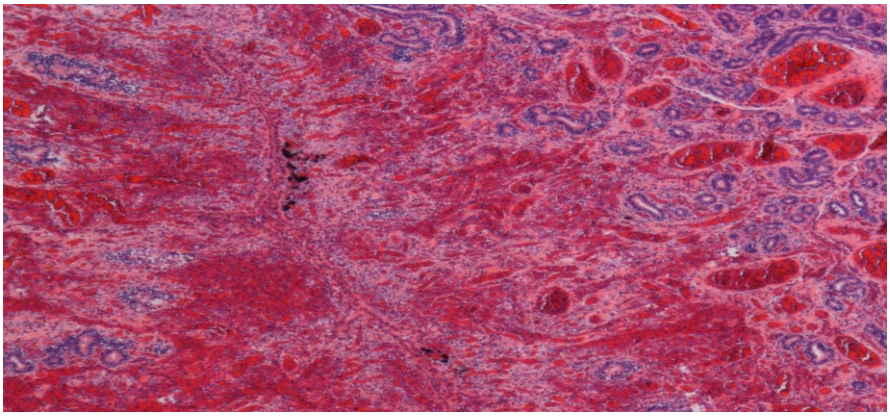
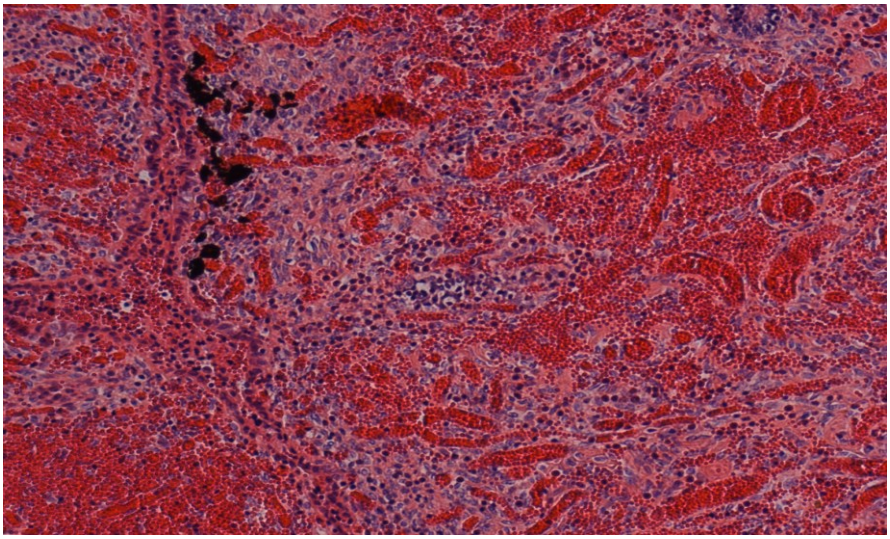
Figure 2b.1i Left oviduct. x40, H&E. Marked congestion and inflammation, consistent with ischaemia.	
Figure 2b.1j Left oviduct. x100, H&E. Marked congestion and inflammation, consistent with ischaemia. (Black pigment = formalin pigment artefact).	

Figure 2b.2 UTx #4 - Cause of early demise

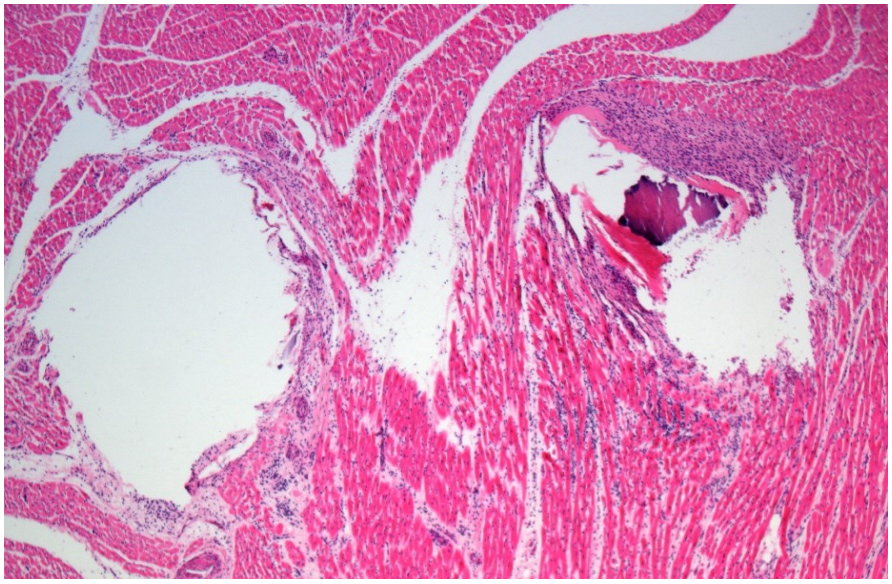
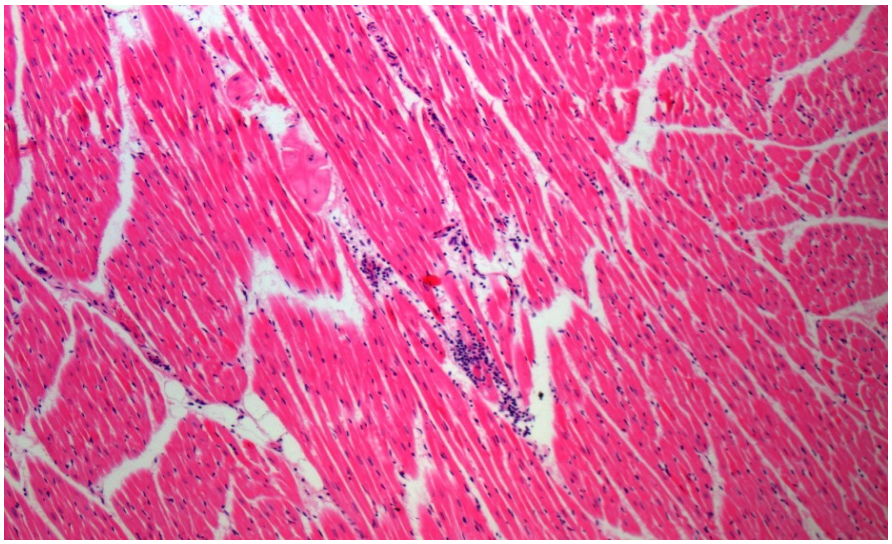
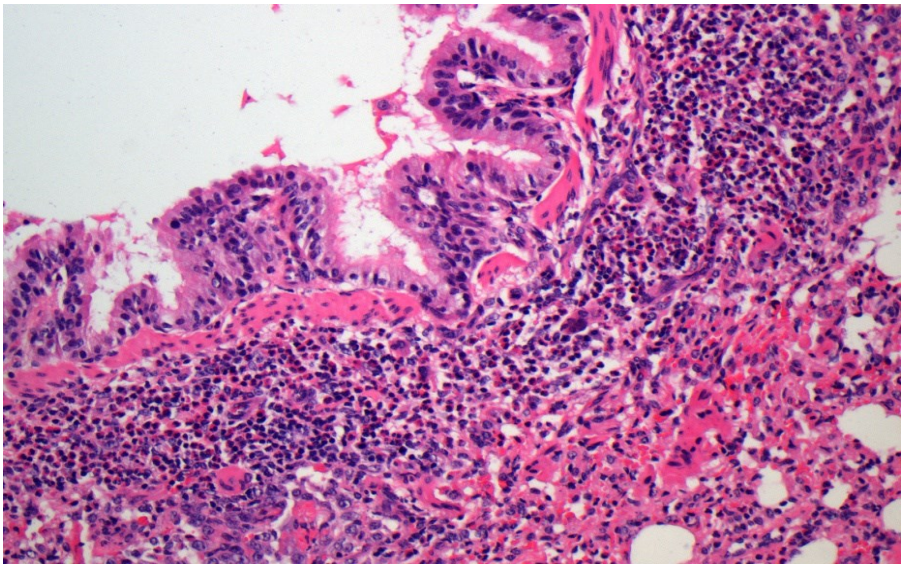
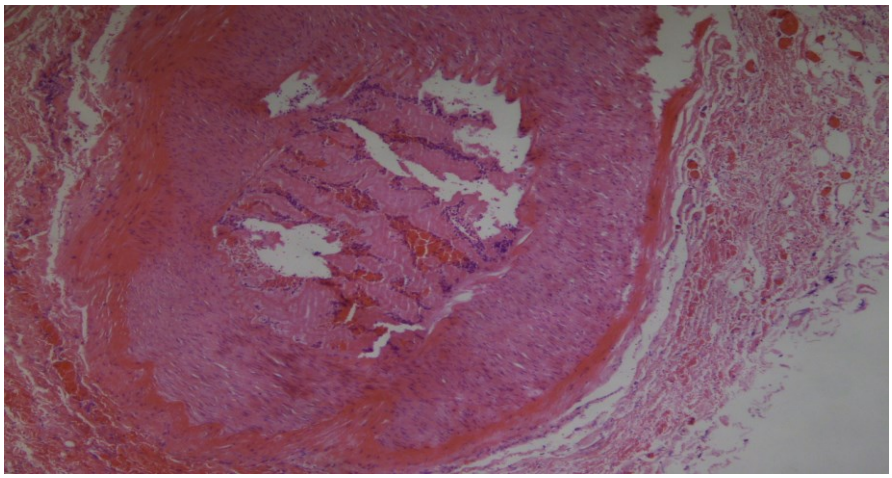
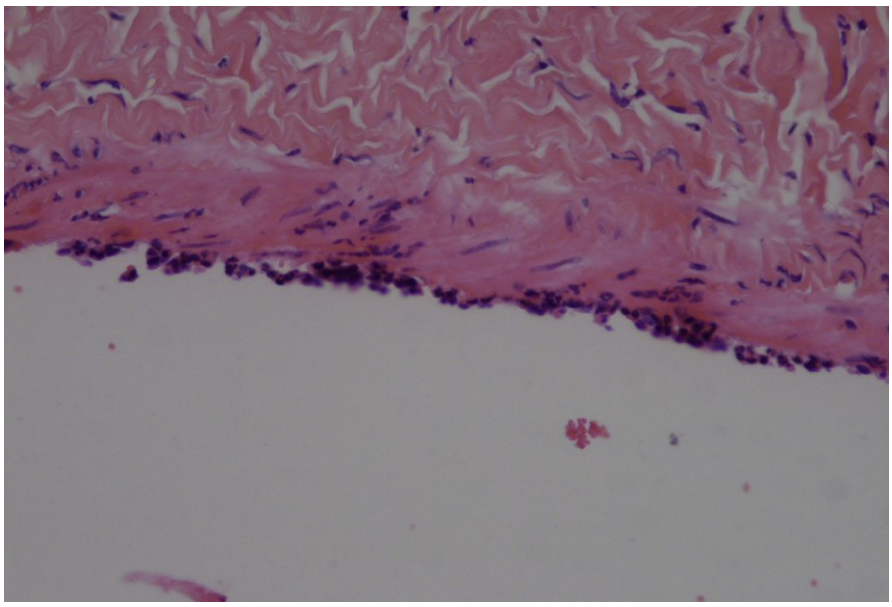
Figure 2b.2a Left ventricle lesion. x 40, H&E.	
Figure 2b.2b Right ventricle. x 100, H&E. Patchy chronic inflammation.	
Figure 2b.2c Left lung. x 200, H&E. Peribronchiolar cuff of eosinophils	

Figure 2b.3 UTx #5 - Demonstration of a patent left vascular anastomosis

Figure 2b.3a Right vascular anastomosis. Original x 40, H&E. Intravascular thrombosis.	
Figure 2b.3b Left vascular anastomosis. x 200, H&E Inflammatory cells adherent to the vascular lumen (possible coagulation activation or margination) Intact anastomosis	

2b.4. Discussion

A natural progression from the animal model towards the human setting involves performing UTx first in the small animal model followed by a large animal model, and finally working with primates. Work with animal models allows the team to address various aspects of the whole UTx procedure, thus ensuring that future human UTx attempts may be clinically appropriate and have an adequate likelihood of a positive outcome. To date, the UK Team has worked with both a small animal model (rabbit) and a large animal model (pig) but the macrovascular model was only attempted in the rabbit. This study describes the 'transfer' of that macrovascular technique to a sheep model, with the main objective being the success of the external iliac-internal iliac anastomotic technique, allowing for normal uterine perfusion. The sheep model is perfectly suitable for this as the size of its pelvic organs and associated vessels is similar to those of a woman, and especially when compared with other large experimental animal models such as pigs and dogs. An autotransplant model was used (as opposed to an allogeneic) in order to judge accurately and exclusively the effects of the anastomosis in maintaining a well-perfused uterus. Therefore, by applying an autotransplant model, one can isolate the potential damage that may be caused by an acute rejection, as well as immunosuppressive medications that need to be administered.[162]

This is only the seventh study at the time of writing whereby a uterine transplant has been attempted in a sheep model. Our previous attempt to achieve a successful orthotopic auto-UTx in a large animal model failed. The model was porcine and the vascular model applied was microvascular. Uterine arteries were anastomosed back together which resulted in subsequent thrombosis and graft failure post-operatively, most likely as a result of the small diameter of the uterine vessels and the associated high rate of thrombosis at these sites.

Five autotransplants were performed. Three were completed. The first autotransplant was not completed because of the inappropriate size of the ewe, which resulted in a ewe of correct weight being chosen for the subsequent four autotransplants. The fourth autotransplant was also not

completed as the animal arrested just as the pelvic dissection began during retrieval. The uteri in the three autotransplants that were completed all demonstrated macroscopically appropriate viability 45 minutes following re-establishment of reperfusion. This is despite only one of the autotransplants (#3) having all four vessels anastomosed (left and right internal-external iliac veins and left and right internal-external iliac arteries). Good surgical access was achieved to the external and more importantly internal iliac vessels, especially where the uterine vessels meet the anterior division of the internal iliac. In UTx #5, a surgical error resulted in accidental transection of the left internal iliac artery. To overcome this problem, the left uterine artery was anastomosed to the left external iliac artery. Despite this, macroscopically, the end-point was considered a success: a viable uterus with adequate perfusion. Microscopically, no ischaemia was seen in UTx #3 and UTx #5, with good viability in the uterus, cornua and oviducts. Thrombosis was demonstrated as hypothesised intra-operatively in the left internal-external iliac anastomosis of UTx #2, secondary to the spasm of the left internal iliac artery. This led to early ischaemia of the left cornua and marked ischaemia of the left oviducts. However as the right internal-external iliac anastomosis was intact, the uterus did not ultimately fail and demonstrated good peristalsis, colour and tone by the end of the 45 minute perfusion period. Therefore, from a technical as well as learning point of view, this case series is deemed to have been overall successful, where success is deemed to be uterine viability after 45 minutes of perfusion following re-anastomosis. When provided with a suitable and healthy model, the uterus was considered viable in two out of the three transplants (UTx #3 and #5), and partially viable in one (UTx #2). Out of the 12 potential anastomoses (four per transplant), eight anastomoses were achieved and considered patent.

The advantage of using external and internal iliac vessels is primarily the large diameter of the vessels (when compared to both small animal models and the uterine vessels). Furthermore, the size of the vessels is equivalent to those in a human. Third, the external iliac vessels can be easily accessed and mobilized. In both human attempts to date, the graft vessels were anastomosed to the external iliac vessels.[61,261] Extending vein grafts however were not required in the second attempt.[61] From this study, one can also conclude that the uterus can be transplanted orthotopically, with bilateral

anastomoses, and no requirement for vascular extensions. These should be avoided as they predispose to thrombosis, as is what occurred 90 days after the operation was performed on the Saudi case, which led to subsequent graft necrosis.[61]

Lessons

The study itself is a valuable lesson in the importance of choosing an appropriate animal to carry out surgical work and having the correct equipment. The team wished for ewes with proven fertility to ensure easier access to the pelvis but the size of the first ewe meant that it was impossible to reach the point whereby the uterine artery meets the anterior division of the internal iliac artery. A smaller sized ewe (suggested by Professor Brannstrom's team) that was used in the next four transplants allowed the team to test the vascular anastomotic model and from that make decisions as to how to approach a future human UTx attempt. Also, from problems encountered during the first attempt, the subsequent four procedures were performed with the correct equipment.

First, having learnt from auto-UTx #1, the uterus had to be retrieved in such a way as to remove a large proportion of the vagina - as far as the posterior aspect of the bladder as well as the recto-vaginal septum in order to allow the uterine artery to be supported by the vagina to which it is attached. The anatomy at this juncture is much different from a woman's. In a woman the uterine artery comes off at the level of the cervix, whereas in a ewe the uterine artery comes off approximately 8cm below the cervix and carefully dissection is paramount. The surgeons managed to gain access to the 'meeting point' described above, and thus ligate caudally to it. This ensured that the uterine graft could be retrieved with its vascular supply i.e. uterine vessels, anterior division of iliac vessels and the internal iliac up to the common iliac vessels. The ligaclips proved utterly invaluable for removing the anterior division of the internal iliac and the operation would not have been completed without this device. Second, a smaller ewe meant that it was much more straight-forward to skeletonize the external iliac vessels and retrieve the whole uterus because of the lower amount of adipose tissue. This also meant a lower volume of estimated blood loss. Third, an animal of appropriate size also meant that the

surgical model may be deemed successful (**Table 2b.1**). Graft retrieval and subsequent grafting demonstrated a significant decrease in duration (retrieval: 1st transplant - 478 minutes, 2nd transplant - 375 minutes, 5th transplant - 205 minutes; grafting: 2nd transplant - 120 minutes, 5th transplant - 90 minutes) whereas the vascular anastomosis was below 30 minutes in all but two cases (8/10 attempted were performed in under 30 minutes). Estimated blood loss was only 100ml following the last case (<5% of a ewe's total blood volume). The surgeon responsible for this section reported that the technique and ease of operation was no different to what he is accustomed to in a human with regards to vascular anastomosis of similar-sized vessels. Fourth, from **Table 2b.3**, it is apparent that the uterus demonstrated appropriate colour, texture and size 45 minutes following reperfusion in completed transplants #2, #3 and #5, with an improvement in colour in the latter two. Finally, it is worth highlighting that two out of the three ewes which completed the auto-transplantation process suffered from acute renal failure during the operation (**Table 2b.2**). Vascular supply to the kidneys was not manipulated, and pre-operative renal function was normal. The most likely explanation was that 'third-space tissue losses' as well as the gastrointestinal secretions were not replaced adequately with intravenous fluids. **Table 2b.5** summarises the lessons gained from this study.

Limitations

Five cases were attempted, out of which only three were completed. Importantly, the reasons for the non-completion of autotransplants #1 and #4 were non-surgical. A larger cohort would have been preferable. Furthermore, each case was terminated 45 minutes following reperfusion. Ideally, the next study should assess long-term morbidity and especially uterine function, both in an autogeneic and allogeneic model. Uterine function would be ideally assessed by attempting conception, pregnancy and delivery, with analysis of fetal weight and well-being. Finally, fluid balance must be recorded and if so, accurately to prevent future occurrences of renal failure.

Conclusion

The present study was the first time that the UK team attempted a macrovascular UTx large-animal model. In particular, internal to external iliac vessel anastomoses. The surgical technique was very similar to what would be used in a human model, with an obvious improvement with regards to procedure duration and skill. In the three (out of five) ewes where grafting was possible, at least two out of four vessels were joined and the grafted uteri demonstrated immediate perfusion. Therefore, the external iliac vessels may definitely be used as recipients of the graft vessels in order to allow for a secure blood flow. The following chapters analyse the level of perfusion. A future experiment should attempt to achieve normal pregnancies after removal and subsequent autotransplantation of the uterus in a sheep.

Table 2b.5 Modifications to Surgical Technique: UTx #1-5

Transplantation Number	Learning Points
UTx #1	1) Too big an animal (80kg) was the main issue which resulted in an incomplete experiment. The size meant a difficult dissection, inability to locate the point where the uterine artery meets the anterior division of the internal iliac artery. It was even challenging to skeletonize the external iliac vessels for the latter anastomosis. This was mainly secondary to the amount of adipose tissue present. 2) A further cadaveric dissection is necessary to see whether this procedure can actually be carried out i.e. whether one can arrive to the appropriate part of the internal iliac artery described above to make the experiment feasible. 3) The following instruments will be necessary for the next operation: ring retractor, medium and large sized swabs, long scissors and forceps and medium sized ligaclips. 4) It is also necessary to utilize a longer mid-line incision.
UTx #2	1) Much improved in comparison to UTx #1. 2) With the correct equipment and a lighter animal (40-60kg, rather than >80kg), this procedure can be carried out in a sheep model. This includes graft retrieval together with the iliac vessels as well as subsequent internal iliac to external iliac vessel anastomosis. 3) In the next three procedures, the uterine artery should be skeletonized less to prevent spasm. This can be achieved during dissection so that the uterine artery is dissected away with adventitial support. 4) In theatre for approximately 10 hours, so next step is try to decrease that time by improving surgical speed. 5) The following instruments will be necessary for the next operation: all three types of 'ligaclip' (small, medium, large) and one self-retaining retractor.
UTx #3	Above lessons all applied. All four vascular anastomosis completed. No particular issues to highlight.
UTx #4	Animal died early on the operating table. To that point, no problems were encountered during the operation.
UTx #5	1) Above lessons all applied. 2) Satisfactory anastomosis. Comfortable operative technique. 3) This transplant concludes this case series. The next experiment should focus on human work, in particular, fresh human cadaveric dissection.

STUDY 3

3. Study 3: Imaging of Organ Viability during Uterine Transplantation Surgery

This study has now been submitted for peer-review and potential publication.

Abstract

Background Uterine transplantation surgery has been proposed as a treatment for permanent absolute uterine factor infertility in the case of loss of the uterus. Due to the complexity of the vasculature correct re-anastomosis of the blood supply during transplantation surgery is a crucial step to ensure reperfusion and viability of the organ. While techniques such as fluorescent dye imaging have been proposed to visualise perfusion there is no gold standard for intraoperative visualisation of tissue oxygenation. In this paper results from pulse oximetry, a liquid crystal tuneable filter (LCTF)-based multispectral imaging (MSI) laparoscope and laser speckle contrast analysis are described.

Methods The system was used to monitor uterine oxygen saturation (*SaO2*) before and after transplantation. Results from surgeries on two animal models (rabbits and sheep) are presented. A feature-based registration algorithm was used to correct for misalignment induced by breathing or peristalsis in the tissues of interest prior to analysis. An absorption spectrum was calculated at each spatial pixel location using reflectance data from a reference standard, and the relative contributions from oxy- and deoxyhaemoglobin were calculated using a least squares regression algorithm with non-negativity constraints.

Results Results acquired during animal surgeries show that cornual oxygenation changes are consistent with those observed in point measurements taken using a pulse oximeter, showing reduced *SaO2* following re-anastomosis. Values obtained using the MSI laparoscope were lower than those taken with the pulse oximeter, which may be due to the latter's use of the pulsatile arterial blood signal.

Conclusion Future work incorporating immunological test results will help to correlate *SaO2* levels with surgical outcomes.

3.1. Introduction

The literature review has already been completed in an earlier chapter titled 'Literature Review'.

Aims

The aims of this study were to explore and compare the above imaging modalities, pulse oximetry, optical spectroscopy, and endoscopic laser speckle contrast analysis. In particular, their suitability for assessing uterine perfusion, extent of ischaemia and circulatory function of the uterus in order to provide image information relevant to uterine perfusion, function, as well as operative success. This was carried out in two animal uterine transplant models: rabbit (small-animal model) and sheep (large animal model).

3.2. Materials and Methods

Oxygen Saturation and Perfusion Index

This model has been applied previously by this team to assess uterine perfusion and, therefore viability, immediately following UTx.[80,156] A normal finger pulse oximetry probe was modified to allow the jaws to open wider so as to fit around the uterine horns (**Figure 3.1**). It was placed in a sterile endoscopic bag/drape to maintain aseptic technique. Once in direct contact with the uterine tissue, it measured O_2Sat and PI, two independent variables.[262,263] This was possible as the oximeter (Datex-Ohmeda 3600P, Louiseville, USA) has the capacity to measure both variables (**Figure 3.2**). The heart rate recorded on uterine oximeter was constantly compared to that of the anaesthetic oximeter to ensure accuracy. The probe was placed on both the medial and lateral aspects of the right and left uterine horns. This area of the organ is relatively thin allowing infra-red light needed in oximetry to pass through the structure. Use of a superficial marker suture on the broad ligament allowed for consistency in the probe site application.

In the allogeneic rabbit model, we modified the application in comparison to its previous use by applying it on three separate occasions: (i) prior to graft retrieval from donor - donor uterus; (ii) prior to recipient hysterectomy - recipient native uterus; and (iii) post-transplantation - recipient donor uterus. In the first two instances, readings were taken once normal pelvic anatomy had been established and uterine vasculature was skeletonised. Readings were taken post-UTx following visual confirmation of uterine viability by restoration of its pink pre-transplant appearance.

In the autogeneic sheep model, measurements were taken on two separate occasions: prior to graft retrieval, and 20-30 minutes post-transplantation.

PI is a relative indicator of blood volume at the probe site. PI measurements were only used to compare volume in a longitudinal fashion in the same animal because blood volume at the probe site is relative to individual patient tissue and vessel elasticity. The age, weight, stage of the menstrual cycle or any anatomic variants of the rabbit should not have interfered with the comparisons as PI was used to compare volume in a longitudinal fashion in the same animal. Blood flow studies in a longitudinal manner have been successfully utilised previously in the description of variation of blood flow in the testis [112] and ovary.[264]

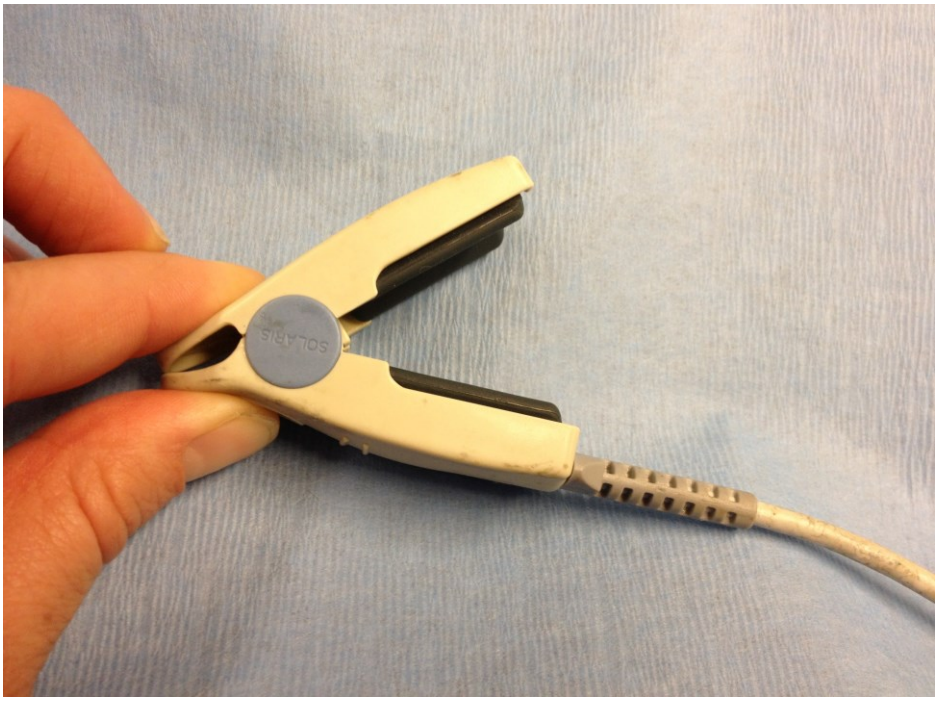


Figure 3.1 Modified finger probe to allow wider opening of the jaws to fit satisfactorily around the uterine cornua.

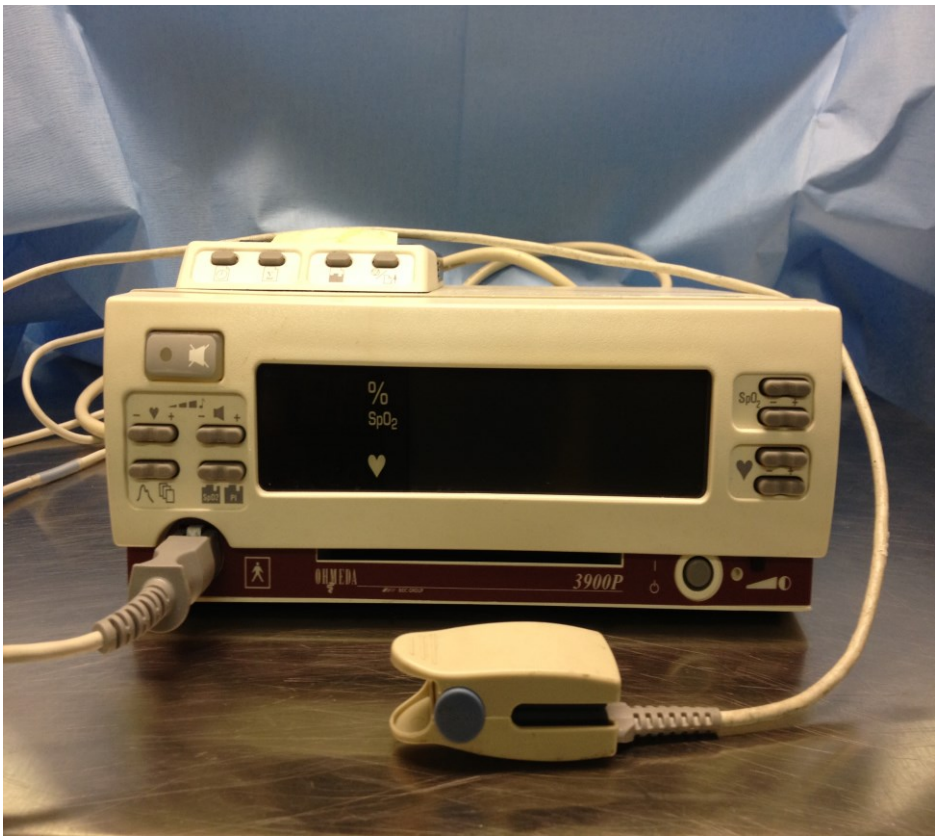


Figure 3.2 Datex-Ohmeda 3600P pulse oximeter which also measures perfusion index, a relative indicator of blood volume at the probe site independent of oxygen saturation.

Minimally Invasive Optical Spectroscopy

The protocols for both optical spectroscopy and eLASCA were devised by Drs N Clancy, L Song (MSI) and M Tziraki (eLASCA) (Department of Surgical Imaging in the Hamlyn Centre for Robotic Surgery). As per references #170 and #186, the methodology for spectroscopy were also included in Drs V Sauvage's and L Song's PhD theses. The hyperspectral laparoscope (**Figures 3.3 and 3.4**) consisted of a Liquid Crystal Tuneable Filter (LCTF, VariSpec, Cambridge Research & Instrumentation, Inc., USA) that transmitted light with an average bandwidth of 15nm as well as a computer controlled central wavelength between 400-720nm. The light emitted by the Xenon light source (model 20133020, Storz GmbH, Germany) ranged between 400nm and 700nm. It was then subsequently transmitted by a fibre bundle light guide and coupled into the 12mm rigid 30 degree laparoscope (Storz GmbH, Germany). The sample was usually placed 50mm from the distal tip of the laparoscope. The central Hopkins rod lens channel of the laparoscope imaged the reflected light in the usual way. To form an image on a monochrome charged-coupled device (CCD) camera (DCU223M, Thorlabs, Inc., USA), an additional 50mm focal length lens was inserted at the proximal end. This lens was then mounted in a helicoid barrel (1 inch linear travel) to adjust the image plan to the laparoscope-sample distance. The LCTF was placed between the lens and the CCD with the laparoscope attached to the LCTF via a clip. This allowed easy removal of the LCTF/CCD block.[170,186,265]

Control of the camera settings was enabled via a LabVIEW programme. It also provided a choice of wavelength range and increment at which images could be acquired and saved. The combination of three images at wavelengths of 450nm, 550nm and 650nm allowed for the display of a live RGB video. With respect to the key element of understanding the principle behind multispectral imaging, the measured reflectance spectrum at each pixel is a linear combination of the oxy- and deoxy-haemoglobin spectra. This is only possible if the scattering is assumed to be approximately constant across the wavelength range and the dominant mechanism of light attenuation in the field of view is absorption by blood. A multiple linear regression algorithm was applied in Matlab (The Math Works,

Inc, USA) to calculate the relative fractions of both of the above haemoglobin species [177] and also to minimise the difference between the experimental reflectance and the predicted value. The programme enabled both generation and display of the spectrum at each pixel of the image by storing the intensities for all the images of what was 'loaded-up'. Therefore, it was possible to draw the spectrum by plotting the two variables (wavelength, intensity) for every pixel of the image because each image was acquired at a known wavelength.[170,186,265]

The instrument spatial and spectral resolutions were measured. The spatial resolution was quantified by acquiring an image of a paper 1951 USAF test pattern (Edmund Optics, Inc., USA) (**Figure 3.5**). It consists of sets of horizontal and vertical bars separated by decreasing spacings when going clockwise from the left bottom of the target to its centre.

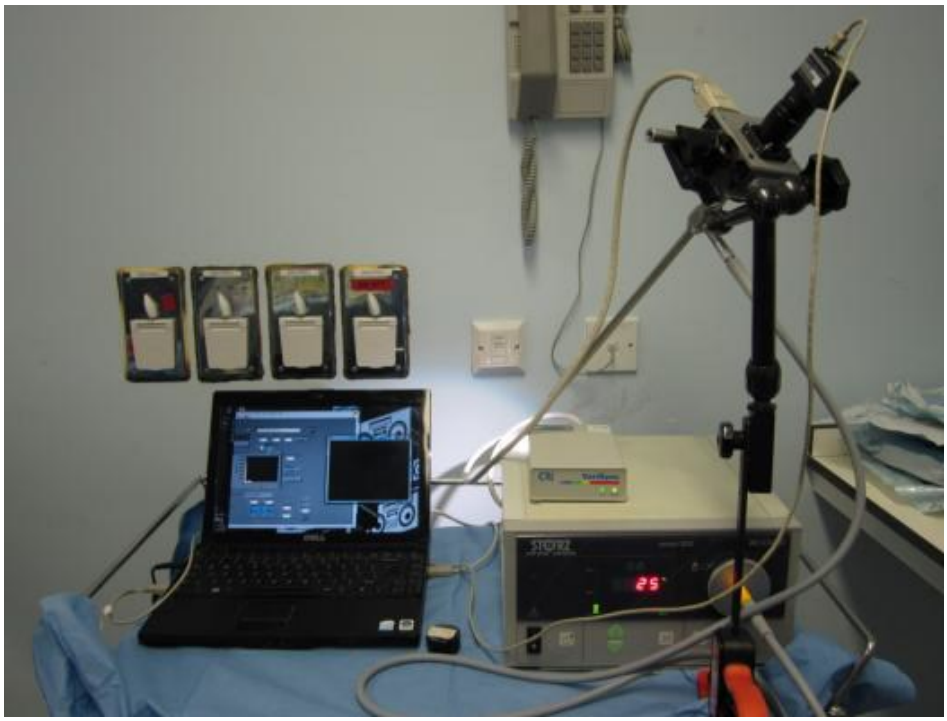


Figure 3.3 Multispectral imaging laparoscope with xenon light source and control computer

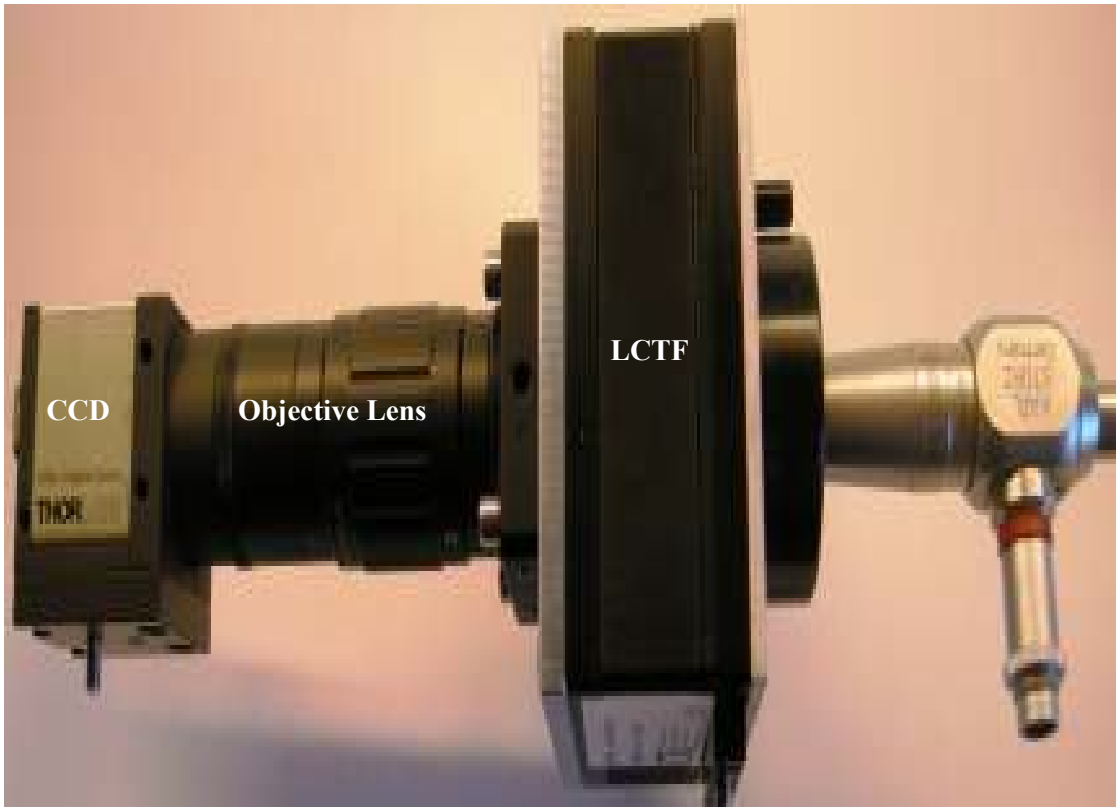


Figure 3.4 Hyperspectral laparoscopic system consisting in a CCD camera and a LCTF attached to a regular rigid laparoscope

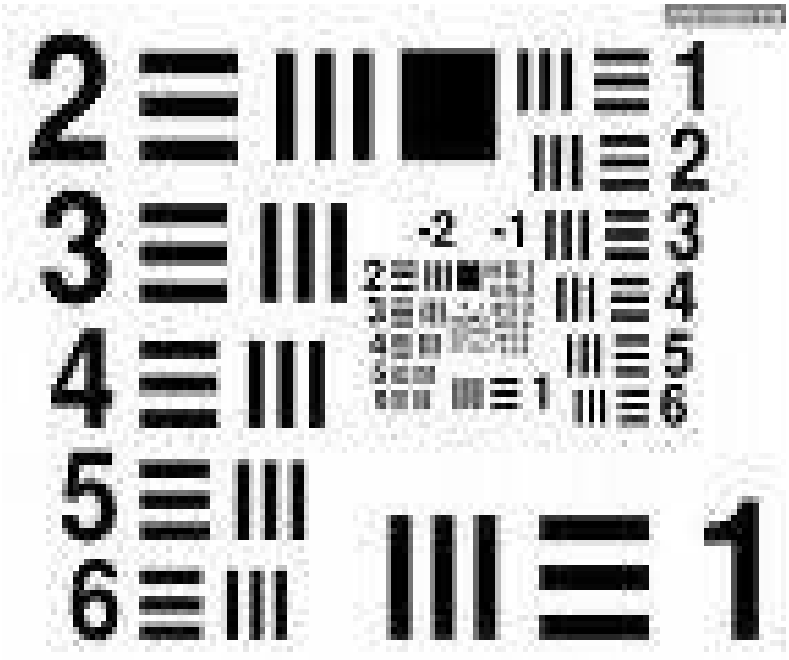


Figure 3.5 1951 USAF test patterns. This target was used to determine optical system resolution

The spectral resolution is best described as the bandwidth of the LCTF transmission spectra. It was evaluated every 10 nm, from 420nm to 680nm, by recording the light reflected by the Xenon lamp from a spectralon reflectance standard (Labsphere, Inc., USA), with a spectrometer (HR4000, Ocean Optics, Inc., USA) through the hyperspectral laparoscope (minus the camera). The bandwidths were calculated at the Full Width at Half Maximum (FWHM) of the transmission spectra. It was crucial to ensure the system response was proportional to the sample emitted light and the CCD exposure time because the principle of measurement of haemoglobin is based on the intensity of the reflected light. This would allow a comparison of the stack of images acquired with different exposure time. The system linearity with sample emission was assessed by recording the light from the standard reflectance target and by inserting neutral density filters of increasing optical density. The linearity with exposure times was checked by acquiring images at increasing exposure times. O_2Sat was then calculated as the quantity of oxyhaemoglobin which is a fraction of the sum of oxy- and deoxyhaemoglobin.[170,186,265]

The key element of multispectral imaging is a tuneable filter. Its band-pass filter, a device capable of passing frequencies within a certain range and rejecting frequencies outside that range, can be electronically tuned. This generates more finely spaced band-pass wavelengths than filter wheel are able to, as they have a lower filter switching time. Hyperspectral imaging is put into practice by three main technologies: Fourier interferometry, acousto-optic tuneable filter, and LCTF. A LCTF consists of several stages, each of them made up of a quartz birefringence retarder and layers of liquid crystal, sandwiched between two polarisers. The principle of the LCTF is to select the desired band-pass by adjusting the retardance of the polarized light.[170,186]

Both the rabbit and sheep uteri were imaged with the left and right cornua, and Fallopian tubes visible. In the rabbit, this was performed (i) prior to transplantation - donor uterus; (ii) prior to recipient hysterectomy - recipient native uterus; and (iii) post-transplantation - recipient donor uterus. In the sheep, measurements were taken prior to retrieval and 20-30 minutes following commencement of -reperfusion (i.e. post-UTx completion).

eLASCA

eLASCA was performed after multi-spectral imaging in both the rabbit and sheep models. Details with respect to the operations are described in studies 2 and 4. For the rabbit transplants, the aim was to carry out eLASCA prior to the recipient hysterectomy only in order to simply trial the technique and apparatus. In the sheep cohort, eLASCA was performed prior to graft retrieval and once more, 30 minutes following establishment of perfusion. **Figure 3.6** illustrates a rabbit uterus. The green circle marks the imaging area containing the uterine artery and the vein.

Prior to the surgery the heart rate and the O_2Sat were measured using an oximeter. The field of view of the probe was 6mm in diameter, thus altered in order to obtain a higher spatial resolution. The experiment for the rabbit animal model consisted of two parts: (a) detection of the heart beat (with single wavelength and high CCD frame rate), and (b) detection of a change in blood flow and tissue oxygenation following a vessel occlusion. In the sheep model experiment no vessels were occluded.

In the rabbit experiment, a single laser diodes (λ=660 nm) was turned on. The heartbeat of the rabbit ranges from 130-220 beats per minute (bpm). Therefore to capture the structure of the contrast change in one pulse period, the frame rate for the rabbit required the value used in the finger test to be doubled (this was not the case with the sheep experiments, where the heart rate is comparable to the human). This in turn meant a decrease in the exposure time leading to a decrease the signal intensity and spatial resolution. In order to limit the effects of the latter two, the exposure time was set to 0.5ms, thus reaching a compromise between the frame rate and the intensity. Frame rate was 22fps. 30 frames were recorded at a time, the process lasting 1.4 seconds. In the latter experiment, both the laser Dopplers were used. Data was acquired initially for 25 seconds, with normal circulation throughout; subsequently the artery was occluded for 25 seconds while the CCD continued to record images. Finally, the artery was released and the speckle images were recorded for another 15 seconds. The acquisition time of every frame was also recorded into an excel document. A customized Labview

programme controlled and synchronized the filter wheel. The exposure time was 20ms and the average frame rate was 1.6fps for each wavelength.

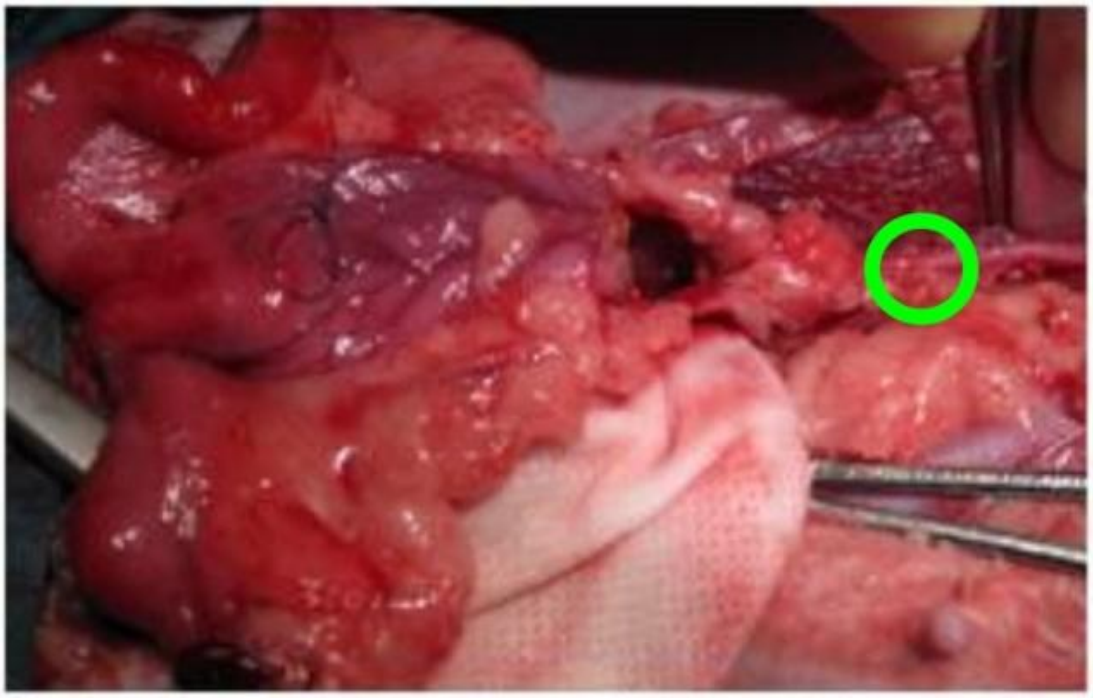


Figure 3.6 Rabbit uterine graft following the IVC and AA anastomosis (green circle indicates the imaging area)

Figure 3.7 shows eLASCA performed in the sheep model. The imaging area is approximately 6mm^2 and is marked by the illumination of the red laser as it shines from the imaging probe placed 3cm above the tissue.

The optical experimental system is shown in **Figure 3.8**. The two laser lights were coupled into the fibre and were directed onto the sample by the probe. The speckle image of the target was transmitted through the leached fibre image guide (LFIG), and passed through a magnification system before reaching the CCD camera. The magnification system consisted of a microscope objective (x10, Olympus), and an achromatic lens (f=100mm, Thorlabs) to give a x7 magnification. The images were magnified and captured by a 12bit CCD camera (Retiga Exi cooled CCD, Qimaging). An electronic

data acquisition card (DAQ, National Instruments) was introduced to control the laser excitation and the acquisition of the images. The lasers were switched on alternatively and an image was captured each time with a given acquisition time controlled by the shatter of the camera. A customized LabVIEW programme was used to synchronise the data acquisition card, the laser controllers and the exposure of the CCD. An exposure time and a delay time were characterised for each recording. The slower recording condition was 5 frames per second (fps) for each wavelength and 10fps for both. The imaging probe was guided over the imaging area ($6mm^2$). The lasers, magnification system and the CCD camera were set on a portable optical bench. The stack of raw images were processed, and post-acquisition were written in a Matlab programming language with custom algorithms.

The probe used in both the rabbit and sheep models is depicted in **Figure 3.9**. The laser beam from two laser diodes (ML101J27 660nm and DL8142 830nm, Thorlabs) were coupled into two polarization maintaining fibres (PM fibre, Thorlabs). The two fibres were passed through channels in a custom made adapter to direct the light exiting from the fibres at an incident angle of ±3.6° to the axis so that the illumination area of both wavelengths overlaps. The polarization directions of the lasers from the PM fibre were adjusted so as to beam in the same direction. This way the direct reflected light could be eliminated together by a single polarizer thus increasing the speed contrast sensitivity. The plano-convex lens (f=6mm, D=3 mm, Edmund Scientific) imaged the sample onto the entrance end of the LFIG (Schott North America).

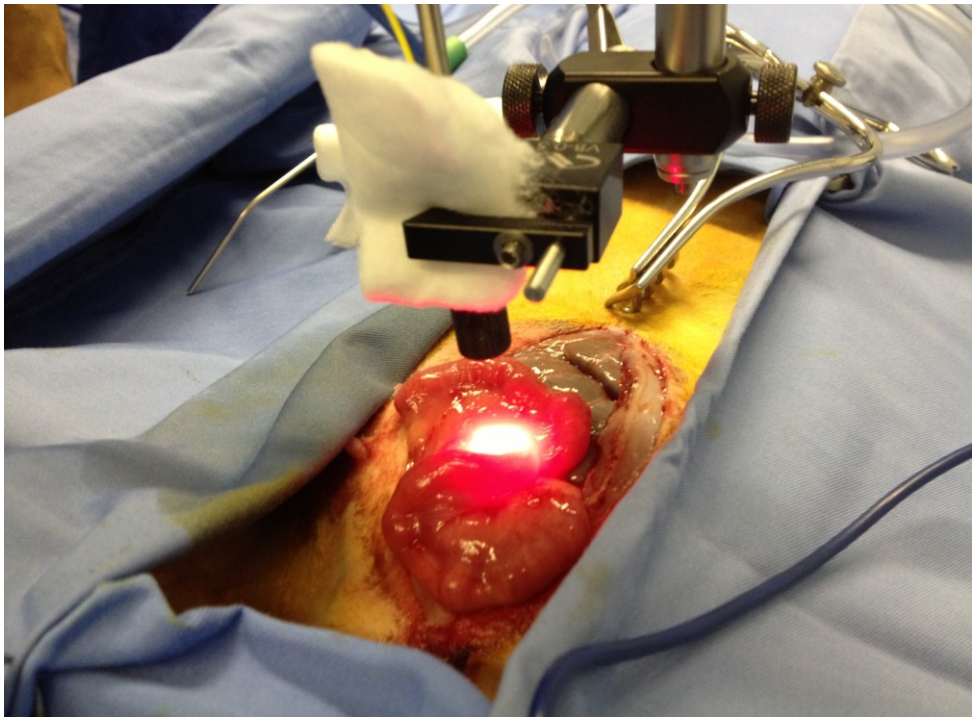


Figure 3.7 Measurement site for eLASCA performed on a sheep uterus. This photograph was taken shortly after the abdomen was opened and the uterus was exteriorized.

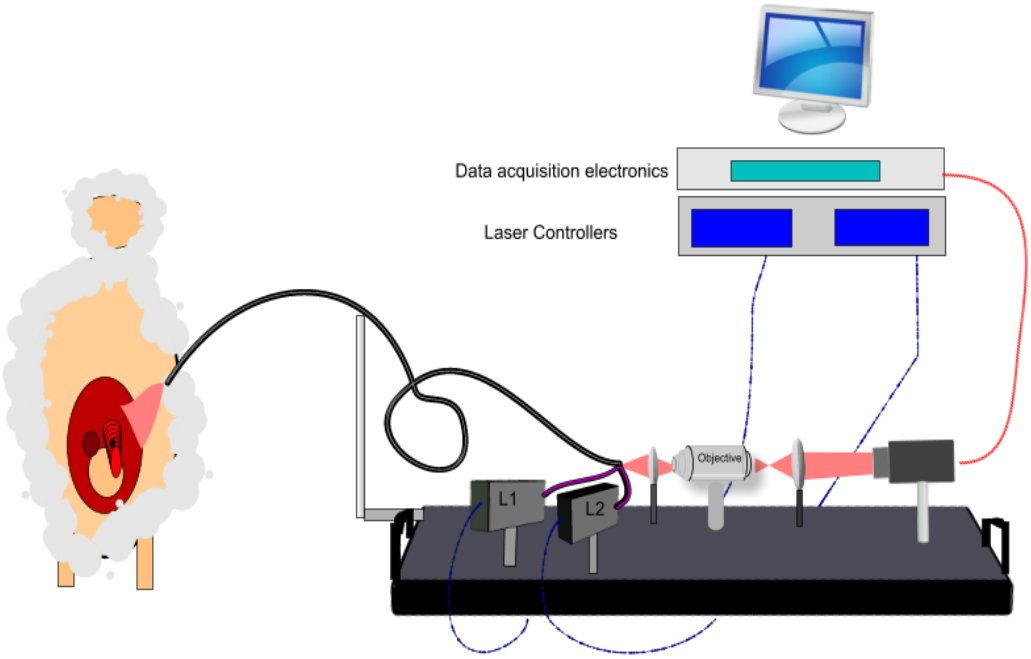


Figure 3.8 Experimental model set up

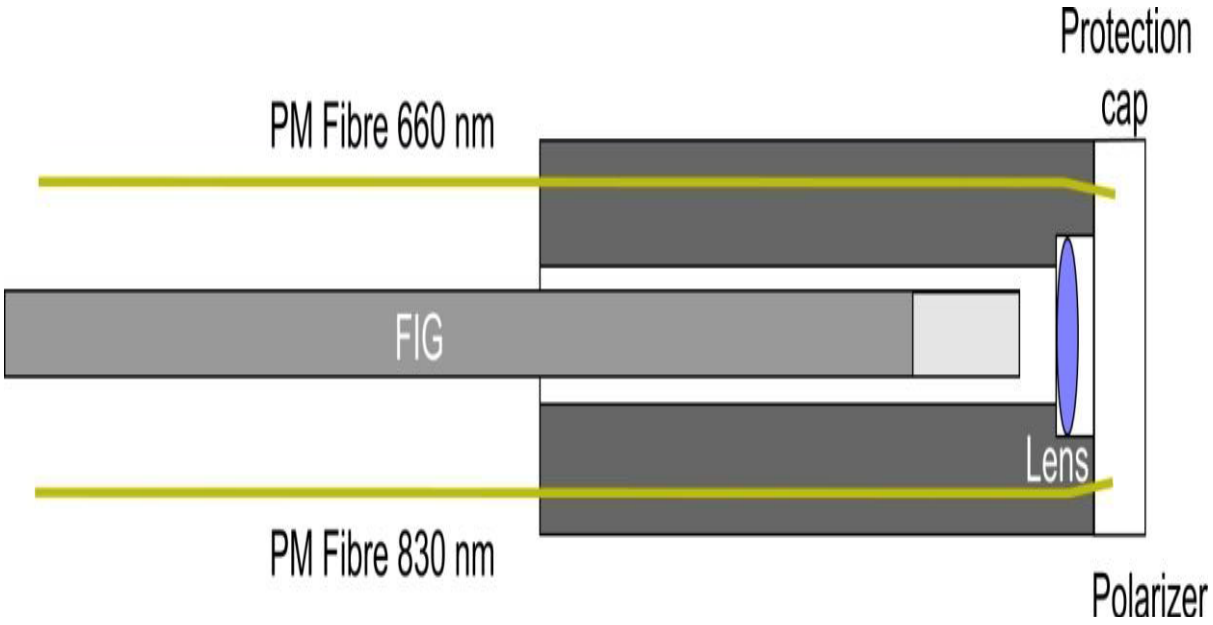


Figure 3.9 Diagram showing a cross-section design of the probe used in eLASCA

Oxy-haemoglobin and deoxy-haemoglobin have different absorption coefficients at most wavelengths and can be therefore used to detect the haemodynamics of blood circulation. Oxygenized blood is supplied continuously to the tissue whereas deoxygenized blood flows back to the heart. When the blood flow is stopped in the vessels, the concentration of the oxy- and deoxy-haemoglobin varies which results in a change in the light intensity absorbed and reflected back by the blood. Therefore the blood supply can be monitored by the change of the intensity of the reflected light. The modified Beer-Lambert law is used to calculate the change of the oxygenation.[191,266] In this experiment two wavelengths, 660nm and 830nm, were used.

The acquisition of the data with respect to initial experiment and equipment provision and set-up to subsequent data presentation and depiction as shown in the results section below was done by Drs N Clancy, V Sauvage (MSI), L Song (MSI) and M Tziraki (eLASCA) (Department of Surgical Imaging in the Hamlyn Centre for Robotic Surgery). As per reference #238, results for UTx #3-7 (rabbit) were also included in Dr L Song's and Dr V Sauvage's PhD theses.

Data Presentation and Analysis

In general, data has been presented using 2D graphs, 2D images, histopathology slides, figures and descriptive tables. Data related to O_2Sat and PI was defined as non-parametric and *Mann-Whitney U test* was therefore carried out for analysis. A statistically significant difference was applied for a p-value <0.05. All statistical analysis was done using the Statistical Package for the Social Sciences version 19 (SPSS Inc, Chicago, Illinois, USA).

3.3. Results

3.3.1. Rabbit model

Pulse oximetry was applied in all nine transplantations. As the does died intra-operatively in UTx #3, 4 and 6, no values for O_2Sat and PI were obtained on the recipient donor uteri (post-UTx). The averages and standard deviations for these measurements are provided in **Table 3.1a and 3.1b**. Comparison of O_2Sat values between the pre-UTx donor and post-UTx recipient, and pre-UTx recipient and post-UTx recipient revealed a statistically significant decrease in saturation levels post-UTx. A similar comparison of PI values did not demonstrate any fall in PI level post-UTx, and importantly no statistical difference. This variation in O_2Sat and PI values is depicted in **Figure 3.10a-c**.

Optical Spectroscopy was applied in UTx #3-9. Images of the donor uterus, recipient native uterus and recipient donor uterus were obtained in all seven transplants. Following the re-anastomosis of the IVC and AA, the uterus was observed to undergo a colour shift during reperfusion from its blanched appearance after flushing to a more reddish colour. However, as the does died intra-operatively in UTx #3 and #4, O_2Sat measurements were calculated with a multispectral imaging laparoscope only on the recipient donor uteri #5-#9 (post-UTx). Even though recipients #6 and #8 also died intra-operatively, measurements were obtained in these cases as deaths occurred 18 minutes and 23 minutes

after the donor uterus had been transplanted and re-perfusion had been established. Recipient #5 was the only doe to survive long-term and underwent an embryo transfer process to see if pregnancy could be achieved following allogeneic UTx. As a result an additional measurement was taken on day 89 post-operatively just prior to the embryo transfer. The averages and standard deviations for O_2Sat are provided in **Table 3.1c.**

The processed O_2Sat images for the donor uterus pre- and post-UTx, along with colour images reconstructed from the multispectral data after integration under the RGB filter transmission response of a digital colour camera are shown in **Figure 3.11a-g**. The pre-UTx images show that the graft is well-perfused in the donor doe, with high oxygen content in both cornua. The area of low O_2Sat in the centre may correspond to a less vascularised area or possibly an area with a higher venous to arterial supply ratio. The post-UTx images show similar features after blood supply is re-established from the AA. Areas of low O_2Sat correspond to connective tissue where re-perfusion is expected to last the longest. Here, the comparison of O_2Sat values between the pre-UTx donor and post-UTx recipient, and pre-UTx recipient and post-UTx recipient revealed a statistically non-significant decrease in saturation levels post-UTx.

Rabbit	Side	Cornua	Oxygen Saturation									Mean ± S.D.
			UTx 1	UTx 2	UTx 3	UTx 4	UTx 5	UTx 6	UTx 7	UTx 8	UTx 9	All UTx
Donor (pre-UTx)	Right	Medial	99%	99%	93%	98%	99%	96%	100%	100%	100%	**98±0.02%**
		Lateral	98%	98%	95%	99%	99%	99%	100%	100%	97%	**98±0.02%**
	Left	Medial	99%	97%	96%	100%	100%	97%	100%	98%	98%	**98±0.02%**
		Lateral	99%	97%	87%	96%	100%	98%	96%	98%	97%	**96±0.04%**
Recipient (pre-UTx)	Right	Medial	100%	99%	98%	99%	97%	100%	100%	100%	100%	**99±0.01%**
		Lateral	100%	100%	96%	98%	97%	97%	97%	99%	98%	**98±0.01%**
	Left	Medial	100%	99%	92%	97%	98%	100%	98%	100%	100%	**98±0.03%**
		Lateral	100%	100%	99%	99%	95%	92%	96%	100%	98%	**98±0.03%**
Recipient (post-UTx)	Right	Medial	90%	87%	**NA**	**NA**	92%	**NA**	91%	95%	90%	**91±0.03%**
		Lateral	89%	80%	**NA**	**NA**	96%	**NA**	92%	91%	83%	**89±0.06%**
	Left	Medial	87%	88%	**NA**	**NA**	95%	**NA**	94%	95%	80%	**90±0.06%**
		Lateral	85%	83%	**NA**	**NA**	95%	**NA**	93%	95%	90%	**90±0.05%**

Key: **NA,** Non applicable; **S.D,** Standard Deviation

Table 3.1a Oxygen Saturation values of the Donor and the Recipient rabbit (measured with a pulse oximeter)

Rabbit	Side	Cornua	Perfusion Index									Mean ± S.D.
			UTx 1	UTx 2	UTx 3	UTx 4	UTx 5	UTx 6	UTx 7	UTx 8	UTx 9	All UTx
Donor (pre-UTx)	Right	Medial	0.38	0.17	0.44	0.41	0.41	0.19	0.36	0.77	0.44	**0.40±0.17**
		Lateral	0.33	0.16	0.15	0.36	0.36	0.13	0.32	0.25	0.39	**0.27±0.10**
	Left	Medial	0.54	0.15	0.15	0.35	0.35	0.38	0.43	0.33	0.42	**0.34±0.13**
		Lateral	0.31	0.14	0.25	0.41	0.41	0.28	0.47	0.33	0.47	**0.34±0.11**
Recipient (pre-UTx)	Right	Medial	0.27	0.42	0.25	0.40	0.40	0.59	0.44	0.20	0.36	**0.37±0.12**
		Lateral	0.25	0.73	0.13	0.41	0.41	0.43	0.32	0.18	0.25	**0.35±0.18**
	Left	Medial	0.37	0.63	0.35	0.41	0.41	0.56	0.30	0.21	0.38	**0.40±0.13**
		Lateral	0.24	0.46	0.23	0.22	0.22	0.17	0.29	0.29	0.22	**0.26±0.08**
Recipient (post-UTx)	Right	Medial	0.16	0.30	**NA**	**NA**	0.30	**NA**	0.33	0.14	0.43	**0.28±0.11**
		Lateral	0.19	0.56	**NA**	**NA**	0.29	**NA**	0.43	0.32	0.23	**0.34±0.14**
	Left	Medial	0.19	0.52	**NA**	**NA**	0.35	**NA**	0.32	0.29	0.15	**0.30±0.13**
		Lateral	0.12	0.35	**NA**	**NA**	0.37	**NA**	0.24	0.27	0.24	**0.27±0.09**

Key: **NA,** Non applicable; **S.D,** Standard Deviation

Table 3.1b Perfusion Index values of the Donor and the Recipient rabbit (measured with a pulse oximeter)

Rabbit	Side	Cornua	O_2 Saturation						Mean ± S.D.
			UTx 5	UTx 5 pre-ET	UTx 6	UTx 7	UTx 8	UTx 9	All UTx
Donor (pre-UTx)	Right	Medial	83%	NA	76%	86%	93%	81%	**80±5%**
		Lateral	89%	NA	92%	79%	77%	86%	**91±2.1%**
	Left	Medial	90%	NA	88%	80%	89%	92%	**89±1.4%**
		Lateral	82%	NA	86%	83%	84%	83%	**84±2.8%**
Recipient (pre-UTx)	Right	Medial	89%	NA	83%	84%	85%	89%	**86±4.2%**
		Lateral	89%	NA	88%	77%	67%	82%	**89±0.7%**
	Left	Medial	93%	NA	88%	85%	56%	90%	**91±3.5%**
		Lateral	78%	NA	81%	64%	68%	81%	**80±2.1%**
Recipient (post-UTx)	Right	Medial	60%	84%	44%	21%	57%	66%	**55±0.21%**
		Lateral	58%	84%	51%	46%	68%	51%	**60±0.14%**
	Left	Medial	59%	82%	68%	24%	51%	58%	**57±0.19%**
		Lateral	63%	82%	93%	7%	58%	59%	**60±0.30%**

Key: **ET,** Embryo Transfer

Table 3.1c Oxygen Saturation values of the Donor and the Recipient rabbit when measured with a multispectral imaging laparoscope

Figure 3.10a-c Variation in Oxygen Saturation and Perfusion Index values when measured with a pulse oximeter and a multispectral imaging laparoscope

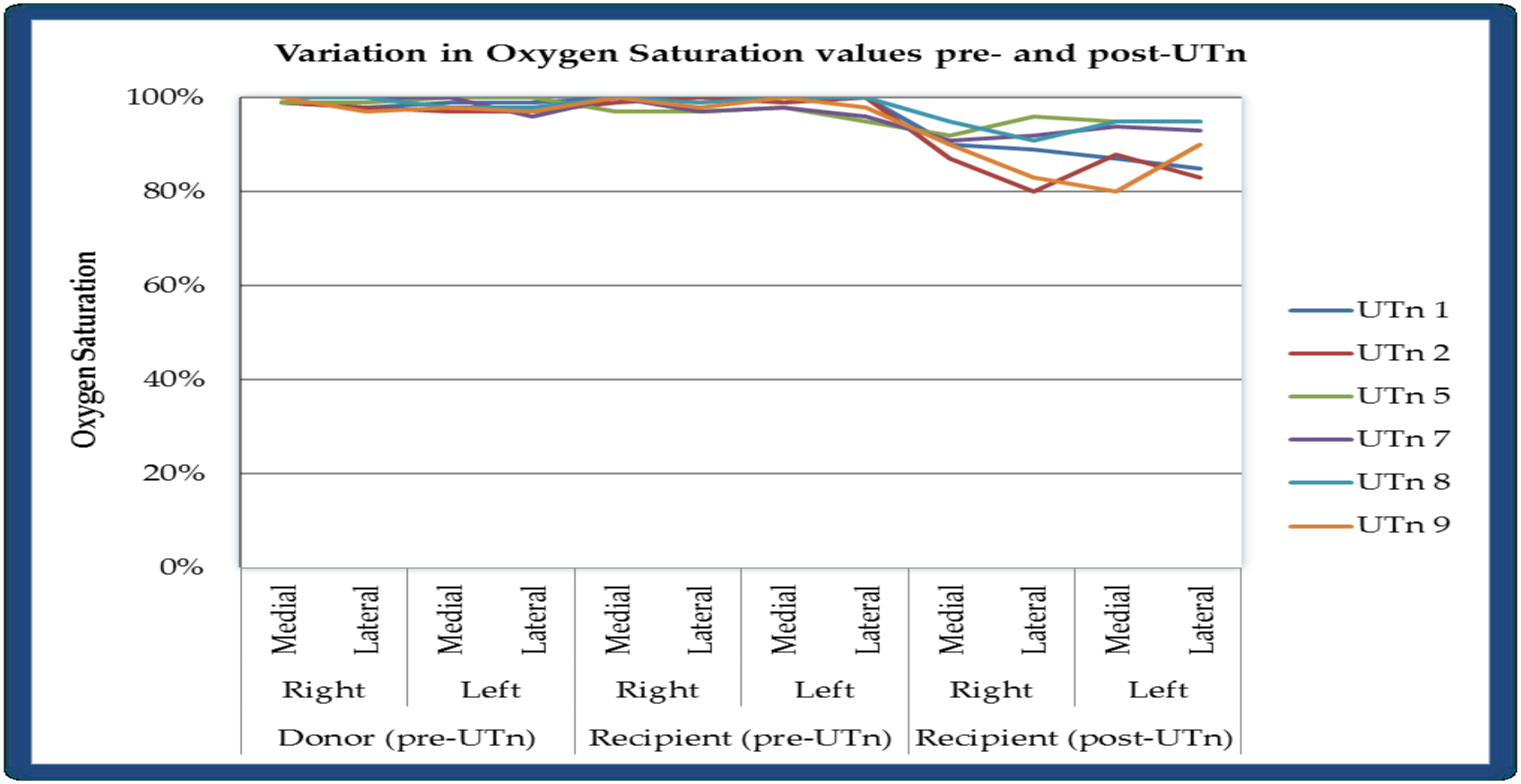


Figure 3.10a Oxygen Saturation values of the Donor and the Recipient rabbit (measured with a pulse oximeter)

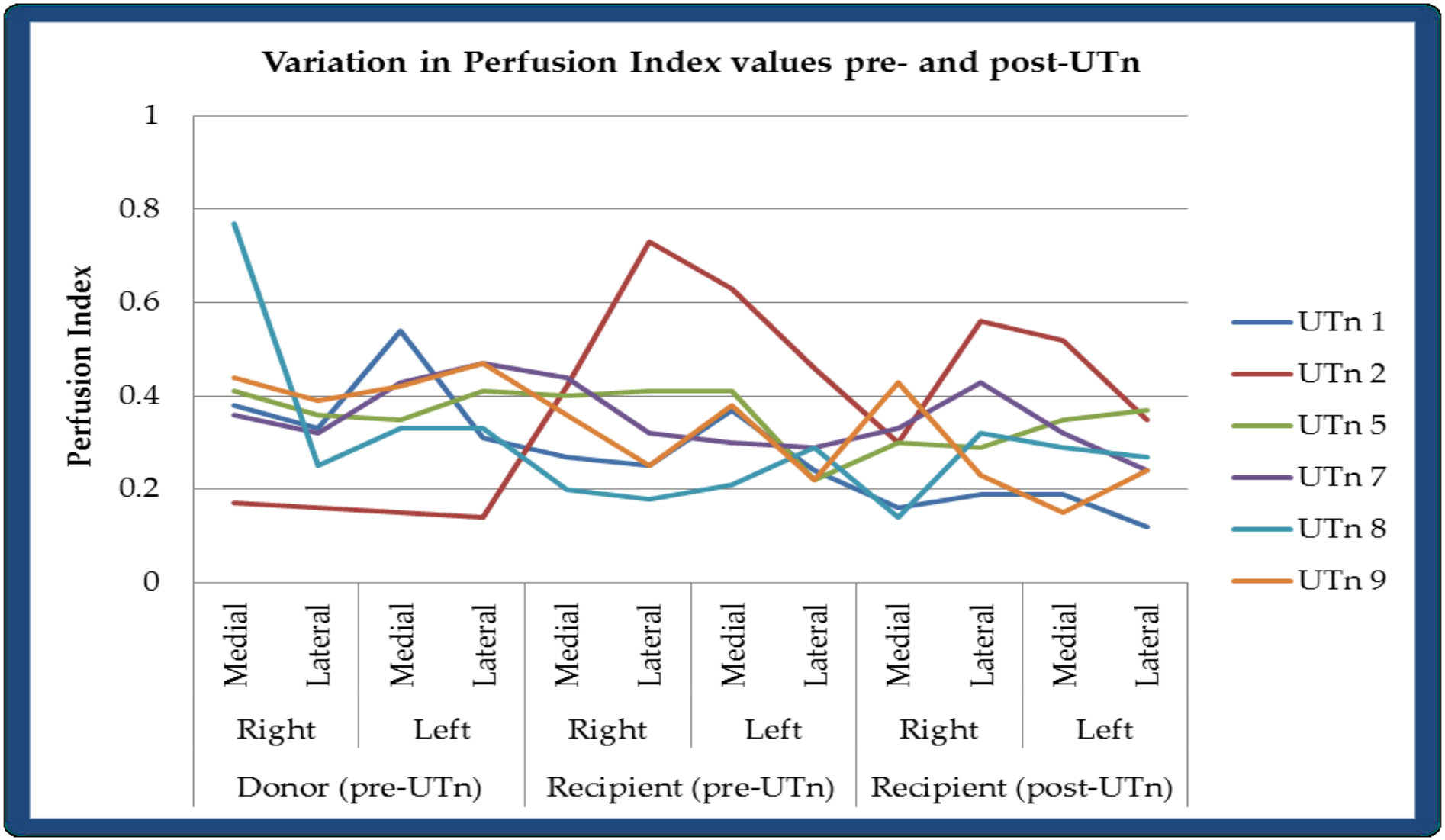


Figure 3.10b Perfusion index values of the Donor and the Recipient rabbit (measured with a pulse oximeter)

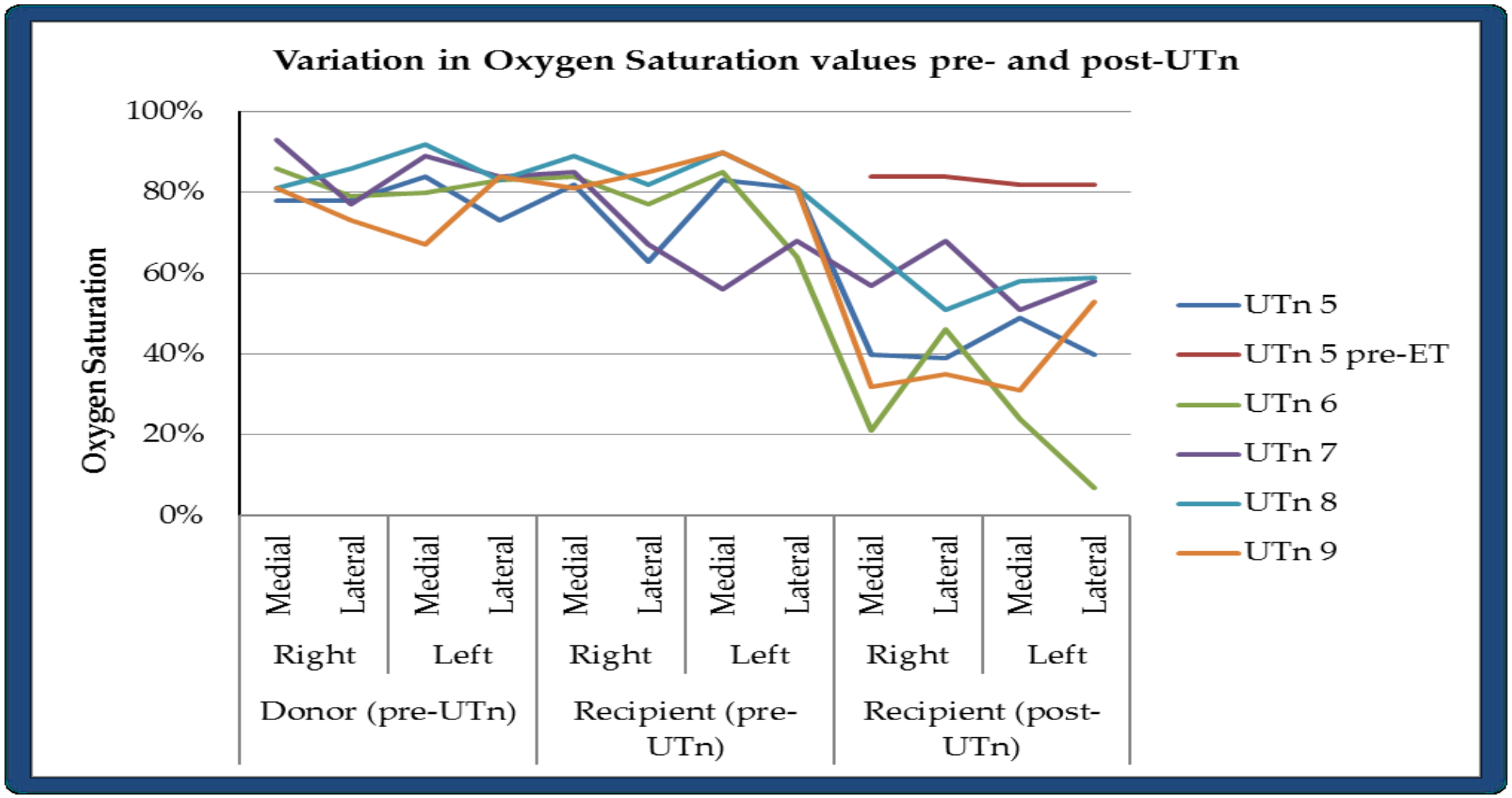
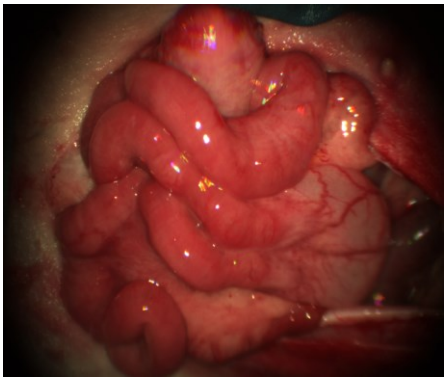
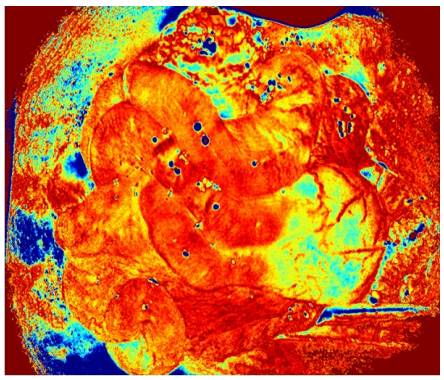


Figure 3.10c Oxygen Saturation values of the Donor and the Recipient rabbit measured with a multispectral imaging laparoscope

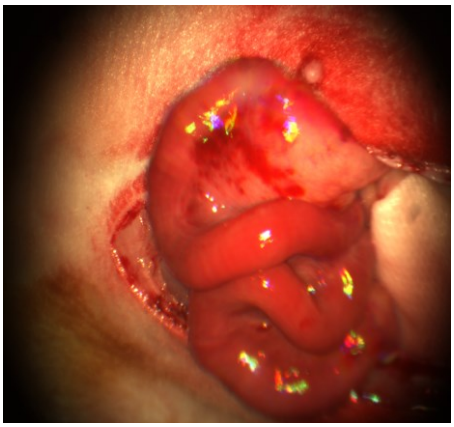
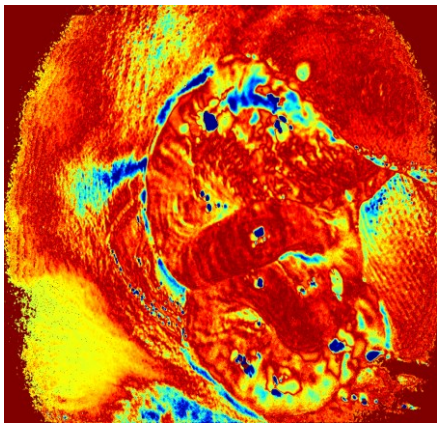
Figure 3.11a-h The images on the left are standard images of the rabbit cornua produced using a RGB filter. The images on the right are reference tissue O_2Sat images. The O_2Sat images are displayed using a colour scale that ranges from '0' (dark blue) to '1' (bright red).

Figure 3.11a Images correspond to UTx #3

Donor (Pre-UTx #3)

Recipient (Pre-UTx #3)

Recipient (Post-UTx #3)

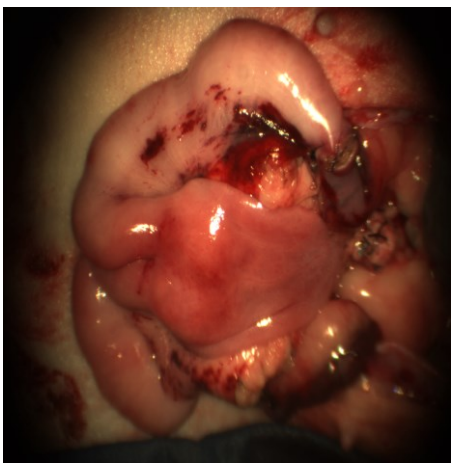
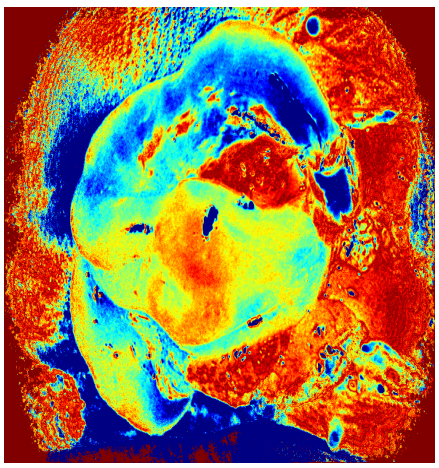
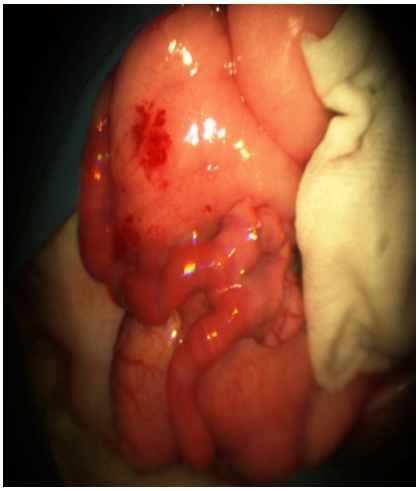
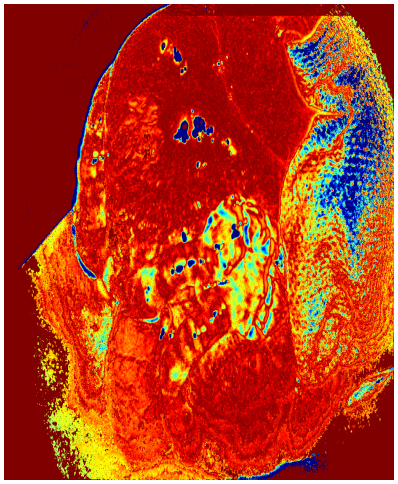



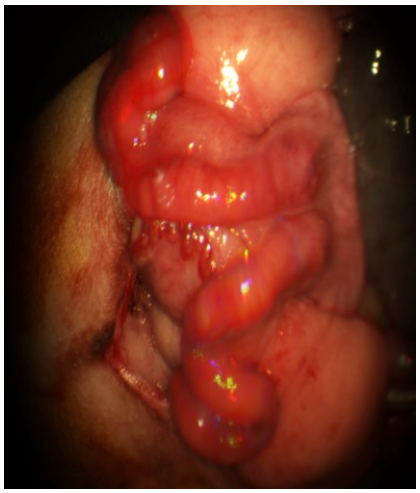
Figure 3.11b Images correspond to UTx #4

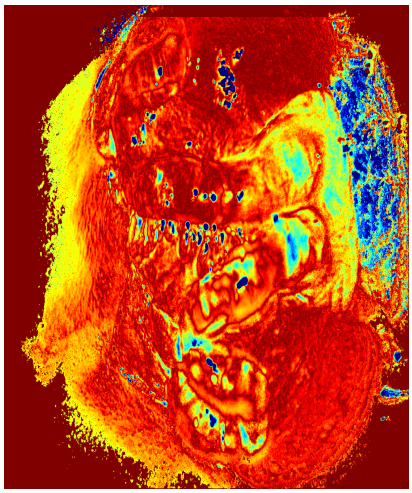
Donor (Pre-UTx #4)



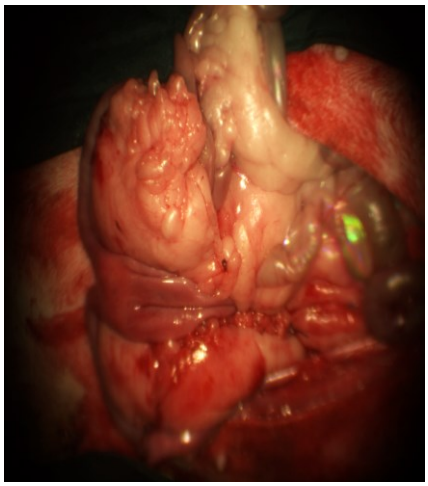


Recipient (Pre-UTx #4)





Recipient (Post-UTx #4)



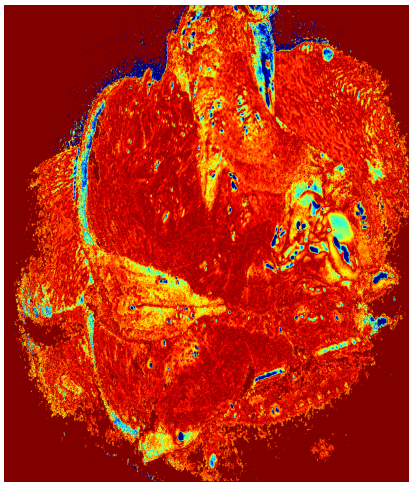
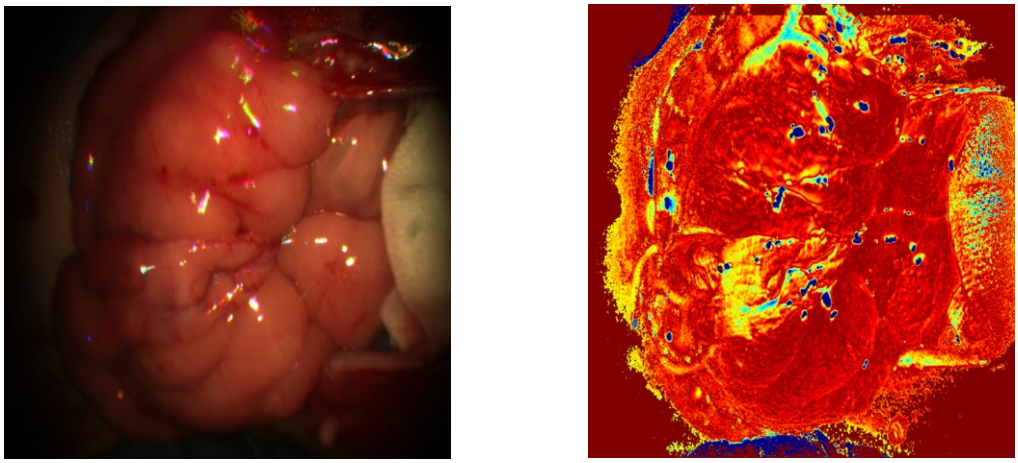
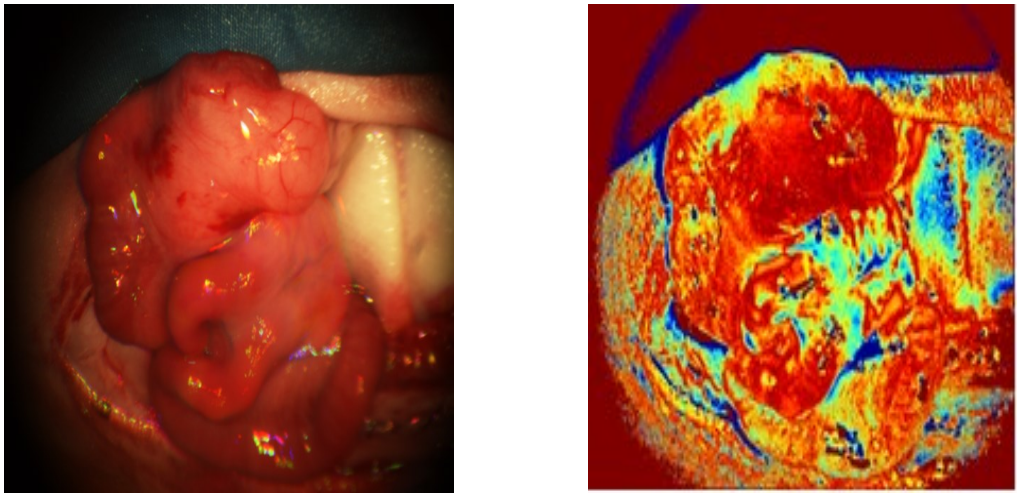


Figure 3.11c Images correspond to UTx #5

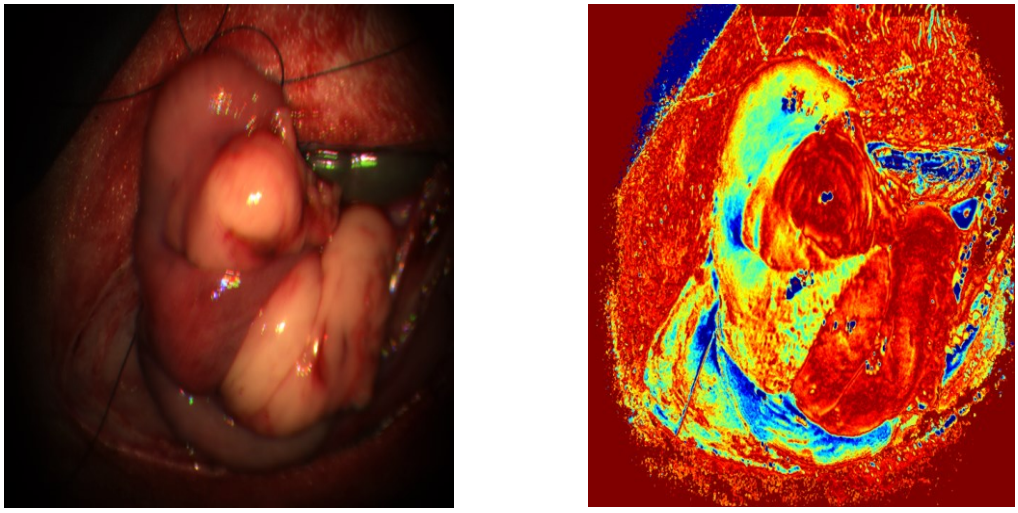
Donor (Pre-UTx #5)



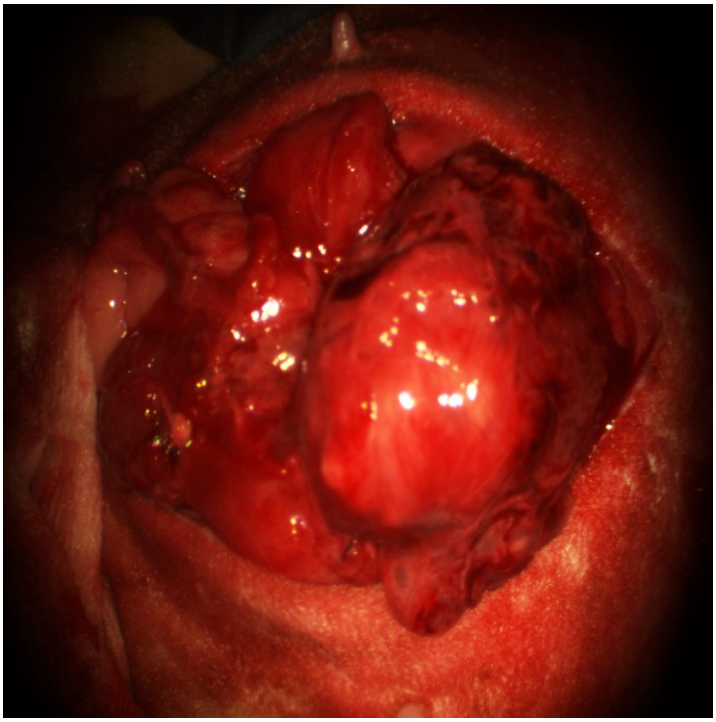
Recipient (Pre-UTx #5)



Recipient (Post-UTx #5)



Recipient (Day 89 post-UTx #5)



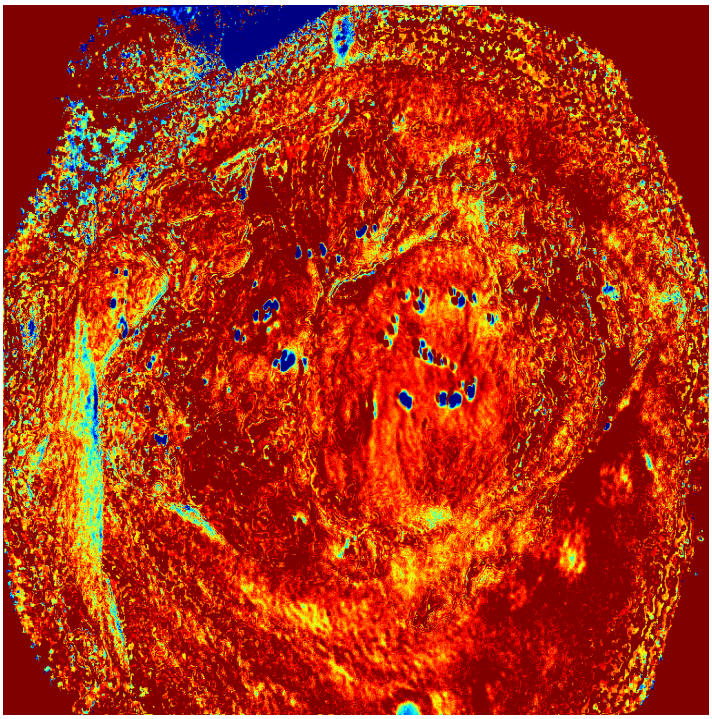
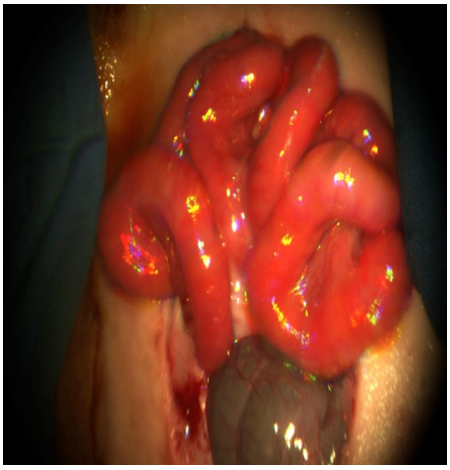
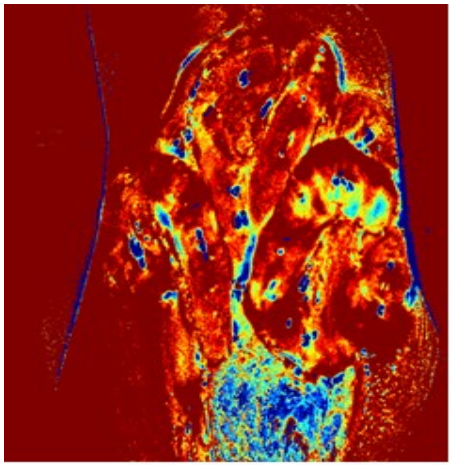
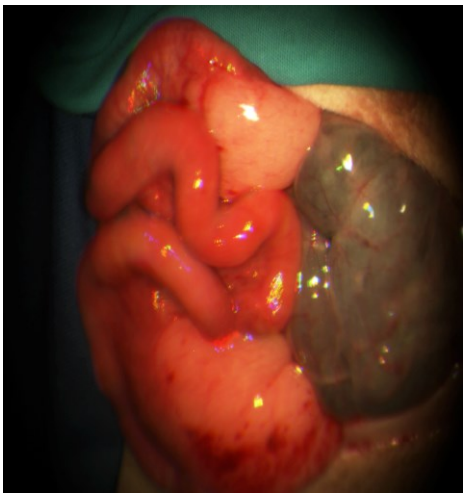
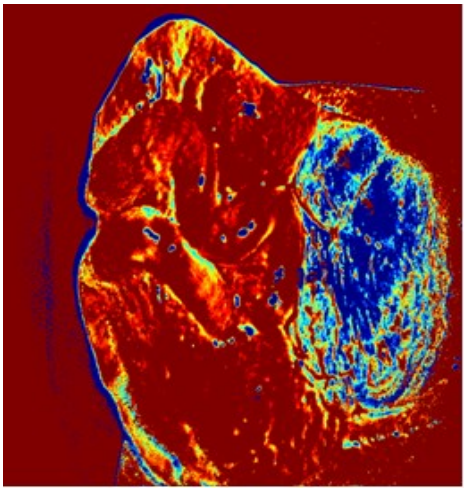


Figure 3.11d Images correspond to UTx #6

Donor (Pre-UTx #6)

Recipient (Pre-UTx #6)

Recipient (Post-UTx #6)

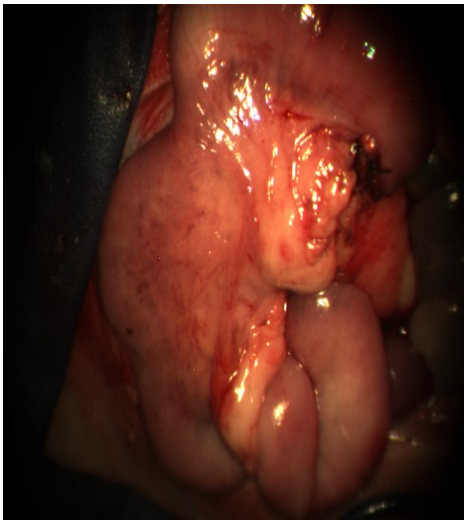
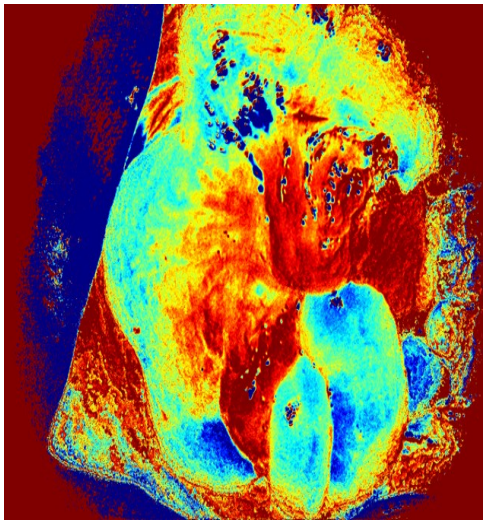
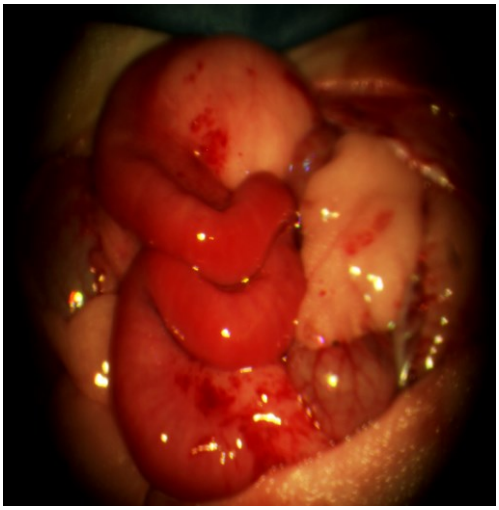
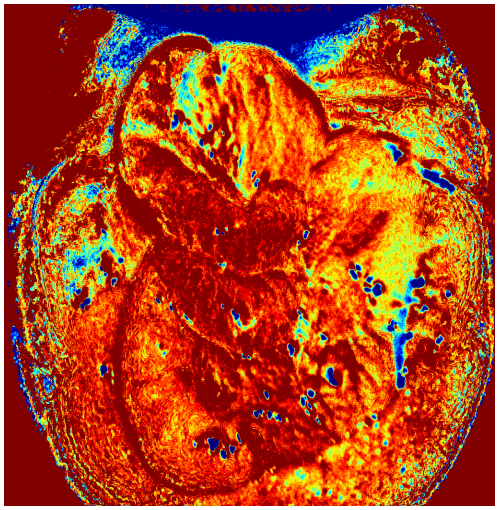
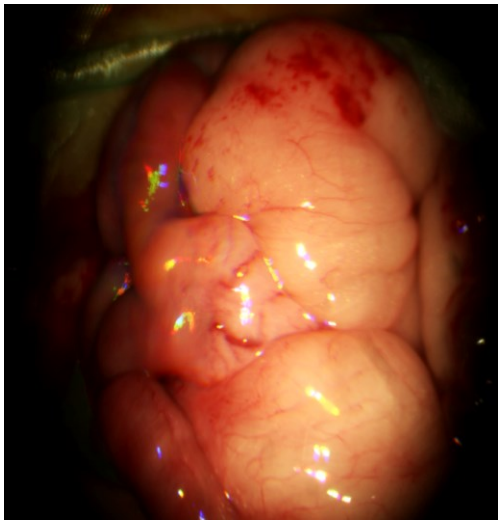
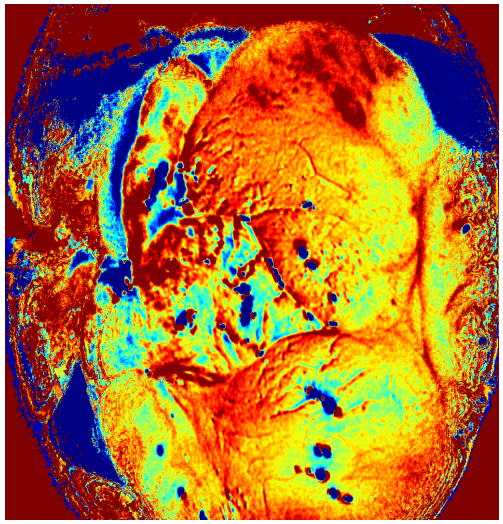



Figure 3.11e Images correspond to UTx #7

Donor (Pre-UTx #7)

Recipient (Pre-UTx #7)

Recipient (Post-UTx #7)

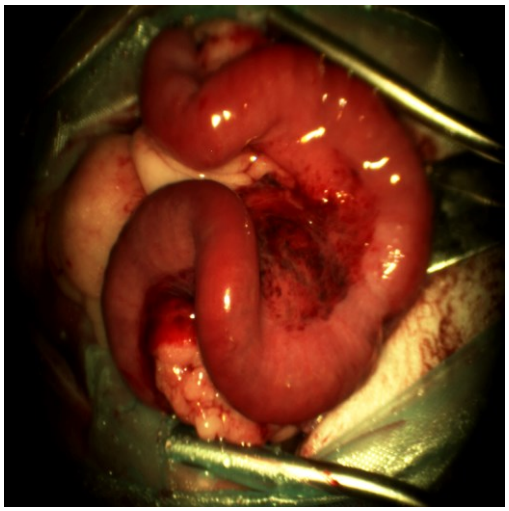
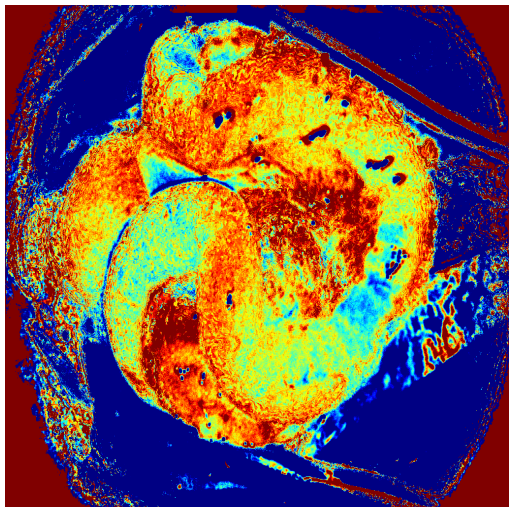
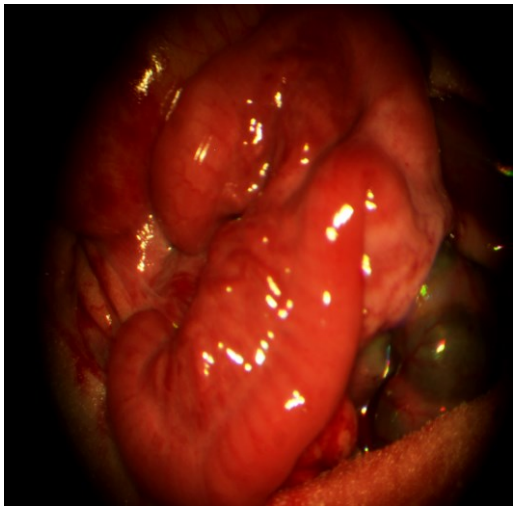
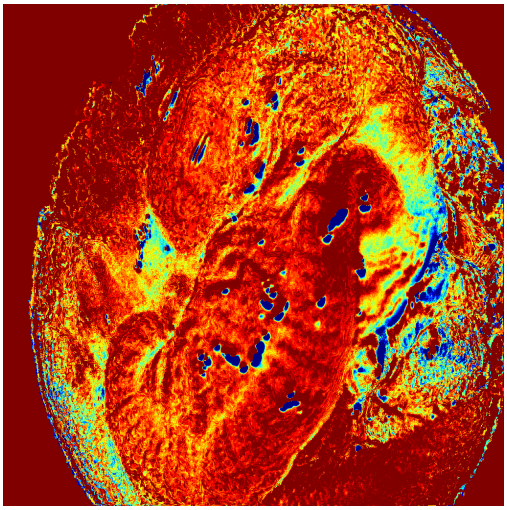
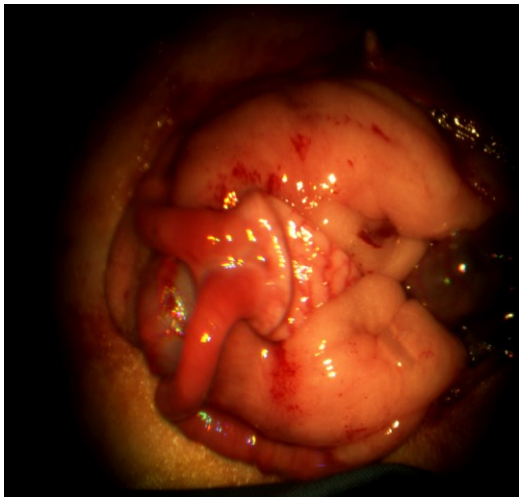
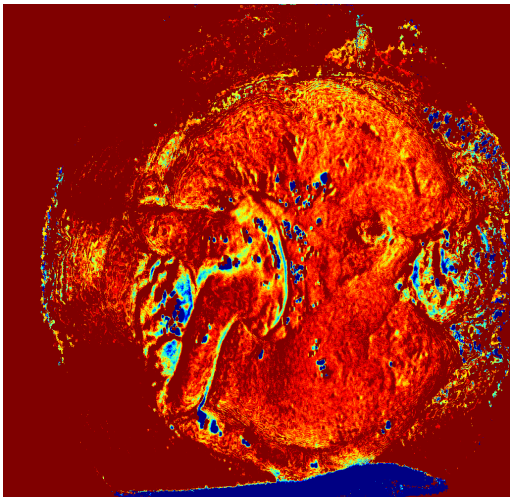



Figure 3.11f Images correspond to UTx #8

Donor (Pre-UTx #8)

Recipient (Pre-UTx #8)

Recipient (Post-UTx #8)

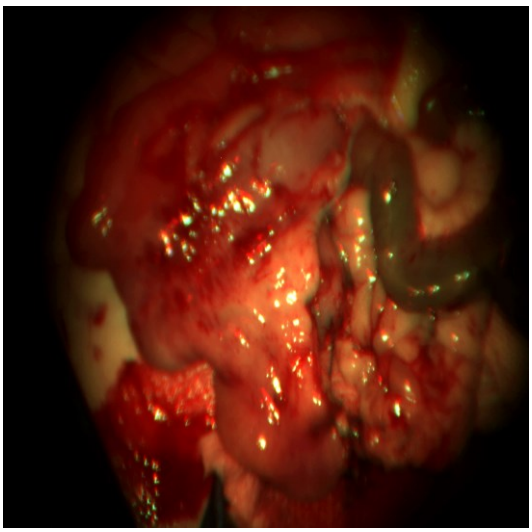
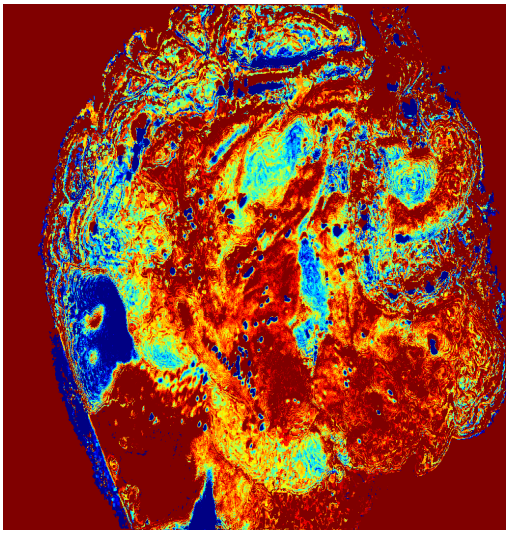
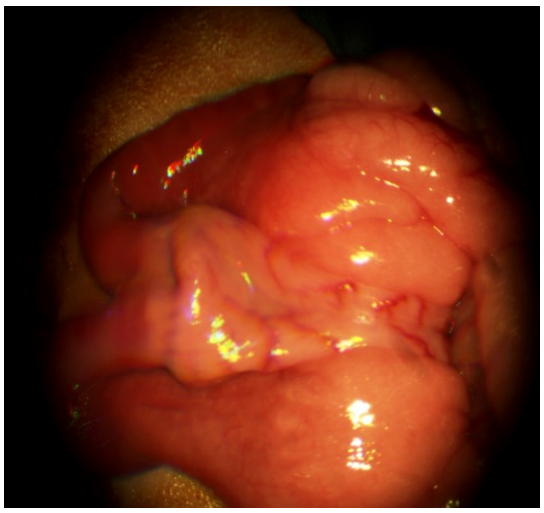
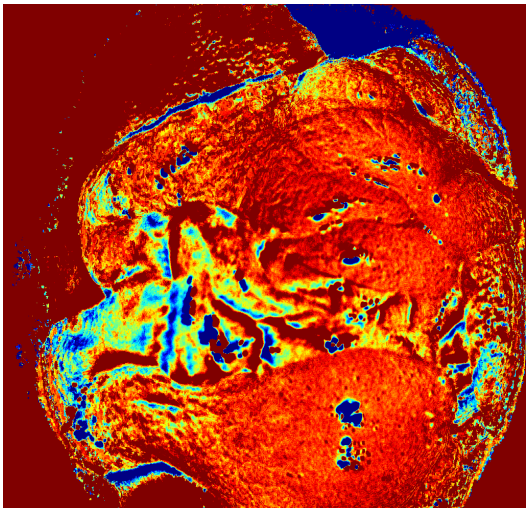
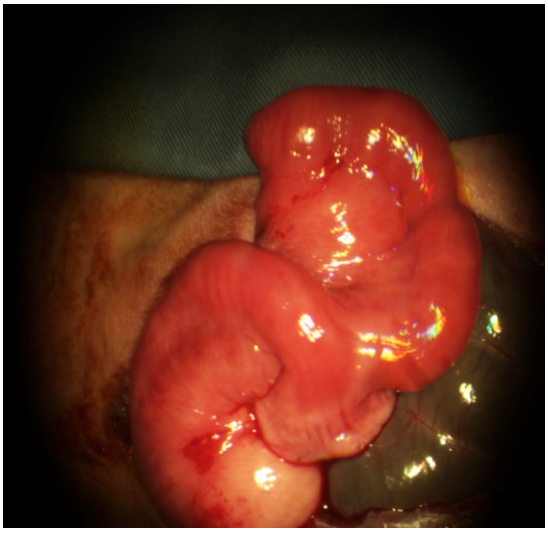
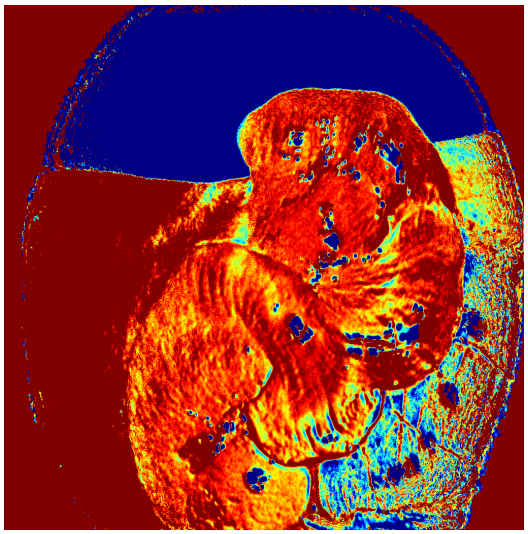



Figure 3.11g Images correspond to UTx #9

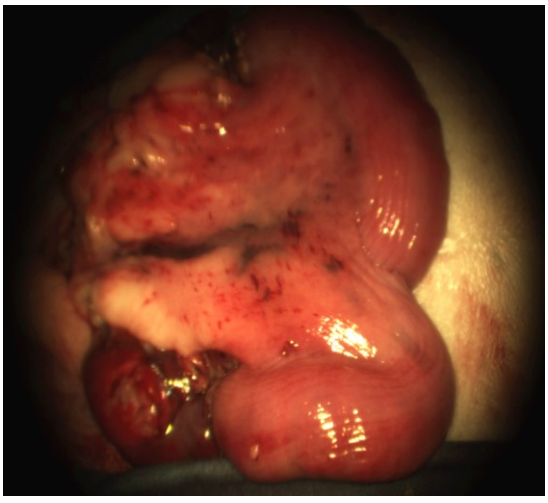
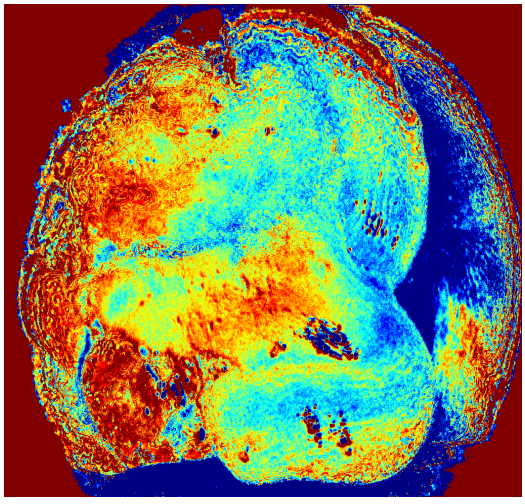
Donor (Pre-UTx #9)

Recipient (Pre-UTx #9)

Recipient (Post-UTx #9)

eLASCA

Rabbit model (UTx #7 and #9)

Rabbit experiment - UTx #7

Figure 3.12 showed the rotated contrast image. After rotation, the AOI was chosen to be a rectangle, which was marked in red in **Figure 3.13**. The vessel area showed slightly lower contrast in blue colours than the surrounding area.

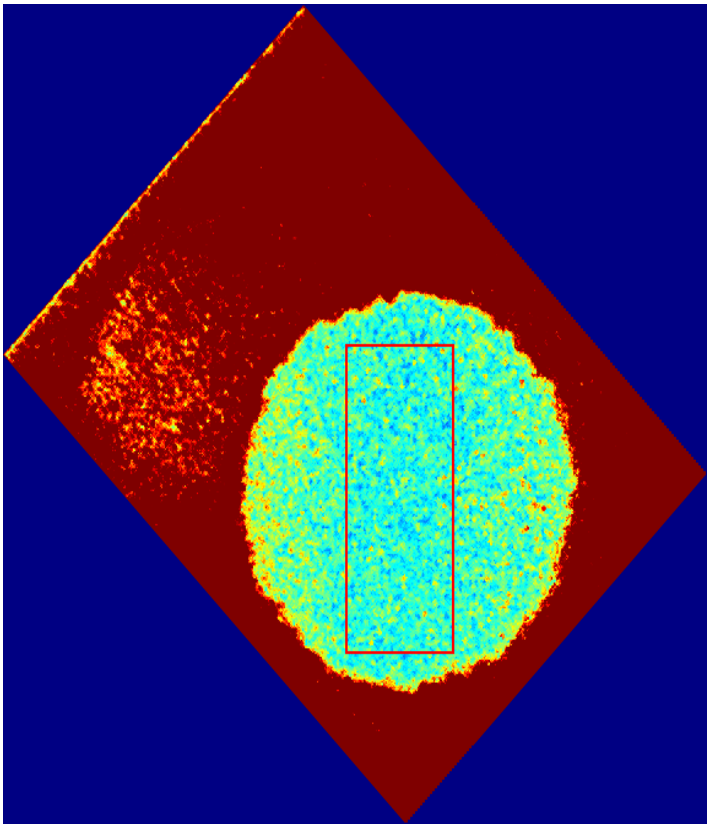


Figure 3.12 Rotated contrast image with the red rectangular marking the AOI

The averaged contrast from the AOI was detrended to remove the DC component and interpolated with a double sampling frequency. Then a FFT was applied on the resulting contrast used to calculate the frequency at which the contrast changed. **Figure 3.13a-b** depicts the raw contrast as a function of time and the contrast after being detrended and interpolated. The contrast displayed a periodic change.

The frequency spectrum of the contrast is shown in **Figure 3.13c**. The peak frequency was around 3.5Hz, corresponding to 210bpm, which is slightly higher than 160-180bpm measured using an oximeter at the commencement of the trial.

Figure 3.13a-c The contrast and the spectrum of the contrast [186]

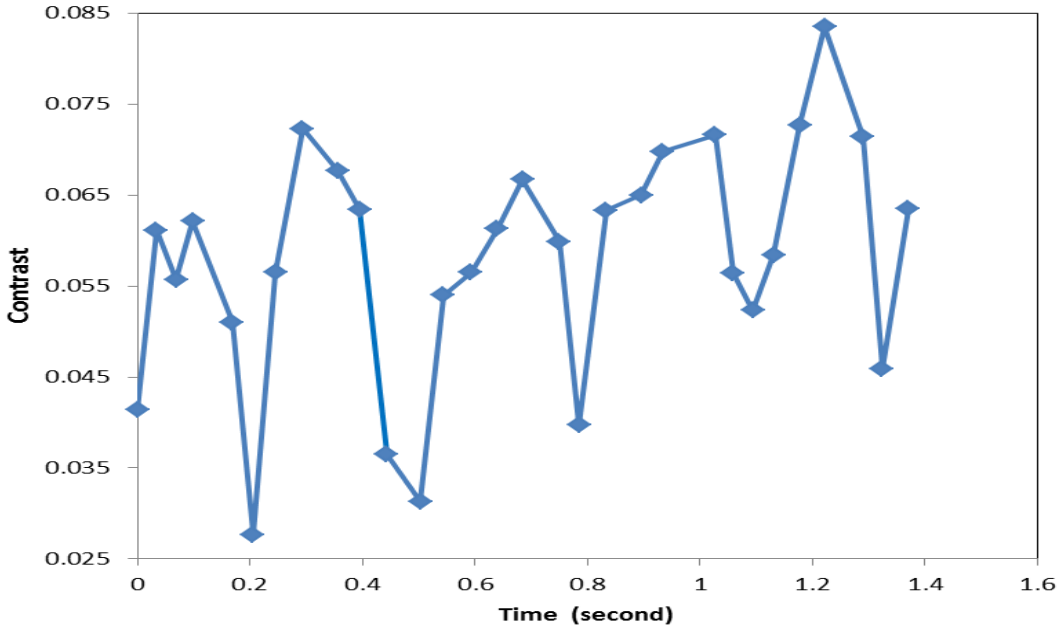


Figure 3.13a Graph showing raw contrast as a function of time

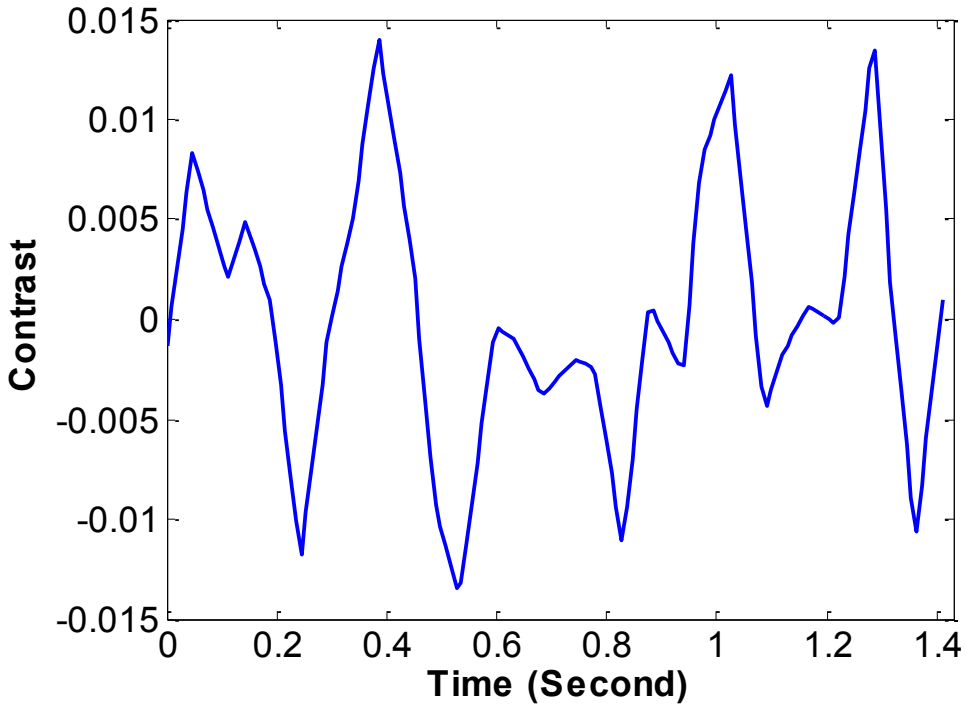


Figure 3.13b Graph showing contrast after detrending and interpolation

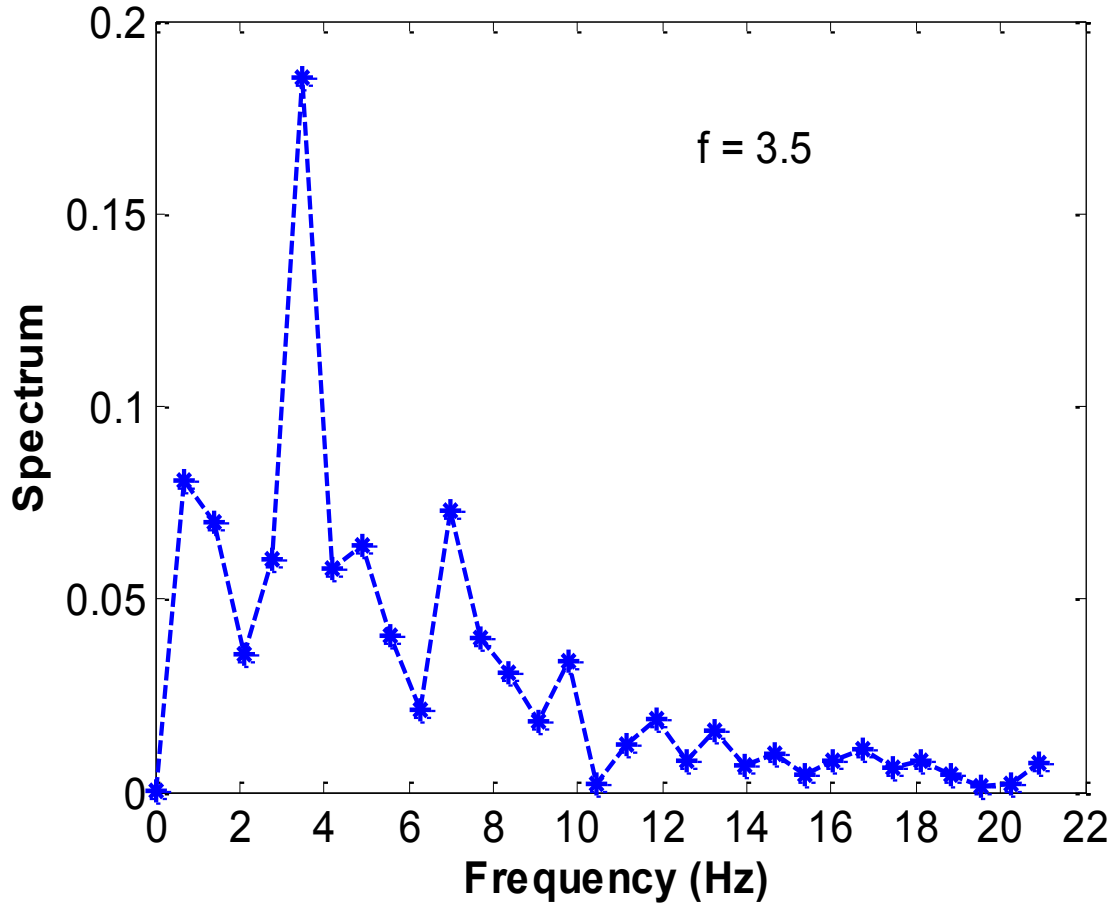


Figure 3.13c Graph showing frequency spectrum of the interpolated contrast

As outlined in the methods section, the second experiment which was performed on the recipient in UTx #7 involved an occlusion of the aorta.

Figure 3.14a-b demonstrates the contrast change using both wavelengths, following the occlusion of the blood flow. The two figures depict a matching increase in contrast after the 25th second when the occlusion was applied, thus confirming that the blood flow was blocked, and a drop in contrast at the 50th second when the blood flow had been re-commenced. The spikes show a periodic change of two seconds, most likely brought about by the breath cycle as the respiration rate of the rabbit is 30-60bpm.

Figure 3.14a-b Graph showing contrast portrayed as a function of frames [186]

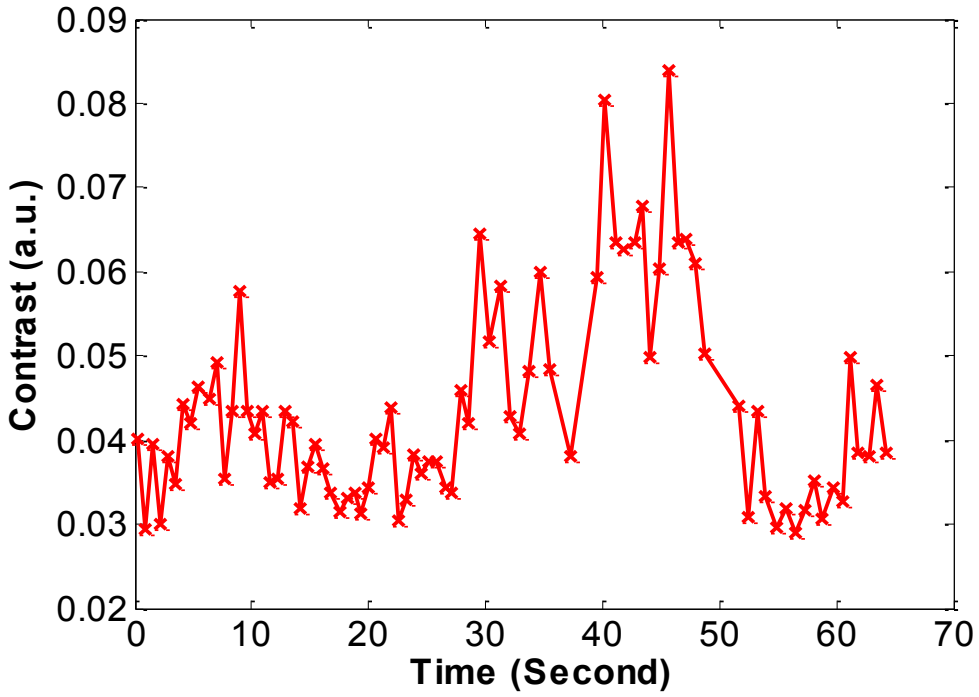


Figure 3.14a Data acquired at 660 nm

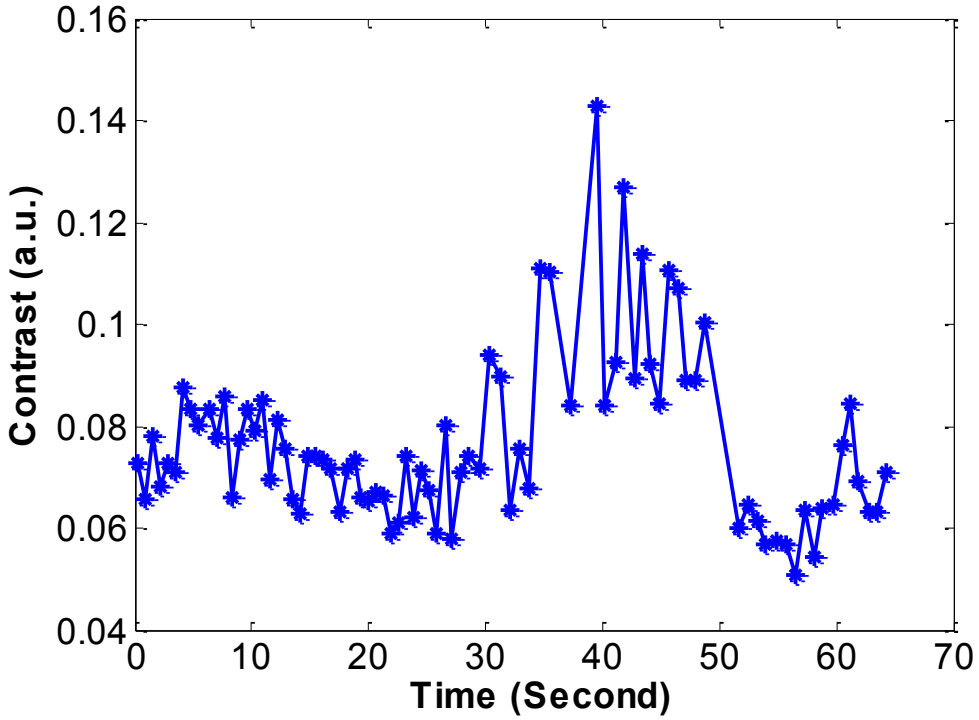


Figure 3.14b Data acquired at 830 nm

A change in the [Hb] is shown in **Figure 3.15a-b**. Large fluctuations are apparent in the first 20 seconds in all three figures and are shaded **Figure 3.15b**. This fluctuation was brought about by the power fluctuation of one of the lasers with a wavelength 830nm prior to it reaching a stable state. In **Figure 3.15a**, the concentration of oxyHb increased during the occlusion, whereas **3.15b** shows that the concentration of deoxyHb decreased. The total Hb increased overall during the occlusion as shown in **Figure 3.15c**.

Figure 3.15a-c Demonstration in the change of Hb concentration [186]

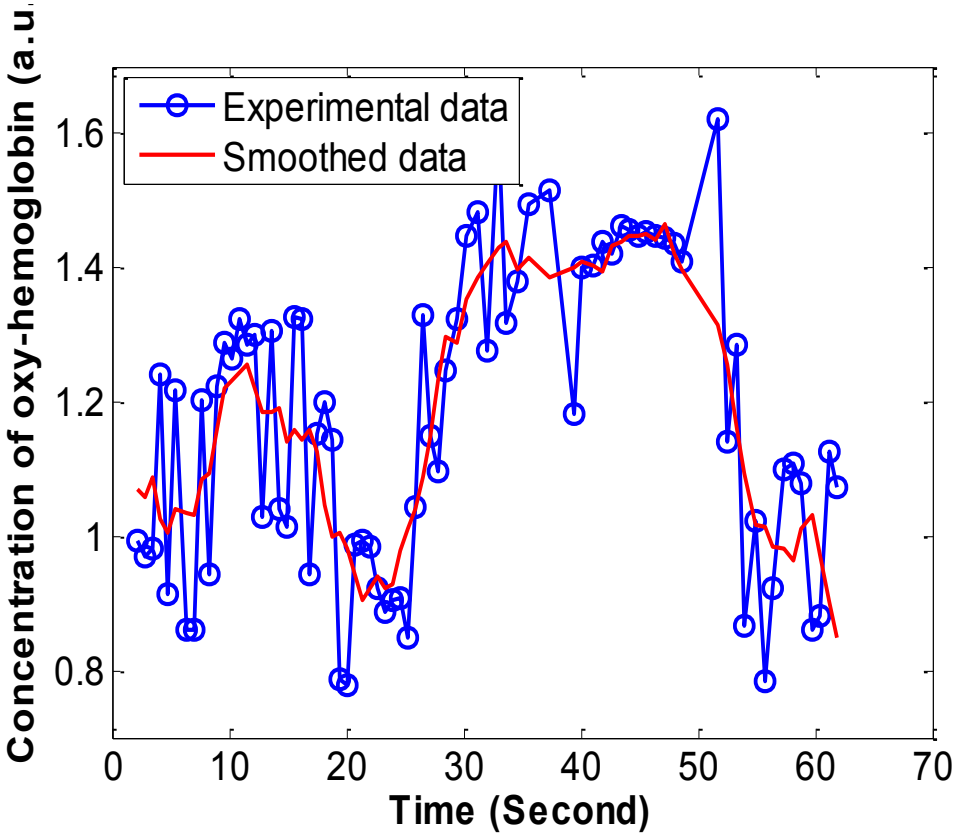


Figure 3.15a Graph showing the variation of Oxy-haemoglobin concentration with time

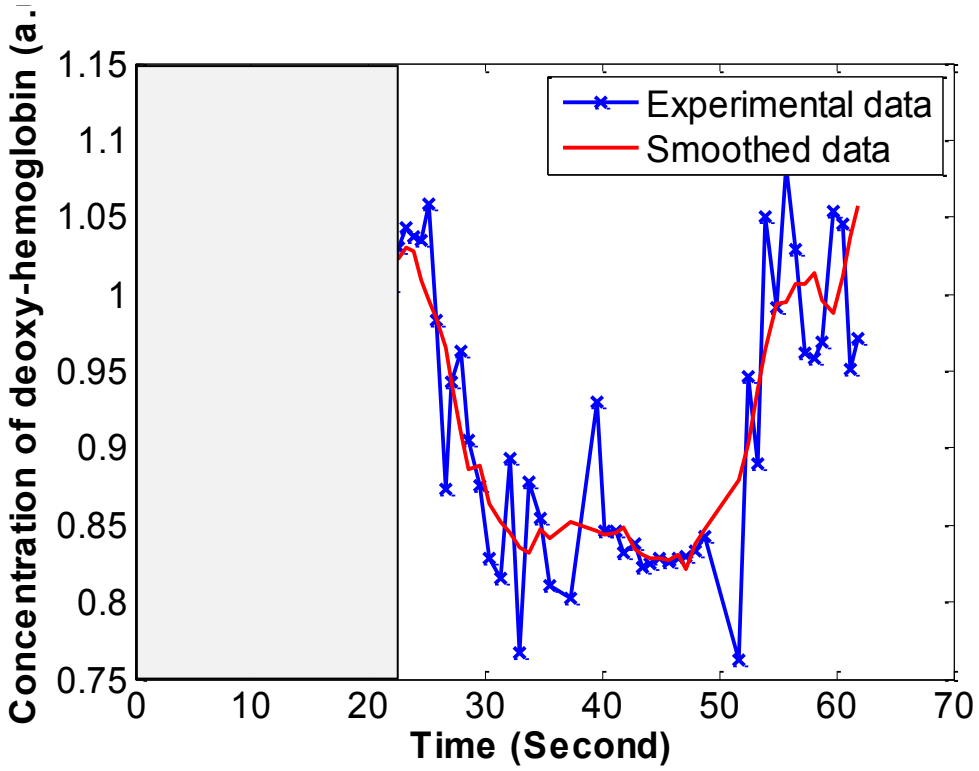


Figure 3.15b Graph showing the variation of deoxy-haemoglobin concentration with time

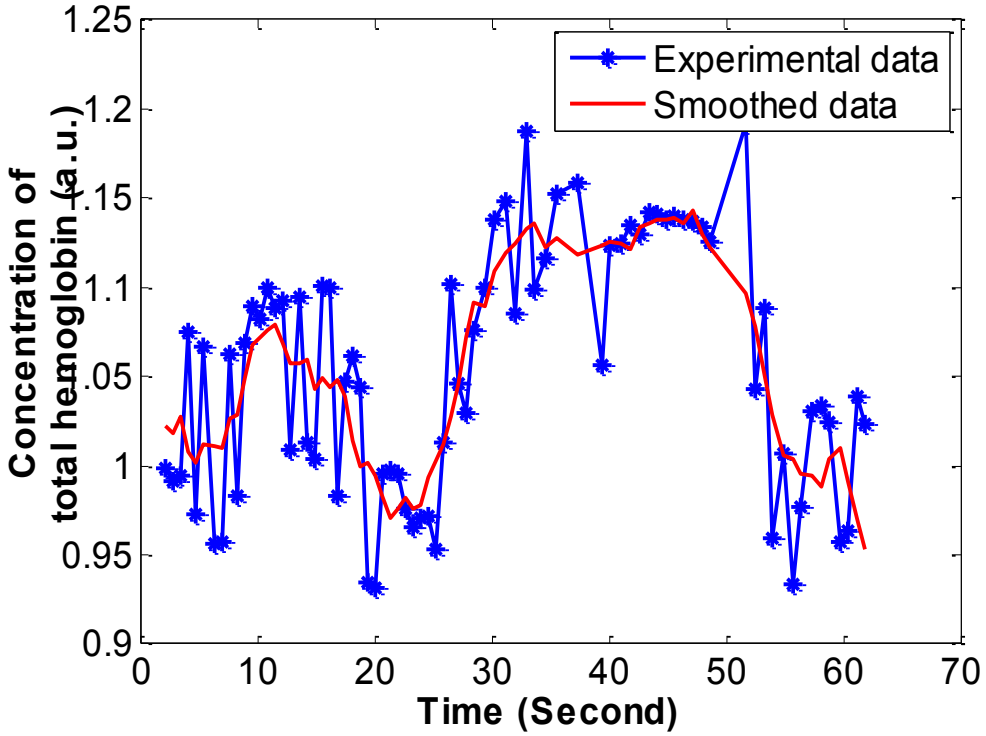


Figure 3.15c Graph showing the variation of total haemoglobin concentration with time

NB The shaded area in **Figure 3.15b** marks the error of the concentration induced by the intensity fluctuation of the laser 880 nm.

Rabbit experiment - UTx #9

Two exposure times were used for the rabbit does: 500μs and 10ms. Frequency was set at 0.5Hz.

Donor: Exposure time 10ms; Frame rate: about 21fps; Frame: 0-199 frame

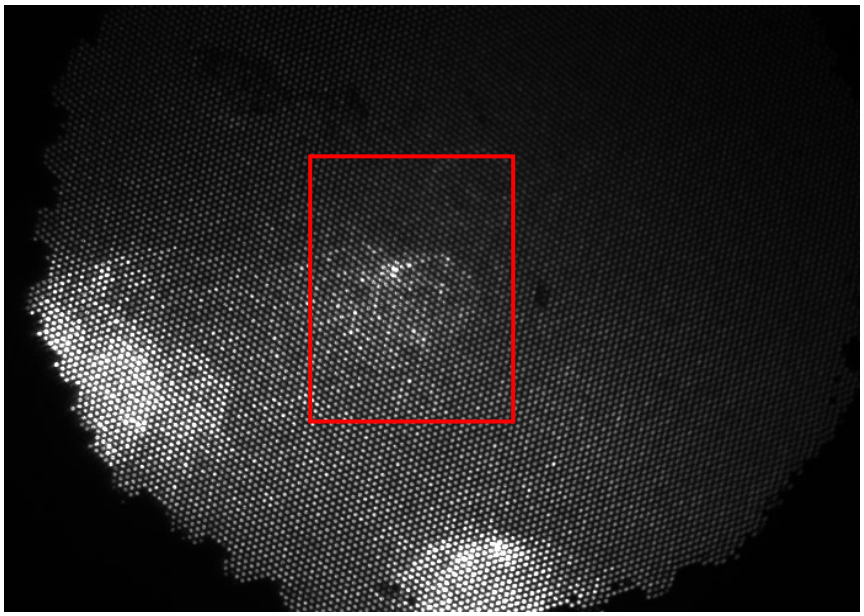


Figure 3.16a A focused and imaged fibre bundle pattern

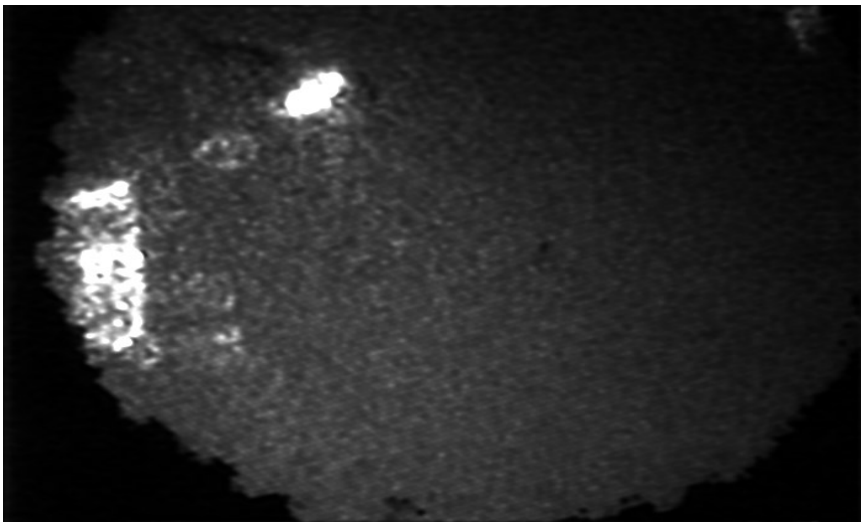
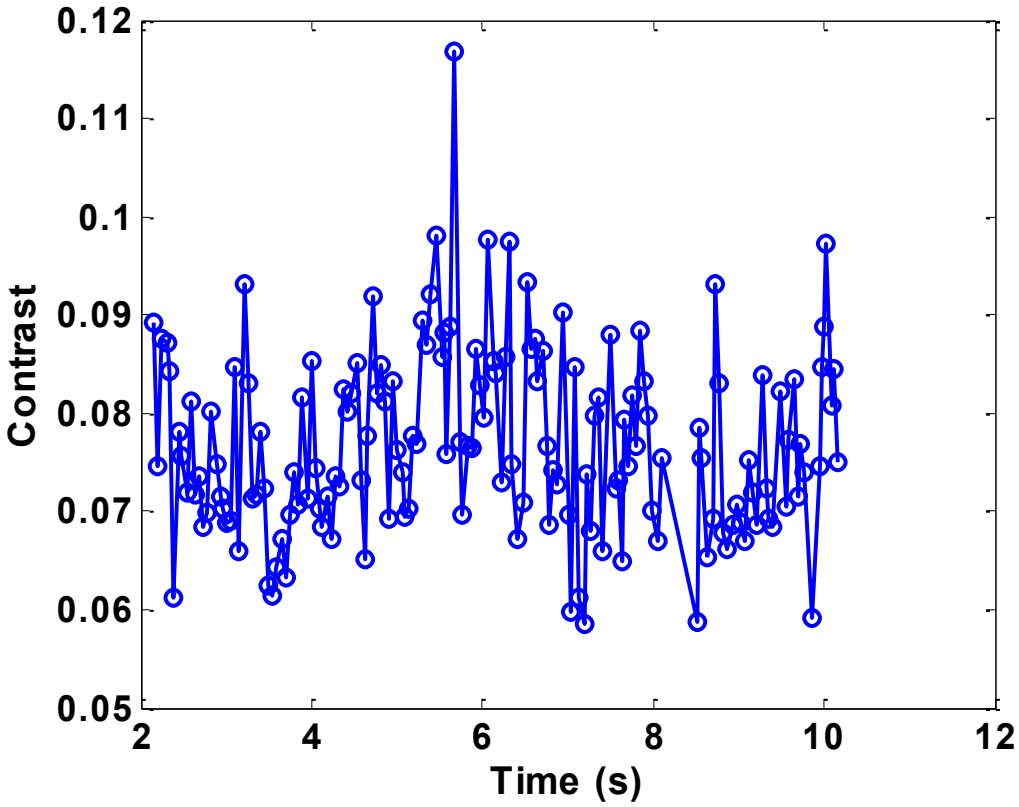
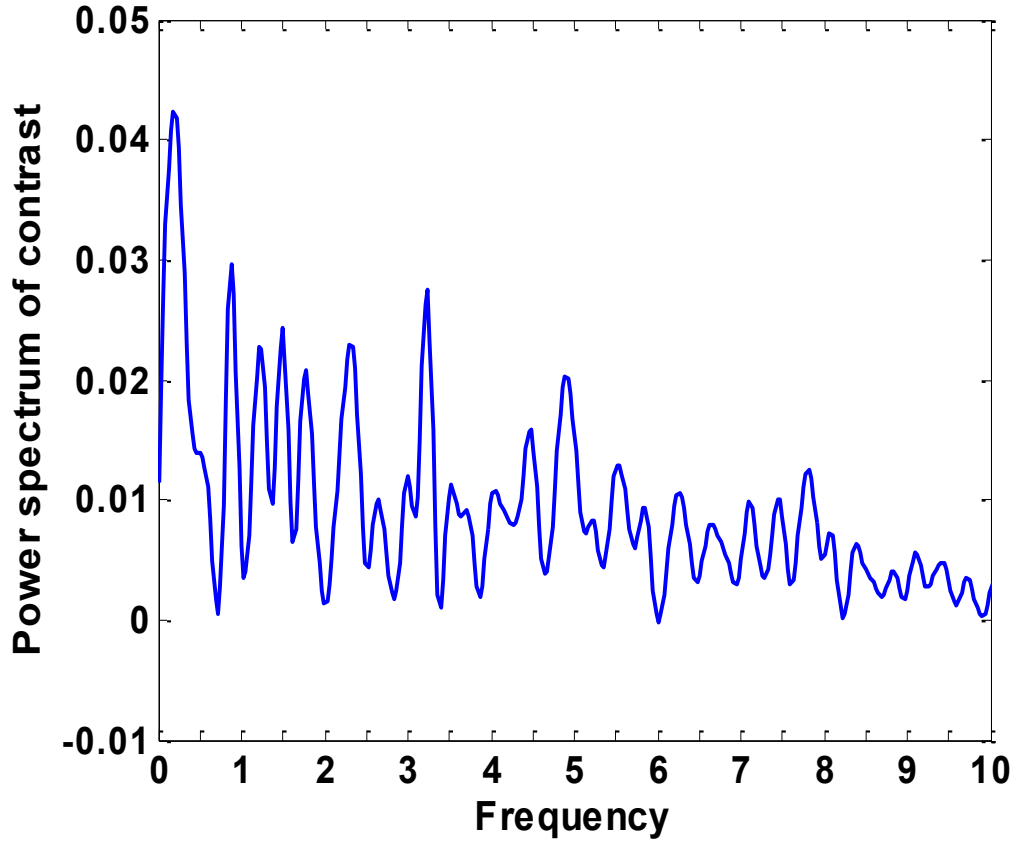
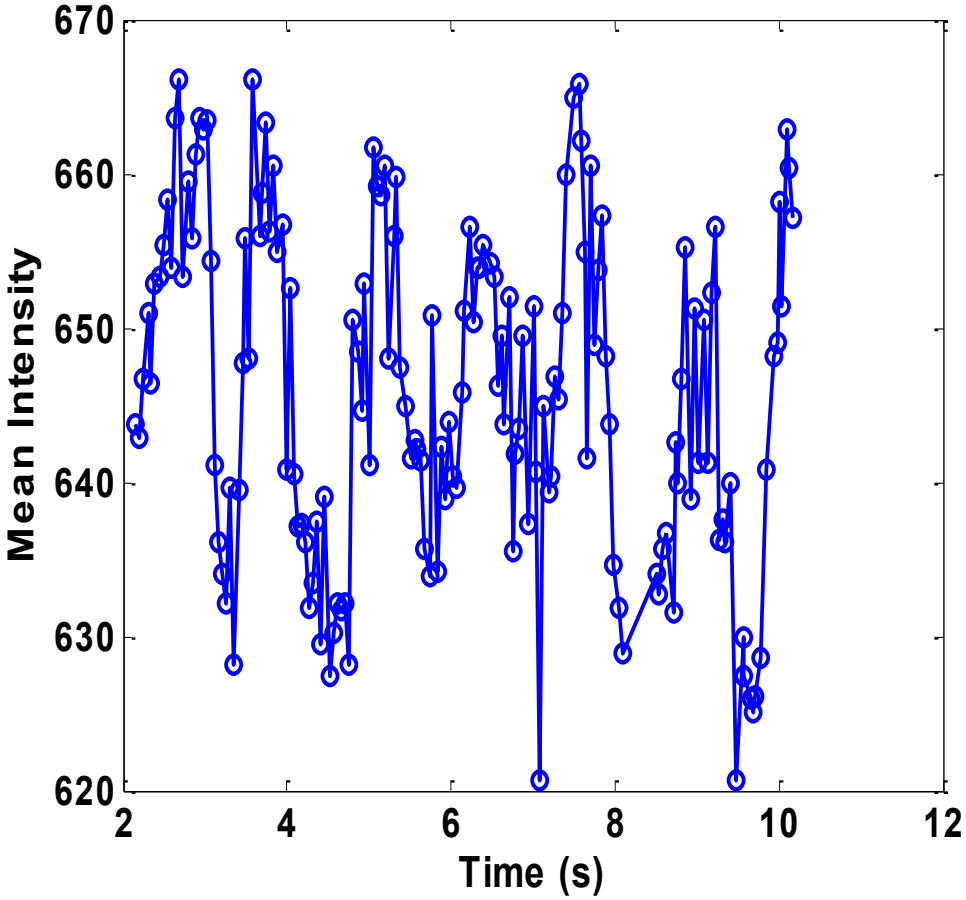


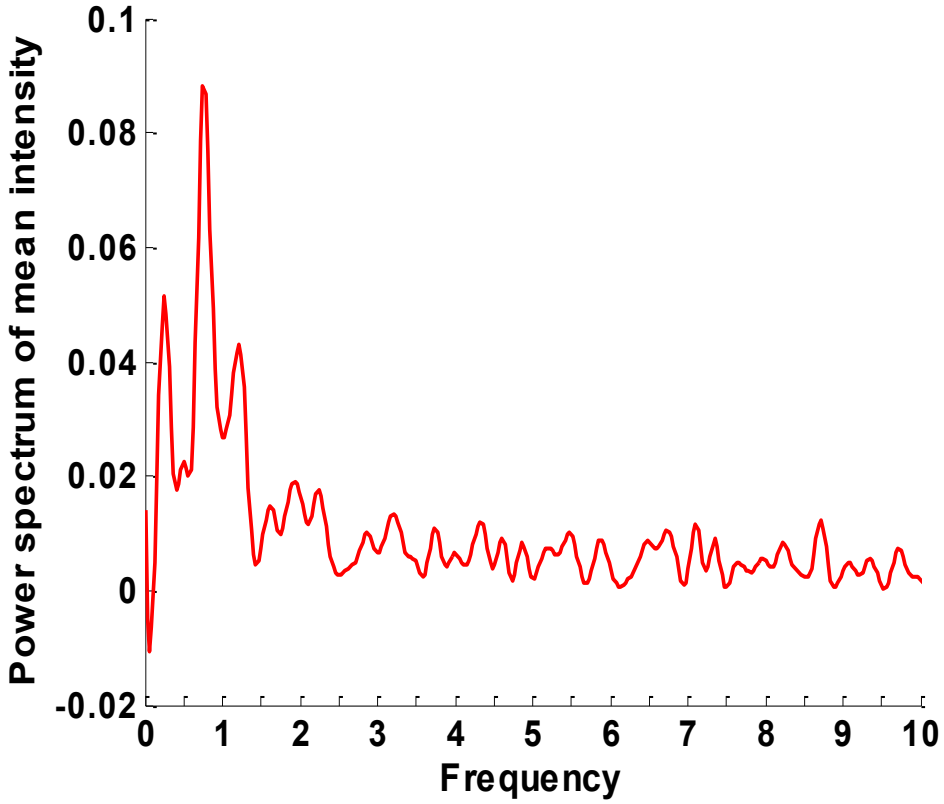
Figure 3.16b Image of interest when the low pass Butterworth filter was applied. It removed the fibre bundle pattern thus ensuring that the contrast is mainly from the fibre pattern and not from the sample.

Figure 3.17a-d Graphs of contrast and mean intensity over time and frequency



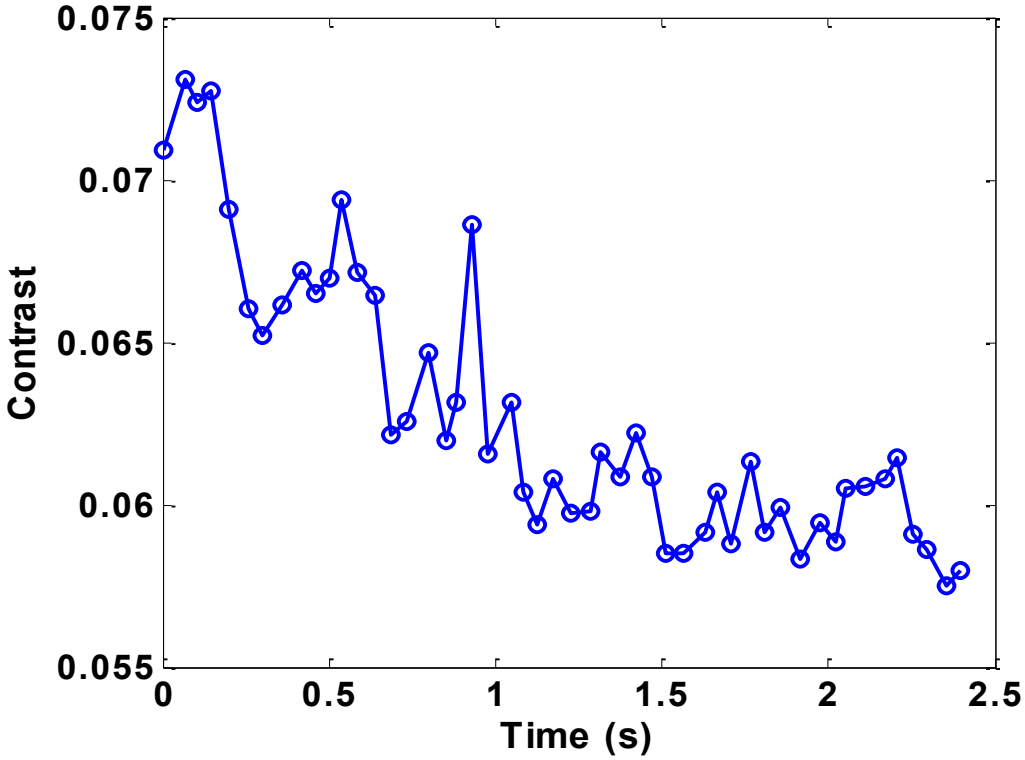


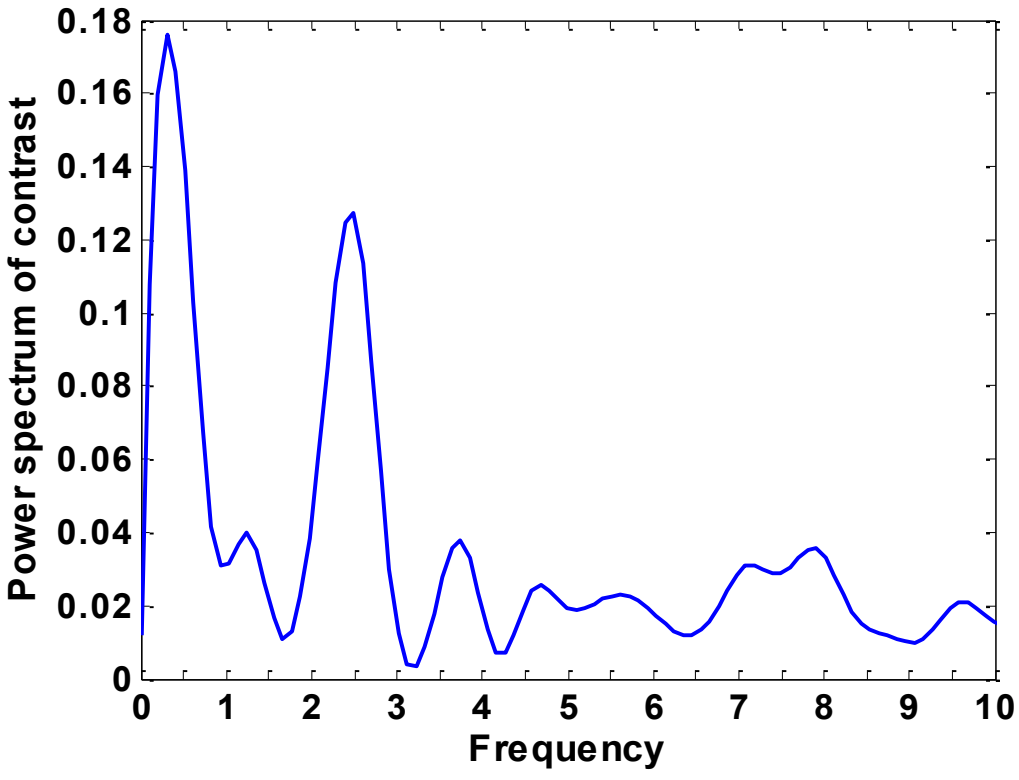

Mean Intensity
670
660
650
640
630
620
2
4
6
8
10
12
Time (s)

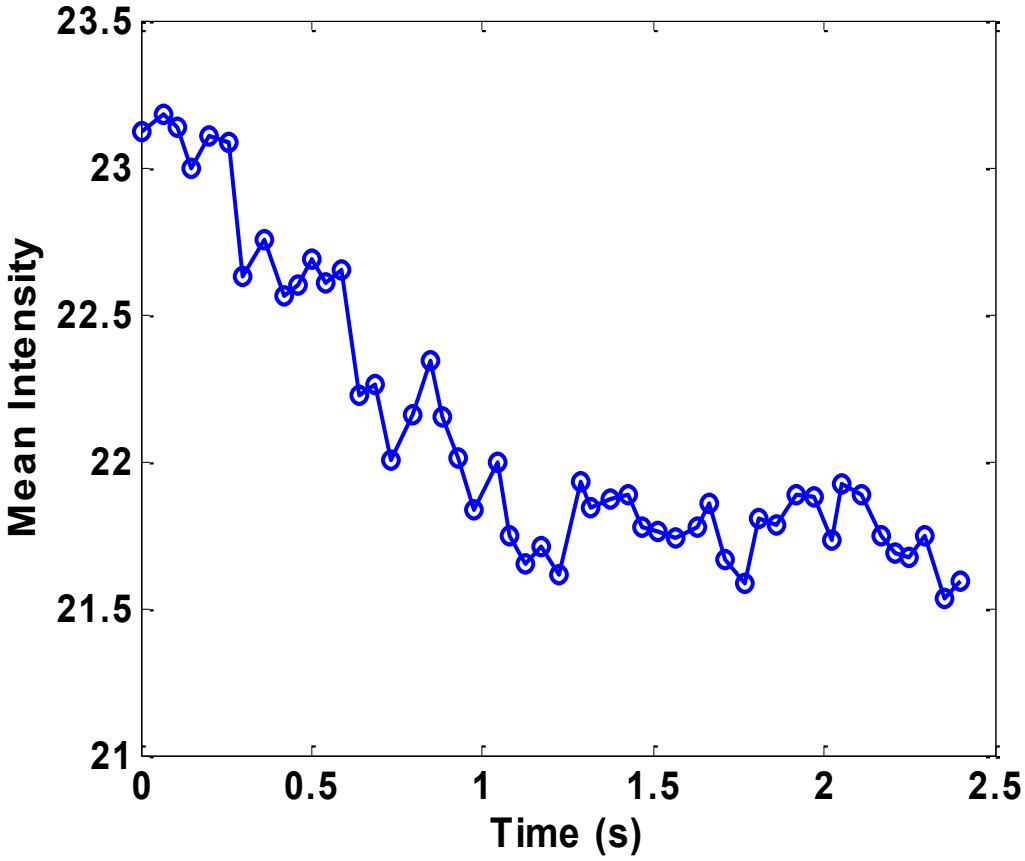

Power spectrum of mean intensity
0.1
0.08
0.06
0.04
0.02
0
-0.02
0
1
2
3
4
5
6
7
8
9
10
Frequency

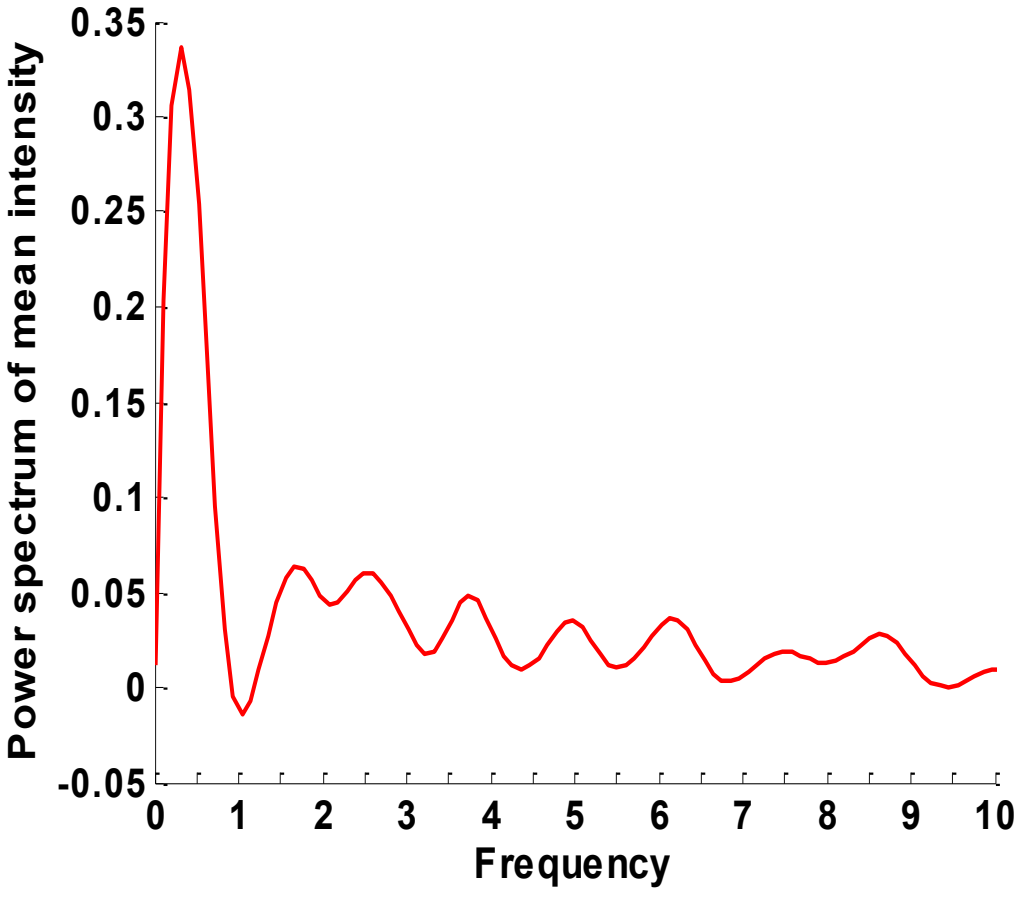
Recipient (1): Exposure time 500µs; Frame rate: about 21fps; Frame: 0-60 frame

Figure 3.18a-d Graphs of contrast and mean intensity over time and frequency





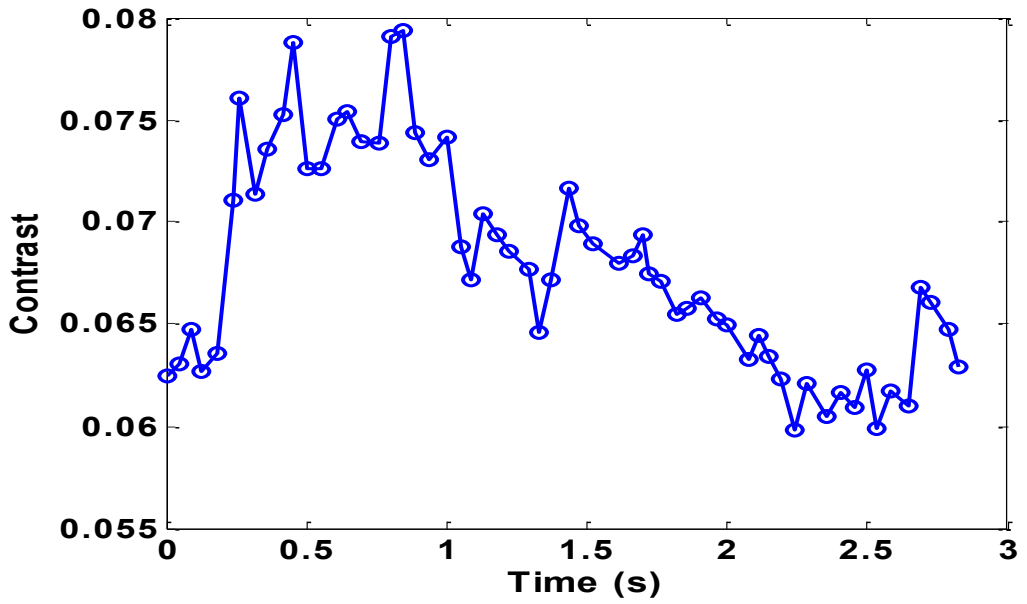

23.5
23
22.5
22
21.5
21
Mean Intensity
0
0.5
1
1.5
2
2.5
Time (s)

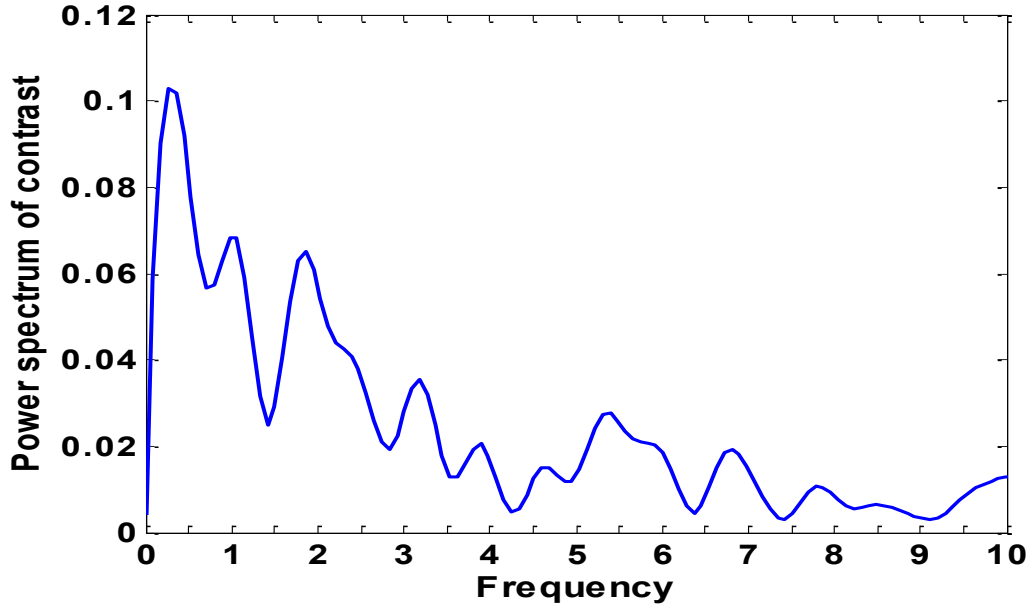

0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
Power spectrum of mean intensity
0
1
2
3
4
5
6
7
8
9
10
Frequency

Recipient (2): Exposure time 10ms; Frame rate: about 21fps; Frame: 0-60 frame

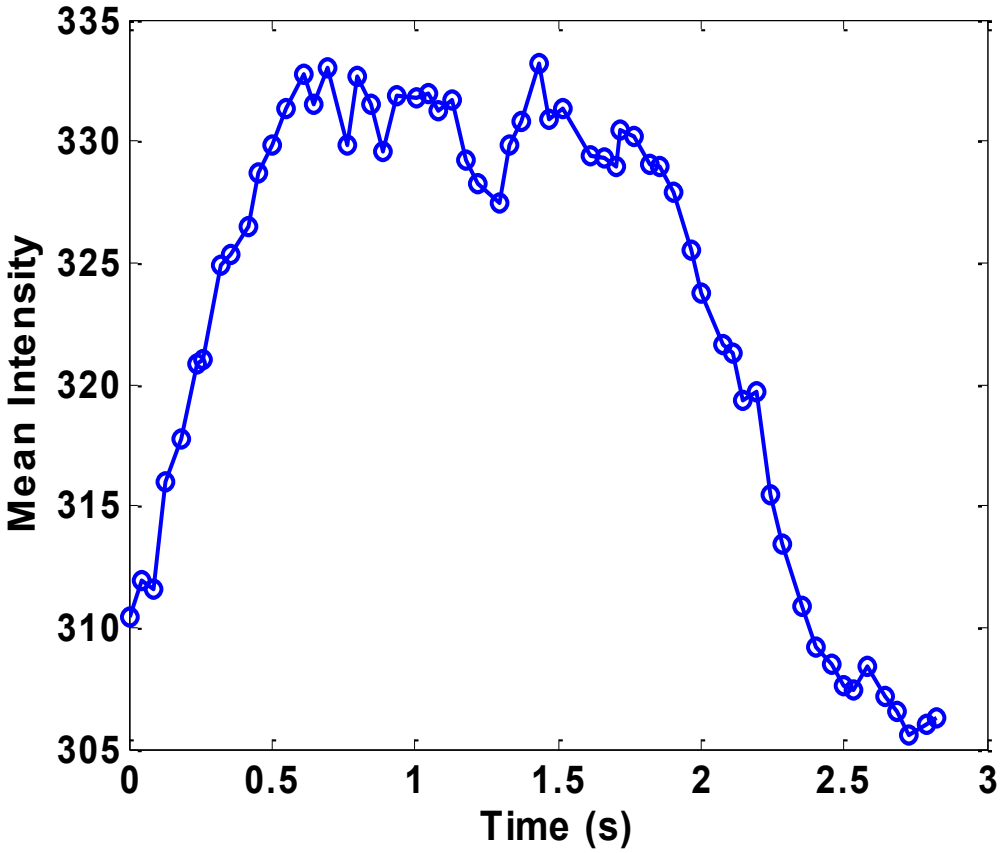
According to the timing of the frame capture, a sudden delay of frame rate at the 65th frame arose. We chose therefore to process the first 60 frames. Calculation was the same as for the donor doe: a low pass filter-contrast calculation-frequency.

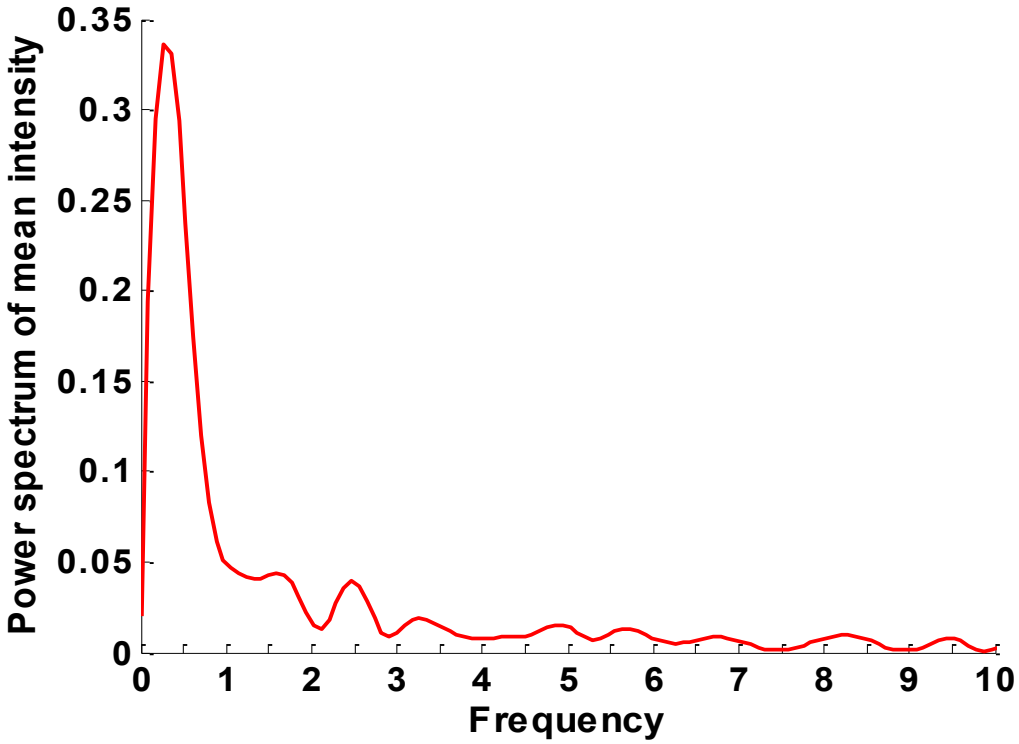
Figure 3.19a-d Graphs of contrast and mean intensity over time and frequency





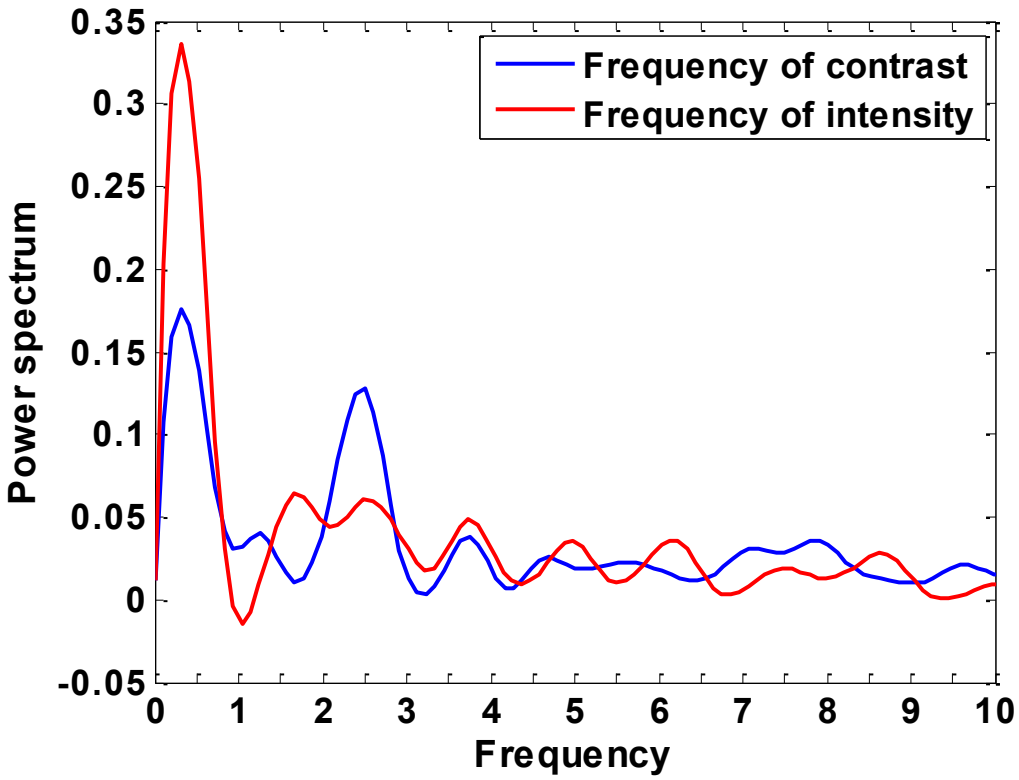
NB Peak at 0.25Hz and 2Hz





NB Frequency at 0.5Hz

Figure 3.20 Graph depicting the variation of contrast and intensity simultaneously for comparison



3.3.2. Sheep model

Pulse oximetry was applied in all five autotransplants. As the uterus was not retrieved in UTx #1 and the ewe died intra-operatively in UTx #4, no values for O_2Sat and PI were obtained post-UTx. The averages and standard deviations for these measurements are provided in **Table 3.2a**. Comparison of O_2Sat values prior to graft retrieval and post-UTx revealed a statistically significant decrease in saturation levels post-UTx. A similar comparison of PI values did not demonstrate any fall in PI level post-UTx, and importantly no statistical difference. This variation in O_2Sat and PI values is depicted in **Figure 3.21a-b**.

Optical Spectroscopy was applied for all five transplants. However for reasons explained above, images of the uterus prior to retrieval and post-UTx were obtained only in UTx #2, #3 and #5. Following the anastomosis of the internal to external iliac vessels (details are outlined in study 4), the uterus was observed to undergo a colour shift during reperfusion from its blanched appearance after flushing to a more reddish colour. The averages and standard deviations for O_2Sat are provided in **Table 3.2b**, with variation depicted in **Figure 3.21c**.

The processed O_2Sat images of the uterus prior to retrieval and post-UTx, along with colour images reconstructed from the multispectral data after integration under the RGB filter transmission response of a digital colour camera are shown in **Figure 3.22a-e**. The pre-retrieval images show that the graft is well-perfused in the donor doe, with high oxygen content in both cornua. The area of low O_2Sat in the centre may correspond to a less vascularised area or possibly an area with a higher venous to arterial supply ratio. The post-UTx images show similar features after blood supply is re-established from the external iliac arteries. Areas of low O_2Sat correspond to connective tissue where re-perfusion is expected to last the longest. Here, the comparison of O_2Sat values between the uterus prior to retrieval and post-UTx revealed a statistically non-significant decrease in saturation levels post-UTx.

Sheep	Side	Cornua	O_2 Saturation					Mean ± S.D.	Perfusion index					Mean ± S.D.
			UTx 1	UTx 2	UTx 3	UTx 4	UTx 5	All UTx	UTx 1	UTx 2	UTx 3	UTx 4	UTx 5	All UTx
Ewe (pre-retrieval)	Right	Medial	94%	100%	95%	96%	95%	**96±0.02%**	0.34	0.58	0.38	1.25	0.52	**0.61±0.37**
		Lateral	92%	88%	95%	95%	95%	**93±0.03%**	0.44	0.61	0.38	1.33	0.48	**0.65±0.39**
	Left	Medial	94%	96%	97%	94%	95%	**95±0.01%**	0.23	0.64	0.47	1.55	0.52	**0.68±0.51**
		Lateral	96%	88%	92%	96%	96%	**94±0.04%**	0.55	0.78	0.39	1.50	0.37	**0.72±0.47**
Ewe (post-UTx)	Right	Medial	**NA**	94%	77%	**NA**	94%	**88±0.10%**	**NA**	0.42	0.55	**NA**	0.63	**0.53±0.11**
		Lateral	**NA**	82%	77%	**NA**	87%	**82±0.05%**	**NA**	0.53	0.67	**NA**	0.93	**0.71±0.20**
	Left	Medial	**NA**	78%	86%	**NA**	94%	**86±0.08%**	**NA**	0.24	0.36	**NA**	1.63	**0.74±0.77**
		Lateral	**NA**	76%	90%	**NA**	90%	**85±0.08%**	**NA**	0.38	0.23	**NA**	1.31	**0.64±0.59**

Key: **NA,** Non applicable; **S.D,** Standard Deviation

Table 3.2a Oxygen Saturation and Perfusion Index values when measured with a pulse oximeter

Sheep	Side	Cornua	O_2 Saturation					Mean ± S.D.
			UTx 1	UTx 2	UTx 3	UTx 4	UTx 5	All UTx
Ewe (pre-retrieval)	Right	Medial	76%	63%	53%	74%	76%	68±0.10%
		Lateral	70%	54%	50%	73%	72%	64±0.11%
	Left	Medial	65%	67%	42%	64%	76%	63±0.13%
		Lateral	70%	60%	49%	69%	74%	64±0.10%
Ewe (post-UTx)	Right	Medial	**NA**	62%	72%	**NA**	60%	65±0.06%
		Lateral	**NA**	55%	71%	**NA**	61%	62±0.08%
	Left	Medial	**NA**	58%	66%	**NA**	58%	61±0.05%
		Lateral	**NA**	51%	74%	**NA**	61%	62±0.12%

Table 3.2b Oxygen Saturation values when measured with a multispectral imaging laparoscope

Figure 3.21a-c Variation in Oxygen Saturation and Perfusion Index values when measured with a pulse oximeter and a multispectral imaging laparoscope

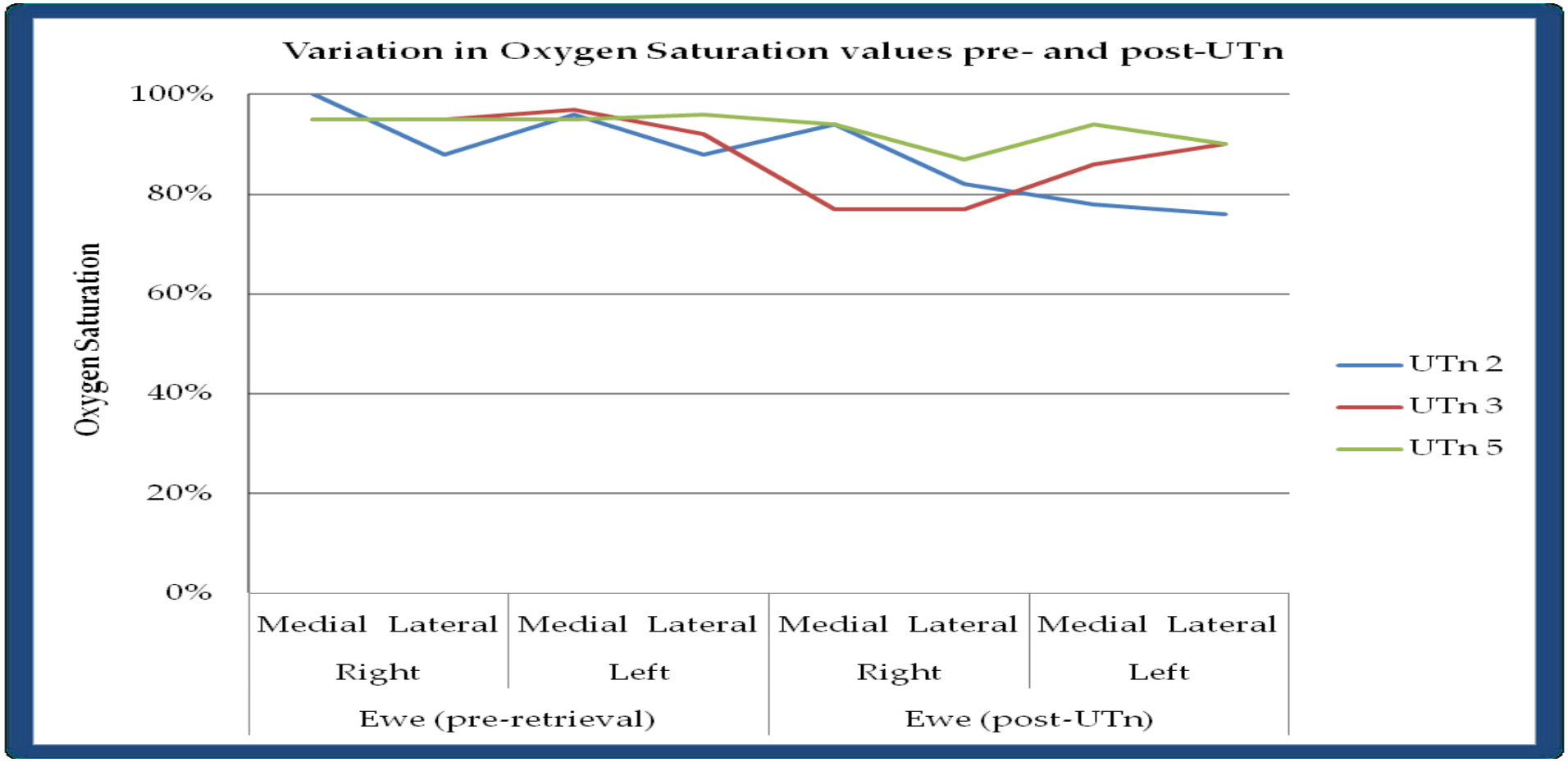


Figure 3.21a Oxygen Saturation values measured with a pulse oximeter

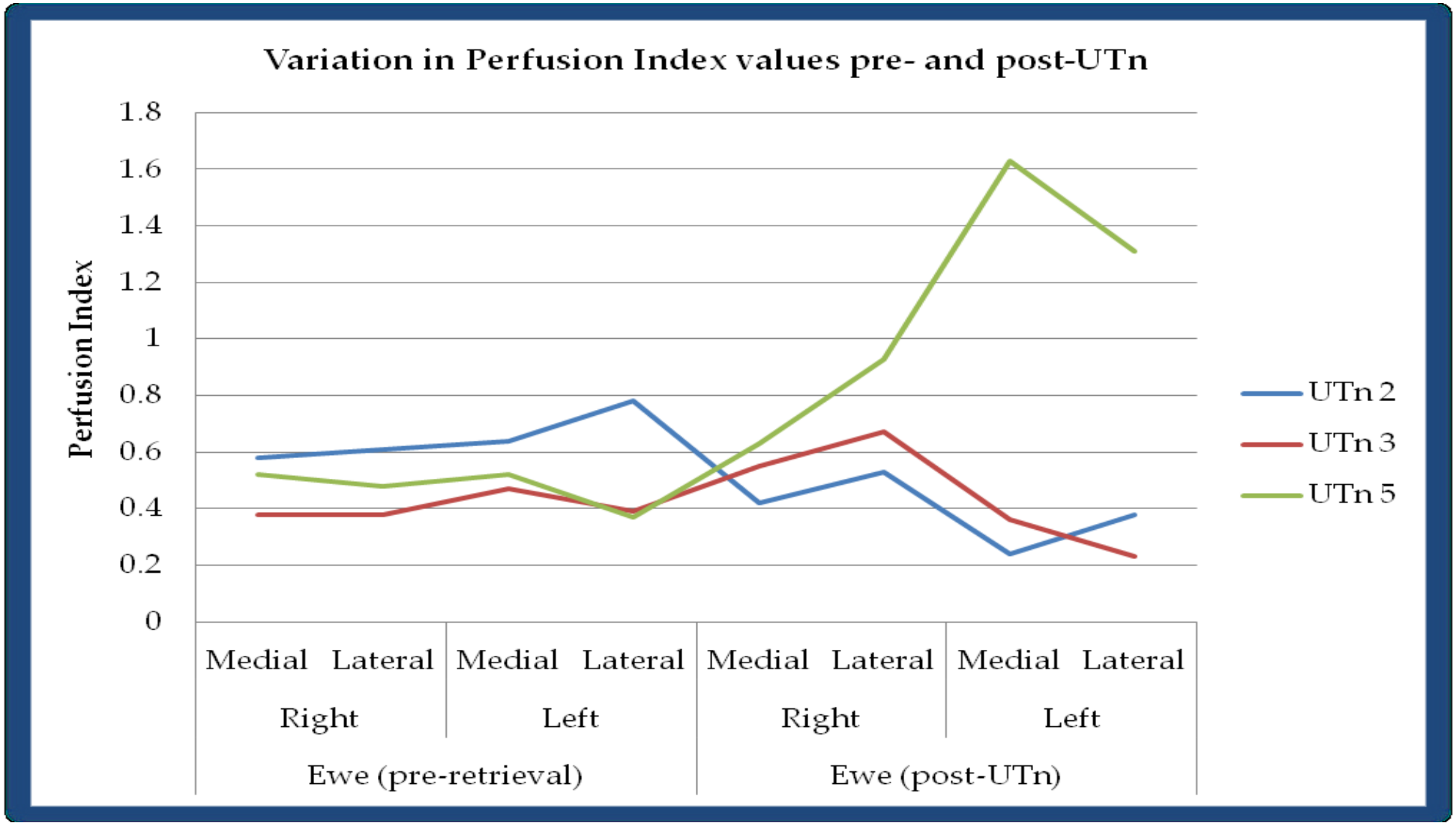


Figure 3.21b Perfusion index values measured with a pulse oximeter

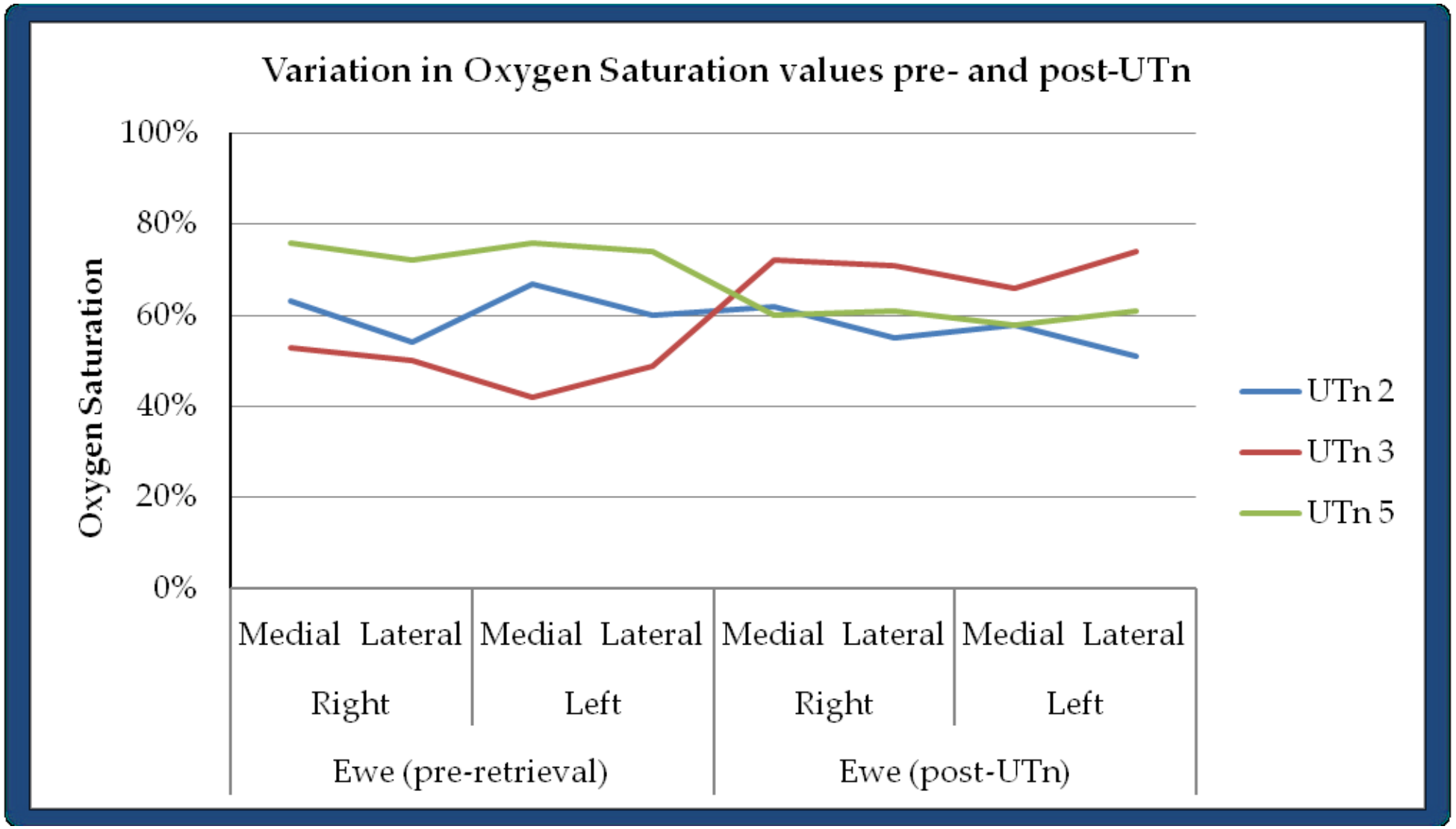
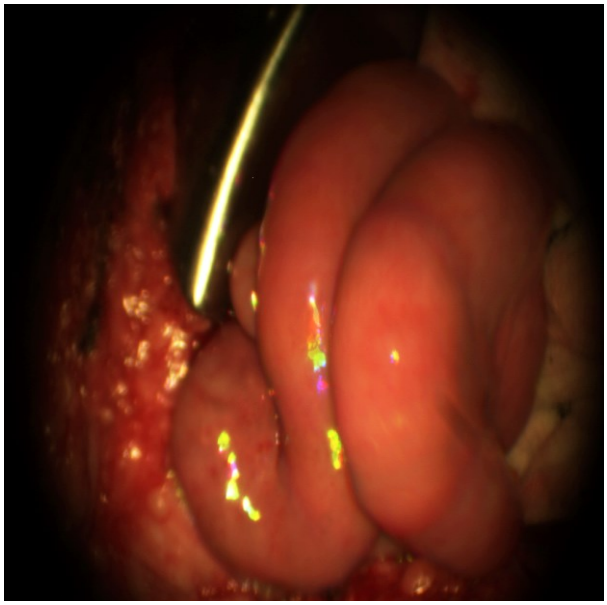


Figure 3.21c Oxygen Saturation values measured with a multispectral imaging laparoscope

Figure 3.22a-e The images on the left are standard images of the sheep cornua produced using a RGB filter. The images on the right are reference tissue O_2Sat images. The O_2Sat images are displayed using a colour scale that ranges from '0' (dark blue) to '1' (bright red).

Figure 3.22a Images correspond to UTx #1

Pre-retrieval



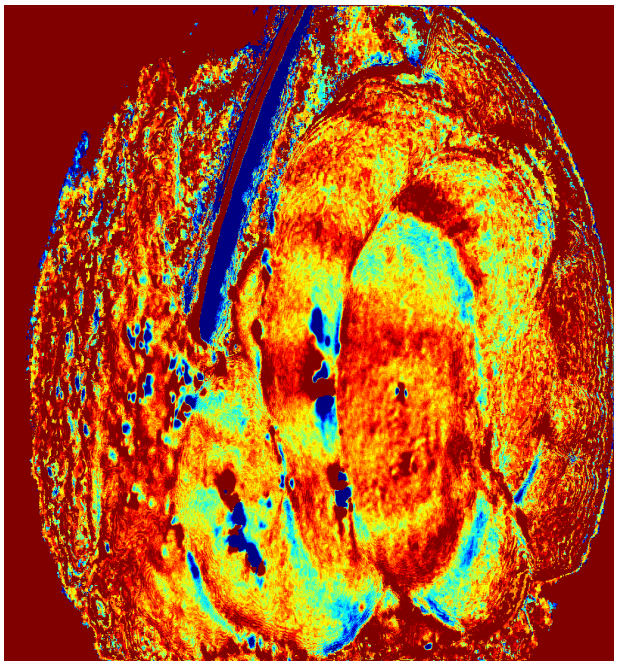
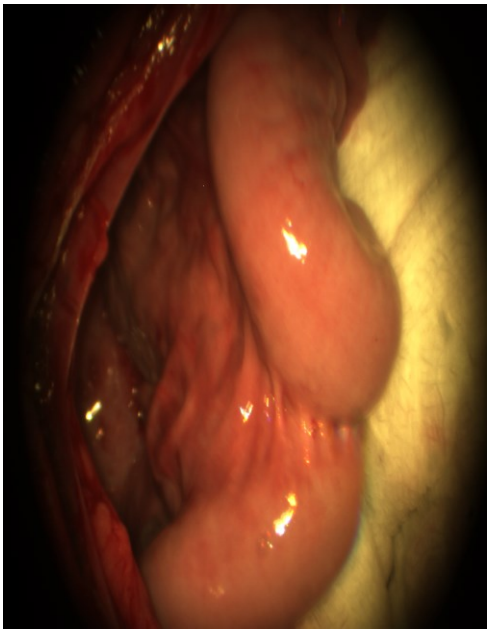
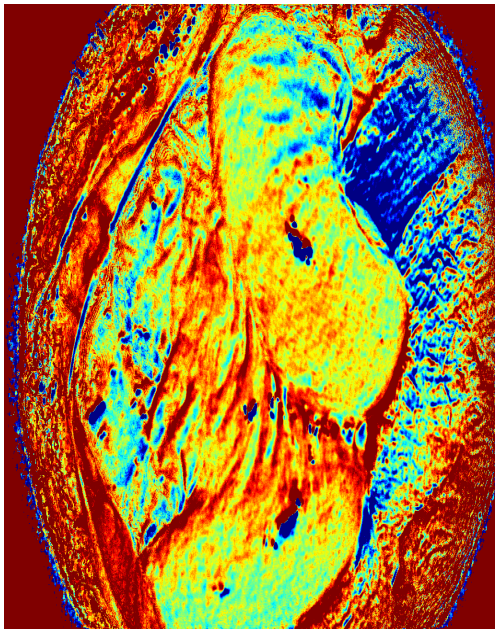


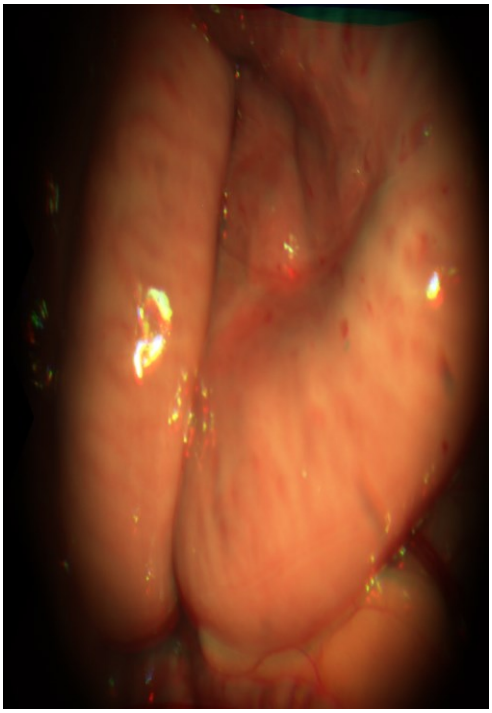
Figure 3.22b Images correspond to UTx #2

Pre-retrieval





Post-UTx



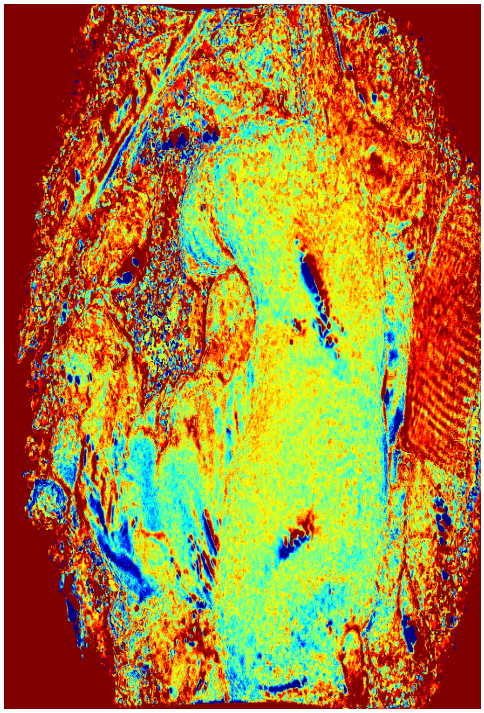
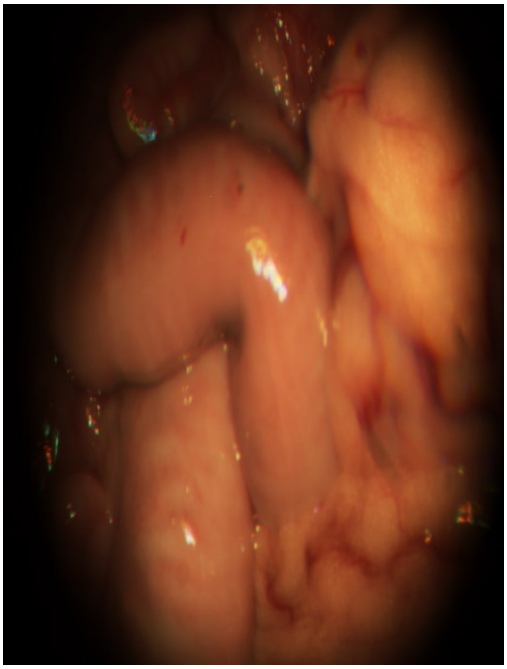
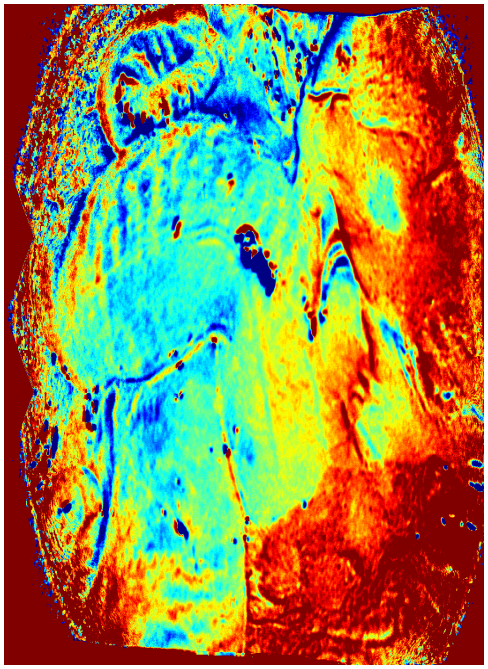


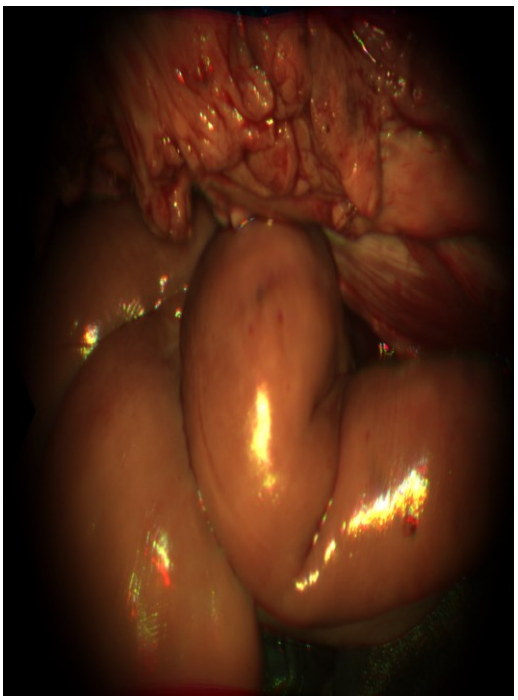
Figure 3.22c Images correspond to UTx #3

Pre-retrieval





Post-UTx



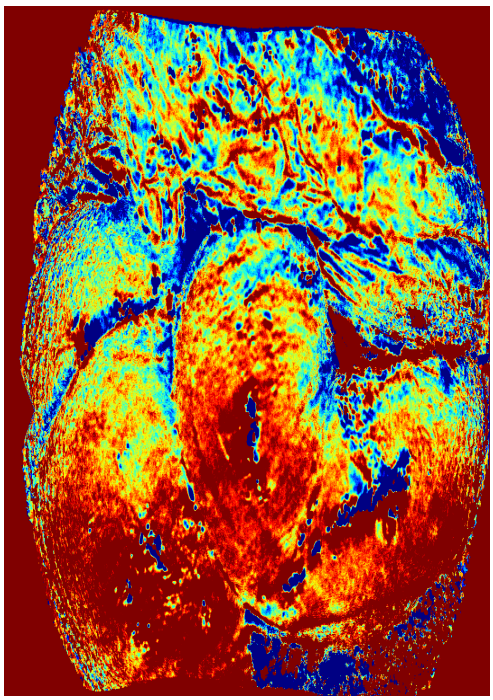
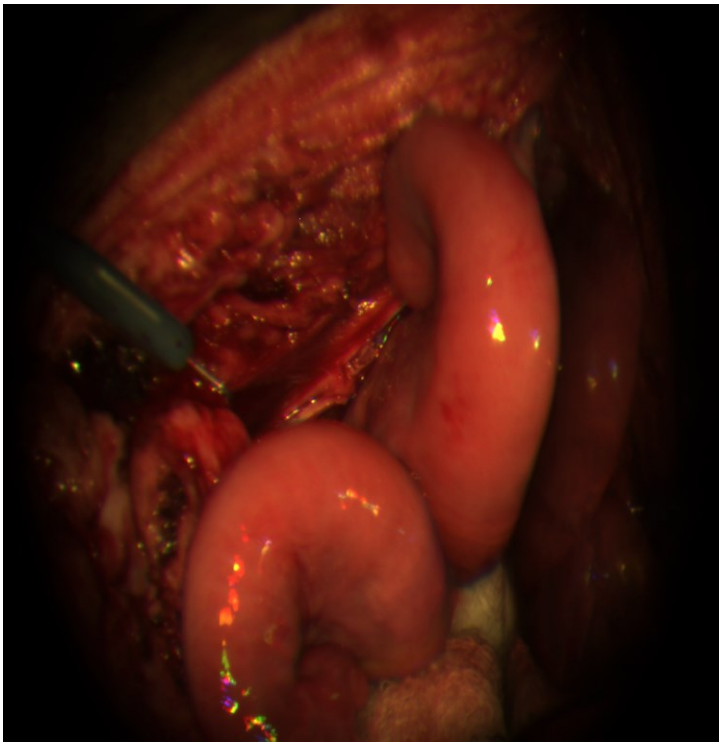


Figure 3.22d Images correspond to UTx #4

Pre-retrieval



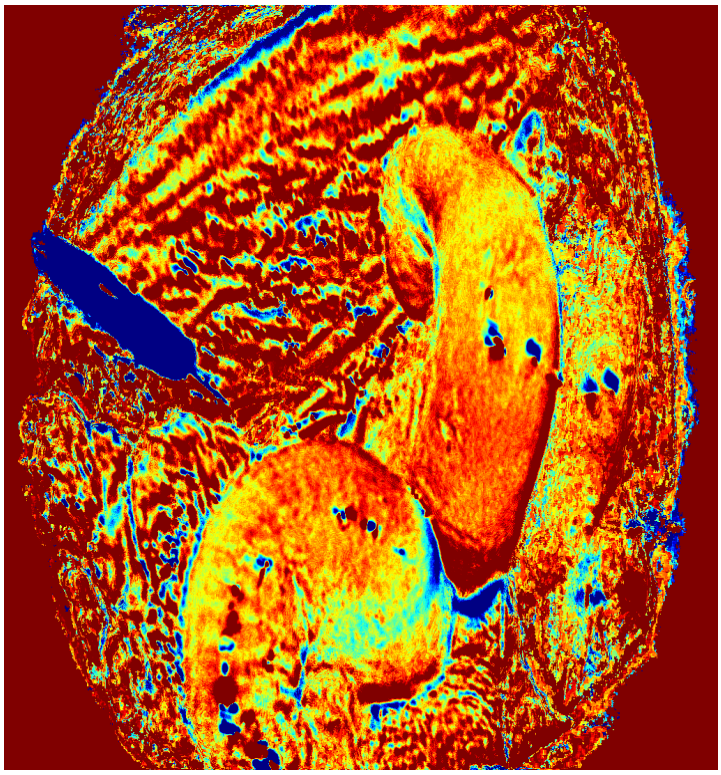
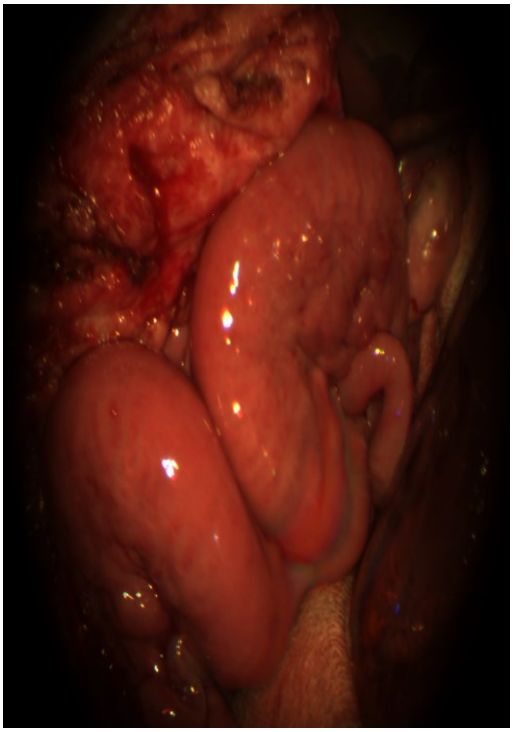
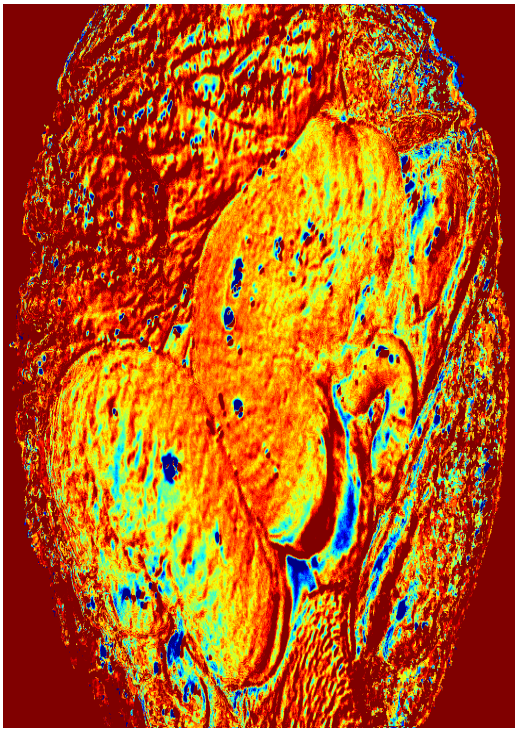


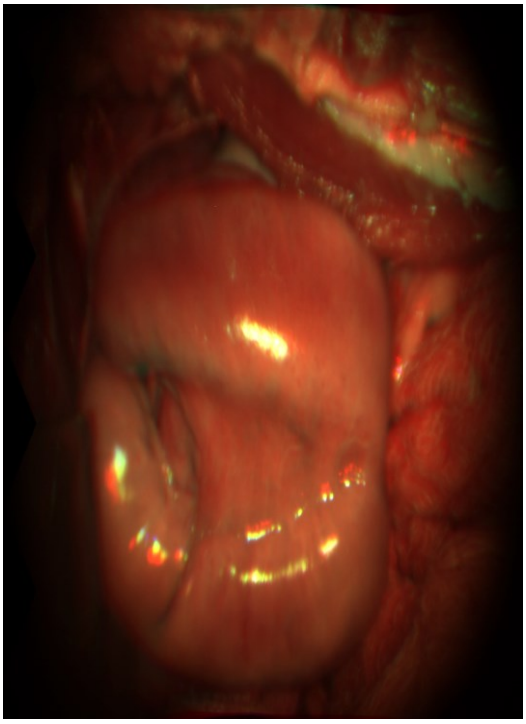
Figure 3.22e Images correspond to UTx #5

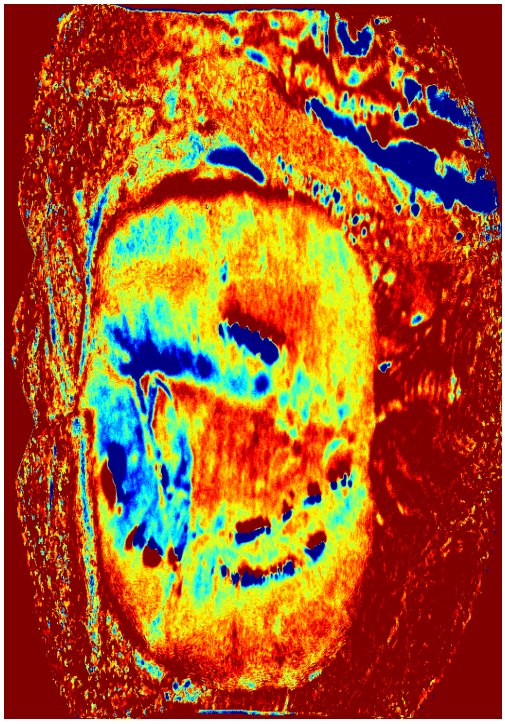
Pre-retrieval





Post-UTx





eLASCA

Sheep model (UTx #2-#5)

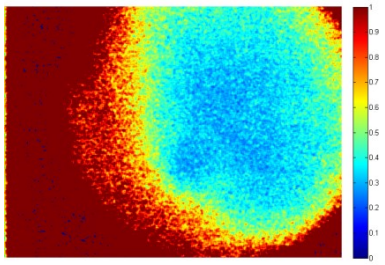
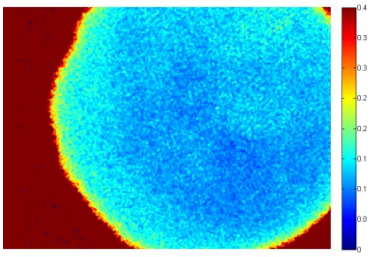
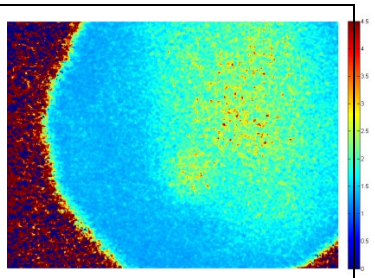
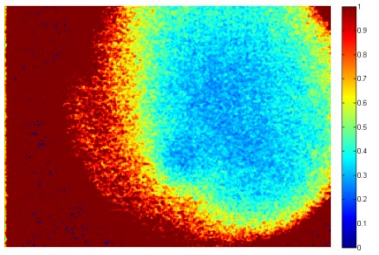
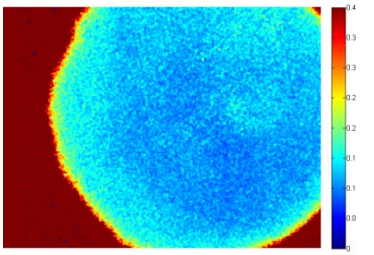
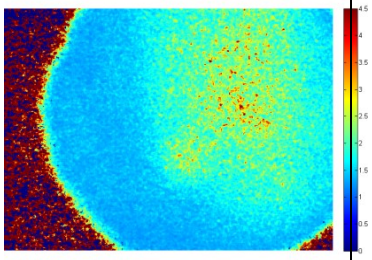
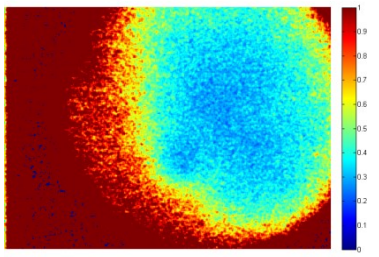
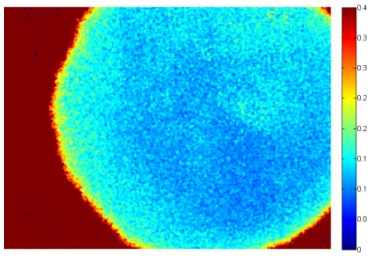
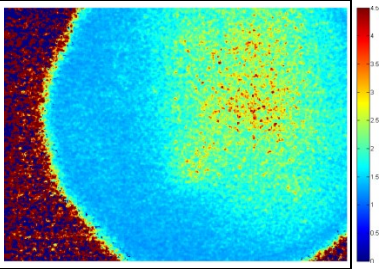
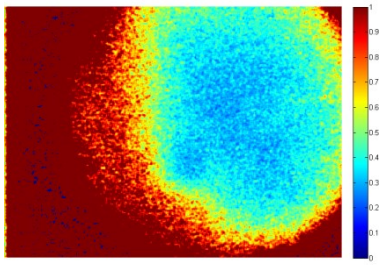
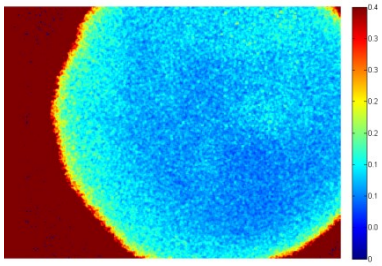
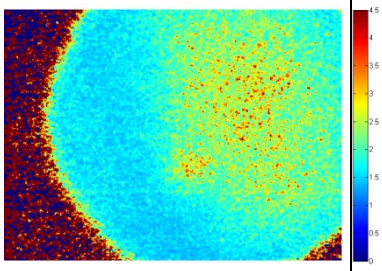
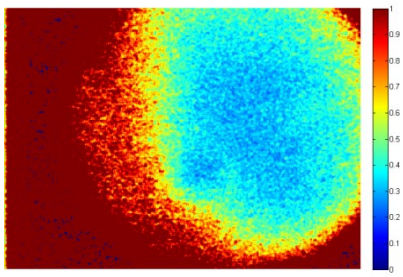
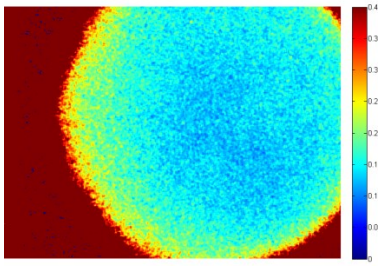
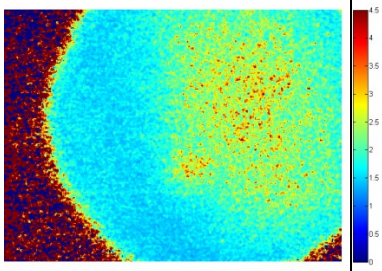
Sheep experiment - UTx #2

Pre-retrieval. Acquisition time: 1ms and delay time: 100ms

The exposure time of the camera is 1ms. The delay corresponds to the time period from the moment that the camera is shut to the moment that the programme gives a signal to switch on the laser controller and thus open the camera for the next acquisition. The time period from one picture to the other is ~100ms. As the exposure time alternates from 660nm to 830nm the acquisition for a single wavelength is 200ms, which corresponds to 5 fps. The acquisition for both wavelengths is 10fps.

Figure 3.23 Image acquisition pre-retrieval. The Speckle Contrast images (C660 and C830) demonstrate blood circulation, which is depicted by the blue areas. The lower the contrast (a bigger area in blue), the faster is the blood flow. The oxygenation image is ratio-metrically calculated by the images at wavelengths of 660nm and 830nm based on the difference between absorption coefficients of the tissue chromophores. Blood circulation is indicated by the colour red. These results are qualitative and show that the speckle contrast images are a good indicator of blood flow.

One full set of acquired images is shown below. With respect to the other transplants, only the best acquired image is presented here.

		
C660_fr40	C830_fr40	Oxygenation image_fr40
		
C660_fr41	C830_fr41	Oxygenation image _fr41
		
C660_fr42	C830_fr42	Oxygenation image _fr42
		
C660_fr43	C830_fr43	Oxygenation image _fr43
		
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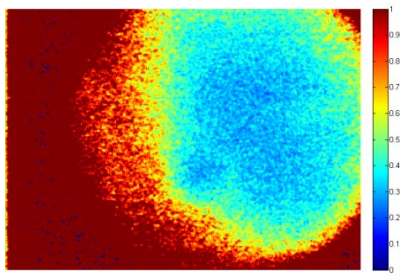
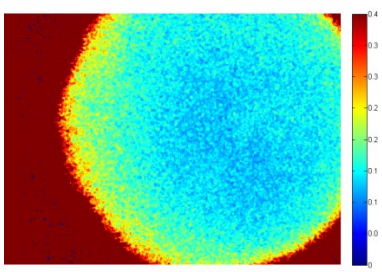
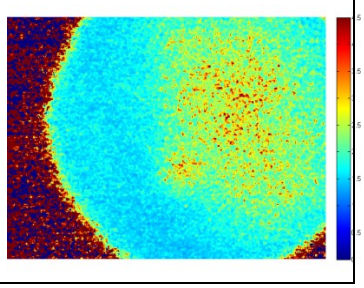
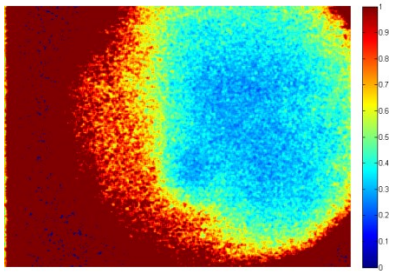
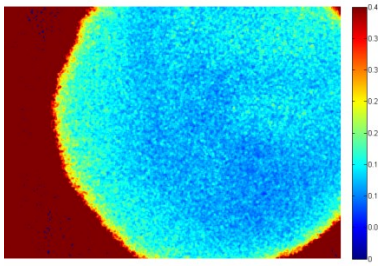
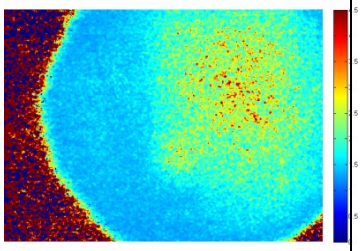
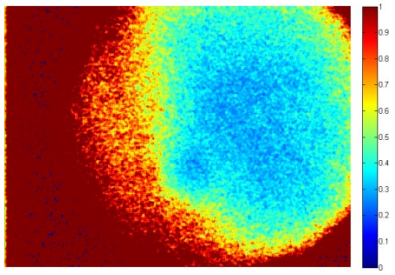
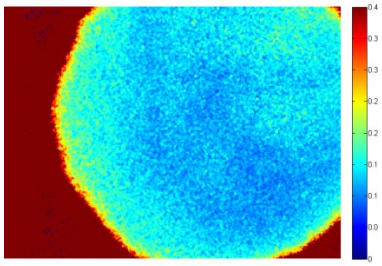
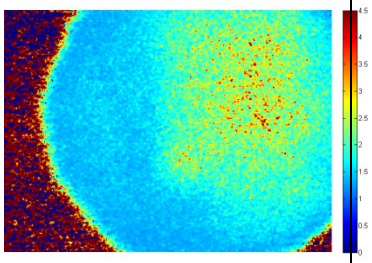
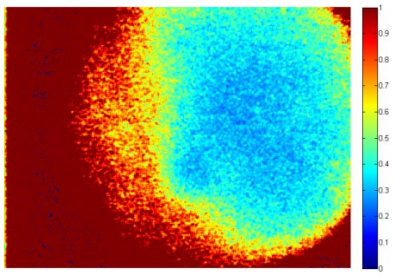
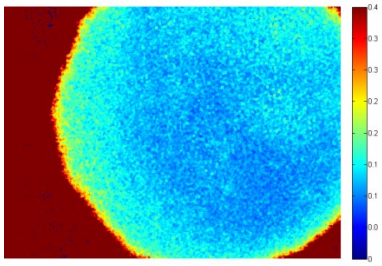
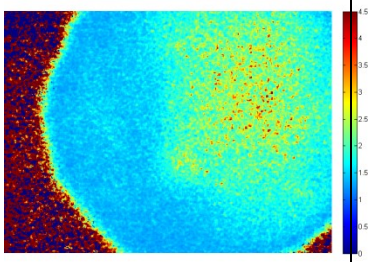
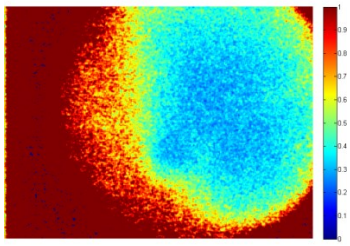
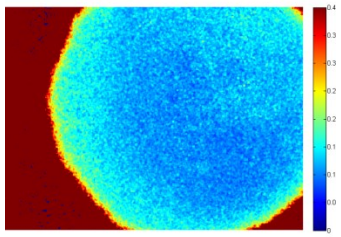
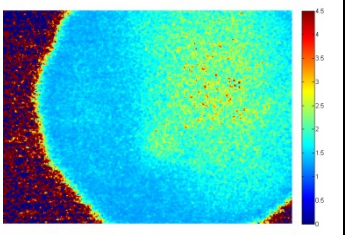
		

C660_fr45	C830_fr45	Oxygenation image _fr45
		
C660_fr46	C830_fr46	Oxygenation image _fr46
		
C660_fr47	C830_fr47	Oxygenation image _fr47
		
C660_fr48	C830_fr48	Oxygenation image _fr48
		
C660_fr49	C830_fr49	Oxygenation image _fr49

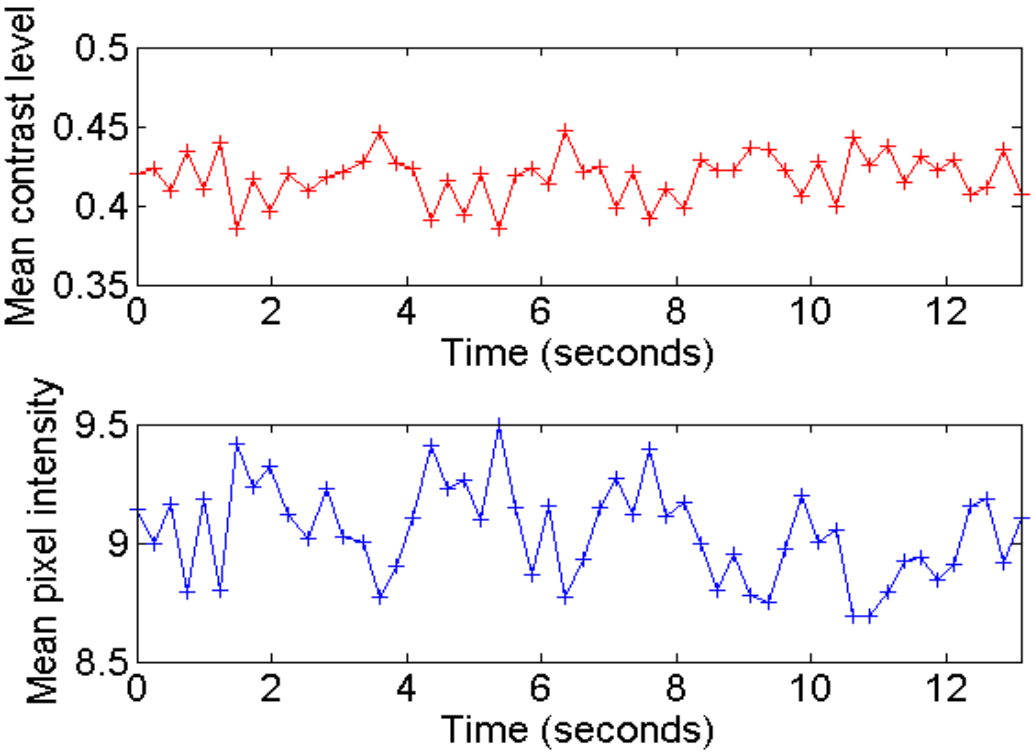


Figure 3.24 The mean intensity and mean contrast calculated for every image of the time sequence during the acquisition at 660nm. It shows that the contrast is inversely proportional to the intensity.

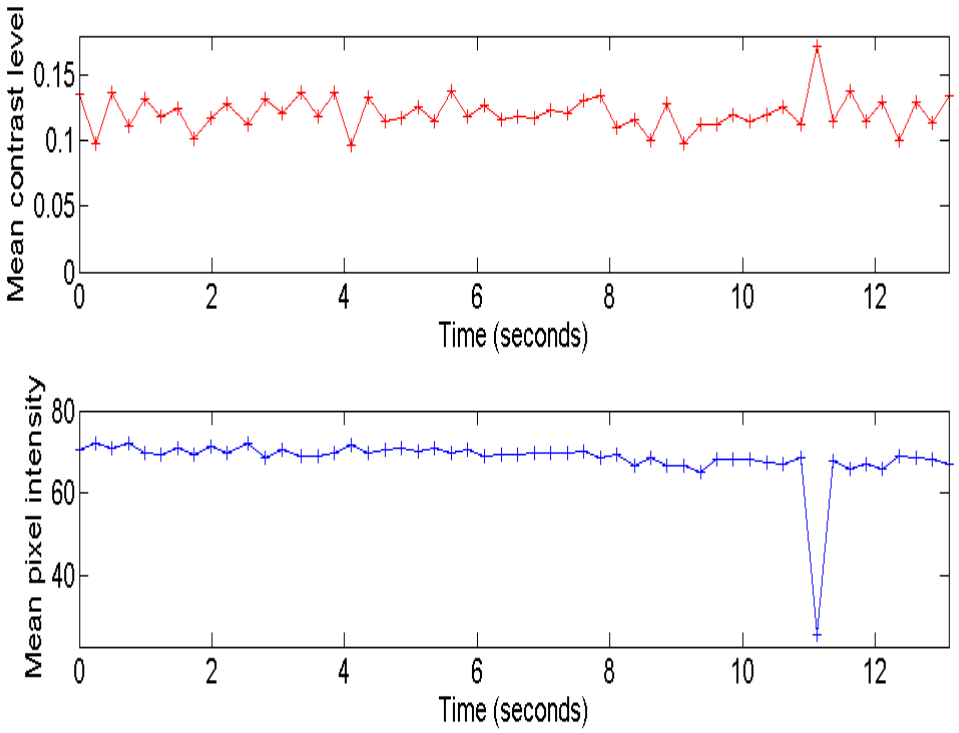


Figure 3.25 The mean intensity and the mean contrast calculated for every image of the time sequence during the acquisition at 830nm

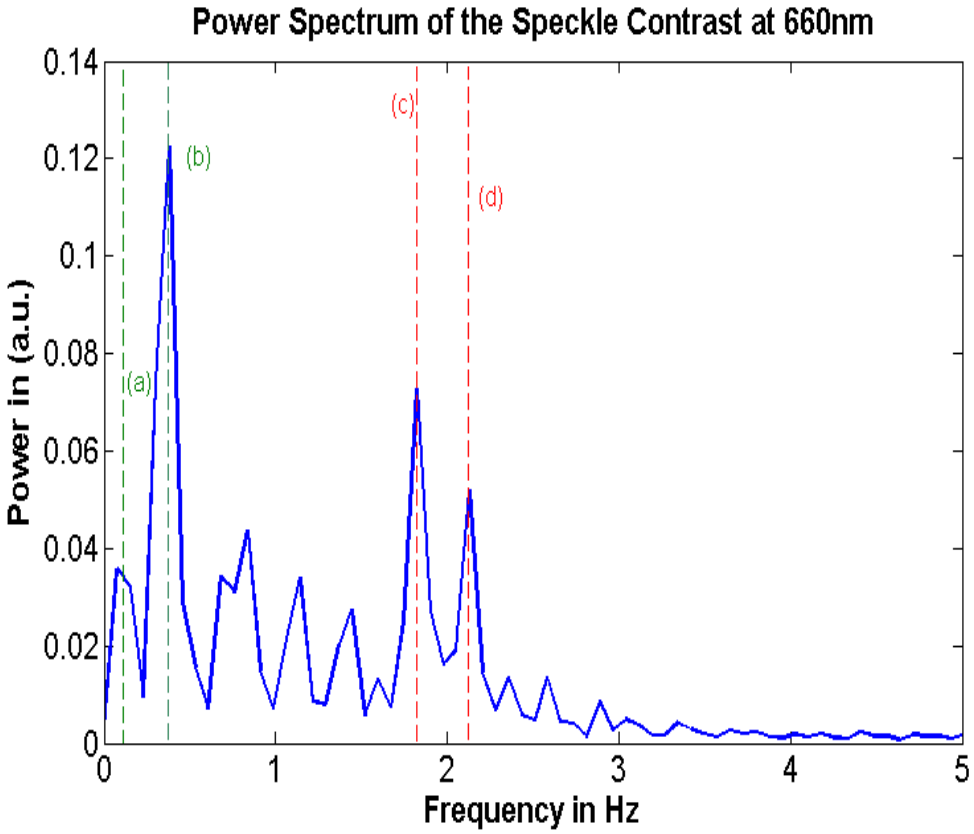


Figure 3.26 Intensity distribution of the various frequencies of Speckle Contrast at 660nm

a) 0.13Hz attributed to a breathing rate of 7.8 breaths per min
b) 0.38Hz attributed to a breathing rate of 22.8 breaths per min or to any vibration of the system
c) 1.83Hz attributed to a cardiac rate of 109bpm
d) 2.13Hz attributed to a cardiac rate of 127bpm

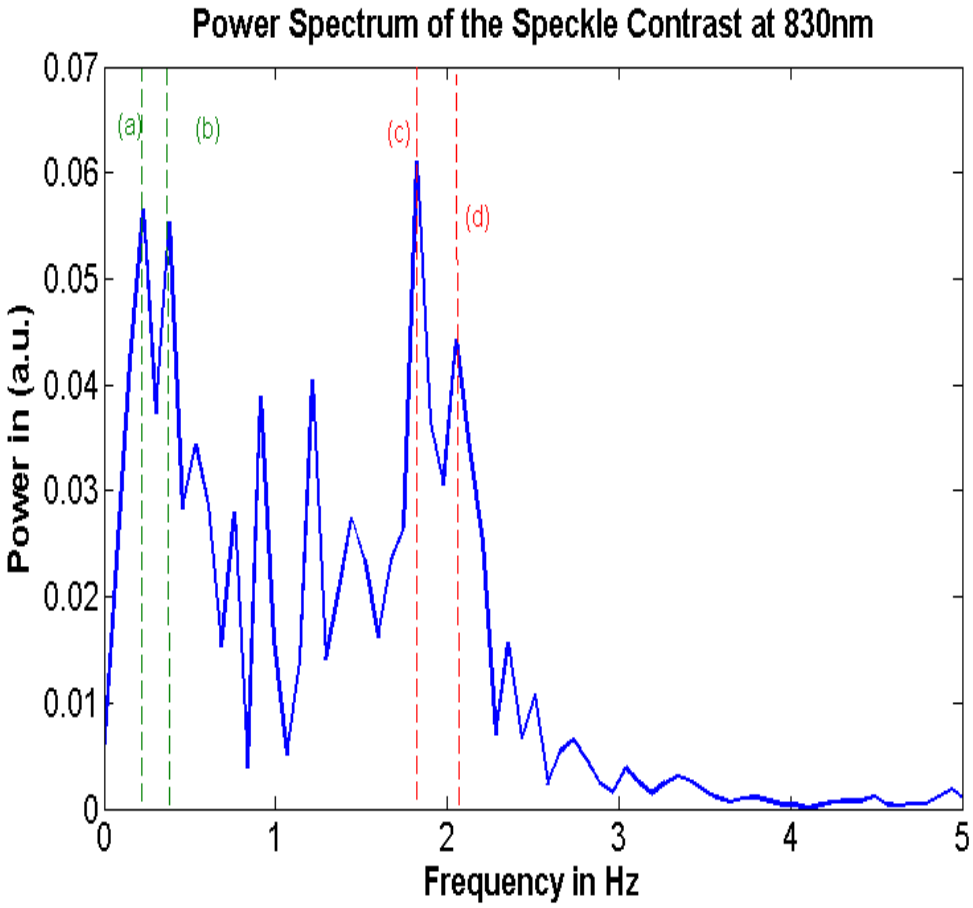


Figure 3.27 Intensity distribution of the various frequencies of Speckle Contrast at 830nm

a) 0.23Hz attributed to a breathing rate of 13.8 breaths per min
b) 0.38Hz attributed to a breathing rate of 22.8 breaths per min or to any vibration of the system
c) 1.82Hz attributed to a cardiac rate of 109.2bpm
d) 2.06Hz attributed to a cardiac rate of 123.6bpm

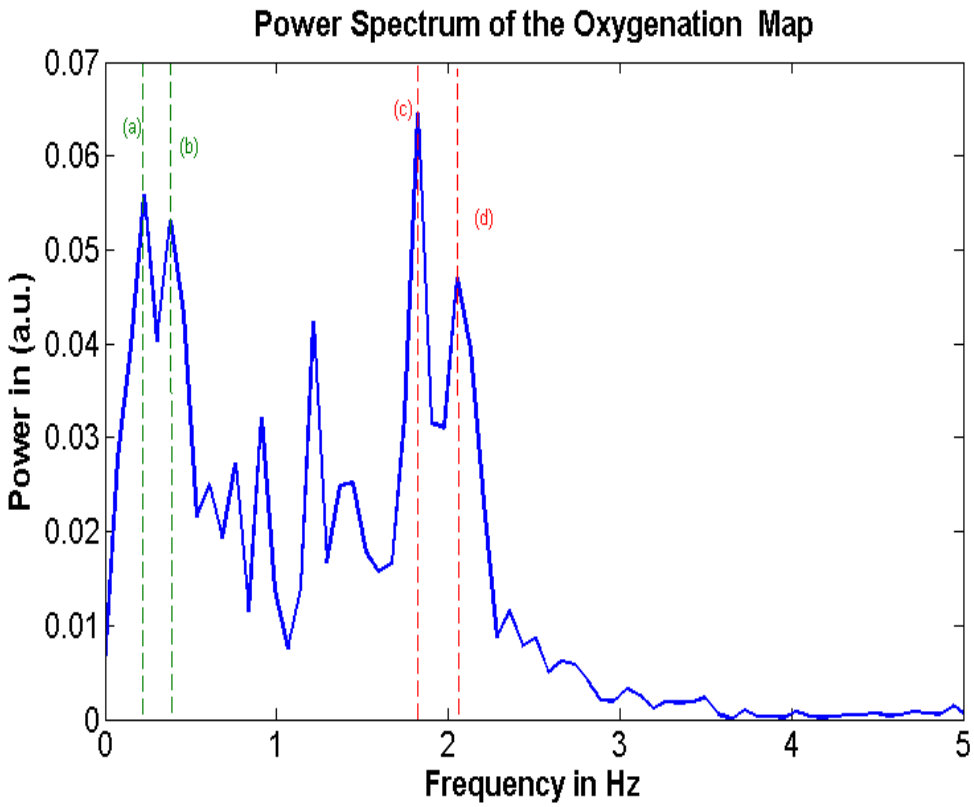
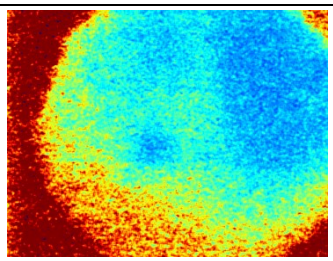
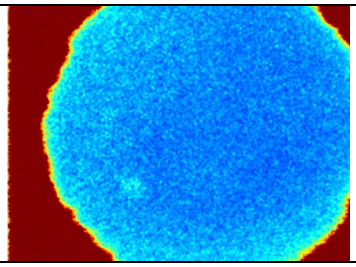
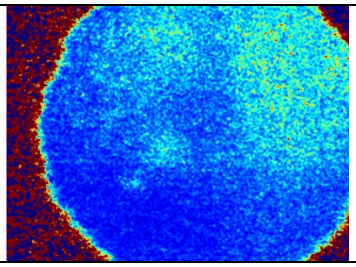
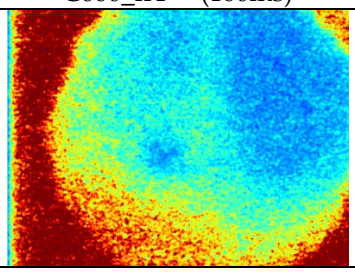
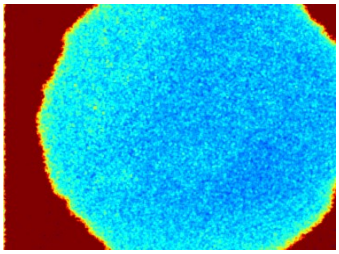
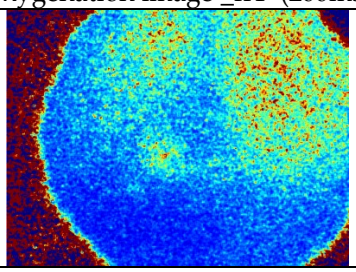
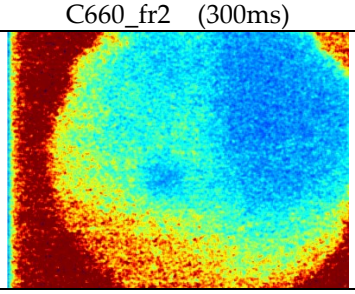
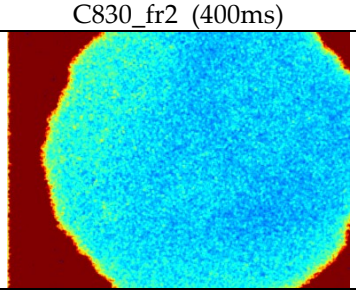
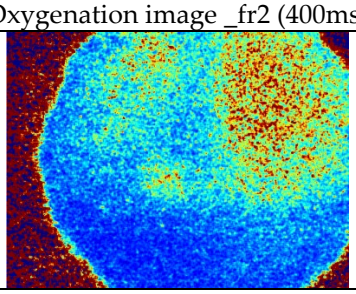
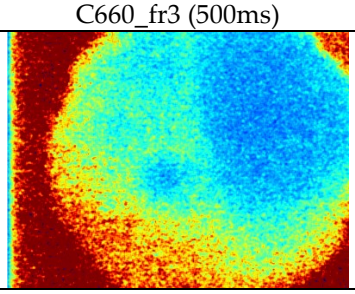
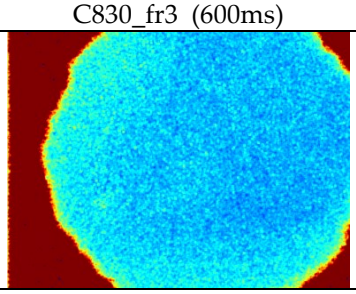
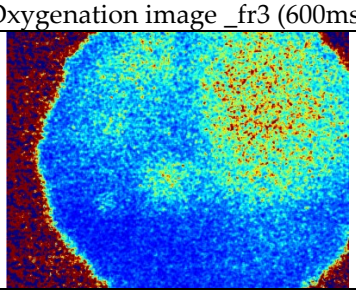
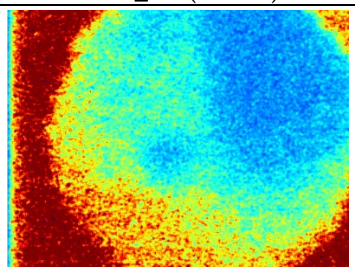
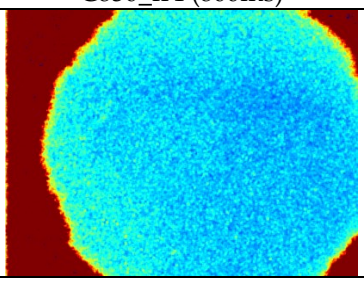
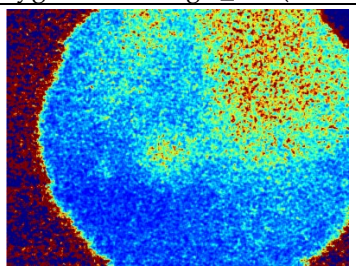


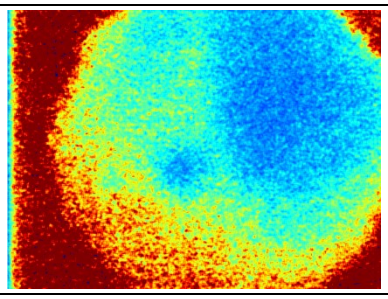
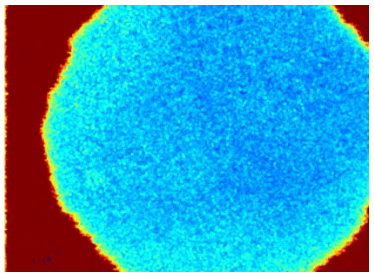
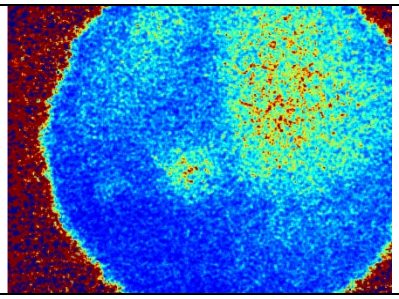
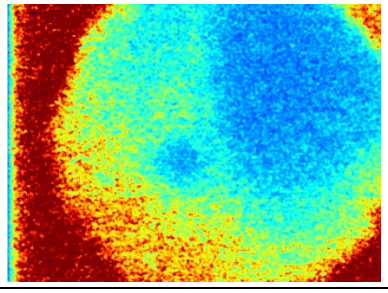
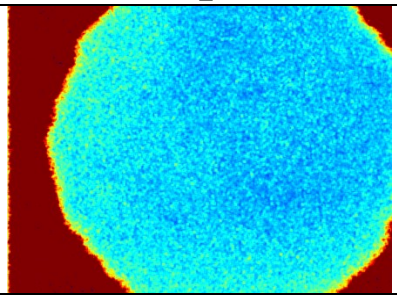
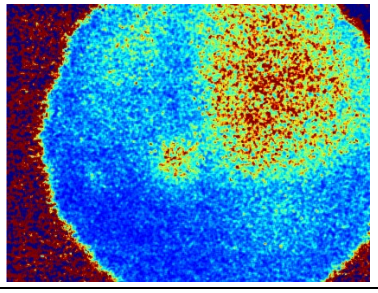
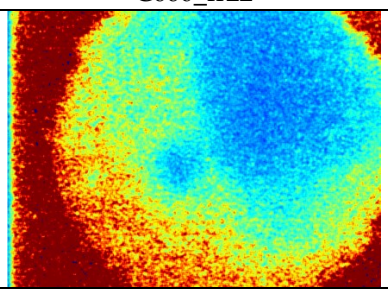
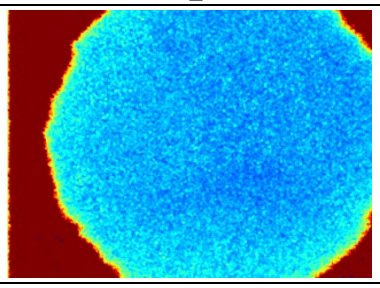
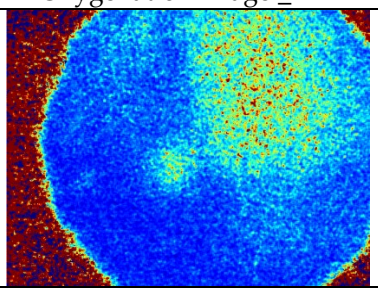
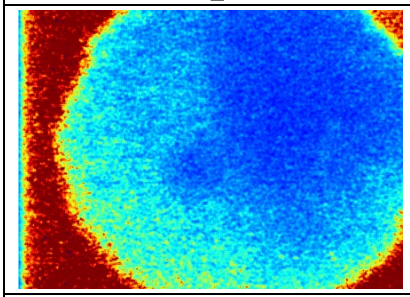
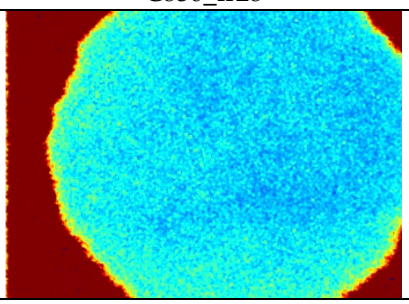
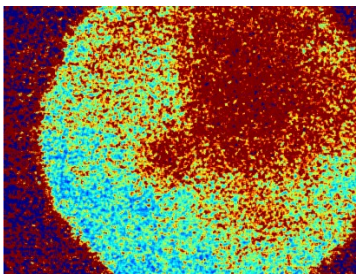
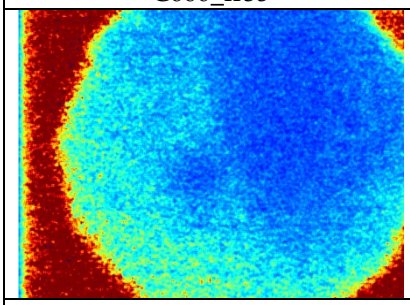
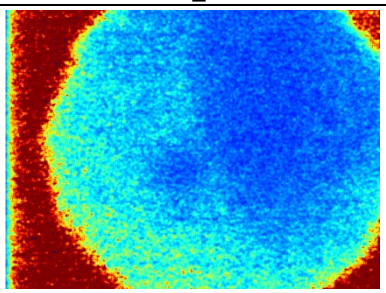
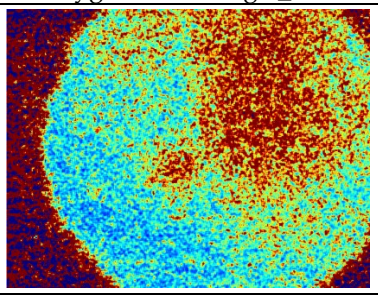
Figure 3.28 Intensity distribution of the various frequencies of the Oxygenation map

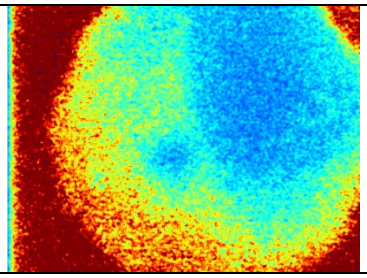
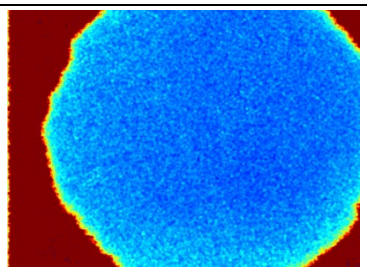
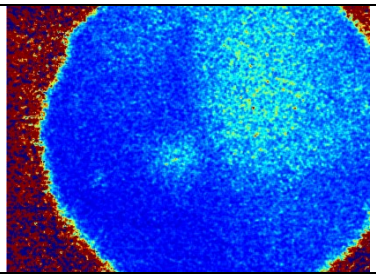
a) 0.23Hz attributed to a breathing rate of 13.8 breaths per min
b) 0.38Hz attributed to a breathing rate of 22.8 breaths per min or to any vibration of the system
c) 1.815Hz attributed to a cardiac rate of 109bpm
d) 2.08Hz attributed to a cardiac rate of 123.6bpm

Post-transplantation Acquisition time: 1ms and delay time: 100ms

Figure 3.29 Image acquisition post-transplantation

		
C660_fr1 (100ms)	C830_fr1 (200ms)	Oxygenation image _fr1 (200ms)
		
C660_fr2 (300ms)	C830_fr2 (400ms)	Oxygenation image _fr2 (400ms)
		
C660_fr3 (500ms)	C830_fr3 (600ms)	Oxygenation image _fr3 (600ms)
		
C660_fr4 (700ms)	C830_fr4 (800ms)	Oxygenation image _fr4 (800ms)
		
C660_fr6	C830_fr6	Oxygenation image _fr6 1200ms

		
C660_fr11	C830_fr11	Oxygenation image _fr11
		
C660_fr22	C830_fr22	Oxygenation image _fr22
		
C660_fr28	C830_fr28	Oxygenation image _fr28
		
C660_fr35	C830_fr35	Oxygenation image _fr35
		
C660_fr36	C830_fr36	Oxygenation image _fr36

		
C660_fr37	C830_fr37	Oxygenation image _fr37

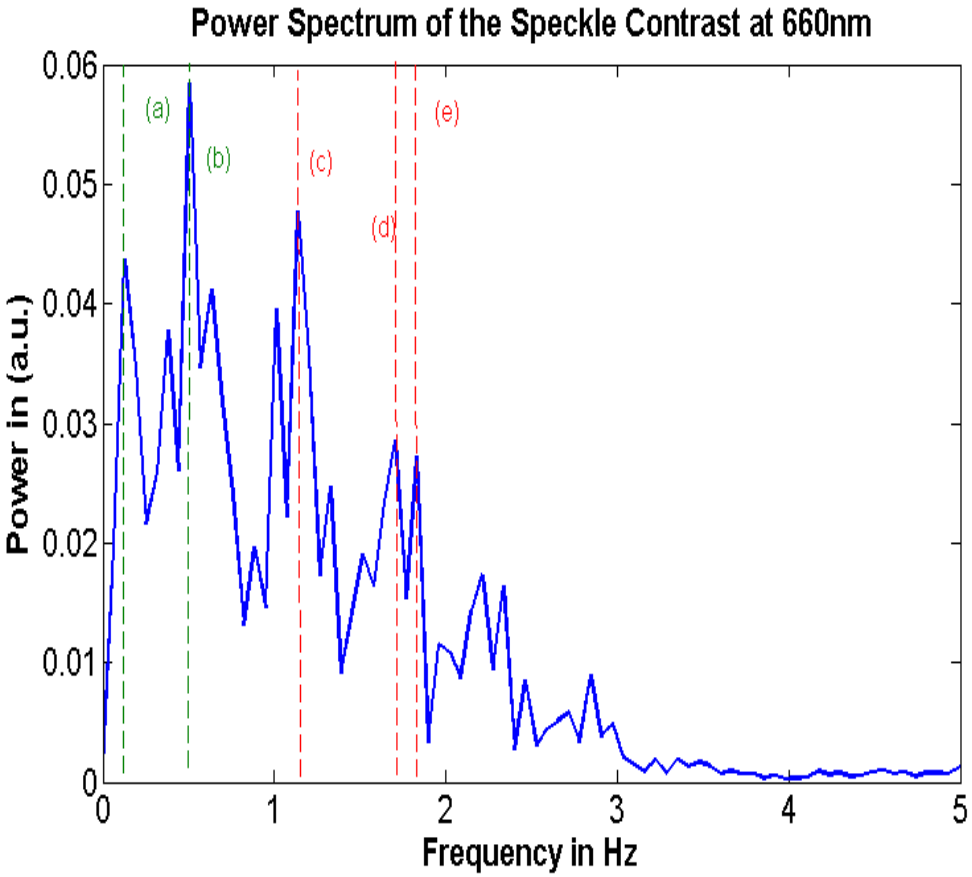


Figure 3.30 Intensity distribution of the various frequencies of Speckle Contrast at 660nm.

(a) 0.13Hz attributed to a breathing rate of 7.8 breaths per min
(b) 0.50Hz attributed to a breathing rate of 22.8breaths per min or to any vibration of the system
(c) 1.15Hz attributed to a cardiac rate of 69bpm
(d) 1.70Hz attributed to a cardiac rate of 102bpm
(e) 1.82Hz attributed to a cardiac rate of 109bpm

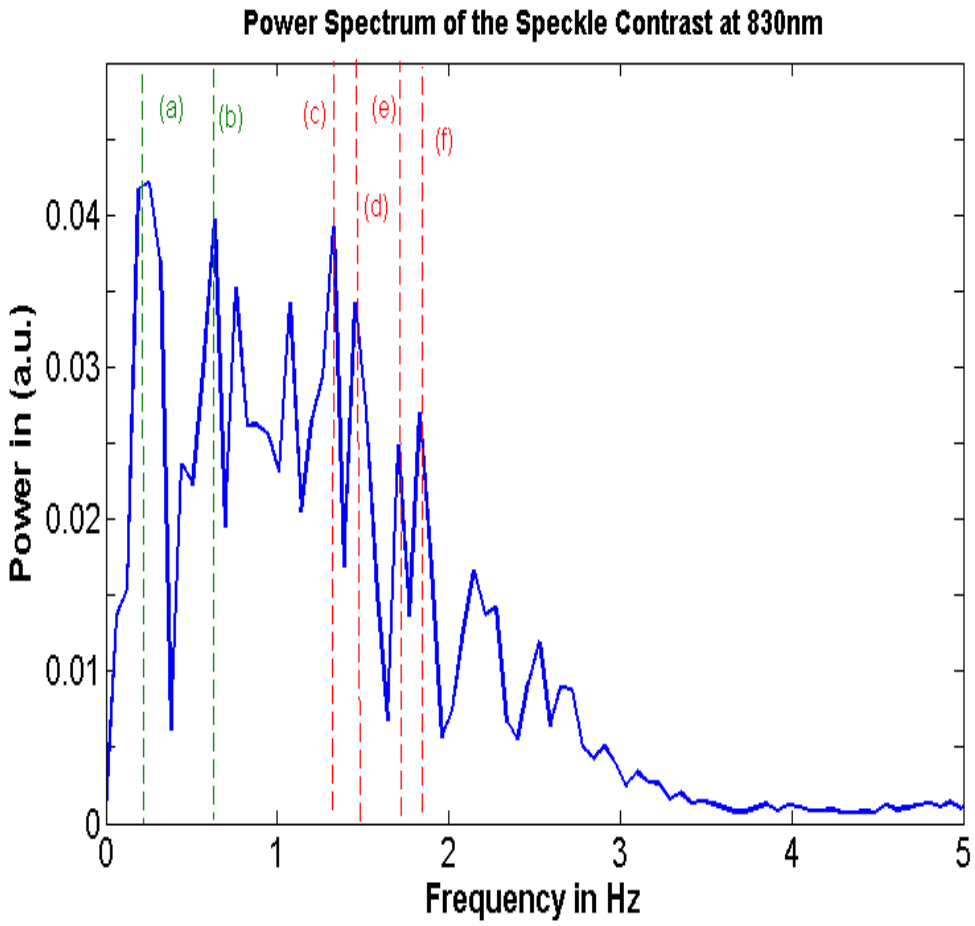


Figure 3.31 Intensity distribution of the various frequencies of the Speckle Contrast at 660nm

(a) 0.24Hz attributed to a breathing rate of 14.4breaths per min
(b) 0.63Hz attributed to a breathing rate of 37 breaths per min or to any vibration of the system
(c) 1.33Hz attributed to a cardiac rate of 79.8bpm
(d) 1.46Hz attributed to a cardiac rate of 87.6bpm
(e) 1.71Hz attributed to a cardiac rate of 102.61.84Hz attributed to a cardiac rate of 110.4bpm

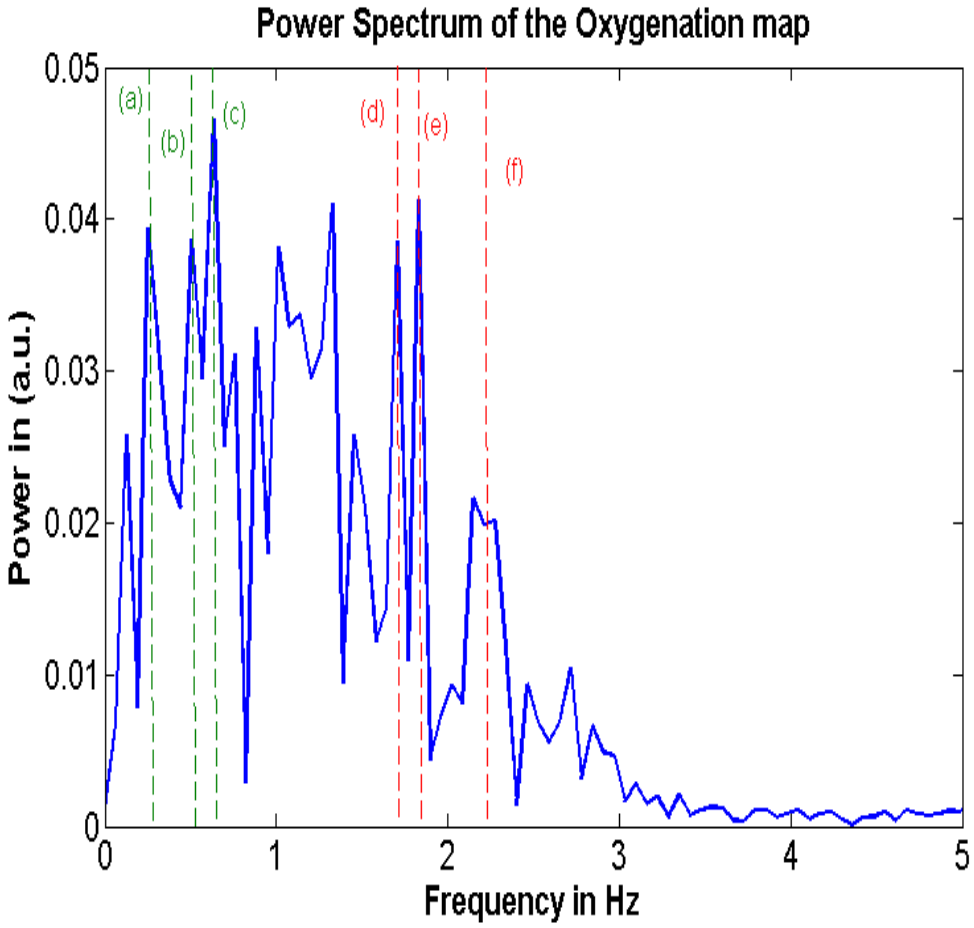
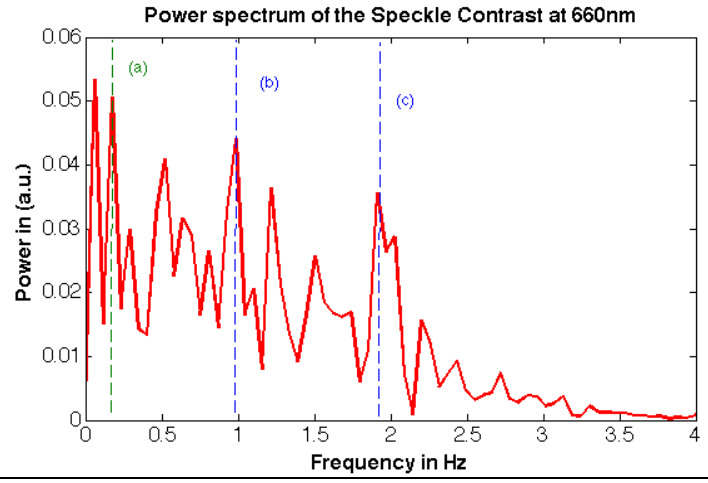


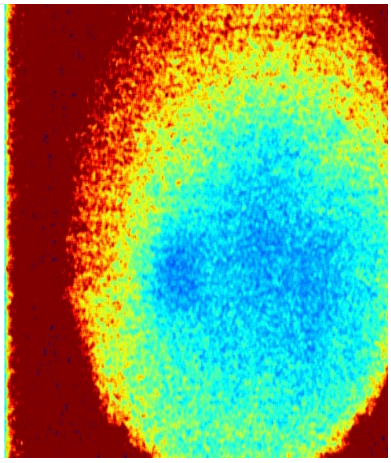
Figure 3.32 Intensity distribution of the various frequencies of Speckle Contrast of the Oxygenation map

(a) 0.27Hz attributed to a breathing rate of 16.2 breaths per min
(b) 0.51Hz attributed to a breathing rate of 30.6 breaths per min or to any vibration of the system
(c) 0.62Hz attributed to a cardiac rate of 37.2bpm
(d) 1.72Hz attributed to a cardiac rate of 103.2bpm
(e) 1.81Hz attributed to a cardiac rate of 108.6bpm
(f) 2.22Hz attributed to a cardiac rate of 133.2bpm

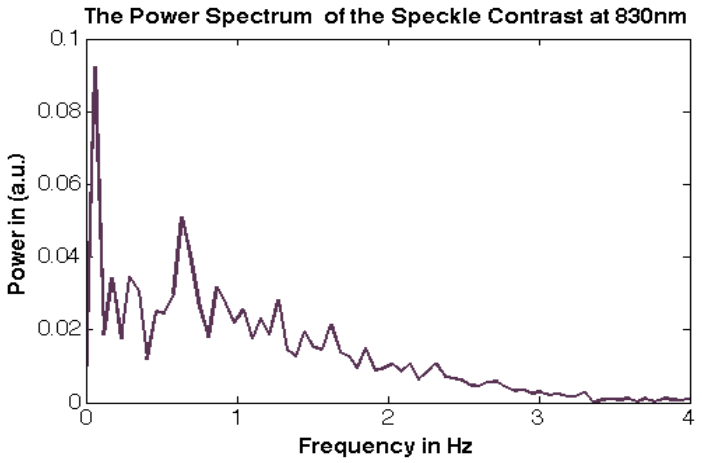
Sheep experiment - UTx #3

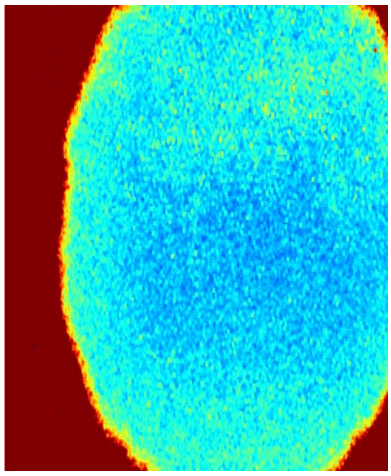
Exposure time: 1ms; Delay: 100ms; Frame rate: 5frps for a single wavelength and 10frps for two wavelengths



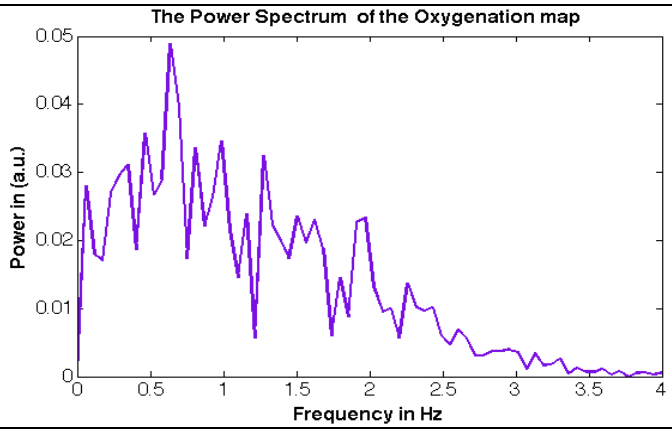


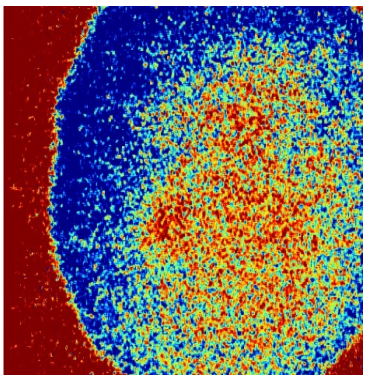
C660__Frame17





C830_ Frame17





Oxygenation image _Frame17

660nm: peak at (a) 0.17Hz corresponding to a respiratory rate of 10 breaths per minute; peaks also at (b) 1Hz and (c) 1.93Hz corresponding to heart rates of 60bpm and 116bpm respectively. 830nm: nil significant. Oxygenation map frequencies: peak at 2Hz corresponding to 120bpm.

Figure 3.33 Pre-retrieval Speckle Contrast results (UTx #3)

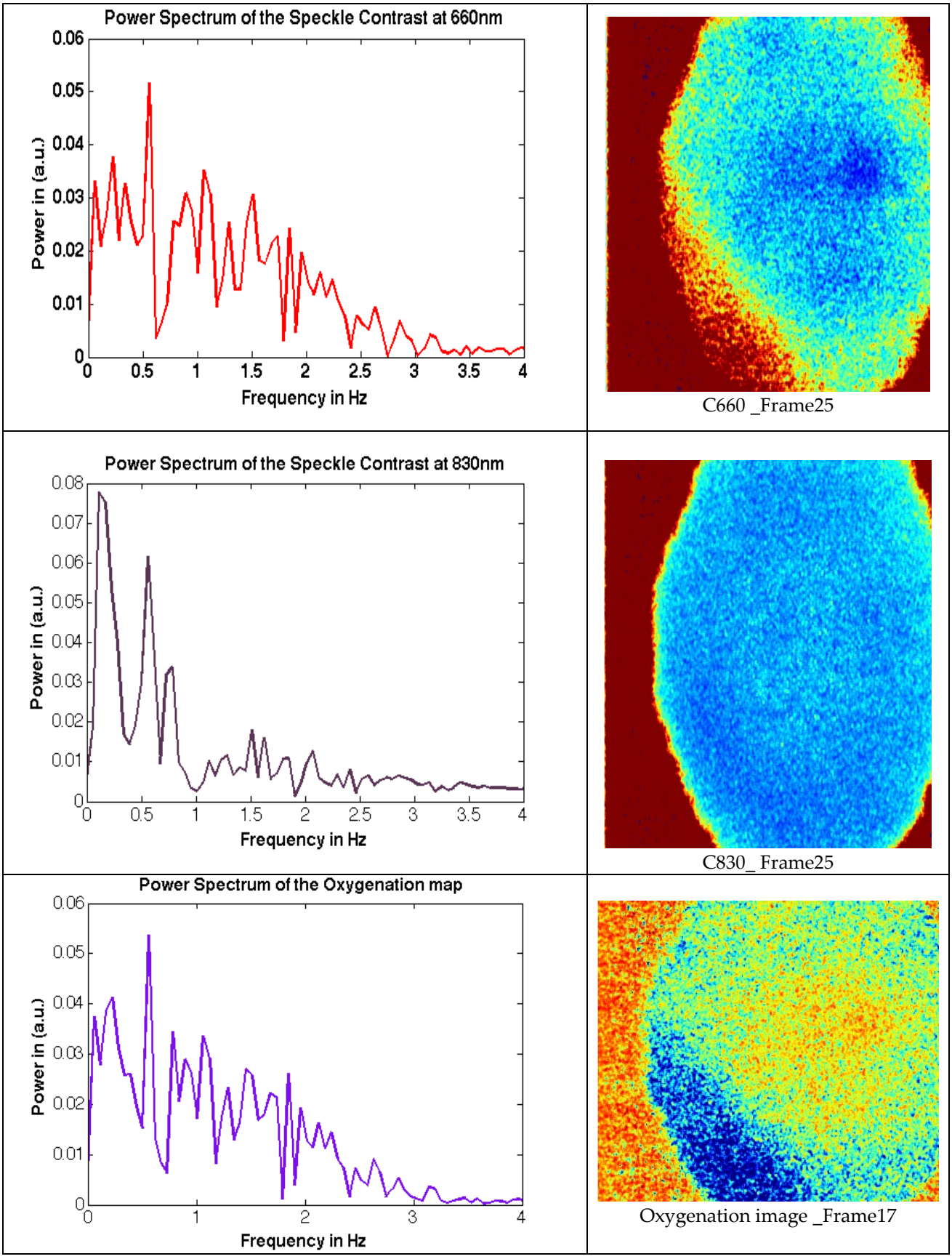


Figure 3.34 Post-transplant Speckle Contrast results (UTx #3)

The following set of data is recorded with illumination set at 660nm, exposure time at 1ms and delay at 40ms. This corresponds to acquisition frame rate of 25fps. The faster recording allows better sampling, therefore more precise frequencies measurements.

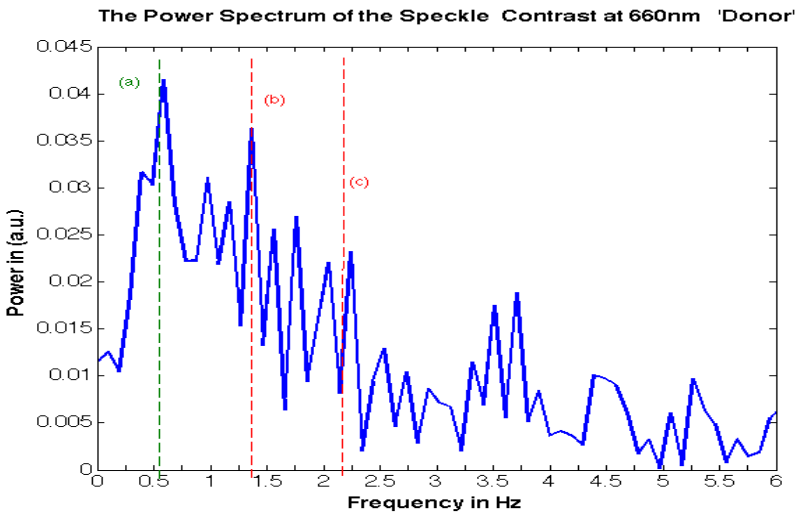


Figure 3.35 Power spectrum of the frequencies of the speckle (pre-retrieval, 'donor'). Peaks can be seen at: (a) 0.51Hz corresponding to a respiratory rate of 16 breaths per minute; (b) 1.24Hz corresponding to a heart rate of 75bpm; and (c) 2.12Hz corresponding to a heart rate of 135bpm.

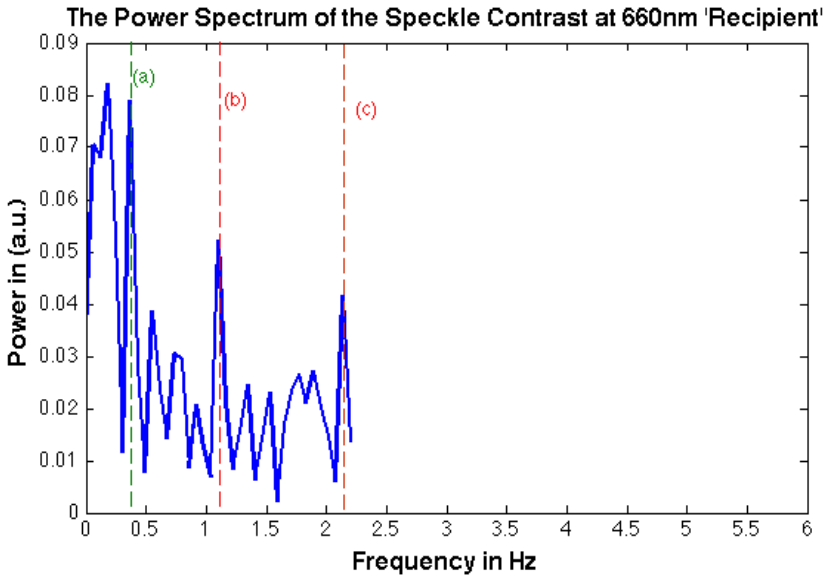
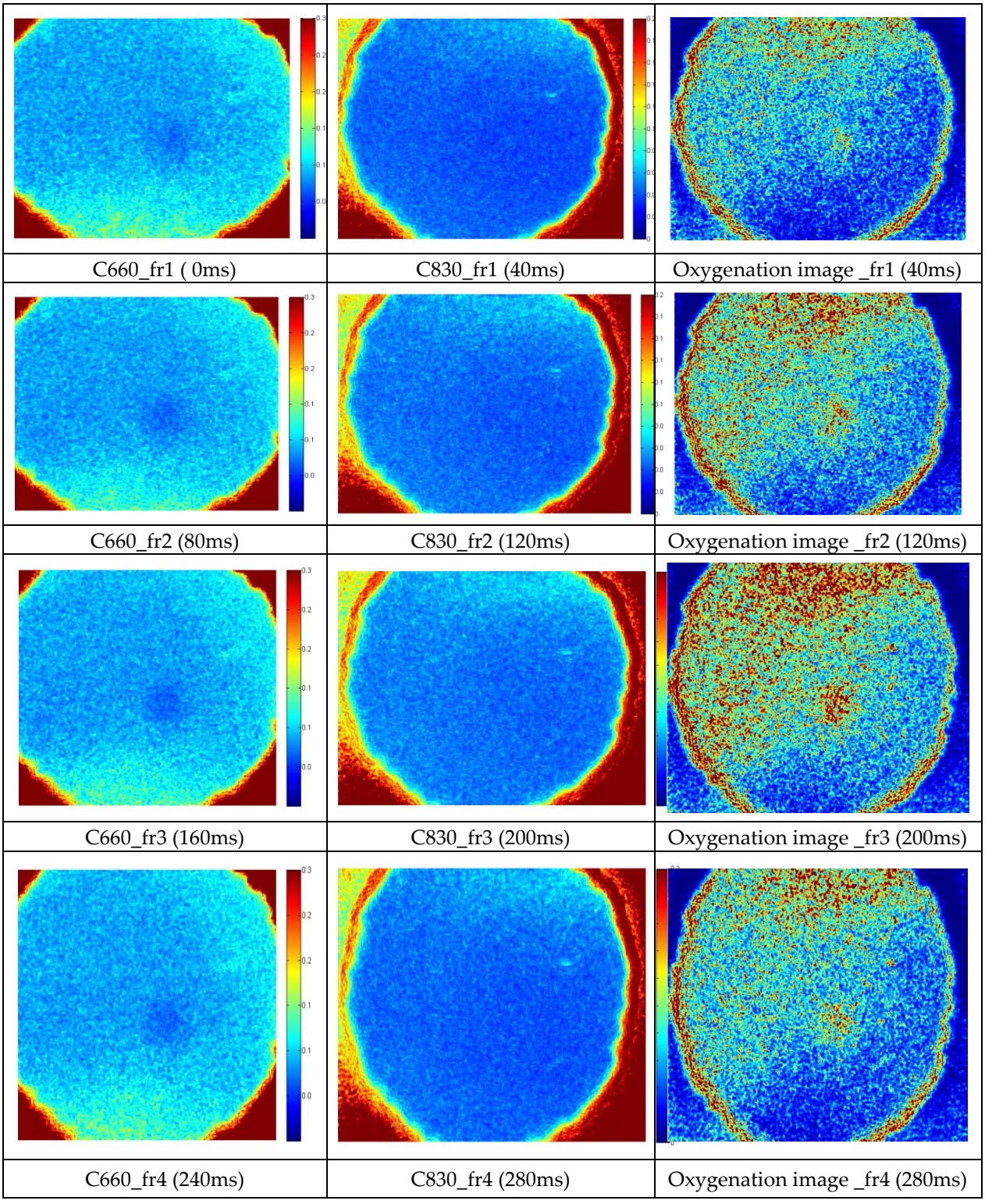


Figure 3.36 Power spectrum of the frequencies of the speckle (post-transplant, 'recipient'). Peaks can be seen at: (a) 0.32Hz corresponding to a respiratory rate of 19 breaths per minute; (b) 1.16Hz corresponding to a heart rate of 70bpm; and (c) 2.18Hz corresponding to a heart rate of 131bpm.

Sheep experiment - UTx #4

Only pre-retrieval data was recorded as the ewe arrested before retrieval was commenced. Exposure time was 20ms and delay 40ms at 10fr/sec.

Figure 3.37 Image acquisition pre-retrieval



C660_fr1 (0ms)	C830_fr1 (40ms)	Oxygenation image _fr1 (40ms)
C660_fr2 (80ms)	C830_fr2 (120ms)	Oxygenation image _fr2 (120ms)
C660_fr3 (160ms)	C830_fr3 (200ms)	Oxygenation image _fr3 (200ms)
C660_fr4 (240ms)	C830_fr4 (280ms)	Oxygenation image _fr4 (280ms)

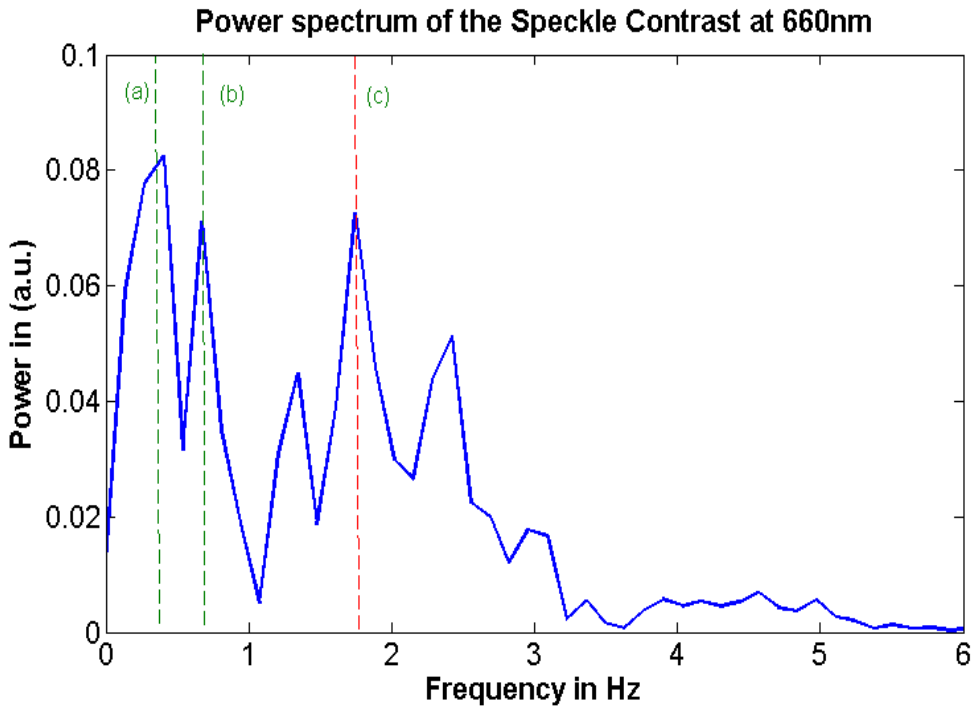


Figure 3.38 Intensity distribution of the frequencies of the speckle as calculated when applying all data. Peaks can be seen at: (a) 0.35Hz corresponding to a heart rate of 21bpm; (b) 0.675Hz corresponding to a heart rate of 40bpm, and (c) 1.75Hz corresponding to a heart rate of 105bpm.

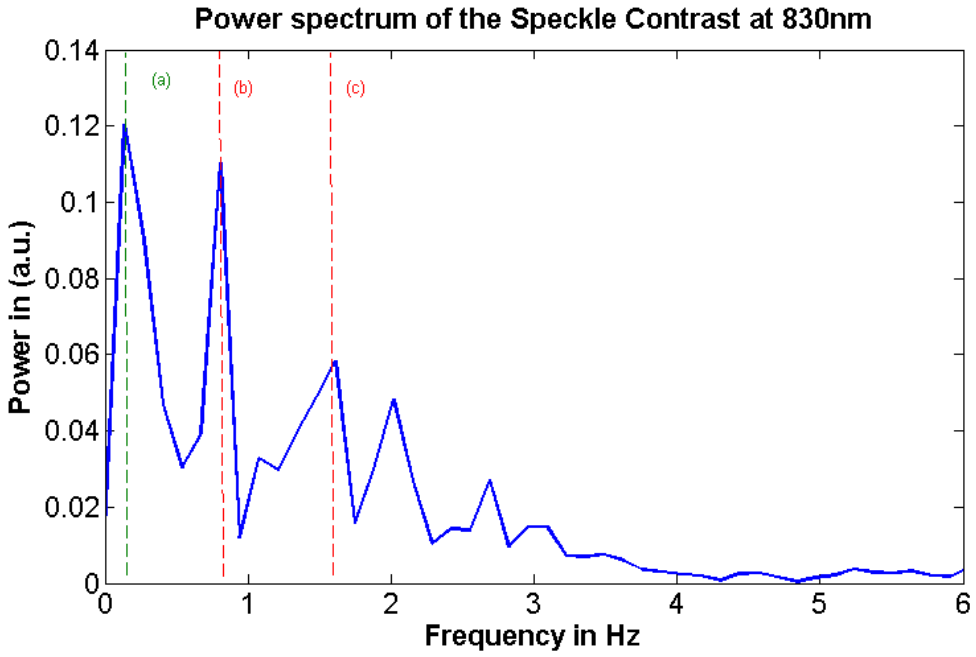


Figure 3.39 Peaks can be seen at: (a) 0.15Hz corresponding to a respiratory rate of 9 breaths per minute; (b) 0.8Hz corresponding to a heart rate of 48bpm and (c) 1.57Hz corresponding to a heart rate of 94bpm.

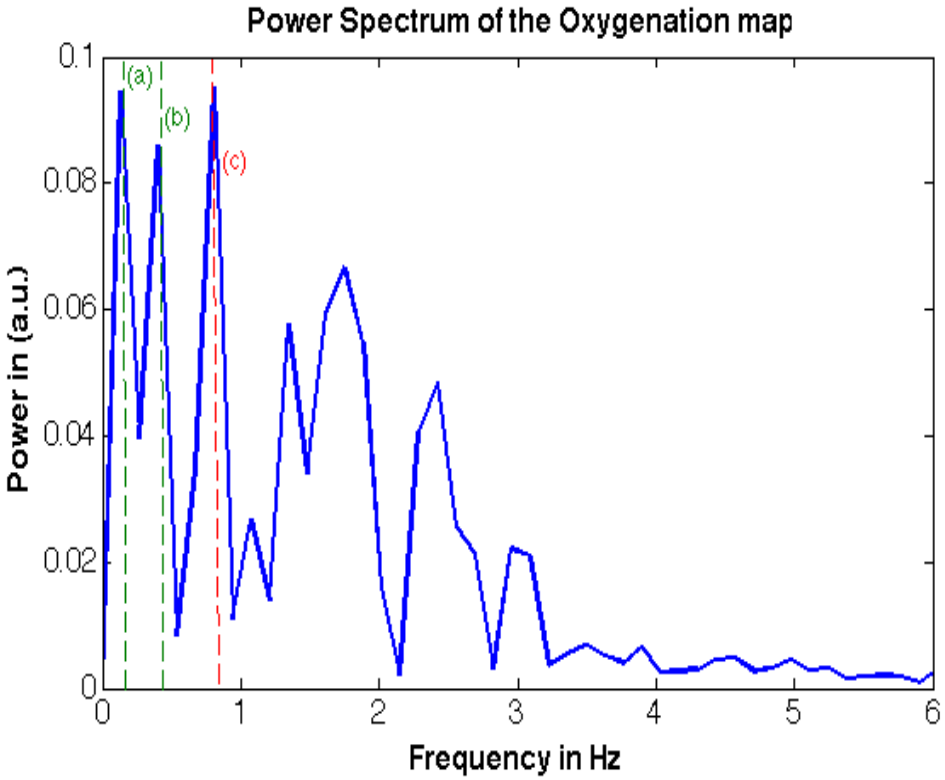
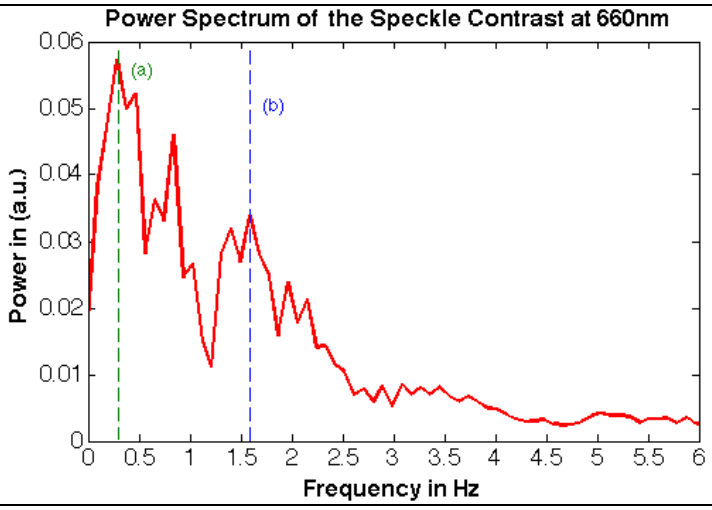
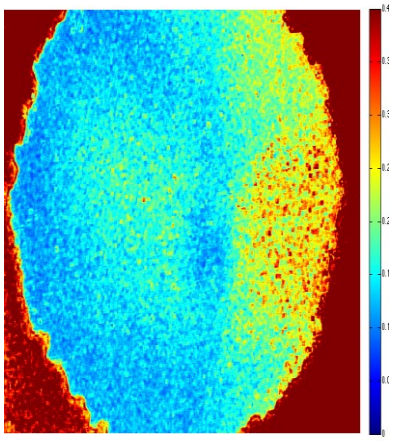


Figure 3.40 Intensity distribution of the speckle frequencies as derived from the oxygenation map images. Peaks can be seen at: (a) 0.13Hz corresponding to a respiratory rate of 7.8 breaths per minute; (b) 0.4Hz corresponding to a heart rate of 24bpm; and (c) 0.8Hz corresponding to a heart rate of 48bpm.

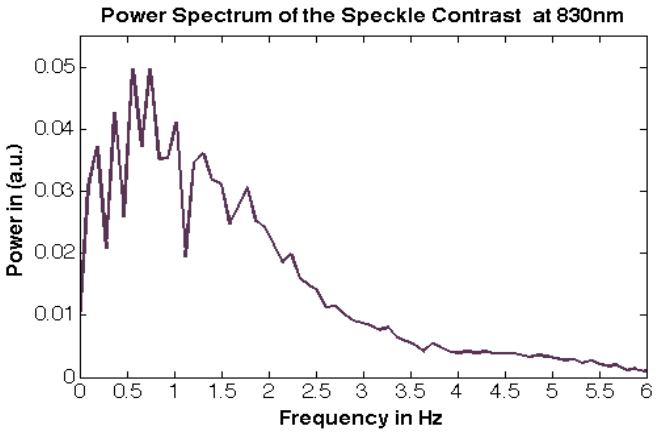
Sheep experiment - UTx #5

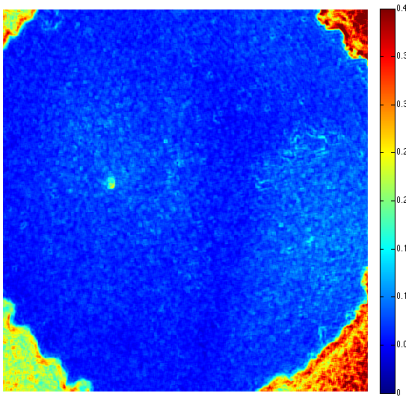
Exposure time: 20msec; Delay: 30mseC; Frame rate: 10 fps for both wavelengths



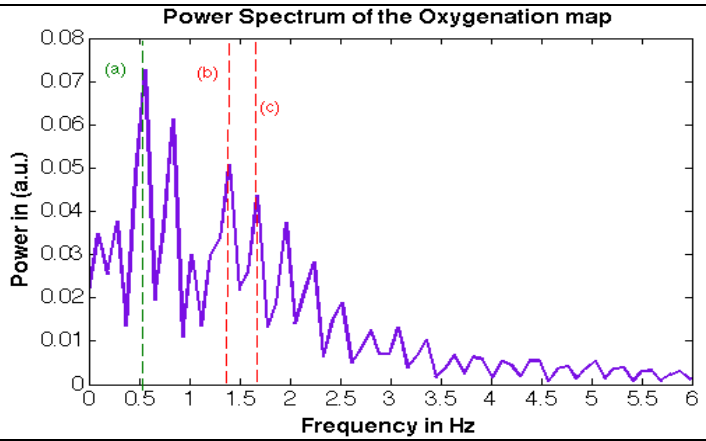


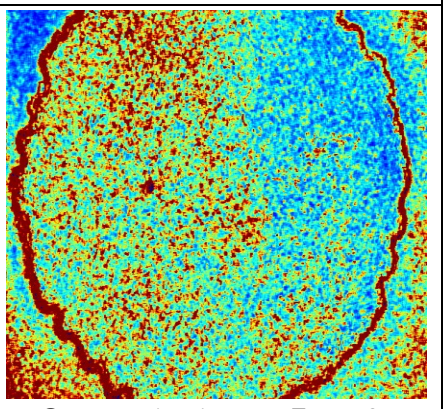
C660 _Frame35





C830_ Frame35

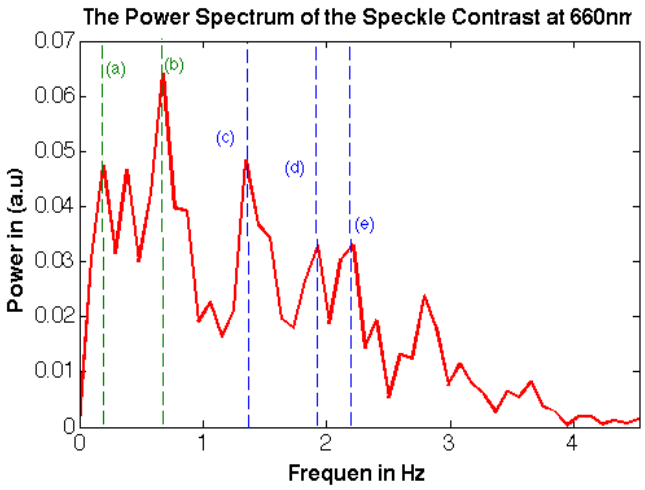


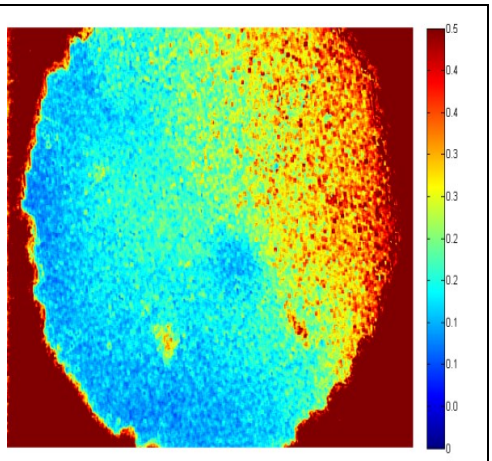


Oxygenation image _Frame35

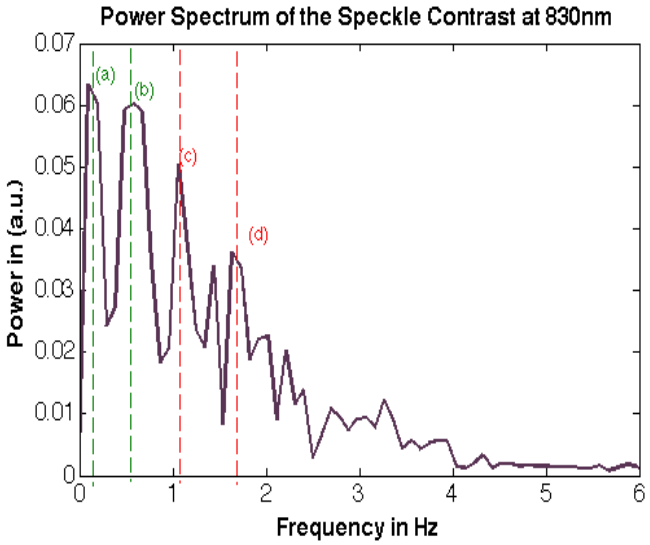
660nm: peaks at 0.3Hz and at 1.55Hz corresponding to a heart rate of 93bpm. 880nm: broad continuum from 0.5Hz to 2.5Hz and thus not much to conclude. Oxygenation map: peak at 0.5Hz corresponding to a breathing rate of 16 breaths per minute, and peaks at 1.4Hz, 1.7Hz, and 2Hz corresponding to 85bpm, 105bpm and 120bpm respectively.

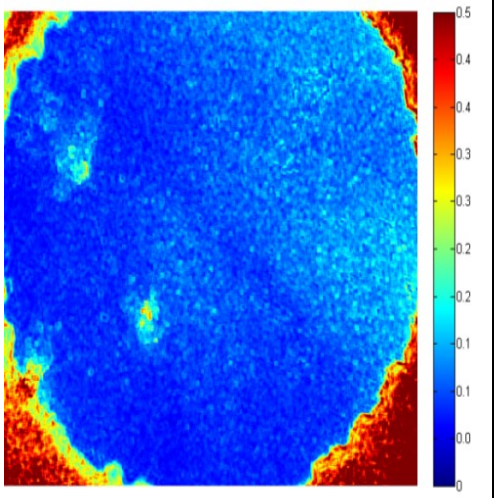
Figure 3.41 Pre-retrieval Speckle Contrast results (UTx #5)



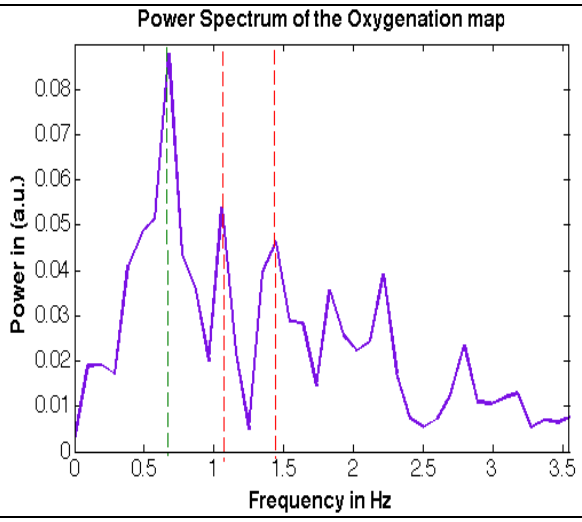


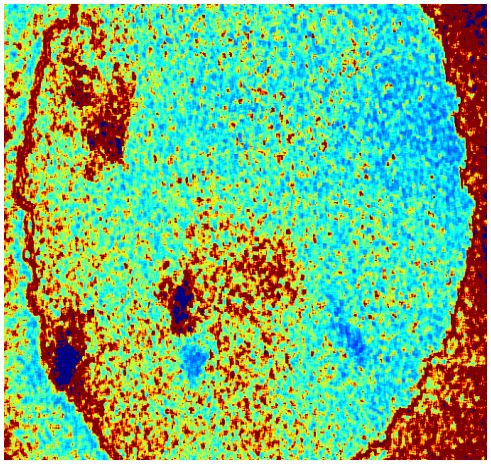
C660 _Frame28





C830_ Frame28





Oxygenation image _Frame28

660nm: peaks at 0.4Hz corresponding to a respiratory rate of 12 breaths per minute and 1.52Hz corresponding to a heart rate of 93bpm. 880nm: peaks at 0.15Hz corresponding to a respiratory rate of 9 breaths per minute, and 1.7Hz corresponding to a heart rate of 102bpm. Oxygenation map: peak at 0.5Hz corresponding to a breathing rate of 16 breaths per minute, and peaks at 1.4Hz, 1.7Hz, and 2Hz corresponding to 85bpm, 105bpm and 120bpm respectively.

Figure 3.42 Post-Transplant Speckle Contrast results (UTx #5)

3.4. Discussion

The ultimate aim of UTx is to enable a feasible allogeneic transplant with respect to anatomy and vascular viability, in order to allow for embryo transfer and pregnancy at a later date. The visual appearance of the graft, O_2Sat and PI measurements and optical spectrometry were used to ensure adequate graft reperfusion immediately after UTx. Prior to abdominal closure in those animals that survived the operation, the uterine graft appeared well perfused each time. Overall, both pulse oximetry and multispectral imaging recorded a reduction in O_2Sat following completion of the transplant i.e. anastomosis of the relevant vessels. Perfusion index levels were not different when competing pre- and post-levels.

Pulse Oximetry and Perfusion Index

In the rabbit, uterine perfusion was assessed using a pulse oximeter at three different time points: donor (pre-UTx), recipient (pre-UTx, native uterus) and recipient (post-UTx, donor uterus). It is worth highlighting that the timing of each measurement was standardised. Donor and recipient native uteri were scanned immediately following laparotomy, with the recipient donor uterus scanned just prior to closure. In UTx #1, #2, #5, #7-#9, where the recipient did not die intra-operatively, O_2Sat measurements taken after graft perfusion had been re-established were statistically different to the pre-transplant values of both donor and native uteri. The appliance was sensitive enough to detect a statistically significant 10% reduction in O_2Sat when comparing the cornua at four different points (right and left medial and lateral) pre- and post-UTx. Promisingly, however, the mean absolute O_2Sat values were still 89-90%. This is a level perfectly adequate to maintain a viable uterus. PI was statistically no different before retrieval and after completion of the transplantation. *Moxey et al* designed a study to establish if a pulse oximeter could assess the contribution of both the uterine and ovarian vessels to the overall perfusion of the uterus.[176] The two markers used for the study were O_2Sat and PI which were measured over the right and left uterine cornu. Clamping both sets of vessels alone, first the ovarian and the uterine, decreased O_2Sat and PI by a statistically significant amount.

The conclusion was that as the level of reduction in O_2Sat and PI was the same whether the ovarian or uterine vessels were ligated, then both pairs of vessels contribute almost equally to uterine perfusion.[176] During an abdominal radical trachelectomy procedure, the ovarian arteries are the only two arteries that are maintained as the uterine blood supply. Despite this, a woman can still have a normal menstrual cycle as well as fall pregnant following this operation. Therefore, an O_2Sat level of 40-50% appears to be perfectly adequate to ensure a healthy and viable uterus. This percentage was recorded upon ligation of both uterine and ovarian vessels, thus leaving the uterus supplied with only two arteries (as would be post-UTx), which are the left and right vaginal. Furthermore, the idea of using a pulse oximeter to assess uterine perfusion and therefore, the success of uterine and ovarian vessel re-anastomosis via O_2Sat and PI following UTx has its origins in this experiment.

The sheep cohort produced results which confirmed the above findings. Only three out of five sheep received the autograft (UTx #2, #3 and #5), and from those three the O_2Sat post-UTx ranged from 82-88%. Perfusion index was again statistically no different before retrieval and after completion of the transplantation. This means that in the lager animal model, where technically the team had a much easier access to the vessels, and a larger surgical field to work on, uterine perfusion, as well uterine blood flow were adequately high at the end of the surgery. This is reassuring as the sheep model closely resembles the female pelvis, and the type of vessel anastomosis (internal to external iliac vessels) applied will be exactly the same in a human model.

Our results also suggest that the surgical expertise necessary for adequate vessel anastomosis has improved in comparison to the previous rabbit and pig cohorts. The latter view is also supported by the fact that there was no significant change in PI level pre- and post-UTx. The difference in the pattern of O_2Sat and PI values pre- and post-UTx can be explained by two factors. First, the anastomosis appears to be patent in the immediate post-operative period which would explain the consistency in PI value. PI is an indicator of blood volume as explained previously. However, the delay in vessel vasodilatation secondary to both cold and warm ischaemia as well as a minimal

reperfusion time between re-anastomosis and pulse oximetry would have contributed to a reduction in O_2Sat.

The limitations of this model are self-evident from the methodology. Positioning and manipulating the probe and cornua is a practically difficult task, especially in smaller animals such as rabbits. It is a model which requires constant contact with the target in question i.e. cornua, can be 'trial and error' at times, and the actual oximeter is calibrated on human volunteers. It also only provides a point measurement of arterial oxygenation giving no spatial information on the overall supply to the tissue. Furthermore, blood volume differs with each animal, vessel elasticity and probe site. For that reason, O_2Sat and PI have been compared in a longitudinal fashion in the same animal. Finally, probe size and positioning must be correct, with excessive pressure avoided at all times so as to prevent cornual damage. The number of separate factors which can affect data derived from pulse oximetry calls for an improved and more precise tool.

Optical spectrometry

A multispectral imaging laparoscope has been demonstrated as a potentially useful tool during UTx. On comparison of its methodology with pulse oximetry, it appears to be more accurate and scientific. The advantages were the ability of the technique to monitor cornual O_2Sat over the entire visible section of the organ in a fast and non-contact method. Furthermore, one does not have to 'wrestle' with the cornua to find a strong pulsatile signal. All these might be important for stages of the operation where time is critical.

Image warping allowed correct alignment of the multispectral data in order to remove artefacts because of cornual peristalsis during acquisition. As with previous measurements using pulse oximetry in the rabbit model, measured values of both cornual O_2Sat show a statistically significant decrease after transplantation, as well as a greater one. The absolute values of O_2Sat are therefore much lower, on average ~25%. This is lower than the decrease recorded with a pulse oximeter.

Reasons for this could be that multispectral methodology is simply more accurate and precise. Also it may reflect the fact that pulse oximeters measure arterial O_2Sat only, while this imaging method also takes the venous side into account in addition to areas of the tissue that are not well-perfused, reducing the mean value. This also explains the lower oxygenated area in the centre. With regards to the most important part of the grafting process, re-anastomosis of IVC and AA, the O_2Sat map demonstrated a successful anastomosis as highlighted by the O_2Sat regions close to the region where AA was re-connected. The O_2Sat map was processed from a hyperspectral image stack acquired immediately after the re-anastomosis. Finally, it is worth highlighting that the average value of 58% is still acceptable in ensuring a perfused uterus, based on the above discussion.

Multispectral imaging was performed post-operatively only once and on one rabbit recipient only. This was the only rabbit that had survived long-term, with the measurement taken on the day of the embryo transfer (day 89). Interestingly, the level of perfusion in the one long-term surviving doe (#5) was almost back to pre-operative levels when measured on day 89 post-UTx (83% versus 85% respectively). It is difficult to draw conclusions based on just one result but it should be highlighted that a pregnancy was achieved with that specific uterine graft, thus adding to certainty regarding the result. In addition, it seems that small vessel neovascularisation of the uterus occurs once the abdomen is closed, which restores the uterine oxygen saturation to pre-operative levels (or from 58% in the immediate post-operative period to 83% on day 89). This is promising for a future human uterine attempt and perfusion of a human uterine graft which would have only two arteries supplying it - therefore, generation of new vessels supplying the graft would be beneficial.

The results demonstrated with the sheep model are extremely promising. There was no statistically significant difference between the pre-retrieval and post-UTx O_2Sat values, which ranged from 63-68% to 63-68% respectively. Again the most obvious explanation lies in the anatomical similarity when comparing the pelvis of a sheep and a woman, and therefore a surgery with which the operators felt more at ease and more comfortable. A perfusion level above 60% would be more than acceptable

to ensure future fertility, especially as we now that the level is likely to increase in the long-term secondary to uterine vascular neo-generation.

This data also comes with its limitations. One of the major disadvantages that arises with optical spectrometry is motion artefacts. They are introduced by breathing, peristalsis and relaxation of tissue during acquisition of the image stack, with peristalsis of cornua the major issue in our model. This, together with the current assumption of a flat scattering spectrum may be contributing to underestimating O_2Sat as the optical path length is longer in the red end of the spectrum than the blue. Motion artefacts were corrected using a registration algorithm based on 'sparse feature correspondences'. These techniques are applied to detect and track change induced by unwanted motion of deformable surfaces such as a peristalsing cornua.[267] The effectiveness of the approach has been demonstrated for tracking the motion of the beating heart endoscopically.[111]

In this study, where motion of the organ was significant enough to cause misalignment of the multispectral stack and introduce artefacts, the image registration approach described above was used. This could successfully correct for movements which caused blurring of blood vessels, for example, as shown in **Figure 3.43.** In summary, multispectral imaging could be an efficient tool to temporally and spatially follow tissue re-oxygenation subsequent to re-anastomosis of blood vessels required to sustain a viable uterus.

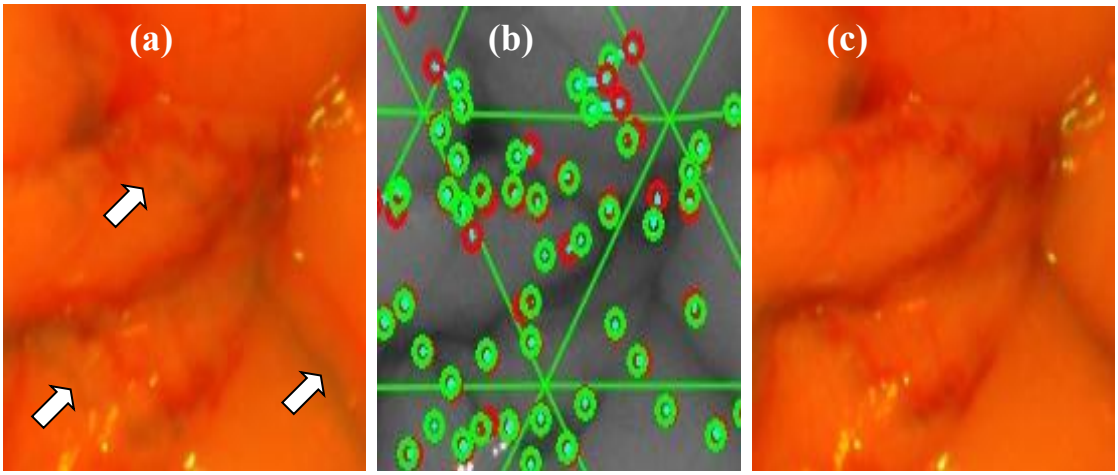


Figure 3.43 Colour images of a section of uterus reconstructed from the full multispectral stack. (a) Organ motion is visible as blur and is especially noticeable around the blood vessels (indicated by arrows); (b) Feature detection and tracking in multispectral space; (c) After registration the vessels are correctly aligned and blur reduced.

When assessing the overall picture, hyperspectral imaging may be an efficient tool to assess uterine tissue re-oxygenation in both time and space in the period immediately following the re-anastomosis, as well as in the post-operative period overall. Mapping of O_2Sat is now possible over the entire uterine graft and visible range, as opposed to a few selected points. Minor movements of the operators could be corrected for, without hindering the overall result, and thus allow for the registration of the complete stack of images. From the physics aspect, more work needs to be done on the system in order to continue the transportation of biophotonics into the surgical sphere. In conclusion, the use of multispectral imaging in this scenario is the first such case with respect to gynaecological surgery and has demonstrated promise of possible future use in a human model. It is also the first time it has been applied in a rabbit and a sheep model.

eLASCA

Whereas multispectral imaging assessed the level of uterine perfusion, the plan here was to see whether a novel technique (eLASCA) could primarily assess uterine blood flow and circulatory function and subsequently, use that data to provide information on other crucial physiological variables: respiratory and heart rates and O_2Sat. The principle of eLASCA is based on the interaction

of light and tissue in terms of refraction, absorption and scattering. This is the first time that this experiment has been attempted in the field of gynaecology. The ultimate aim was to see whether the current range of available investigations involving minimal or non-invasive techniques could be expanded by eLASCA.[184]

eLASCA was introduced in the rabbit transplants from UTx #7 onwards and was attempted in UTx #7 and #9. It was not possible to perform it in UTx #8 as a result of recipient demise. With respect to the sheep studies, the aim was to perform eLASCA in all five autotransplants. However, it was only attempted pre-retrieval and post-UTx in UTx #2, #3 and #5, and pre-retrieval only in UTx #4 because the first autotransplant was abandoned and the fourth ewe demised very early during the surgery. The figures demonstrated that some data was collected and contrast and intensity was plotted against time, with the power spectrum plotted against frequency. For UTx #9, the range of results increased as two exposure times were used for the rabbit does: 500μs and 10ms, with frequency set at 0.5Hz. In **Figure 3.15a-c**, which refers to UTx #7, the oxy-haemoglobin concentration as well as the total haemoglobin increased following active cessation of arterial blood flow. In theory the opposite is supposed to occur.[186] The main reason for this may be that during the occlusion of the abdominal aorta, a significant section of the inferior vena cava was also occluded as the two major vessels are found adjacent and parallel to each other. This would then result in a greater proportion of venous blood flow rather than arterial being blocked off during the occlusion because the uterine artery and the uterine vein are parallel with each other and the vein is close to the surface.[186] The experiment had to be curtailed because the rabbit became haemodynamically unstable. Therefore, the rabbit's abdomen had to be closed promptly allowing for no time to repeat the results.

In UTx #9, when exposure was 500μs, the intensity of the image as well as the contrast fell with identical trends. However here the frequency of the contrast peaked at 2.5Hz, which allows us to calculate the heart rate: (2.5x60) 150bpm. When exposure was 10ms, no obvious frequency peak was seen in the contrast frequency. This is most likely because of too long an exposure time resulting in an inability to catch the contrast changes induced by the heartbeat.

Figure 3.13b demonstrates suitably the rather experimental nature of eLASCA. When plotted against time, it is the contrast which indicates the heart rate. At increased heart rates, for example 200bpm, the frame rate needs to be higher than 27fps (real frame rate was 22fps).[186] Therefore the normal triphasic change associated with a single heart beat could only be captured when the frame rate was adjusted accordingly. In addition, the interaction between light and the tissue upon which it is shone is complex. In a single cardiac period, blood volume, blood flow speed and O_2Sat can all alter, and additionally, the vessel walls move.[268] All of these factors may potentially affect the contrast value. Therefore the heart beat induces the frequency of the contrast change but other cardiac-related periodic changes in the blood circulation may also contribute to this.[186]

Future experiments can be improved by adding a well-established system to monitor the change of O_2Sat simultaneously and thus compare the two sets of results, one obtained from an established system and the other from a trialled procedure.[186]

The sheep model differed to the rabbit model. The aim in the rabbit model was to see whether LASCA could pick up blood flow in the pelvis of a small animal model. In the sheep the experiment was set up as would be in a human model in order to test whether LASCA may have some use in human UTx. Therefore measurements were taken prior to retrieval and post-transplantation, and occlusion was unnecessary.

The preliminary results show that LASCA has the potential to determine blood flow, as well heart rate, respiratory rate and oxygen saturation. At this early stage accuracy is questionable, but not whether these parameters were recorded. The oxygenation saturation images are ratio-metrically calculated by the images at 660nm and 830nm based on the difference of the absorption co-efficients of the oxy and de-oxy haemoglobins. In the maps above, blood circulation was indicated with the red colour. The importance of such findings must not be underestimated. In medical practice, an innovation such as LASCA does not exist. What we have here is a system which is non-contact and

therefore does not cause any tissue damage, and also provides real-time data. This data gives us information related to presence of adequate blood flow to a particular tissue, and from that information, heart and respiratory rates and oxygen saturation is calculated. The latter parameter is given in a numerical and a pictorial form. In UTx #2, #3 and #5, the results obtained allow us to conclude that (a) blood flow was present in the uterine graft following transplantation; (b) post-UTx, the animal had heart and respiratory rates, and oxygen saturation compatible with a normal haemodynamic status, and (c) pre-retrieval and post-UTx oxygen saturation of the tissue was comparable. This last point confirms the findings form multi-spectral imaging.

Even though the ewe in the fourth transplant arrested before retrieval could be commenced, it proved useful in assessing LASCA. The technique managed to pick up the falling heart rate of an animal that was suffering and beginning its demise. The heart rate was 21bpm at a frequency of 0.4Hz. At a signal of 0.8Hz, the heart rate was 48bpm most probably because of the second harmonic of equal to 21bpm. A second harmonic can be explained as follows. In a simple sinusoidal signal, the negative values of the sine curve may sometimes be received as positive values. Therefore a signal at 1Hz can be counted as 2Hz if the negative values are added. Therefore, 0.4Hz may be a signal of the reduced cardiac rate of 24bpm which appears as a second harmonic at 0.8Hz. The actual heart rate of the ewe at that time was around 50bpm.

The eLASCA system currently remains a prototype for further experimental investigation. The data acquired was limited and difficult to use in order to make any definitive conclusions. However, images were acquired and as seen from the results section, a number of graphs could still be drawn. In the sheep model, data related to the heart and respiratory rates and oxygen saturation was acquired both pre-retrieval and post-UTx allowing for comparisons. Blood flow was determined in all sheep transplants. Therefore, as a first attempt to trial an experimental technique in a previously untested area of medicine, the experience may be considered as useful. With further trials in the future in both the animal and human models, a more definitive conclusion may be arrived at.

These preliminary results demonstrate vast potential of LASCA to surgery and importantly UTx. An accurate, real-time imaging modality, which uses a mathematical model to process the final results, is an improvement on current tools. The LASCA system is in principle a non-contact and real-time tool for the observation of flow in this case blood from which other parameters can be derived: heart rate, respiratory rate and oxygen saturation (level and map). Therefore its application is both qualitative and quantitative. Its strengths were revealed when applied to the sheep model. However there are still many challenges that need to be resolved before it can be reliably introduced in clinical practice.

The challenges are mostly related to the data acquisition methodology. The movements of the target sample *in vivo*, brought on by pulsation, respiration or uterine contraction, lead to faulty contrast images with a low 'signal-to-noise ratio'. A faster acquisition system is required for more precise diagnostic information. Further improvement of the hardware and the synchronisation of the various electronic devices may be the answer.

STUDY 4a

4. Study 4a: Psychological Issues Associated with Absolute Uterine Factor Infertility and Attitudes of Patients towards Uterine Transplantation

This study has now been submitted for peer-review and potential publication.

4a.1. Introduction

The challenges that have arisen as UTx has slowly crept towards accepted human practice have included the development of multidisciplinary teams, with transplant surgeons, physicians and ethicists working together in a systematic way. Technical aspects regarding the surgery, immunological factors as to the optimal 'anti-rejection' regimen and practical features relevant to embryo harvest and transfer have been steadily overcome. However the psychology of a potential recipient of a uterine graft in order to bring about fertility has not been adequately explored or reviewed scientifically. This is a glaring omission which requires rectification. The scenario is this: 'A potential UTx patient who has already faced elements of depression and self-examination brought on by her existing infertility is now preparing herself both physically and mentally to undergo an experimental procedure with an unquantifiable level of risk to herself and her unborn child without the certainty of future success'. One can immediately see how psychology now takes a front seat.

The literature review has already been completed in an earlier chapter bearing the same name as its title.

Areas that require exploration

Despite the progress made towards managing the psychological sequelae for these patients, the barrier of infertility remains. UTx has been proposed as a potential solution. This study was therefore designed to explore patients' knowledge of and attitudes (A), motivations (M) and feelings (F)

towards UTx before and after a short educational intervention via video and 'question and answer' session.

4a.2. Materials and Methods

The study sample included only women previously diagnosed with AUFI. They had volunteered to participate in the study having heard about the project either via our website or from the health-care team at the National Centre for Adolescent and Adult Females with Congenital Abnormalities of the Genital Tract, Queen Charlotte's Hospital. They contacted us via email or telephone, expressing an interest in managing their AUFI diagnosis. The sole inclusion criterion was age between 18 and 50. Permission to conduct the study was granted by the NRES West London Ethics Committee (study ID: 11/LO/1057).

Interview

Participation in the study lasted approximately two to three hours and consisted of the following parts (**Appendix B**):

a) Meeting and Introduction

b) Agreement to conduct interview with investigator and a chance to for the potential participants to ask questions about the study.

c) Consent form signed

d) **Part 1:** Completion of questionnaire (15 minutes)

e) **Part 2:** Discussion/Q&A session (30 minutes)

f) **Part 3:** Video viewed by participant (30 minutes)

g) **Part 4:** Completion of the above shortened questionnaire (15 minutes)

h) **Part 5:** Structured interview (25 minutes); conversation to be recorded using audio recording equipment or answers to open-ended questions written down depending on patient preference.

Part 1: Completion of a guided questionnaire completed together with patient

Part 1 involved the completion of a questionnaire. The researcher goes through each question in order and records the answers given to him by the participant on the same sheet as the questions.

Part 2: Discussion of topics relevant to UTx/Q&A session

All the main topics relevant to UTx (surgery, immunology, IVF, timeline, ethics, human attempts, animal research) were discussed with the participant. The options available to the participant with regards to fertility are discussed as well as how UTx may aid the patient in the future. Any questions that the participant has are answered.

Part 3: Patient watches a 20 minute video whereby Mr JR Smith and Dr S Saso discuss UTx

Following the discussion, the participant was left alone in the room to watch a 20 minute video which shows a discussion between Mr JR Smith (Chief Investigator) and Dr S Saso (researcher) about UTx (history, reasons for doing it, advantages, risks and other options).

Part 4: Patient completes a shorter version of the guided questionnaire

Part 5: Patient answers a number of semi-structured questions (audio-recorded)

After the patient completed a shortened version of the above questionnaire, the participant was asked to respond to a series of open-ended questions. The participant either wrote down the answers on a sheet of a paper which is stored by the researcher or if she preferred, her answers are recorded on a tape to allow for the content of the conversation to be transcribed in verbatim.

The interview schedule consisted of seven structured questions as follows:

a) How does the diagnosis of infertility affect your day-to-day life?

b) Have you given any thought to having children in the future? If you have, which methods have you considered (surrogacy, adoption)? What are your thoughts about such options?

c) Why is the idea of 'having children' important to you?

d) In the questionnaire, you had to answer questions regarding uterine transplantation. Following that, you watched a video on this topic. Have you ever heard of this proposed procedure before and what are your thoughts regarding it?

e) Do you feel that uterine transplantation can ever become a valid option? Can you explain your answer?

f) Can you describe what the process of uterine transplantation might entail (e.g. patient selection, surgery, IVF, pregnancy, delivery, immunosuppression, support)?

g) Do you have any other questions? Is there something regarding the questionnaire or this interview that you would like to discuss?

The presence of the participant's family or partner was always welcomed. Support is necessary and important. They were only asked to leave while the participant completes the questionnaire in order to prevent any undue influence with answers.

No such study has been performed before although the suggested patient assessment has been used by the UK face transplantation team when attempting to select a potential patient for the UK's first face transplant. Professor Alex Clarke (Professor of Clinical Psychology at UCL, closely involved with the UK Face Transplantation team) and Miss Maria Jalmbrant (Clinical Psychologist, Imperial College London)) were involved in creating the questionnaires. The questionnaires also underwent numerous revisions as suggested by the West London Ethics Committee (made up of physicians, obstetricians and psychologists) so as to obtain ethical approval.

Methodology

The study employed a quantitative (**Parts 1 & 4** - questionnaire) and qualitative (**Part 5** - interview) design. **Part 5** involved a structured interview with the participants in order to fully and sensitively explore the issues that are pertinent to them in relation to UTx. The reason for the qualitative aspect (**Part 5**) is because qualitative data provides more detail, can capture constructs and narratives, is often popular amongst participants as answers are not constrained and is suitable for hypothesis generation. The data was transcribed in verbatim.

Data Presentation and Analysis

Attitudes, motivations and feelings of UTx patients (**Tables 4a.1-2**) and the sub-group analysis (**Table 4a.3**) were analysed using Fisher's exact test. A bar graph was used to demonstrate ranking. A statistically significant difference was applied for a p-value <0.05. All statistical analysis was done using the Statistical Package for the Social Sciences version 19 (SPSS Inc, Chicago, Illinois, USA).

4a.3. Results

General

Forty women were interviewed. Each interview took between 2-3 hours to complete. No patients felt upset by the questions posed, and all interviews were completed. Median age of interviewees was 29 (range: 19-55). 92.5% (n=37) were in stable relationships and considered to have a found a partner with whom they would consider starting a family. All reported a strong support system around them, including family and friends. Age of AUFI diagnosis ranged from 7-39 years; median age was 18 years. Cause of AUFI in this cohort to patients was as follows:

(a) 65% (n=26) were MRKH sufferers, diagnosed during their teenage years;

(b) 20% (n=8) underwent a hysterectomy as a treatment for cancer (cervical carcinoma - n=4; rhabdomyosarcoma - n=2; placental site trophoblastic tumour - n=1; uterine cancer secondary to *in utero* diethylstilboestrol exposure - n=1);

(c) 7.5% (n=3) underwent a Caesarean hysterectomy to halt severe post-partum haemorrhage;

(d) 7.5% (n=3) suffered from a benign cause of AUFI (hypoplastic uterus, adenomyosis and multi-fibroid uterus).

17.5% (n=7) have children of their own, either via surrogacy or adoption, whereas the remaining (n=33) do not have any children (genetic or adopted). Three of the seven women achieved motherhood through surrogacy. Two women delivered their own child just prior to the Caesarean hysterectomy. One woman had a child prior to being diagnosed with cervical cancer and undergoing a hysterectomy as a result. Finally, one woman had two children via adoption. All women have to date expressed a wish to have a child or in case of women with children already, to add to the family. All women but one deemed the ability to have a child of their own as (very) important to them and expressed a strong wish to have children from the time of AUFI diagnosis to the time of the interview. This desire was based solely on their own feelings, with no external influence from a partner or family (again all women but one). The sole woman who differed from the majority stated that she had found a way to cope without children following her diagnosis and was content to lead her life without children. Surrogacy and/or adoption offered a viable solution to 52.5% of women (n=21).

All patients had heard and read about fertility options that may be relevant to them: adoption, surrogacy and UTx, from a variety of sources: internet, GP, and gynaecology clinic. Each had contacted Mr J. Richard Smith (Head of Uterine Transplant UK) in order to find out more about the subject and participate in this project. Knowledge of UTx prior to the interview was divided with 52.5% of participants stating that they either knew nothing or had heard UTx discussed only a few times and thus had minimal knowledge.

Attitudes towards UTx

All found UTx an interesting and relevant topic. 82.5% (n=33) understood fully the benefits of UTx and 42.5% (n=17) stated that they were either undecided or did not comprehend the risks of UTx. 42.5% were undecided as to whether the benefits outweighed the risks. 57.5% (n=23) thought that the benefits did outweigh the risks. 97.5% (n=39) thought that UTx research would benefit the fields of medicine and surgery whereas 95% (n=38) believed that it would be of benefit to obstetrics and gynaecology even if the final goal is not achieved (healthy pregnancy post-human UTx). All patients thought that funds should be channelled towards UTx research. 92.5% (n=37) would be happy to donate a deceased family member's uterus to the Organ Donation register.

An overwhelming majority, 85% (n=34) would accept a uterine graft if it could lead to a healthy pregnancy and are of that opinion that it should take place as soon as possible. 90% (n=36) thought that UTx is achievable.

Motivation towards UTx

90% (n=36) found UTx relevant to their quality of life, whereas 10% were undecided. 97.5% believed that UTx would lead to greater personal happiness (one patient was undecided) and all thought that it should be offered as soon as it was proven to be safe. 95% (n=38) of patients felt that UTx would suit them the most. One patient strongly disagreed with this and one was undecided. Crucially, 92.5% (n=37) would undergo UTx (or would have in the past) as a first choice treatment (ahead of surrogacy and adoption) for AUFI, provided it was a safe and accepted procedure in the medical literature. When the idea of graft failure and potential risk to fetus were introduced, that proportion fell to 87.5% (n=35) in both cases. Finally, all reported that they had a strong support system around them, including family and friends and would therefore not be alone experiencing UTx, with their motivation remaining undiminished.

Ranking of UTx-related issues

With regards to the ranking of issues relevant to UTx (surgery, graft rejection, immunosuppression, achieving and maintaining pregnancy, risk to fetal and child development and donor family issues), all patients found issues related to the donor family the least important as there was no genetic link. The other most important issues were, in order, the ability to achieve pregnancy, risk to fetal/child development, and related to graft rejection, including immunosuppression.

Following the three areas above, the next issue thought to be of importance prior to the video and discussion, was the surgical aspect of UTx. Interestingly, following the video and discussion, this issue was considered the second least important after donor family issues. Importantly, difference in ranking of each issue before and after discussion was statistically non-significant. Please refer to **Figure 4a.1** for p-values.

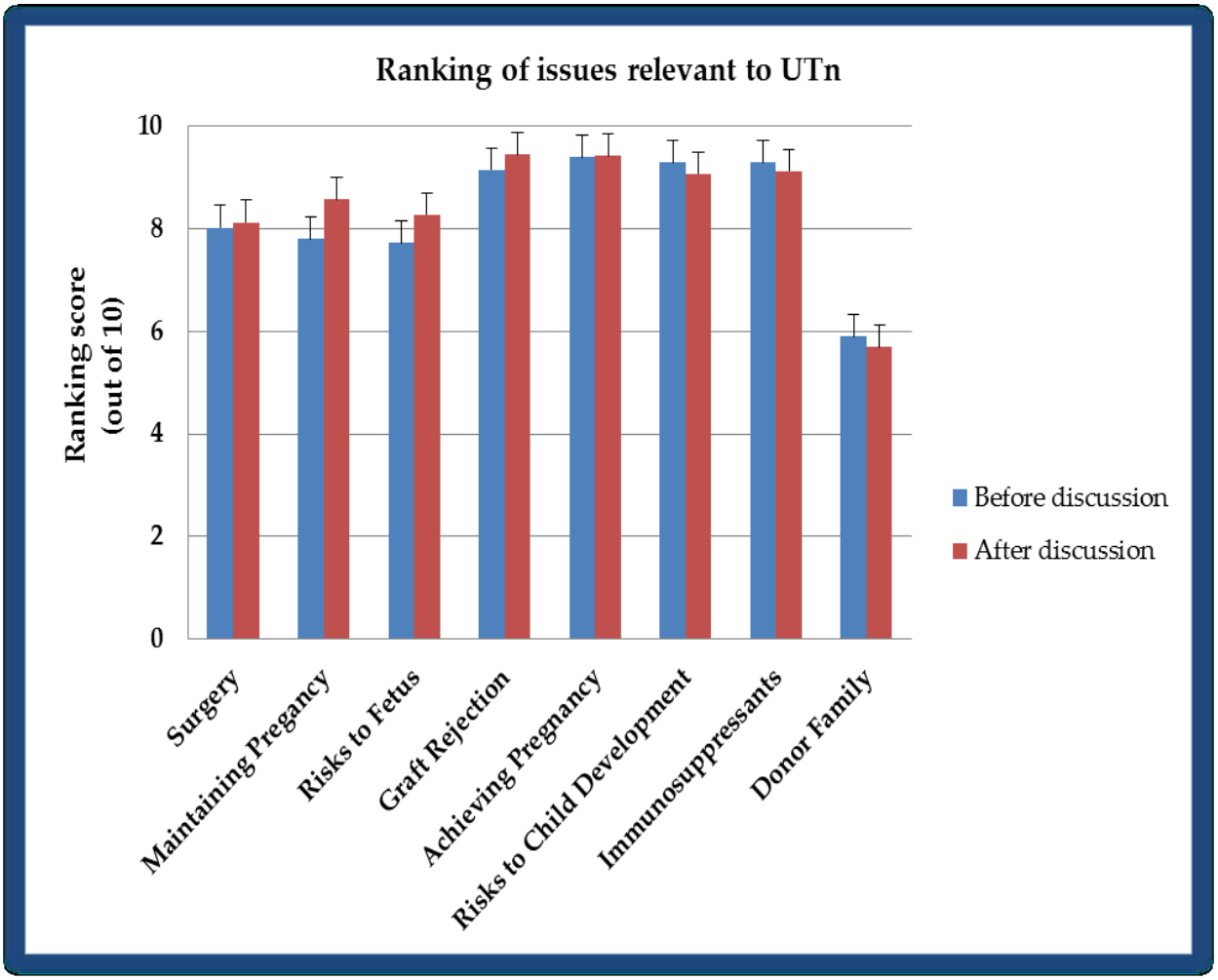


Figure 4a.1 Mean ranking of topics related to Uterine Transplantation before and after discussion. The topics are shown on the x axis. They were scored according to how important the patients felt they were in relation to each other (score: 1-10, y axis; 1=least important and 10=most important). Topics were permitted to be scored equally.

Difference in ranking of each issue before and after discussion was statistically non-significant. P-values are as follows (1-tail):

Surgery	**0.42**
Maintaining pregnancy	**0.21**
Risk to fetus	**0.16**
Graft rejection	**0.34**
Achieving pregnancy	**0.37**
Risks to Child Development	**0.23**
Immunosuppressants	**0.25**
Donor Family	**0.44**

Opinions following Video Presentation

Following a 20 minute discussion of UTx involving the interviewer (Dr S Saso) and the interviewee, aided by a video presentation, the above results were re-assessed. Some significant changes in the patients' answers were noted. All now felt that they understood the benefits and only one woman was undecided as to whether she fully understood the risks of UTx. Similarly, only two women were undecided as to whether they believed that the benefits outweighed the risks; the rest of the cohort (95%) was in agreement that this was the case.

All women now felt that UTx research and the whole process of attempting to bring it about in humans would be of benefit to the fields of medicine, surgery, obstetrics and gynaecology. Similarly, all thought further funds and grants should be made for infertility research including UTx. All other answers remained the same.

With respect to the most important conclusions, only two women (5%) would not accept a uterine graft if it could lead to a healthy pregnancy. The patient, who was undecided as to whether she would undergo UTx ahead of surrogacy or adoption, provided UTx was a safe and acceptable procedure, now felt that she would not have chosen UTx. She has already experienced surrogacy and that would remain her first choice. The second patient was concerned about the potential risk to her well-being and fetus and would wait prior to making a final decision. 97.5% (n=39) would undergo UTx ahead of surrogacy and adoption in full knowledge that the latter two options would be ultimately safer for their own wellbeing and the fact that the graft could fail even prior to conception. All felt that UTx should take place, but 92.5% saw UTx as achievable. Importantly, four women felt that they could not undergo UTx with current risks posed to the fetus. For them, the risk posed by the surgical process and the immunosuppression to themselves were not an issue but the fetal risks still need further clarification before they could fully decide upon undergoing UTx.

Results are summarised in **Tables 4a.1 and 4a.2.**

Key findings	Percentage, % (n)		
Interview completion	100 (40)		
Age	Median: 29; Range: 19-55		
In a stable relationship	92.5 (37)		
Have children	17.5 (7)		
Surrogacy/Adoption as a viable solution	52.5 (21)		
Minimal Knowledge of UTx	52.5 (21)		
Attitudes, Motivations, Feelings towards UTx			
	Pre-Video	**Post-Video**	**Significance**
Understanding of benefit	82.5 (33)	100 (40)	**<0.001**
Understanding of risks	57.5 (23)	97.5 (39)	**<0.001**
Benefit outweighs risks	57.5 (23)	95 (38)	**<0.001**
UTx as option	85 (34)	95 (38)	0.5
UTx as a 1st choice option	92.5 (37)	90 (36)	0.44
UTx as an option with current risks to mother	87.5 (35)	97.5 (39)	0.10
UTx as an option with current risks to fetus	87.5 (35)	90 (36)	0.20
Human UTx is achievable	90 (36)	92.5 (37)	0.32

Table 4a.1 Summary of results

Questions	Average Score (out of 10)		
	Pre-Video	**Post-Video**	**Significance**
1. If uterine transplantation became possible in humans, I would undergo this procedure in order to have a child.	9.8	9.7	0.40
2. If uterine transplantation became possible in humans, I would not undergo this procedure, as I am fully aware that adoption or surrogacy would be ultimately safer for my wellbeing.	1.6	1.5	0.44
3. If uterine transplantation became possible in humans, I would undergo this procedure, fully aware that it could fail and the ultimate goal of achieving successful pregnancy would not be achieved.	9.1	9.6	0.10
4. If uterine transplantation became possible in humans, I would not undergo this procedure, fully aware of significant risks to my future baby.	2.3	1.9	0.10

Table 4a.2 Mean scores to four key questions assessing patient motivation (key: 1 = strongly disagree; 10 = strongly agree)

Sub-group Analysis

a) Women with MRKH

Overall, no statistical difference was found when comparing the responses between women diagnosed with MRKH and those women diagnosed with other types of AUFI. The only response that differed was whether surrogacy or adoption may be a viable option for the patient. MRKH patients were more in favour of these two treatments, currently the only two accepted in practice, than non-MRKH patients.

b) Women who were already mothers at the time of the interview

Seven women were already mothers at the time of the interview. Median age was 40 (range: 29-43 years). Overall, the responses in this small sub-set did not statistically differ to the answers provided by the whole cohort. One patient differed in her replies and her case was described above. The patient was initially undecided as to whether she would undergo UTx ahead of surrogacy or adoption, provided UTx was a safe and acceptable procedure, but following the video, felt that she would not have chosen UTx. She has already experienced surrogacy and that would remain her first choice. However she did feel that UTx was achievable whereas she was undecided previously. One other patient also changed her mind following the video and felt that UTx was not achievable although she was supportive of the whole process and would undergo the procedure as a first choice as oppose to surrogacy and adoption.

Only two responses differed statistically. First, the 'Mothers group' was strongly in favour of surrogacy and adoption as alternative options, in comparison to the 'Non-Mothers' group. Second, the two groups also differed significantly as to whether they would proceed with UTx fully aware of the potential risks to the fetus, with the 'Non-Mothers' group appearing a little more cautious. This difference arose following the discussion and the video.

c) Women with minimal knowledge of UTx prior to the interview

Again, overall the responses between these two groups did not differ. Those that did are as follows: (a) understanding of potential benefit of UTx to women suffering from AUFI prior to the video and discussion; and (b) understanding of how the benefits of UTx outweigh the risks.

All results above are summarised in **Table 4a.3**.

Sub-Group Analysis			
	MRKH v. non-MRKH (pre-video / post-video)	**Mothers v. Non-Mothers** (pre-video / post-video)	**UTx non-awareness v. awareness** (pre-video / post-video)
Understanding of benefit	0.22 / 0.44	0.53 / 0.55	**0.004** / 0.34
Understanding of risks	0.43 / 0.31	0.10 / 0.23	0.28 / 0.16
Benefit outweighs risks	0.45 / 0.45	0.43 / 0.44	0.18 / **0.03**
UTx as option	0.65 / 0.20	0.24 / 0.29	0.20 / 0.41
UTx as a 1st choice option	0.57 / 0.48	0.36 / 0.14	0.21 / 0.15
UTx as an option with current risks to mother	0.30 / 0.13	0.12 / 0.35	0.39 / 0.053
UTx as an option with current risks to fetus	0.40 / 0.37	0.47 / **0.048**	0.24 / 0.08
Human UTx is achievable	0.56 / 0.57	0.45 / 0.45	0.34 / 0.40

Table 4a.3 Statistical significance of Sub-Group Analysis

4a.4. Discussion

Before a patient can be selected for UTx, she will have to undergo an appropriate psychological assessment. This will include a series of appraisals looking at, for example, cognitive function, mood, stress disorder, quality of life and beliefs regarding medicine. One of these assessments should gauge the motivations, attitudes and reasons why a patient would choose UTx ahead of surrogacy or adoption. This study aims to assess perceptions of AUFI patients to UTx. No such study exists yet in the literature and thus no attempt to quantitatively or qualitatively report what AUFI patients feel about UTx. After almost 50 years of research, our evidence with regards to this topic is still anecdotal.

The goal here is not to influence a patient; there is recognition among UTx researchers that UTx is a procedure which may be suitable for a small number of AUFI sufferers only. The ultimate aim of this research programme is to understand the patient perspective in considering UTx as one of the list of possible solutions to AUFI, including surrogacy or adoption.

The actual study clearly demonstrated an overwhelming level of interest and support from AUFI patients towards the continuation of UTx research, its benefit to medicine and surgery in general and most importantly the application of UTx to clinical practice.

Interpretation

The forty patients interviewed were from a diverse sample group, with a wide age range (19-55), comprising of mothers and non-mothers, and MRKH and non-MRKH patients. There was considerable support from all these groups about UTx as a concept, a research field, an area that may lead to advancement of the medical field as a whole, and a possible treatment option in the future. They considered UTx as an option which could lead to greater personal happiness and should therefore be offered as soon as it proven to be safe. Most crucial of all, and possibly rather surprising, was the statistic that 92.5% of the women interviewed would undergo UTx ahead of surrogacy and

adoption. When made fully aware that these two options would be safer for their own and their fetus's wellbeing and the graft could fail even prior to conception, the percentage still in favour of UTx as a first choice was 87.5%. The immediate reaction to such a high percentage following the video discussion is a positive one as it clearly supports a human UTx attempt. However, this statistic should be treated with caution. One can easily conclude that rather worryingly, despite being warned about the risks of UTx, patients are potentially distancing themselves from them in order to achieve their goal of fertility. They are so focused on improving their QoL that their views on UTx and whether it may be right for them become one-dimensional to the extent where they would undergo UTx ignoring the potential harm to themselves and the unborn fetus. A question then arises - does the patient require further time and evidence in addition to the 20 minute video to gage for herself whether the benefits truly outweigh the risks and if so how much more time and what additional evidence? A patient undergoing a radical hysterectomy, an operation similar to UTx in scope and duration, will be consented following, from my experience a 10-20 minute discussion. To the UTx patient, having a child is a paramount issue and is as important to them as is continuation of life to a end-stage renal failure patient requiring a renal transplant. They are simple prepared to face the risk to achieve their aims, which would considerably improve their life. A solution may be the setting-up of a series of teaching session whereby the selected patients would undergo one hour classes focused on the main topics related to UTx, taught by transplant surgeons, donor co-ordinators and transplant nurses for example. This would give them the necessary one-to-one teaching and adequate time to study the process in its entirety, as well as have all their questions answered.

90% felt that UTx was achievable within the next three years. The explanation for this elicited from the women was that UTx, if deemed safe in humans, would enable them to have their own genetic child and allow them to be in control throughout the pregnancy. It would also be the cheapest option and one where they did not have to rely on anybody else for results. The former point raises another ethical dilemma - one of patients choosing a high-risk operation because it is cheaper. To answer this point, it is important to clarify that the procedure may be cheaper only at its initial stage, as the aim is

to ensure UTx is funded through private, charitable donations. Future funding (NHS or private) will greatly depend on the outcomes of the initial 10-20 human UTx worldwide.

For some of the patients, their nationality played a part. Here is a transcript from one of the patient's interviewed:

'We have given a lot of thought to having children and have looked at both options, surrogacy and adoption. Given our nationality and poor legislation in Ireland in relation to surrogacy and adoption, the pursuit of either option for us was/ is fraught with red tape and bureaucracy, not to mention any legality issues that could arise with a surrogate mother. While we are ready and willing to go down these roads if left with no other alternative, we feel that either option would have to be approached with great caution as both options are filled with trepidation.'

It is important to note that this was a highly motivated patient group who all felt deeply distressed and devastated when initially diagnosed with AUFI. One patient had to see a psychologist, and issues related to femininity and sexuality affected all our MRKH sufferers. Even the three patients who had their own biological child prior to losing their uteri (Caesarean or standard hysterectomy) still felt that '*something was missing*' as they could not complete their respective families. The concept of not being able to have a child was initially overwhelming. For them, '*a life without a child was empty and simply not worth it*'. Eventually all found ways of coping with this diagnosis, and one patient managed to completely block out the idea of having children. 52.5% considered surrogacy and adoption perfectly acceptable options, but only one would still choose surrogacy if UTx was a safe and accepted procedure with minimal risk to the mother and fetus. The main problems with surrogacy listed by the patients were the lack of control, costs ranging from £15,000-£30,000 and no actual experience of gestation. Adoption also seemed like a laborious, bureaucratic and long-winded process, and it could not meet the need to have a biological child.

This agrees with the prior assessment that UTx would fit the need of a select, highly motivated group of patients, for whom being a mother was of the utmost importance. Even though they found surrogacy and adoption perfectly acceptable options, these women would only consider them if UTx were not an option in humans. In particular, this enthusiasm for UTx improved following an open discussion and video presentation about UTx. Once the participants knew more, they felt more confident and thus, UTx was consolidated even further as a 'number one' option. One result, however, should be highlighted. One participant out of the seven stated that she would still choose surrogacy over UTx, as she had gone through it and now has two healthy biological children. This decision is perhaps based on the success of surrogacy in her case.

The benefit of having a discussion and showing the patients a video on UTx was highlighted most clearly when seeing whether they understood the benefits and risks of UTx. In both cases an obvious and statistically significant increase was seen following the discussion and video, from 87.5% to 100% in the former and 52.5% to 97.5% in the latter. Following the video, 95% felt that the benefits outweighed the risks. It is important for the team to realise how valuable in the consenting process will be this one-to-one discussion with the patients. This will serve to answer their questions and try to gage how ready they may be.

Following the video, 36 women would choose UTx ahead of surrogacy and adoption, having been briefed on all possible consequences that they or their fetuses may have to endure. These women present the optimum patient group from which to choose a potential recipient. Apart from being fully independent women with at least secondary school education to the age of 18 as well as a supportive family and partner, one can find no fault for their motivation to have a UTx. They were able to weigh up the benefits and risks of UTx and think carefully about which areas they felt to be the most important (in this study, surgery, maintaining pregnancy and risks to fetus).

The limitations of this study are, firstly, as discussed above 40 patients is not a large cohort. This may appear as a small sample size and therefore a drawback. However, the results are so definitive that a

greater sample would not have altered the drawn conclusions. Secondly, the patients were not drawn randomly from an AUFI clinic. They all contacted us expressing an interest in UTx. Therefore, one could argue that this cohort is a biased group in favour of UTx. In my opinion, this is not a valid point. An interest in a novel procedure does not automatically mean a wish to undergo it or in this case, find it more to one's liking than surrogacy or adoption. It is the right of all couples who desire to have children to investigate all possibilities that may bring this about. Those who have heard about it decided to contact us and the expectation is that the vast majority of women who hear of our team and UTx will contact us in the future.

The sub-group analysis focused on three groups. One of them, women who were aware of UTx prior to the discussion and those women who were not, is relevant to the previous point. When comparing the answers of those two groups, one can see that almost all answers do not differ significantly. This was also the case with the other two sub-groups: MRKH and non-MRKH women; and mothers and non-mothers. This suggests that having a child is the most important factor for an AUFI patient when it comes to deciding whether UTx should be her first option, and the differences between AUFI sufferers become unnoticeable at that point.

Psychological Assessment

Upon deciding on a potential patient, the UTx team should initially investigate her motivations for undergoing UTx, as well as her attitudes and expectations following the operation. The patient ought to have considered and ruled out surrogacy and/or adoption as her main choice. Prior to commencing any aspect of the UTx process, the team should feel confident that the patient fully understands the plan proposed, the reasons behind it and the potential risks, and in her ability to make an independent decision.

The parameters described below provide a conceptual framework for the proposed AMF assessment tool. They are based on published material related to other recent 'life-improving' transplantation

research, i.e. face transplantation.[206,207] It is important to understand that this tool will form a significant and first part of an overall psychological assessment prior to UTx.

Motivation to seek treatment, goals and expectations of outcome

The transplant team should have a clear understanding of the problems that motivate the candidate to seek a uterine transplant in the first place, and her expectation of the potential of surgery in alleviating them. Unrealistic pre-operative expectations of fertility outcomes from the procedure are likely to be associated with poor post-operative psychological adjustment. Discussions between the patient and members of the transplant team should focus on surgical risks of UTx, as well as risks of viral infections and neoplastic changes caused by use of immunosuppressants. Type and duration of treatment should be clarified beforehand - a transplanted uterus should stay in for a maximum time of 2-3 years to allow a successful pregnancy. If the patient wishes, she could retain her uterus for a repeat pregnancy. However, such wishes should be noted prior to the surgery.

Pre-operative information into other fertility solutions

An evaluation should be made as to whether the prospective patient has been offered any alternative interventions (which offer a lower level of risk) such as surrogacy and adoption. Surrogacy offers genetic parenthood and benefits over transplantation by excluding risks related to the transplant recipient such as surgery and immunosuppressants. For example, following the first ever hand transplant, the patient, a convicted con-man, failed to follow the post-operative drug and physiotherapy program, and as a result the donated hand was rejected.[269]

However, disadvantages exist: one cannot control for lifestyle factors of the surrogate mother such as smoking/alcohol. In addition, the pregnancy-morbidity risk is placed on another person, and legal conflicts about the motherhood of the newborn may emerge.[65]

Psychological stability and adjustment

The prospective patient should be assessed as to her likely resilience in coping with potential negative outcomes associated with the transplant. These would also include several 'unknown' variables, such as outcomes associated with complex drug regimens, risks of graft rejection, poor fertility outcomes and intrusive media interest.

Level of cognitive functioning

This should be at a sufficient and appropriate level, thus enabling the patient to have full understanding of the risk/benefit information, which is likely to be detailed and complex in a procedure of this nature. Cognitive testing should therefore be performed on potential recipients.

Adherence "credentials"

Experience from other organ transplants has demonstrated that strict adherence to a complex post-operative drug regimen is crucial to a successful graft and would be even more paramount in obtaining a subsequent successful fertility outcome. It is worth noting that around 30% of organ transplant recipients are non-adherent at some point during the post-transplant period.[270] A screening test that could highlight this group prior to transplantation would be useful, as it would allow the transplant team to advise a candidate against having the procedure. Furthermore, if the candidate were still to be considered suitable, the team could prepare her adequately so she would cope well psychologically following the procedure.

Candidate's social support network

A consideration of the level and quality of support from the candidate's family and friends should be made.

Support for those deemed unsuitable for uterine transplantation

The possibility of fertility restoration through UTx may raise the hopes of many, whilst only a small number of women may be suitable for the procedure. An investigation will be carried out as to how a

support network for these women deemed unsuitable to have the procedure may be implemented in the future.

Potential psychological responses to transplantation

UTx may give rise to psychological 'stressors' found following other solid organ transplants such as liver and kidney, and also potentially novel stressors unique to the procedure. These stressors include fears relating to the viability of the transplanted organ and a number of emotional responses associated with the experience of receiving a transplanted organ; for example, feelings of gratitude or guilt towards the donor.[206]

Conclusion

In conclusion, this study, apart from being the first to demonstrate a qualitative relationship between AUFI patients and their curiosity and desire for UTx, also paves the way for forming the introduction into the psychological assessment of a potential patient. There is a keen interest in this new technique by potential UTx patient, partly because surrogacy and adoption seem difficult to access currently , but primarily because UTx gives a patient total control from start to finish as well as a child which genetically they can call their own. However, the results must be treated with caution. Paitents appear to be distancing themselves from the risk and this issue will need very careful assessment in any clinical programme.

The argument whether we should try to improve surrogacy and adoption services to make them more successful instead of focusing on UTx seems redundant. There is no ethical argument against UTx research and if it were to become a safe and accepted procedure, it would be unethical not to offer it to patients in addition to the other two options.

STUDY 4b

Study 4b: A UK Survey Assessing the Perceptions of Healthcare Professionals towards Uterine Transplantation

This study has now been peer-reviewed and accepted for publication as per following reference: **Saso S,** Clarke A, Bracewell-Milnes T, Al-Memar M, Hamed AH, Thum MY, Ghaem-Maghami S, Del Priore G, Smith JR. A UK Survey Assessing the Perceptions of Healthcare Professionals towards Uterine Transplantation *Prog Transplant* 2014.

Abstract

Context: Uterine Transplantation

Objective: To investigate the opinions and views of UK healthcare professionals towards uterine transplantation (UTx) and create a rank order of importance of UTx-related issues in order to make UTx transparent and understandable to colleagues.

Design: Large, in-depth survey investigating health care professionals' opinions on UTx.

Setting: UK Locations. Analysis done at Imperial College London.

Participants: UK transplant professionals (surgeons, nurses, operating room staff, and donor coordinators) and obstetricians and gynaecologists (trainees, members and fellows of the Royal College of Obstetricians and Gynaecologists).

Intervention: Questionnaires were given out at hospital grand-rounds, trainee teaching days, and conferences (national and international).

Main outcome measures: Should UTx takes place? Is UTx achievable? What is the rank order of importance of key issues related to UTx?

Results: 528 participants participated in the study. With respect to overall support for UTx and as a possible future therapeutic option for Absolute Uterine Factor Infertility, 93.8% (n=495) felt that UTx should take place if considered appropriate medically, surgically and ethically. 57.2% (n=302)

thought it was an achievable objective. Issues related to immunology of UTx and pregnancy post-UTx were unanimously thought of as most important.

Conclusion: This study is the first in literature to try to sample the opinions and concerns of health-care professionals towards UTx. A substantial majority of the participants support the development of the UTx research programme and believe that UTx should be performed in the near future. More effort is required to educate health care professionals about all aspects of UTx.

4b.1. Introduction

Any innovative and new surgical procedure brings with it a potentially polarized ethical debate. One of the ways of moving that innovation closer to reality i.e. the clinical setting is by assessing and gathering support amongst colleagues or to use a better term, health care professionals. This group is well placed to understand what the aim of that innovation is, its benefits and risks, its long-term outcome and most importantly, likelihood of success. To demonstrate face transplantation as an ethically accepted procedure, the UK team assessed the level of support amongst colleagues as well as professionals who deal with transplant surgery (i.e. transplant community).[271] They surveyed 170 transplant professionals by means of a questionnaire generated by a focus group of transplant coordinators. A high level of support for facial transplantation was revealed, with 76% of respondents in favour and none opposed to the procedure in principle. Areas of concern were also highlighted, mainly factors that affect organ retrieval and the impact on the retrieval team and the donor family.[271] *Clarke et al* used closed-ended questions to allow for appropriate statistical analysis of quantitative data as well as open-ended questions to allow for qualitative data which can explore particular issues in more depth.[272] A similar pragmatic approach, applying the methodology of the UK face transplantation team to deal with ethical issues, is necessary with respect to UTx.

By sampling such diverse groups, one can gather the following information: attitudes to practical issues related to organ retrieval, professionals' concerns for the recipient, her baby and donor families, and perceived impact on the transplant program as a whole. The UK face transplantation team was able to explore a number of different issues (**Box 4b.1**).[163,271,272]

Published material from research related to the other recent 'life-improving' transplantation, i.e. face transplantation can form the model for the above experiments. A significant overlap between face transplant and potential uterine transplant patients is evident, as both sets of patients are planned to undergo a 'QoL' improving transplant rather than a life-saving transplant.

Box 4b.1. Issues that require investigation amongst health-care professionals and donor co-ordinator teams [271]

- **Uterine retrieval & grafting**
  - patient wellbeing after retrieval
  - development of donor criteria
  - liaison with other retrieval teams
  - surgical and technical issues

- **Pregnancy with a transplanted uterus**
  - wellbeing of mother and fetus
  - immunosuppressant effects

- **Retrieval team**
  - education of other health professionals about UTx
  - development of a specific patient UTx team
  - impact of UTx on operating room and intensive care unit staff
  - support for health professionals
  - negative impact on other transplant programs
  - press intrusion for health professionals

- **Donor co-ordinators**
  - likelihood of benefit for recipient
  - long-term support for donor family
  - discussion of process involved
  - interest in baby from donor family
  - consent issues and consent form
  - press intrusion

4b.2. Materials and Methods

A large, in-depth survey investigating health care professionals' opinions on UTx was devised. The survey consists of a simple questionnaire which takes a maximum of 15 minutes to complete. The questionnaire was of a similar format to the one used and validated by the face transplantation team in the exercise described in the introduction. Professor Alex Clarke (Professor of Clinical Psychology at University College London, closely involved with the UK Face Transplantation team) and Miss Maria Jalmbrant (Clinical Psychologist, Imperial College London)) were involved in creating a questionnaire specific for UTx (**Appendix C**). Permission to conduct the study was granted by the NRES West London Ethics Committee (study ID: 11/LO/1057).

The target populations are:

- **Group I:** transplant professionals (surgeons, nurses, operating room staff, transplant and donor coordinators);
- **Group II:** obstetricians and gynaecologists (trainees, members and fellows of the Royal College of Obstetricians and Gynaecologists)
- **Group III:** fellows of the Royal College of Obstetricians and Gynaecologists only

While sampling three different groups, the following information was gathered: attitudes to practical issues related to uterine retrieval and grafting, professionals' concerns for the recipient, her baby and donor families, and perceived impact on the UTx program as a whole. With regards to Group I, this population came from teaching and non-teaching hospitals in the UK, as well as study days organized by transplant coordinators. Mainly because of geography, the London Donor Transplant Coordinators Team helped with organising the relevant information days. As this is a gynaecological project, our biggest group was obstetricians and gynaecologists who make up Group II. To obtain a national sample distribution, members and fellows of the Royal College of Obstetricians and Gynaecologists (RCOG) were sampled on a national basis. Regarding the groups, opportunities to give out questionnaires to participants was at hospital grand round days, trainee teaching days, and conferences (national and international). A letter explaining our project (**Appendix D**) with the questionnaire and an envelope (with our address stamped on it) attached to it was sent out to all the fellows of the RCOG.

Questionnaires were designed using closed-ended questions to allow for appropriate statistical analysis for quantitative data. The following issues are explored:

a) general concepts related to UTx (operation technique, time length, complications; immunology;

achieving pregnancy post-transplantation**;** maintaining pregnancy; risks to fetus and child development)

b) uterine retrieval and grafting (development of donor criteria; liaison with other retrieval teams; surgical and technical issues);

c) pregnancy with a transplanted uterus (wellbeing of mother and fetus; immunosuppressant effects),

d) retrieval team (education of other health professionals about UTx; development of a specific patient UTx team; impact of UTx on operating room and intensive care unit staff; support for health professionals; negative impact on other transplant programs; press intrusion for health professionals and patients);

e) donor family (likelihood of benefit for recipient; long-term support for donor family; discussion of process involved; interest in baby from donor family; consent issues and consent form; donor family press intrusion).

Our ultimate aim was to create a rank order of importance of each set of described issues so that information could be obtained as to which target areas had to be focused on in order to make UTx more transparent and understandable to the colleagues within our own profession. Target population number is 500.

Data Presentation and Analysis

A bar graph was used to demonstrate ranking. The ranking data for the 'Health-care Professionals' survey was analysed using a one-sample Kolmogorov-Smirnov test. A statistically significant difference was applied for a p-value <0.05. All statistical analysis was done using the Statistical Package for the Social Sciences version 19 (SPSS Inc, Chicago, Illinois, USA).

4b.3. Results

528 participants took part in the study; 59.8% were female (n=316) and 40.2% (n=212) were male. 398 participants were surveyed after presentations on the topic of UTx given by Dr S Saso and/or Mr J. Richard Smith at a national and international level. More than a 1000 questionnaires were sent out to the Fellows of the RCOG, out of whom 130 responded and sent them back. All participants took a maximum of 15 minutes to complete the survey and their entries were anonymised. **Table 4b.1** shows the break-down of participants according to profession. The cohort was evenly split according to age: (a) < 30 years: 25% (n=132); (b) 30-40 years: 33.5% (n=177); (c) 40-60 years: 32.2% (n=170); and (d) > 60 years: 9.3% (n=49). Also extracted was the level of experience within the medical profession: (a) < 1 year: 3.8%; (b) 1-5 years: 27.4%; (c) 5-10 years: 15.5%; and (d) > 10 years: 53.0%.

73.4% (n=388) know someone (woman/couple) who has experienced problems with infertility and 17.2% (n=91) have suffered personally with infertility. 31.6% (n=167) stated that they have at least some knowledge of the history and research into UTx. More than two thirds of the surveyed population, 68.6% (n=188), reported that they found the field of UTx interesting. Importantly, only 9.5% (n=50) felt that they did not understand the benefits and 25.2% (n=133) the risks of UTx. 35.8% (n=189) felt those benefits outweighed the risks, 50.8% (n=268) were undecided and 13.4% (n=70) disagreed. Only 11.7% (n=62) felt that UTx will not lead to greater human happiness, although 41.3% (n=218) were undecided. More than 50% of participants felt that research into UTx is of a benefit to medicine (56.6%), transplant surgery (55.9%) and obstetrics and gynaecology (53.0%), even if it does not lead to an eventual healthy pregnancy. However, only 41.9% (n=221) were adamant that more funds and grants should be made available for novel and innovative fields such as UTx, instead of focusing it all on life-saving research programmes e.g. cancer research. 65.0% (n=343) would be happy to donate their own or their family's uterus to the donor registry if this option were to be available.

With respect to overall support for UTx and as a possible future therapeutic option for AUFI, 93.8% (n=495) felt that UTx should take place if considered appropriate medically, surgically and ethically, with 42.6% (n=225) of the surveyed population believing that it should take palace 'as soon as possible'. Therefore, 51.1% (n=270) support the eventual commencement of the human UTx programme in the UK but not in its current state. 57.2% (n=302) thought it was an achievable objective, with the second largest group, 39.2% (n=207), not able to confidently decide (box ticked: 'not sure').

Responses by Group

Group I: Transplant Professionals (transplant surgeons and donor coordinators)

54.1% (40/74) felt undecided as to whether benefits outweighed the risk of UTx, with 24.3% (18/74) believing they did and 21.6% (16/74) stating that in fact the risks outweighed the benefits. Only 9.5% (7/74) felt that development of UTx would not lead to greater patient happiness, but 44.6% were undecided (33/74). With respect to bringing benefit to medicine in general, over 50% of respondents thought that the development of UTx would also lead to a furthering of the medical, surgical, gynaecological and transplantation fields. 66.2% (49/74) thought that UTx is achievable, 32.4% (24/74) were not sure and only 4.1% (3/74) felt that it should never take place. 39.2% (29/74) believed that UTx should take place as soon as possible.

Group II: obstetricians and gynaecologists (trainees, members and fellows of the Royal College of Obstetricians and Gynaecologists)

47.4% (136/287) felt that the benefits of UTx outweighed the risks, 37.3% (107/287) were undecided, with only 15.3% (44/287) stating that in fact the risks outweighed the benefits. 14.3% (41/287) felt that development of UTx would not lead to greater patient happiness, but 31.7% were undecided (91/287). Therefore, the majority (54%) could see clinical UTx would lead to greater human

happiness. With respect to bringing benefit to medicine in general, over 60% of respondents thought that the development of UTx would also lead to a furthering of the medical, surgical and transplantation fields. 68.6% (197/287) thought that UTx is achievable, 27.2% (78/287) were not sure and only 8.4% (24/287) felt that it should never take place. 47.7% (137/287) believed that UTx should take place as soon as possible.

Group III: Fellows of the Royal College of Obstetricians and Gynaecologists only

23.1% (30/130) felt that the benefits of UTx outweighed the risks, 54.6% (71/130) were undecided, with 22.3% (29/130) stating that in fact the risks outweighed the benefits. 24.6% (32/130) felt that development of UTx would not lead to greater patient happiness, but 42.3% were undecided (55/130). Therefore, 33.1% (43/130) stated that clinical UTx would lead to greater human happiness. With respect to bringing benefit to medicine in general, less than 22% of respondents thought that the development of UTx would not lead to a furthering of the medical, surgical, gynaecological and transplantation fields. 54.6% (71/130) felt that UTx is achievable, 40.8% (53/130) were not sure and 12.3% (16/130) thought that it should never take place. 38.5% (50/130) believed that UTx should take place as soon as possible.

Group IV: Respondents who have encountered personal issues with infertility

54.9% (50/91) felt that the benefits of UTx outweighed the risks, 36.3% (33/91) were undecided, with 8.8% (8/91) stating that in fact the risks outweighed the benefits. 13.2% (12/91) felt that development of UTx would not lead to greater patient happiness, but 31.9% were undecided (29/91). Therefore, 76.9% (70/91) stated that clinical UTx would lead to greater human happiness. With respect to bringing benefit to medicine in general, over 60% of respondents thought that the development of UTx would also lead to a furthering of the medical, surgical and transplantation fields. 68.1% (62/91) felt that UTx is achievable, 29.7% (27/91) were not sure and 7.7% (7/91) thought that it should never take place. 53.8% (49/91) believed that UTx should take place as soon as possible.

Ranking issues

The level of importance of a particular issue with regards to achieving a successful UTx as well as its relation to other such issues of relevance has been demonstrated in **Box 4b.2**. **Figure 4b.1** is a more detailed and illustrative assessment of Group 1, ranked as the group containing seven general concepts thought to be the most relevant to UTx. Risks to fetal and child development together with maintaining pregnancy were the three areas considered the most important in bringing about a healthy pregnancy from a transplanted uterus.

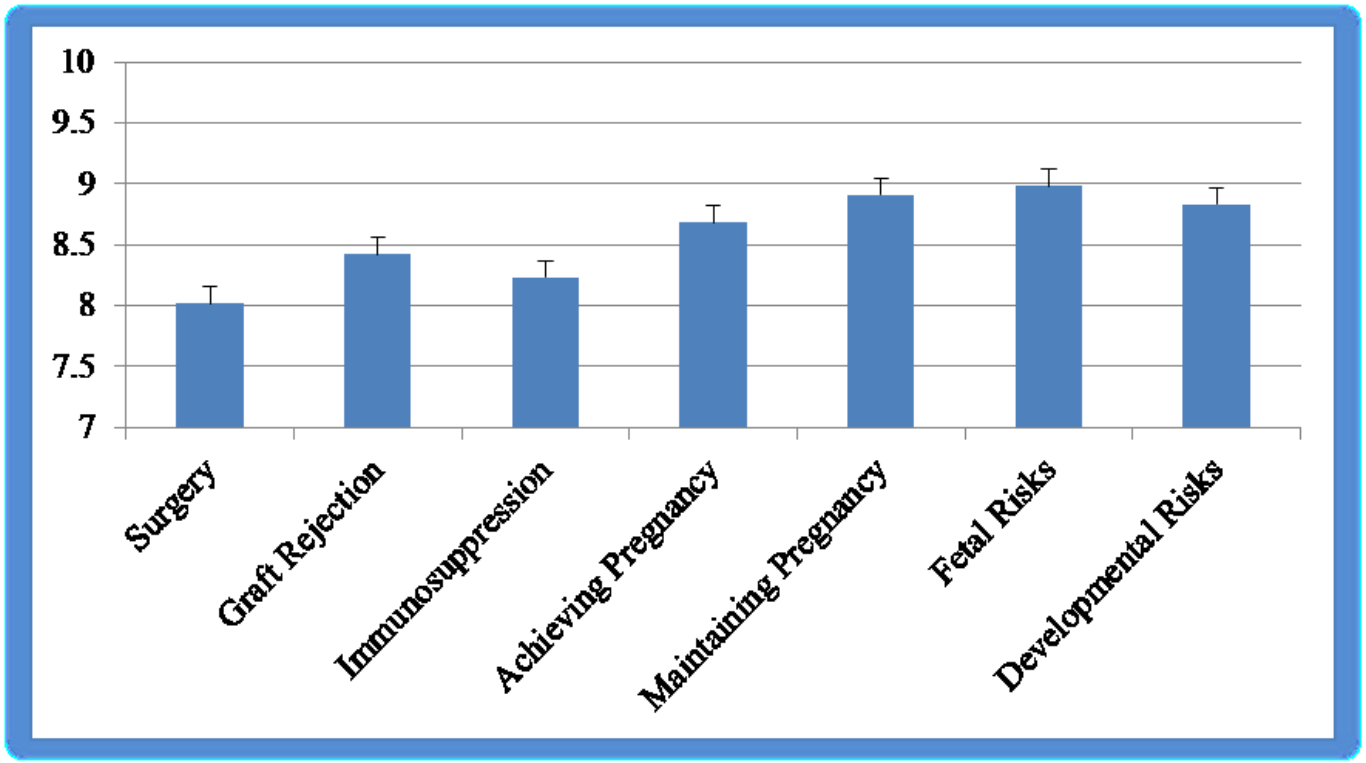
The scores all followed a normal distribution and were of statistical significance ($p<0.05$). The hypothesis score was defined as 5/10 (logical decision as 1=least important and 10=most important).

Box 4b.2 Grading of UTx issues according to how important they are in relation to each other (1=least important; 10=most important). Scores were provided by all 528 participants.

	Mean Score
Group 1: Concepts specific to Uterine Transplantation	**8.58**
Surgery (technique, time length, complications)	8.02
Immunology (graft rejection/tolerance)	8.42
Immunosuppressants (role and side-effects)	8.23
Achieving pregnancy post-transplantation	8.68
Maintaining pregnancy	8.91
Risks to fetus	8.98
Risk to child development	8.83
Group 2: Procedural issues	**7.94**
Developing a specific designated uterine transplantation team (surgeon, anaesthetist, psychologist, obstetrician, immunologist)	6.56
Close link of uterine transplant team with coordinators	7.41
Educating professionals about the procedure	7.84
Support for healthcare professionals	7.62
Impact of uterine transplantation on theatre and ITU staff	8.48
Exposure of healthcare professionals to press intrusion	8.86

Negative impact of uterine transplant on other transplant programs	8.84
Group 3: Retrieval issues	**7.05**
Development of donor/recipient criteria for Uterine Transplantation	8.12
Donor organ retrieval / transplantation time length	7.65
Interruption/delay of daily operating room time in host hospital	7.41
Liaison between other organ retrieval teams	7.07
Issues related to surgical technique	6.65
Patient acceptance of donor uterus (psychological/emotional impact)	6.06
Patient post-operative health status	6.42
Group 4: Recipient issues	**7.57**
Length of operation	7.99
Post-operative complications	7.48
Risk of rejection	6.68
Side-effects of immunosuppressants	7.35
Conception	7.44
Pregnancy	7.93
Risks to fetus/baby	8.12
Group 5: Donor Issues	**7.05**
Educating the donor family about the aim & benefits of uterine transplantation	8.16
Discussing with the donor family the actual process behind uterine transplantation	7.72
Procedure outcome - Likelihood of benefit for the recipient	7.81
Consent and consent form issues	7.90
Is there any relation between donor and recipient's offspring?	5.87
Exposure of donor family to press intrusion	6.28
Opportunities to meet with the recipient and potential baby post-transplantation	5.59

Figure 4b.1 Scoring (score: 1-10, y axis; 1=least important and 10=most important) of general UTx concepts (Group 1) according to how important each concept is (in relation to each other) with regards to achieving a successful UTx. Mean scores were provided by all participants. The concepts are shown on the x axis. Topics were permitted to be scored equally.



4b.4. Discussion

Part of the process of 'transferring' UTx from the animal to the human setting is ensuring that it is scientifically and ethically acceptable to attempt UTx in a woman. Gauging the thoughts and feelings of health-care professionals towards UTx is part of ethical acceptance. The support of fellow colleagues is not a necessary but is definitely a useful tool when it comes to bringing about the acceptance of a new and innovative procedure. Constructive pointers can also be highlighted in a pressure-free environment; for example, how the research process can be improved, areas which need more focus and depth and more emphatic ways for the UK UTx team to get their message across. The 528 participants surveyed fell into five major groups: obstetricians and gynaecologists, nurse and midwives, surgeons, donor co-ordinators and medical students. They all took a maximum of 15 minutes to complete the survey. It was handed out to them at the end of a presentation on aspects of UTx, emailed as a link following such a presentation or posted in an envelope.

The most important finding of the study is that a noticeable majority of the participants (>90%) support the development of the UTx research programme and believe that UTx should be performed in the near future if considered appropriate medically, surgically and ethically. Interestingly, the proportion of the surveyed population who believed that UTx should take palace 'as soon as possible' was significant - 43%. Exactly four years was the figure given as the average time by when UTx would be offered as an acceptable treatment procedure, if funding is in place. However, despite this apparent support for UTx, a smaller proportion - 57.2% - believed that UTx is an achievable objective. The second largest group, 39.2%, was not able to confidently decide, and declared themselves as 'unsure'. These two results when analysed together with the above percentage of the population who felt that UTx should be performed immediately suggests that generally speaking, healthcare professionals can be neatly placed in two camps. Those that feel enough research has been done to date, and thus, there is a real need for application of UTx clinically (43%) and those that, even though they may be supportive and want UTx to be performed provided it passes the ethics review,

think or cannot decide on whether human application of UTx can ever achieved (also 43%). Overall, however UTx appears to be supported by healthcare professionals in the UK with 94% wanting its clinical application provided all the criteria has been met and 57% stating that the afore-mentioned is achievable. Therefore, there is no real objection to UTx in principle.

The proportion of the surveyed population who reported that they were undecided regarding an answer to a particular question was substantial throughout. Exactly half the population were undecided as to whether the benefits exceeded the risks. Approximately 40% were likewise unsure as to whether UTx would increase patient happiness. These two results initially appear difficult to explain if one considers that over 90% of those surveyed believe that UTx should be attempted provided the procedure, the team and the institution meet medical, surgical and ethical criteria. It seems that generally the health-care profession are supportive of UTx as an idea to bring about the restoration of fertility but a significant proportion, 40-50%, are not confident that UTx will ever get to a clinical stage. Three reasons stood out: (a) belief that the risks of UTx to both the mother and fetus are significant enough to prevent human UTx; (b) conviction that transplantation and non-rejection of a uterine graft may be possible, yet the graft will not able to carry a term pregnancy; and (c) financial implications on an already cash strapped National Health Service. With respect to the first reason, the significance of the potential risks certainly cannot be underestimated. However, one needs to realise that human UTx can only occur when granted permission by both the (proposed) institution and ethics committees. This will only occur if those committees believe that the risks are minimal and substantially outweighed by the potential benefits of UTx to the woman and couple. It is understood that until then, a section of the health-care profession will believe that the risks outweigh the benefits. Nevertheless, if human UTx is approved, the number of those professionals who are undecided will greatly decrease, as they instead start to see the risks as minimal rather than significant and UTx as predominantly a procedure performed to bring about personal happiness. Until then, it is reassuring to see that, despite the ‘undecided’ population, only 13% of those surveyed actually think that the risks outweigh the benefits and only 12% feel that UTx will not bring about patient happiness if successful. The second reason is similar in that it constitutes an aspect which cannot be proven either way and in

fact, only actual human attempts will shed light onto whether a transplanted graft can become gravid. No set number of animal experiments that must be performed before human UTx is permitted exist because no objective standards or criteria exist to make such a determination. This is because ultimately animal models cannot fully predict how a clinical experience will play out. Whether a human UTx should be allowed here in the UK will depend on research and advances abroad but most crucially, on the judgment of the researcher and transplant teams, the review boards of the participating institution, and most importantly, the patient diagnosed with uterine factor infertility. Finally, financial shortcomings should never prevent medical research and especially, the trial of a novel and innovative procedure, whether that may be in surgery or medicine, as its real benefit will only become apparent following clinical trials.

When analysing the results according to the sub-groups, no real difference was seen in the percentages when compared to the overall results discussed above. The three sub-groups that were chosen prior to the commencement of the study were transplant team members (surgeons and donor co-ordinators), obstetricians and gynaecologists and fellows of the Royal College of Obstetricians and Gynaecologists specifically. The proportion of those who were undecided as to whether the benefits outweighed the risks revolved around 50%. Around two thirds of those surveyed from groups I and II felt that UTx was achievable, and 40% believed that it should occur as soon as possible. These results are crucial to the UK UTx drive. They demonstrate a broad level of support for this procedure from potential future team members. Obstetricians, gynaecologists, transplant surgeons and donor co-coordinators will all be part of the multi-disciplinary team looking after a UTx patient. Their belief in UTx is vital at the outset of a human attempt. Also, it will allow for any concerns and worries to be recognized before the procedure is officially approved. The results are also a strong indication that UTx research and the direction which the UK human attempt is heading towards have a strong base of support amongst colleagues which will eventually perform this operation and subsequently look after the patient and hopefully her fetus and baby. Group III, made up of fellows, and therefore more senior and experienced obstetricians and gynaecologists, was also overall supportive but definitely more cautious. A small drop in the proportion of those fellows who felt that felt that UTx was achievable

was noticeable - 54.6% in comparison to 66-68% recorded from groups I and II. Also 12.3% thought that it should never take place, which is still a small proportion but definitely higher than what was recorded for groups I and II. However those who believed that UTx should take place as soon as possible numbered the same as in groups I and II (~40%) which is reassuring. The main reason for this slight but still significant shift lies in the age demographic of the sub-population. The seniority of the group means that the participants are more experienced and more knowledgeable yet also more conservative, set in their own practice and less likely to consider novel and innovative ideas. All 16 fellows who stated that UTx should never take place cited ethics as their principle reason, i.e. UTx is an unethical procedure and its 'promise' should not be offered to 'vulnerable' patients. It is interesting that the same criticisms thrown towards our effort were also used in the 1960s and 1970s with the introduction of in-vitro fertilization. Fertility specialists have to date been the most supportive group towards UTx, both the research and the clinical effort, within the field of obstetrics and gynaecologists, most likely as they have faced similar arguments. Finally, in the same manner in which group III was less supportive, so group IV was more supportive than the general population, as well as groups I and II. Group IV was made of participants who have experienced infertility either personally or within a 'couple' setting. They have therefore individually felt the effects on infertility on their life as well as relationships. For this reason, their thoughts and ideas would be more empathic and understanding towards women who are experiencing a problem with their fertility - in this case a type of infertility caused by a uterine factor. Importantly, those familiar with problems that people with infertility encounter may find it easier to justify a radical and novel procedure. This was seen in the responses given. 54.9% felt that the benefits of UTx outweighed the risks, which was around 25% higher than the general population, including the other three groups. A smaller proportion was undecided. 76.9% stated that clinical UTx would lead to greater human happiness and over 60% of respondents thought that the development of UTx would also lead to a furthering of the medical, surgical and transplantation fields. In comparison to the overall proportion of 40%, in group IV, 53.8% believed that UTx should take place as soon as possible.

It goes without saying that being aware of infertility may not automatically mean that the participants would select it over current and accepted non-surgical interventions i.e. surrogacy and adoption. These two options would always be offered first to a potential patient. Only if the patient proved to both medical and psychological teams that surrogacy and adoption would not suit them, would UTx be offered. Therefore, a more appropriate interpretation of the above relationship is that it justifies further more grants and research into the need for UTx. UTx should never be an automatic treatment choice; it will only ever treat a small number of AUFI patients.

Ranking

Participants were also asked to rank various issues related to UTx. These issues were divided into five main groups (**Box 4b.2**). Group 1 included seven issues which together explain the essence of UTx. Group 2 included issues related to the actual UTx procedure and transplant programme. Groups 3-5 incorporated factors related to retrieval, recipient and donor aspects of UTx. The ranking process allowed for grading each issue according to its importance. The meaning of the word 'importance' in this case is as follows: importance with respect to UTx research, a future human UTx attempt and relative importance in comparison to other issues. Despite the number of participants who ranked all areas equally important, the actual ranking proved to be useful. Immunology and pregnancy issues were ranked as the most important, in particular maintaining pregnancy, risks to fetus and risks to development (**Figure 4b.1**). This would be as expected as these issues define the goal of UTx. It is only if they are researched and discussed properly, that one should move towards other issues. By this logic donor and procedure related issues are ranked last. Importantly, analysing the ranking separately for males and females, obstetricians and gynaecologists, fellows and transplant coordinators and surgeons did not alter the overall rank of the five groups.

Of relevance to the UTx team is that despite the multi-factorial nature of UTx, it is still the main topics (group 1) which will decide whether this procedure goes ahead in the clinical setting. If the evidence shows that immunologically the graft will not be rejected in both its pregnant and non-

pregnant states, and that conception, fetal growth and eventual delivery are statistically likely, then the other areas (groups 2-5) will not halt the trial of the procedure. Therefore, both the publicity and presentation of the research should focus on those topics. According to **Figure 4b.1**, conception, fetal progress and neonatal development are the key areas that score the highest rank with respect to importance, yet the only way to assess these areas is by allowing for human UTx in the first place. Also highlighted was the potential impact of UTx on theatre and ITU staff**,** negative impact of UTx on other transplant programmes and exposure of healthcare professionals to press intrusion

The questionnaire also proved valuable in demonstrating how to approach different aspects of UTx. The optimal strategy would be to break up each issue into a number of manageable steps and approach each one separately. Each step of the UTx process (patient selection, embryo storage, surgery, embryo transfer, implantation, gestation) as well as issues related to the operation, recipient and donor ought to be tackled separately. Each set of issues falling under one of these categories (Groups 1-5) must have a clear management pathway developed.

Strengths and Limitations

The most important factor in favour of the results gathered is that 68% of those surveyed have been working within the medical profession for at least 5 years, which gives greater relevance to the answers provided, as they are based on a degree of experience. In addition, 75% of the participants were above the age of 30.

All the conferences were generic 'obstetrics and gynaecology' conferences rather than specific conferences dedicated to fertility which should have minimised the risk of bias. However completing any type of survey poses the risk of 'self-selecting' individuals who will fill it in i.e. persons who are interested in some way or have a vested interest in the topic.

Conclusion

It is clear from the topics addressed and sub-groups whose answers were analysed that for UTx to be successful and accepted, a broad level of support needs to be established from the outset. This will lead to a creation of an environment which shields form unnecessary stress but also one where concerns can be raised and addressed prior to the commencement of UTx. This study is the first in literature to try to sample the opinions and concerns of health-care professionals towards UTx. The most important finding of this study suggests that this 'level of support' is already there with regards to UTx. A substantial majority of the participants support the development of the UTx research programme and believe that UTx should be performed in the near future if considered appropriate medically, surgically and ethically. Those who believe that UTx should take palace 'as soon as possible' were almost half of the population, with a slightly higher proportion believing that UTx is an achievable objective. More effort is required to educate health care professionals about all aspects of UTx. Areas that need further developing and education are relationships of risks and benefits of UTx to the mother and fetus, as well as the likelihood of pregnancy following UTx. Greater exposure of UTx research is a necessity.

Summary

This thesis represents a gradual addition to existing work done by the UK UTx team from 1997-2010. This has been the theme of UTx research since it began in the 1970s - always a steady, continuing accumulation of data towards the final goal of achieving pregnancy in a human transplant. Themes and areas which have been published on before have been added to and a number of novel ideas have been introduced.

Study 1

The two studies which were a continuation of previous research were the retrospective trachelectomy project and the rabbit allogeneic UTx. The point of the former study was to act as an introduction to UTx for this thesis. Trachelectomy is a fertility-preserving procedure and the origins of UTx lie in its discovery. There is an ultimate benefit with respect to UTx research. First, as a fertility-preserving procedure, it can raise awareness of other such procedures such as UTx Second, the surgical techniques required to carry out ART overlap significantly with the graft retrieval and anastomotic aspect of the UTx procedure. Finally, lessons learned from ART with regards to uterine perfusion can be applied to UTx. Namely, the fact that the uterus remains viable if supplied by two vessels out of the six that supply it (three pairs of ovarian, uterine and vaginal arteries). Post-ART, the uterus is most commonly supplied and drained by the left and right ovarian vessels only.

Study 2a

As mentioned the rabbit allogeneic UTx cased series was attempted previously by the UK team. This case series was the largest (nine as opposed to two previous sets of six and five). It highlighted some of the challenges with the choice of animal model and the complexity of the surgical procedure involved. The macrovascular patch technique involves aorto-aorta and veno-vena anastomoses which

would not be attempted in a human because of its highly risky strategy involving the most important blood vessels. The surgical survival rate (when including all 20 rabbit UTx performed by the team) was 80%, thus proving the feasibility of uterine allo-UTx using a macrovascular patch technique with respect to anatomical terms and surgical vascular achievability. Immunosuppression would follow kidney transplantation, with definite use of a steroid and CNI.

The projects which were novel here were (a) IVF in the rabbit model; (b) autotransplantation in a sheep (solely to the UK team); (c) introduction of multi-spectral imaging and eLASCA to both the rabbit and sheep models in order to see if they could offer a better method of assessing uterine perfusion and blood flow and supply; and (d) the two human studies.

The end result of the IVF experiment was a termination of pregnacy as a result of miscarriage. This may be seem rather disappointing but it is important to highlight that the study represents only the third example of conception and pregnacy following an allogeneic UTx, and the first such example in a rabbit model. The surgical anatomical macrovascular model was successful, with an appropriate level of perfusion attained. Post-operative recovery was uneventful, with no adverse effects recorded. The cause of fetal demise is most likely secondary to inadequate prevention of the immunological rejection response. Selecting the correct immunosuppression in a rabbit model proved difficult as a result of the paucity of data in the world literature as to what is deemed safe and appropriate in a rabbit allogeneic transplant model.

Study 2b

Considering the present sheep study, it should be emphasized that this was the first time that the UK team attempted a macrovascular UTx large-animal model. In particular, internal to external iliac vessel anastomoses. The surgical technique was very similar to what would be used in a human model, with an obvious improvement with regards to procedure duration and skill. The final conclusion was that the external iliac vessels may definitely be used as recipients of the graft vessels

to allow for a secure blood flow because in three (out of five) ewes where grafting was possible, the grafted uteri demonstrated immediate perfusion.

Study 3

A multispectral imaging laparoscope has been demonstrated as a potentially useful tool during UTx. On comparison of its methodology with pulse oximetry, it appears to be more accurate and scientific. The advantages were the ability of the technique to monitor cornual O_2Sat over the entire visible section of the organ in a fast and non-contact method. Furthermore, one does not have to 'wrestle' with the cornua to find a strong pulsatile signal. All these might be important for stages of the operation where time is critical. In the rabbit model, the most striking result was the level of perfusion in the one long-term surviving doe which was almost back to pre-operative levels when measured on day 89 post-UTx. This together with the pregnancy achieved in that particular uterus is a true proof of a successful UTx. Furthermore, the results demonstrated with the sheep model were extremely promising with no statistically significant difference between the pre-retrieval and post-UTx O_2Sat values. Again the most obvious explanation lies in the anatomical similarity when comparing the pelvis of a sheep and a woman, and therefore a surgery with which the operators felt more at ease and more comfortable.

The LASCA system is in principle a non-contact and real-time tool for the observation of flow, in this case blood, from which other parameters can be derived: heart rate, respiratory rate and oxygen saturation (level and map). Therefore its application is both qualitative and quantitative. Its strengths were revealed when applied to the sheep model. These preliminary results demonstrate vast potential of LASCA to surgery and importantly UTx. An accurate, real-time imaging modality, which uses a mathematical model to process the final results, is an improvement on current tools. Therefore, as a first attempt to trial an experimental technique in a previously untested area of medicine, the experience may be considered as useful. However, the eLASCA data acquired were limited and difficult to use in order to make any definitive conclusions. Therefore, this system currently remains a

prototype for further experimental investigation. **Table D** summarises the old and novel techniques input with regards to this thesis.

Study 4

The two human studies looked at the UTx conundrum from two different angles. The first study focused on potential UTx candidates and the second study on health-care professionals. The former study looked at attitudes and motivations of potential candidates to UTx. It was the first such study to demonstrate a qualitative relationship between AUFI patients and their curiosity and desire for UTx. In addition, it also formed the first part of what may be a psychological assessment of a potential patient. The health-care professional study is the first in literature to try to sample the opinions and concerns of health-care professionals towards UTx. It is clear from the topics addressed and sub-groups whose answers were analysed that for UTx to be successful and accepted, a broad level of support needs to be established from the outset. This will lead to a creation of an environment which shields form unnecessary stress but also one where concerns can be raised and addressed prior to the commencement of UTx. The most important finding of this study suggests that this 'level of support' is already there with regards to UTx. A substantial majority of the participants support the development of the UTx research programme and believe that UTx should be performed in the near future if considered appropriate medically, surgically and ethically. Those who believe that UTx should take palace 'as soon as possible' were almost half of the population, with a slightly higher proportion believing that UTx is an achievable objective.

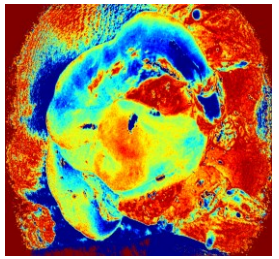
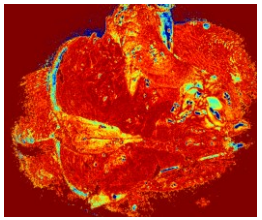
Controls

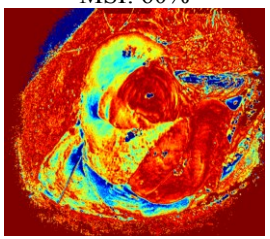
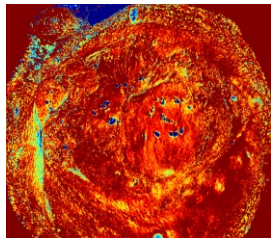
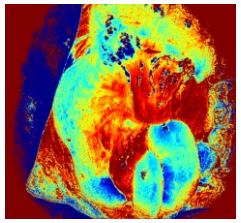
Controls were not used in this thesis as the concept of a control is not appropriate in this type of experimental approach. In the animal studies, the only situation whereby a control would have been suitable was the sole survivor (recipient #5). A pregnant non-transplanted doe would have acted as a control. In the immunology study, the white cell subset counts did have controls (unstained sample

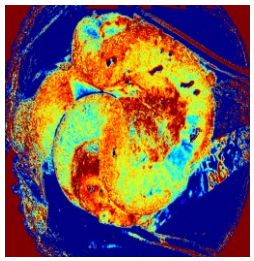
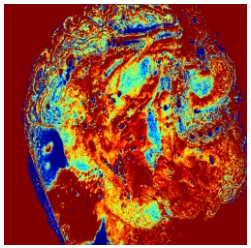
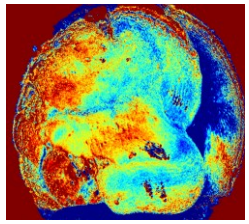
and IgG1). As for the perfusion studies, the uterine graft prior to UTx in both the rabbit and sheep and the non-transplanted graft in the recipient prior to the hysterectomy acted as control-type points of reference. The issue of controls does not apply to the human studies.

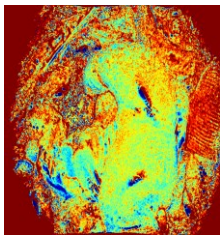
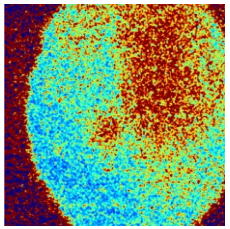
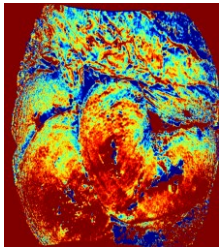
Further material relevant to the UTx story, but which was not part of this thesis, can be found under **Appendix E**.

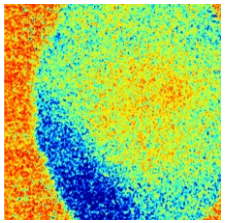
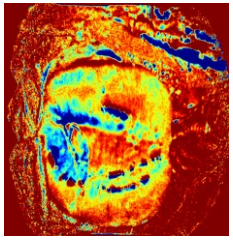
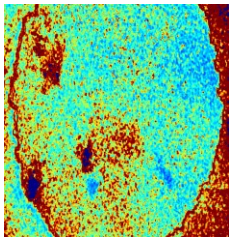
Table D Summary of post-mortem, histopathology and perfusion results

Uterine Transplant	Post-Mortem	Histopathology	Perfusion
Rabbit			
UTx #1	dusky, firm, oedematous anastomotic breakdown total blood volume: 30ml	Cavo-caval & aorto-aortic anastomosis breakdown leading to intraperitoneal haemorrhage	PO: 87% MSI: N/A eLASCA: N/A
UTx #2	dusky, firm, oedematous anastomotic breakdown total blood volume: 20ml	Cavo-caval & aorto-aortic anastomosis breakdown leading to intraperitoneal haemorrhage	PO: 85% MSI: N/A eLASCA: N/A
UTx #3	pink, soft, normal size no anastomotic breakdown	Respiratory compromise following blood aspiration	PO: N/A eLASCA: N/A MSI: 
UTx #4	pink, soft, normal size no anastomotic breakdown	Cardiac damage (Left ventricular rupture)	PO: N/A eLASCA: N/A MSI: 

UTx #5	pink, firm, large size no anastomotic breakdown	Rabbit culled electively	PO: 95% eLASCA: N/A MSI: 60%  **Gravid uterus** PO: N/A eLASCA: N/A MSI: 83% 
UTx #6	pink, soft, normal size no anastomotic breakdown	Widespread pneumonia	PO: N/A eLASCA: N/A MSI: 64% 

UTx #7	dusky, firm, oedematous anastomotic breakdown	Shearing of the aorto-aortic and cavo-caval anastomosis leading to intraperitoneal haemorrhage	PO: 93% eLASCA: N/A MSI: 25% 
UTx #8	pink, soft, normal size no anastomotic breakdown	Features in multiple organs of early ischaemia/hypoxia related injury, consistent with the history of hypovolaemic/haemorrhagic shock	PO: 94% eLASCA: N/A MSI: 59% 
UTx #9	dusky, hard oedematous no anastomotic breakdown	Macroscopic features of a pulmonary embolism Features of early peritonitis which may represent a systemic inflammatory response to recent surgery or early infection	PO: 86% eLASCA: N/A MSI: 59% 

Sheep			
UTx #1	N/A	N/A	N/A
UTx #2	dusky, firm, oedematous elements of breakdown	Uterus and oviduct show evidence of mild to moderate ischaemia. Thrombus in the left internal-external iliac anastomosis but the extent of the vessel lumen occlusion cannot be assessed.	PO: 83% MSI: 57%  eLASCA: 
UTx #3	pink, soft, normal size no anastomotic breakdown	Uterus and oviduct showing no significant abnormalities. No evidence of thrombus in the vascular anastomoses.	PO: 83% MSI: 71% 

			eLASCA: 
UTx #4	N/A	Features of myocarditis and an allergen induced bronchial hypersensitivity reaction. Both of these, alone or in combination would have affected the animal's ability to withstand the physiological stress of anaesthesia.	N/A
UTx #5	pink, soft, normal size elements of breakdown	Possible thrombus in the right vascular anastomosis. Uterus and oviduct showing no significant abnormalities. No evidence of ischaemia.	PO: 91% MSI: 60%  eLASCA: 

Epilogue

A group of experts and other 'stake holders' gathered in London in November 2012 to assess the state of the art of human UTx and address any lingering concerns as women are identified for the next UTx surgeries. Experts involved in UTx research discussed the necessary steps, presented here as a set of 'way markers', that must be considered in order to provide sufficient scientific and ethical justification for taking human UTx from a rare oddity, to a recognized and reasonable addition to the armamentarium of assisted reproductive technologies (ART). Speakers and participants represented areas of law, ethics, medicine, hospital administration, lay public and the social sciences. Specialists present included doctors of transplant medicine and surgery, infertility, gynecology, oncology, maternal-fetal medicine and others.

History of uterine transplantation

UTx was first performed in humans in 2000 on a 26 year old who had her uterus removed as a result of post-partum haemorrhage.[61] The transplanted uterus failed after three months. Although controversial and appearing without precedent at the time, animal work had been performed for decades before.

In the 1960s and 1970s, UTx and other female reproductive organ transplantations, were investigated as potential treatment for ovarian, tubal and uterine infertility.[56] UTx was subsequently ignored for two decades following progress of alternative and modern ART, often equally controversial. UTx came back into focus at the beginning of this century secondary to the development of related fertility-preserving procedures [60] in gynaecological oncology and the realisation that a significant number of infertile women suffered from AUFI.[3,4]

Potential indications

Causes of AUFI include congenital (absence or malformation) or acquired uterine factors (e.g. hysterectomy for uncontrollable haemorrhage) rendering a woman 'unconditionally infertile'. Current estimates are that in USA, up to 7 million women, age 15-34 years, with AUFI may be appropriate candidates for UTx.[3,4] As witnessed similarly with the advent of ART in the 1980s, the extent of the likely disease population enlarges at the introduction of any potential therapy. As a result of this realization, investigators have responded with publications demonstrating pregnancies in various autogeneic, syngeneic and allogeneic UTx animal models.[104,163]

After more than a decade of published research from multiple independent institutions, it is now verifiably possible for a surgeon, with no significant prior research in the area, to successfully perform UTx as shown by a case in August 2011 from Turkey.[261]

UTx may be safely performed today because of important developments in transplantation surgery including complex quality-of-life enhancing procedures. These are exemplified by multi-visceral, hand, larynx and face transplants.[273,274] Unlike in these other non-vital transplants, the grafted uterus (and the necessary immunosuppressants) will only be in place for the 2-3 years that are necessary for pregnancy to be achieved. Early UTx recipients must agree to only one viable birth and no more than one year of attempted conception. This will avoid the risk of immune suppression related neoplasms such as HPV and other cancers. The natural history of HPV in other transplant recipients allows us to confidently counsel our patients in this specific short term, limited risk. Similarly, pregnancy in transplant recipients of every organ reassures us that the effects of local endometrial immune modulation on endometrial embryonic receptivity appears to be better than that before transplantation.

Surgery and alternatives to uterine transplantation

UTx is therefore a 'temporary' treatment for AUFI only for women who cannot otherwise have a child either through adoption, surrogacy or any other method currently existing, or wish to undergo UTx in order to experience gestation. The patient who fits the criteria should be able to provide the team with informed consent under no coercion from partner or family and demonstrate all limitations provided by UTx.

The UTx recipient must have produced an oocyte which has been fertilized in vitro resulting in normal cryopreserved embryos. These embryos will have to be successfully transferred into the uterus as the Fallopian tubes would not have been transplanted with the uterus. Excluding the Fallopian tubes from the transplant should eliminate the risk of pelvic inflammatory disease, ectopic pregnancy and potential infertility brought on by Fallopian tube pathology. Similarly, the donor uterus must be free of overt infection, gross anatomic abnormalities, know HPV or known infertility.

The advantage of a live donor is that she could be a close relative, thus allowing for a better chance of a blood/tissue type match. Furthermore, the uterus and general well-being of the reproductive tract can be investigated extensively pre-operatively because of minimal time constraints. This would include imaging, as well as tests to rule out HPV infection, cervical dysplasia, leiomyoma, and endometrial polyps. On the other hand, the advantage of using a deceased donor lies with zero surgical risk to the donor and a more extensive dissection of the vascular tree on the uterine graft compared to a live donor. A more radical dissection would lead to recovery of larger arteries and veins, thus allowing for a technically easier vessel anastomosis. A disadvantage with using a deceased donor, compared with a live donor, is that graft survival may be negatively affected at brain death by major systemic inflammatory changes.[275]

We envisage that following a successful pregnancy, a woman will decide either to proceed with another future pregnancy or will wish for her uterus to be removed. If it were the latter, a total

abdominal hysterectomy will be performed approximately six weeks post-delivery or at the time of Caesarean section. At the time of Caesarean section, preparation for emergency hysterectomy should be contemplated. There is a theoretical risk of abnormal placentation but this has not been seen in the numerous animal experiments that have addressed altered blood supply, impact of different anastomoses, various immunosuppression regimens and other variables. An alternative option would be to stop the immunosuppressants and simply allow the graft to atrophy within the pelvis with or without abnormal placental invasion. Potential obstetrical complications are not limited to the placenta. Specialist in high risk obstetrics will need to call upon all their experience in balancing the risk to the maternal-fetal UTx patient.

Impaired quality-of-life, brought on by infertility, demands that UTx becomes safer and a more valid treatment option. Surrogacy and adoption will remain treatment alternatives of choice, but the risks associated with the former and the obstacles with the latter means they may not be satisfactory to everyone. Regardless, the majority of women with AUFI should be counselled and encouraged to pursue alternatives to UTx.

Adoption is a worthwhile option but can be a challenging process where parents face substantial expenses and sometimes evolving dilemmas. A current debate in the UK is whether to encourage easier adoption of children from one ethnic background by adoptive parents from a different ethnic background. Surrogate births are mostly uncomplicated but the practice has faced controversy, with it being illegal in most of the world and many states in the USA. In the UK, the surrogate delivers and the genetic parents legally adopt, so there is scope for the surrogate to refuse to give up the baby. A need exists for additional relief options where surrogacy and adoption cannot suffice, in this case UTx. FIGO's 2009 ethics statement advised against a premature move to human application.[276] However by 2012, other ethics groups were calling UTx 'a good option' under certain circumstances.[277] UTx will be ethically acceptable whenever and wherever several recognized criteria are satisfied. We therefore treat the news of other successful UTx with cautious optimism, whilst awaiting news of long-term survival and successful pregnancy.[277]

UTx must satisfy, as any surgical innovation would, criteria as defined by FD Moore i.e. laboratory background, field strength, and institutional stability.[278] Progress in multiple solid organ transplants has made UTx well within the technical capabilities of many transplant centres. For instance the uterus graft from a deceased donor may have only one large anastomosis (aorta/cava). However, even if multiple smaller anastomoses are used (never more than two), they are well within the experience of bowel and multi-visceral and certainly paediatric transplant centres. Paediatric transplant also provides reassurance regarding the vascular supply to the growing gravid uterus. Under physiologic control, paediatric anastomoses grow with the organ in a normal physiologic order. Animal transplants have confirmed that the fetus develops normally with no prematurity or growth restriction regardless of the vascular reconstitution. This is the case even in primates where *Mihara et al* underwent performed auto-UTx in non-human primate model (cynomolgous macaque) with anastomosis of two uterine arteries to the external iliac artery, and achieved pregnancy for the first time in this model.[279]

Equally important, UTx must satisfy accepted bioethical principles (respect for autonomy, beneficence, non-maleficence and justice) and their application: informed consent, appropriate assessment of risk and benefit and fair selection of individuals. Whether seen as innovative surgery or a medical study, eventually the early decisions to proceed in any venue should depend on approval by a duly constituted ethics review committee, the participating institution, the local transplant team and most importantly, the patient to whom the transplant will be offered.

Clearly, some institutions, and many surgeons, are capable and will proceed with UTx without any further research. However, it must be stated that a defined number of transplants should not be exceeded worldwide without a successful term delivery to minimize proceeding in futility using current techniques. From the above mentioned maternal and fetal complications, we have the obligation towards the patients and society, to closely monitor and registrar outcome of the procedure

and define satisfactory outcome no less than a live birth. If not achieved, the procedure should not be allowed to be instituted as treatment option. Towards this end, a registry should be maintained of all recipients and candidates much like the transplant waiting list which is updated for other organs.

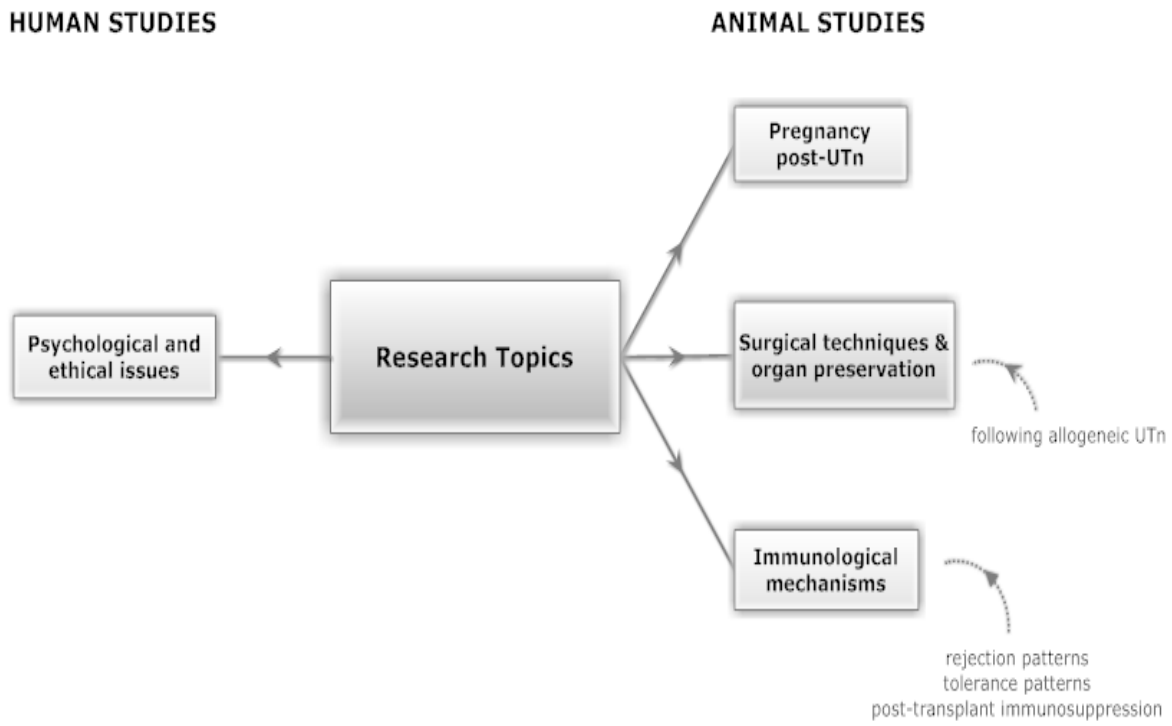
Further research

The following research, most of it covered by this thesis (**Figure J**), is still required to allow application to an IRB for a human UTx:

1) Additional pregnancies in a variety of large animal/primate models (to search for unanticipated consequences);
2) Continuous assessment of women diagnosed with AUFI regarding UTx;
3) Continuous assessment using "borrowed" psychological tools from transplant centres, adoption agencies and ART centres of potential recipients;
4) Continuous careful ethical reflection, assessment and approval.

Our group will continue to meet annually to address these areas and other special challenges that will arise. These challenges will require mature resources from institutions experienced in innovation now engaged in UTx. For instance appropriate 'guaranteed' funding levels will be impossible to ensure for the UTx recipient. Much of the expense, pregnancy, delivery and neonatal care, will be borne by the traditional payers. As best as possible, every contingency should be considered prior to UTx at any specific institution. Similarly, institutions with UTx capabilities must have had experience with appropriate pathways for media engagement for the recipient, her partner, and eventually, the child.

Figure J What must be done prior to proceeding with human UTx?



Conclusion

The second human UTx performed in 2011,[261] as well as a series of 10 cases performed in Sweden in 2012-13,[280] one of which led to a healthy live birth means that UTx is now a recognised feasible procedure.[281] Pregnancy following organ transplantation is complex but now commonplace. Closing on half a century of experience with pregnancy in solid organ recipients, an abundance of data has accumulated indicating satisfactory maternal and neonatal outcomes. Future UTx candidates, likely to represent a group not burdened by multiple co-morbidities, should be the beneficiaries of an even better prognosis.

Further pregnancies after UTx will be challenging, but cannot be unexpected. As news of progress from the Turkish and Swedish cases and of any other human attempts is awaited, the belief is that UTx has become a matter of 'when next' rather than 'if'.

Appendix A - *Statistical analysis*

The analysis of data is described in the Methods section for each study. A brief overview is given here.

Data related to O_2Sat and PI was defined as non-parametric and *Mann-Whitney U test* was therefore carried out for comparison. Data related to the 'Patient Assessment' and 'Health-care Professionals' survey was also defined as non-parametric data and *Mann-Whitney U test* was therefore again carried out for comparison. Attitudes, motivations and feelings of UTx patients (**Table 4a.2**) and the sub-group analysis (**Table 4a.3**) were analysed using Fisher's exact test. The ranking data for the 'Health-care Professionals' survey was analysed using a one-sample Kolmogorov-Smirnov test. A statistically significant difference was applied for a p-value <0.05. All statistical analysis was done using the Statistical Package for the Social Sciences version 19 (SPSS Inc, Chicago, Illinois, USA).

Appendix B - ***Study 4a: Interview***

INFORMED CONSENT FORM

Title Study: Views on Womb Transplantation
Version Number: 2
Name of Principal Investigator: J. Richard Smith
Date: 29/07/2011

1. I confirm that I have read and understand the subject information sheet dated ..... version ..... for the above study and have had the opportunity to ask questions which have been answered fully. ☐

2. I understand that my participation is voluntary and I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that my participation will involve a filling out of a questionnaire and also appreciate that my responses to questions will be taped. ☐

4. I understand that sections of any of my medical and/or research notes may be looked at by responsible individuals from Queen Charlotte's Hospital, Imperial College Healthcare NHS Trust, Imperial College London or regulatory agencies, where it is relevant to my taking part in this research. I give permission for these individuals to access my records. ☐

5. The compensation arrangements have been discussed with me. ☐

6. I agree to take part in the above study. ☐

_____	_____	_____
Name of Participant	Signature	Date
_____	_____	_____
Name of Person taking consent (if different from Principal Investigator)	Signature	Date
_____	_____	_____
Principal Investigator	Signature	Date

Participant Information Sheet

Dear Madam,

Welcome. You are being invited to take part in a research study, organised and funded by Imperial College London. The research project has been reviewed and approved by an official panel involving 3 professors based at our institution, as well as the Hospital's Research Ethics Committee.

We have decided to approach you in view of your history of medical problems related to your womb/uterus. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Background

Our team has been looking into the area of uterine (womb) transplantation as a possible solution to womb factor infertility. This type of infertility describes many young women who were born without a womb or who have lost their womb because of illness. The only human attempt at womb transplantation occurred in 2000 in Saudi Arabia where the transplanted womb unfortunately had to be removed after 99 days. The belief in the scientific community was that this attempt was too early with a minimal amount of prior research. Since then research into womb transplantation (immunological, reproductive and technical aspects) has increased, with the various international teams aiming to arrive at a point where womb transplantation would be considered safe enough for a second human attempt.

Our study

This study is designed to learn more about the views of women with womb infertility and who may one day benefit from womb transplantation. It is important to note that this research is still very much at an experimental stage and is not available in medical practice. Therefore, there would be no direct benefit to you with regards to fertility if you take part in the study.

If you decide to take part, we would like to ask you some questions using a pre-prepared questionnaire. Following this, we will show you a 10 minute video during which the issues related to womb transplantation are discussed. After the video finishes, we plan to talk about some of the issues raised. The whole process should take ~60 minutes. It will be recorded on tape to allow for the interview to flow more quickly and for us to analyse your responses later without worrying that we may have missed important information. There is a small chance that we may contact you again in the future for one further interview, and if this were to occur it would be done only once. In total we hope to interview 30-40 women from this particular clinic.

Deciding to take part

It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. We will then conduct the interview at a time that best suits you. Once the interview has begun, you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

Questions/queries

Please note that all data will be kept confidential and adhere to the Data Protection Act. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised from it.

We cannot promise the study will help you but the information we get might help improve the treatment of people with Womb Factor Infertility in future. The results of study will made available to you, with the data published in a suitable medical journal. You will not be identified in any publication.

If you have any questions or queries after the interview has finished, contact either Mr J. Richard Smith or Mr Srdjan Saso below who will happy to take your questions. In addition, if you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the Investigator (Mr J. Richard Smith, see contact details below). The normal National Health Service complaint complaints mechanisms are also available to you. If you are still not satisfied with the response, you may contact the Imperial AHSC Joint Research Office.

Thank you very much for reading about our project and considering taking part - your efforts are very much appreciated.

With best wishes,

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QUESTIONNAIRE

Title Study: Views on Womb Transplantation
Version Number: 5
Name of Principal Investigator: J. Richard Smith
Date: 28/10/2011

Part 1: General Questions for Patients regarding Uterine Transplantation

1. What is your profession?

Administrative		☐
Advertising		☐
Art related		☐
Business/Entrepreneurship		☐
Catering industry		☐
Finance related	☐	
General Industry		☐
Journalist/writer		☐
Lawyer	☐	
Management Consultant	☐	
Scientist		☐
Teacher/Academic		☐
Trade (builder/plumber/electrician/carpenter etc…)		☐

2. Please indicate your age?
Age …..

3. How old were when you were diagnosed with Absolute Uterine Infertility?
Age …..

4. Would you like to have a child(ren) of your own?
Yes No

5. To what extent would you want to have children (1 - not at all; 5 - very much)?
.....

6. Do you currently have a partner with whom you would consider starting a family?
Yes No

7. Prior to today, how much have you known about Uterine Transplantation?
A lot A fair amount Heard it discussed only a few times Nothing

8. How interesting do you find the field of Uterine Transplantation?
A lot A fair amount Not that much No interest whats-o-ever

9. I do not understand the benefits of Uterine Transplantation.
Strongly agree Agree Undecided Disagree Strongly Disagree
If disagree/strongly disagree, why? ……..……

10. I understand the risks of Uterine Transplantation.
Strongly agree Agree Undecided Disagree Strongly Disagree
If disagree/strongly disagree, why? ……..……

11. I do not believe that the potential benefits of Uterine Transplantation outweigh the potential risks.

Strongly agree Agree Undecided Disagree Strongly Disagree

If disagree/strongly disagree, why? ……..……

12. I believe that the development of uterine transplantation will generally benefit the fields of medicine and surgery.

Strongly agree Agree Undecided Disagree Strongly Disagree

If disagree/strongly disagree, why? ……..……

13. I do not believe that the development of uterine transplantation will benefit the field of obstetrics and gynaecology.

Strongly agree Agree Undecided Disagree Strongly Disagree

If disagree/strongly disagree, why? ……..……

14. Funds and grants should not be made available for novel and innovative fields such as uterine transplantation.

Strongly agree Agree Undecided Disagree Strongly Disagree

If disagree/strongly disagree, why? ……..……

15. This question is hypothetical and we apologise if it causes any distress. You do not have to answer it. If you were to have a functioning uterus and uterine transplantation ever became a reality, would you give permission for your uterus to be added to the Organ Donation register?

Yes No Not sure?

If no, why not? ……..

16. Provided the risks of uterine transplantation were negligible would you accept a uterus if it could lead to a healthy pregnancy?

Yes No Not sure?

If no, why not? ……..

17. In your opinion, should Uterine Transplantation not take place?

Never take place Not yet Should take place as soon as possible If not yet, why not? ……..

If never, why not? ……

18. In your opinion, is Uterine Transplantation achievable?

Yes No

If no, why not? ……..

If yes, how many years? ……

Patient Motivation and Uterine Transplantation

Please give a GRADE from 1 to 10
1 = 'strongly disagree' AND 10 = 'strongly agree'

1. The ability to have a child of my own is important to me ……...

2. Surrogacy and/or adoption offer a valid solution to my infertility …….

3. I have looked at all available options to have a child and feel that uterine transplantation would not suit me the most ……

4. If uterine transplantation became a safe, tried and tested option, I would consider it as my first option i.e. ahead of both of surrogacy and adoption …….

5. I do not find uterine transplantation extremely relevant to my quality of life ……..

6. The development of uterine transplantation will lead to greater personal happiness ……

7. If uterine transplantation became possible in humans, I would not undergo this procedure in order to have a child ……

8. If uterine transplantation became possible in humans, I would undergo this procedure, fully aware that adoption or surrogacy would be ultimately safer for my wellbeing ……

9. If uterine transplantation became possible in humans, I would not undergo this procedure, as I am fully aware that it could fail and the ultimate goal of achieving successful pregnancy would not be achieved ……

10. If uterine transplantation became possible in humans, I would undergo this procedure, fully aware of significant risks to my future baby ……

11. My desire to have a child is based solely on my own feelings and wishes and I am not influenced by anybody else ……....

12. I have a strong support system around me, including family and friends – I would therefore not be alone to go through this ……...

Issues surrounding Uterine Transplantation

Please GRADE the following issues according to how important you feel they are in relation to each other

Grade from 1-10 where 1=least important and 10=most important

Issues can be given the same grade

1. Concepts specific to Uterine Transplantation

Surgery (technique, time length, complications) ……..
Immunology (graft rejection/tolerance) ……..
Immunosuppressants (role and side-effects) ……..
Achieving pregnancy post-transplantation ……..
Maintaining pregnancy ……..
Risks to fetus ……..
Risk to child development ……..
Donor family issues ……..

2. Of all the issues covered, what do you think is the most important issue and why?

……
……
……
……

Part 2: Discussion of topics relevant to UTx/Q&A Session

Part 3: 20 minute video

Part 4: Shortened questionnaire

1. I understand the benefits of Uterine Transplantation.

Strongly agree Agree Undecided Disagree Strongly Disagree
If disagree/strongly disagree, why? ……..……

2. I do not understand the risks of Uterine Transplantation.

Strongly agree Agree Undecided Disagree Strongly Disagree
If disagree/strongly disagree, why? ……..……

3. I believe that the potential benefits of Uterine Transplantation outweigh the potential risks.

Strongly agree Agree Undecided Disagree Strongly Disagree
If disagree/strongly disagree, why? ……..……

4. I do not believe that the development of uterine transplantation will generally benefit the fields of medicine and surgery.

Strongly agree Agree Undecided Disagree Strongly Disagree
If disagree/strongly disagree, why? ……..……

5. I believe that the development of uterine transplantation will benefit the field of obstetrics and gynaecology.

Strongly agree Agree Undecided Disagree Strongly Disagree
If disagree/strongly disagree, why? ……..……

6. Funds and grants should not be made available for novel and innovative fields such as uterine transplantation.
Strongly agree Agree Undecided Disagree Strongly Disagree
If disagree/strongly disagree, why? ……..……

7. This question is hypothetical and we apologise if it causes any distress. You do not have to answer it. If you were to have a functioning uterus and uterine transplantation ever became a reality, would you give permission for your uterus to be added to the Organ Donation register?
Yes No Not sure?
If no, why not? ……..

8. Now that you have watched a video presentation where we discuss the potential risks to you and your baby would you accept a uterus if it could lead to a healthy pregnancy?
Yes No Not sure?
If no, why not? ……..

9. In your opinion, should Uterine Transplantation not take place?
Never take place Not yet Should take place as soon as possible If not yet, why not? ……..
If never, why not? ……

10. In your opinion, is Uterine Transplantation achievable?
Yes No
If no, why not? ……..
If yes, how many years? ……

Patient Motivation and Uterine Transplantation

Please give a **GRADE** from **1 to 10**
1 = 'strongly disagree' AND **10 = 'strongly agree'**

1. If uterine transplantation became possible in humans, I would undergo this procedure in order to have a child ……

2. If uterine transplantation became possible in humans, I would not undergo this procedure, as I am fully aware that adoption or surrogacy would be ultimately safer for my wellbeing ……

3. If uterine transplantation became possible in humans, I would undergo this procedure, fully aware that it could fail and the ultimate goal of achieving successful pregnancy would not be achieved ……

4. If uterine transplantation became possible in humans, I would not undergo this procedure, fully aware of significant risks to my future baby ……

Issues surrounding Uterine Transplantation

Please GRADE the following issues according to how important you feel they are in relation to each other

Grade from 1-10 where 1=least important and 10=most important

Issues can be given the same grade

1. Concepts specific to Uterine Transplantation

Surgery (technique, time length, complications) ……..
Immunology (graft rejection/tolerance) ……..
Immunosuppressants (role and side-effects) ……..
Achieving pregnancy post-transplantation ……..
Maintaining pregnancy ……..
Risks to fetus ……..
Risk to child development ……..
Donor family issues ……..

2. Of all the issues covered, what do you think is the most important issue and why?

……
……
……

Part 5: Post-video interview

a) How does the diagnosis of infertility affect your day-to-day life?

b) Have you given any thought to having children in the future? If you have, which methods have you considered (surrogacy, adoption)? What are your thoughts about such options?

c) Why is the idea of 'having children' important to you?

d) In the questionnaire, you had to answer questions regarding uterine transplantation. Following that, you watched a video on this topic. Have you ever heard of this proposed procedure before and what are your thoughts regarding it?

e) Do you feel that uterine transplantation can ever become a valid option? Can you explain your answer?

f) Can you describe what the process of uterine transplantation might entail (e.g. patient selection, surgery, IVF, pregnancy, delivery, immunosuppression, support)?

g) Do you have any other questions? Is there something regarding the questionnaire or this interview that you would like to discuss?

Appendix C Study 4b: The Healthcare Professionals Survey: Views on Uterine Transplantation

1. What is your medical profession?
Obstetrician/Gynaecologist □
Surgeon □
Physician □
Pathologist/Immunologist □
Paediatrician □
Psychiatrist □
Nurse □
Midwife □
Donor Co-ordinator □
Medical Student □
Others, please specify …….

2. Please indicate your age and gender?
Age ….. Gender …..

3. Please indicate the length of time that you have been working/studying within the medical field (not including research):
Less than 1 year □
Between 1 and 5 years □
Between 5 and 10 years □
More than 10 years □

4. Have you ever experienced any personal issues with infertility?
Yes □ No □ Declined to comment □

5. Do you know anyone (woman or a couple) who has experienced problems with conceiving i.e. infertility?
Yes □ No □

6. Prior to today, how much have you known about Uterine Transplantation?
A lot □ A fair amount □ Heard it discussed only a few times □ Nothing □

7. How interesting do you find the field of Uterine Transplantation?
A lot □ A fair amount □ Not that much □ No interest whats-o-ever □

8. I understand the benefits of Uterine Transplantation.
Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □
If disagree/strongly disagree, why? ……..……

9. I do not understand the risks of Uterine Transplantation.
Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □
If disagree/strongly disagree, why? ……..……

10. I believe that the potential benefits of Uterine Transplantation outweigh the potential risks.
Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □
If disagree/strongly disagree, why? ……..……

11. I do not believe that the development of uterine transplantation will lead to greater patient happiness.
Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □
If disagree/strongly disagree, why? ……..……

12. I believe that the development of uterine transplantation will generally benefit the fields of medicine and surgery.
Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □
If disagree/strongly disagree, why? ……..……

13. I do not believe that the development of uterine transplantation will benefit the field of obstetrics and gynaecology.
Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □
If disagree/strongly disagree, why? ……..……

14. I believe that the development of uterine transplantation will be a useful and welcome addition to the field of transplantation.
Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □
If disagree/strongly disagree, why? ……..……

15. Funds and grants should not be made available for novel and innovative fields such as uterine transplantation but should instead be focused on life saving research programmes e.g. cancer research.
Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □
If disagree/strongly disagree, why? ……..……

16. Would you give permission for your or your family member's uterus to be donated after death?
Yes, for both my uterus and a family member's uterus □
Yes, for my uterus only □
Yes, for my family member's uterus only □
No, not for either my uterus or a family member's uterus □
I cannot make a decision for my family member □
I do not have a uterus □
Not sure □

If no, why not? ……..

17. In your opinion, should Uterine Transplantation not take place?
Never take place □ Not yet □ Should take place as soon as possible □
If not yet, why not? ……..
If never, why not? ……

18. In your opinion, is Uterine Transplantation achievable?
Yes □ No □ Not sure? □
If no, why not? ……..
If yes, how many years? ……

Issues surrounding Uterine Transplantation

Please GRADE the following issues according to how important you feel they are in relation to each other

Grade from 1-10 where 1=least important and 10=most important

Issues can be given the same grade

1. Concepts specific to Uterine Transplantation

Surgery (technique, time length, complications) ……..
Immunology (graft rejection/tolerance) ……..
Immunosuppressants (role and side-effects) ……..
Achieving pregnancy post-transplantation ……..
Maintaining pregnancy ……..
Risks to fetus ……..
Risk to child development ……..

2. Recipient issues

Length of operation ……..
Post-operative complications ……..
Risk of rejection ……..
Side-effects of immunosuppressants ……..
Conception ……..
Pregnancy ……..
Risks to fetus/baby ……..

3. Procedural issues

Developing a specific designated uterine transplantation team ……
(surgeon, anaesthetist, psychologist, obstetrician, immunologist)
Close link of uterine transplant team with coordinators ……..
Educating professionals about the procedure ……..
Support for healthcare professionals ……..
Impact of uterine transplantation on theatre and ITU staff ……..
Exposure of healthcare professionals to press intrusion ……..
Negative impact of uterine transplant on other transplant programs ……..

4. Retrieval issues

Development of donor/recipient criteria for Uterine Transplantation ……..
Donor organ retrieval / transplantation time length ……..
Interruption/delay of daily operating room time in host hospital ……..
Liaison between other organ retrieval teams ……..
Issues related to surgical technique ……..
Patient acceptance of donor uterus (psychological/emotional impact) ……..
Patient post-operative health status ……..

5. Donor Issues

Educating the donor family about the aim & benefits of uterine transplantation ….
Discussing with the donor family the actual process behind uterine transplantation ….
Procedure outcome - Likelihood of benefit for the recipient ……..
Consent and consent form issues ……..
Is there any genetic relation between donor and recipient's offspring? ……..
Exposure of donor family to press intrusion ……..
Opportunities to meet with the recipient and potential baby post-transplantation …

6. Of all the issues covered, what do you think is the most important issue and why?

……
……
……
……

7. Are there any issues that we have not covered? What are they?

……
……
……
……

Appendix D – Letter to the Fellows of the Royal College of Obstetricians and Gynaecologists

Dear Fellows of the Royal College of Obstetrics and Gynaecology,

Mr J. Richard Smith has been the UK lead into Uterine Transplantation for the past 10 years. We thought it was time to bring you up to date with progress and to tell you about the next phase of our research.

Our team, based at The Lister Hospital in Chelsea and at Imperial College London, is about to begin several new projects. We will very soon be carrying out a new series of operations on mammals to prove some new surgical techniques and hopefully to enable some of the animals to become pregnant.

One of these new projects is designed to learn more about health-care professionals' attitudes and importantly the views of our own colleagues regarding uterine transplantation.

It is in connection with this project that we would very much appreciate your help. Dr Srdjan Saso is a clinical research fellow carrying out a PhD in uterine transplantation at Imperial College and has devised a questionnaire which attempts to assess the views of health care professionals

Furthermore, a website is almost up and running. On it, one will be able to find out everything about the project - background, reasons for carrying out the research, future related projects and conferences, forthcoming events, information concerning members of our team and contact details if you wish to raise any issues.

If you would like to help our research, please fill in the questionnaire and either post it back to one of the addresses below or drop it off at the College.

Thank you once again for expressing an interest in our programme. We very much hope we will hear from you again in the near future. Please do not hesitate to contact us if you have any queries.

With best wishes,

Mr J. Richard Smith MD FRCOG and Mr Srdjan Saso MBBS BSc MRCS

Appendix E

Uterine Transplantation in Primates

This thesis has focused on two specific animal models, rabbit and sheep. Lately, UTx research has been focusing on experiments concerning non-human primates and it is worth describing in brief the progress in this area of UTx. The main advantage is the relatively large body size and the close resemblance to the anatomy and physiology of a human. The foremost similarity lies in the straight cervical canal enabling endometrial sampling, embryo-transfer and hysteroscopy. Several reviews and guidelines have stated that primate research should be mandatory before introduction of UTx in humans.[4,64,282-284] Prior to the first ever human UTx in 2000, the only primate research was performed by *Scott et al* in the early 1970s.[67] This was an original experiment, especially when considering the decade in which it was carried out, involving the transplantation of the both the uterus and Fallopian tube. As discussed previously, the transplantation performed was just one of the solutions put forward as a possible cure of infertility. Four rhesus monkeys had auto-transplants and four had cross-transplants with each serving as a donor and a recipient in the latter. With respect to the auto-transplants, following their removal, the uterus and Fallopian tubes were placed back into the same monkey after 20 minutes, with no dissection of vascular pedicles. The surgical technique involved omental wrapping and therefore the use of the omentum as the vascular supply. The blood flow was re-established by neo-agenesis from the omentum. Three out of four autotransplants maintained a normal sized uterus and resumed cyclical menstruation with clomiphene citrate stimulation. One monkey died three months following the operation from an unknown cause. The three surviving monkeys were placed with males for 10 months but no pregnancy occurred. Histopathology carried out one year following the transplants showed a normal secretory endometrium but non-patient Fallopian tubes, most probably caused by an ischaemic period of several days post-autoUTx. This most likely explained the absence of a gravid uterus. In a human UTx, the Fallopian tubes would not be transplanted as conception would occur via embryo transfer and would therefore, not be natural.

The allotransplants underwent regular post-operative biopsies of their internal genital organs. The usual rejection signs were elucidated - oedema and progressive mononuclear infiltration which appeared initially in the endometrium and after 14 days full rejection was present. The endometrium was rejected more rapidly than the myometrium as seen with small-animal studies. Weekly white cell counts and haematocrit levels were no different between the groups. It is worth highlighting that a one year follow-up period in this study is to be commended as that exact period would be applied in human UTx. The omentum technique is today obsolete and would not be practised in a woman. However from the results above it seems that the omentopexy technique may indeed bring about uterine graft re-vascularisation if the uterus is of a smaller size such as in the rhesus monkey. The allotransplants in the above study were not administered any immunosuppression.[67] Despite extensive use of immunosuppression in primate research, results cannot be directly extrapolated.[285] What may be therapeutic in humans i.e. hand transplantation, may be sub-therapeutic in composite tissue allotransplantation in monkeys.[4,286,287]

The next of set primate experiments was performed by *Fageeh et al* as part of preparation for human UTx.[61] These experiments were described only briefly. Hysterectomies followed by auto-transplantations were performed in 16 baboons. Tissue and vascular integrity was preserved. The uteri were flushed with cold Euro-Collins solution. Ischaemic times were not reported. Inspection of tissue texture and colour at exploration 6–12 weeks post-operatively indicated viability. Two types of anastomosis were tried out, first end-to-end uterine vessel anastomosis and subsequently a modification to end-to-side anastomosis to the internal iliac vessels. Occlusion was 75% and 90% respectively. The primate studies demonstrated survival of the uterine graft and indicated that good mid- and long-term vessel patency could be achieved using particular microvascular techniques for uterine arterial and venous anastomosis in an end-to-side fashion.[61]

Since then six further studies have been performed on non-human primates. It is interesting that they have all been published from 2010-2013 as primate research is a very novel addition to the long-standing body of UTx research. *Enskog et al* performed an original experiment whereby basic surgical

techniques were developed in a case series of baboon uterine transplants. Five animals were used for studies of pelvic vascular anatomy.[173] Ten underwent actual surgery - a uterine autotransplant. The graft was utero-tubal-ovarian, with the recovery surgery including bilateral dissection of the uterine arteries and the anterior portions of the internal iliac arteries, and for venous outflow bilateral ovarian veins. The ovaries and the oviducts were kept with the graft as the hormones produced resulted in typical cyclic perineal skin changes of the female baboon which is an easy and non-invasive method to assess graft function.[288] The graft was removed, flushed and kept ex vivo for two hours when the two arterial ends and two venous ends were anastomosed side-to-side to construct one arterial and one venous end. The single arterial and venous ends were anastomosed unilaterally to the external iliac vessels. The animals (n=10) were evaluated concerning cyclicity and later by laparoscopy/laparotomy. The total duration of organ retrieval, and transplantation was recorded by the authors and came to around six hours with an overall ischaemic time of the specimen of about three hours. Nine animals survived with one animal dying from cardiomyopathy. Five out of the nine surviving animals resumed cyclicity which suggested re-established ovarian function. Only two out of these five animals exhibited resumed menstruation, indicating re-established both ovarian and uterine function. Normal-sized uteri were confirmed in these two animals. The conclusions of the authors were apposite. The feasibility of UTx by vascular anastomosis in a non-human primate species was demonstrated in this study. However, as only 20% of the uteri resumed menstruation, it was concluded that UTx is a complex procedure and thus, modifications of the surgical method would be required.[173]

The follow-up study was also performed by the Brannstrom team.[289] The animal model was again a baboon and the transplantation was autogeneic. The authors modified the surgical technique and extensively dissected the ovarian veins to include their inlets into the inferior caval vein and the left kidney vein. This was done so as to achieve a vascular anastomosis with venous walls that are thicker. With respect to anastomosis, the modification was such that the anastomosis was unilateral and end-to-end to the internal iliac artery. The anastomosis surgery was also performed by a transplant surgeon. Ten baboons were operated on using the modified technique whereas six underwent a technique like that described in the previous study.[173] Overall short-time survival of the animals was

88%. Out of the surviving animals, 75 % (80% in the modified group and 66% in the original group) resumed ovarian cyclicity. Regular menstruation after UTx was only demonstrated in the modified group; it was 60 % which is an increase on the previous study (20%). The authors tried to achieve both conception and pregnancy by exposing the menstruating animals (n=6) to timed mating for >5 menstrual cycles. However pregnancy did not occur. This was most likely because of adhesions and tubal blockage seen in the post-mortem analysis. This conclusion is yet another which adds to the growing evidence that conception should be attempted in a woman via IVF and embryo transfer. The final conclusion of the authors is suitable, that the modified anastomosis technique is a safe procedure and leads to resumed long-term uterine function in a majority of the animals.[289]

Longer term non-human primate UTx survival has also been reported with successful immunosuppression. Using rhesus macaques, three uterine allograft transplants were performed. Two animals expired post-UTx but the third transplant was successfully accomplished and maintained for approximately one year.[283] This primate data was also important for demonstrating that acute rejection could be treated with commonly available immunosuppressants that are compatible with human pregnancy. Specifically, immunosuppression of the primate uterus was maintained with CsA targeting serum levels (target CsA trough was 150ng/mL) typically found in other successful human solid organ transplantations. The diagnosis of acute rejection was made based on clinical grounds (i.e. watery vaginal discharge), confirmed by sonogram (oedematous UTx graft) and managed based on clinical human transplant medicine protocols i.e. steroids, CsA dose adjustment and adding other agents such as tacrolimus. These results were important in confirming the potential for robust survival of the human uterus graft under a variety of situations.[104]

A team in Japan has furthered primate UTx research by carrying out two original and highly valuable studies. First, *Mihara et al* evaluated the intra- and post-operative blood flow in the vascular anastomosed regions and the blood-perfused area of the transplanted uterus in a cynomolgus macaque model of uterus autotransplantation.[290] Investigations used were indocyanine green (ICG) fluorescence angiography and Doppler ultrasonography. The former was used during surgery to assess

blood perfusion in the vascular anastomosed region and uterine area. The latter evaluated post-operatively the uterine size, presence or absence of the endometrium and blood flow rates in the uterine artery and vein. Six female cynomolgus monkeys underwent surgery. The first two animals were used to study the pelvic vascular anatomy and the remaining four animals were used for uterus autotransplantation. Uterine arterial and venous anastomoses to the external iliac artery and vein respectively succeeded in all four animals that underwent autotransplantation. Intraoperative ICG fluorescence angiography showed appropriate blood flow in the vascular anastomosed regions. Interestingly, and demonstrated for the first time in large animal and specifically primate research, the entire uterus received sufficient blood supply from a single uterine artery. Doppler studies demonstrated favourable blood flow in the uterine artery and vein immediately after surgery. The 75% mortality rate was not because of poor anastomosis or surgical complications. The animals died within three months because of reduced feeding and loss of body strength. Importantly, the authors' conclusion that ICG fluorescence angiography can be used for simple evaluation of real-time blood flow conditions in the anastomosed uterine artery, vein and uterine area is important as it can bring about a greater chance of successful uterus transplantation.[290]

The second study by the same team again utilized a UTx primate auto-model and evaluated the patency of the microsurgical anastomoses and the perfusion of the transplanted uterus with the aim of achieving pregnancy.[279] Two female cynomolgus monkeys underwent surgery. For the anastomosis, the arteries used were the uterine arteries which were joined in an end-to-side fashion to the external iliac artery. For the first case, two arteries and one vein were anastomosed and for the second, two arteries and two veins. As with the previous study, uterine arterial blood flow and uterine size were determined by intraoperative ICG angiography and ultrasonography. The biopsy of the uterine cervix was taken post-operatively. In the first case, ICG angiography demonstrated the occlusion of one of the anastomosed arteries during the operation and the uterus atrophied two months post-UTx. In the second case however, the transplanted uterus survived and normal menstruation occurred. The animal achieved a natural pregnancy and was delivered via an emergency Caesarean section as a result of early placental separation. The newborn suffered fetal distress. Importantly, this is the first report of a

natural pregnancy in a primate following uterine autotransplantation. It seems that in this study, bilateral uterine arteries and the unilateral ovarian vein are required for a successful UTx. However drawing a conclusion is difficult as a result of only two primates used in the research.[279]

The final study in the primate series is also the most recent and of the highest quality.[291] It mimicked what would happen in a human attempt in Sweden. The plan put forward by the Brannstrom team is to use a live donor as a result of a paucity of suitable heart-beating brain stem dead donors. 18 female baboons were used as uterus donors and as uterus recipients, thus making up a total of 36 female baboons. Donation was live for the reason explained above. The actual transplant was allogeneic. Blood type was investigated by the authors; out of all the donor/recipient combinations, 55% were ABO identical and additionally 45% were ABO compatible. The follow-up time was between five and eight weeks. The uterine graft retrieved included (bilaterally) the main trunk of the internal iliac artery with the largest branch on each side and ovarian veins dissected all the way to their inlets into the IVC and the left kidney vein. Anastomoses were unilateral to the left external iliac artery and vein. The round ligaments of the uterine graft were attached to the corresponding ligaments of the recipient for graft fixation and the vagina was re-anastomosed by continuous suture. With respect to immunosuppression, the baboon recipients were placed in one of the following groups: (a) no immunosuppression (n=4); (b) monotherapy (oral slow release tacrolimus; n=4); and (c) induction therapy (antithymocyte globulin) followed by triple therapy (tacrolimus, mycophenolate, corticosteroids; n=10). Variables that were measured included surgical parameters, survival, immunosuppression and rejection patterns. The durations of uterus retrieval and recipient surgery were acceptable and would be considered ‘normal’ in humans. They were around three and three and a half hours respectively. The total ischaemic time was around three hours. Importantly, all the recipients and the donors survived the surgery. Rejection was seen in all recipients to varying degrees, with only one baboon displaying a uterus of normal appearance at the end of the study period. High blood levels of tacrolimus (.60 ng/ml) were recorded but interestingly, no evidence of nephrotoxicity.
In summary, the study is the most important to date. It used the biggest number of non-human primates, it described an allogeneic transplant, and donation was of a live nature. The transplants were

surgically successful, with allogeneic UTx in the baboon demonstrated as a donor- and recipient-safe and thus feasible surgical procedure. The immunosuppression protocol that should be applied must involve induction therapy and a triple protocol. Further research is needed however to optimize immunosuppression protocols in order to avoid uterine rejection.[291]

Human Uterine Transplantation

The first ever UTx performed in a woman occurred in 2000 in Saudi Arabia as outlined in detail in Chapter 1. In a fair comparison with other firsts in transplantation history, the first human uterus transplant was a relative success.[61] In summary, UTx was carried out on a 26-year-old female who lost her uterus at the age of 20 as a result of a post-partum haemorrhage. The donor was a 46-year-old patient diagnosed with multi-loculated ovarian cysts. She had undergone a modified hysterectomy, performed in such a way to preserve tissue and vascular integrity. Surgically, the uterine graft was connected in an orthotopic position to the recipient's vaginal vault. The uterosacral ligament was shortened in order to achieve additional fixation. The immunosuppression regimen was oral cyclosporine A, azathioprine and prednisolone. Allograft rejection was monitored by Echo-Doppler studies, magnetic resonance imaging and measurement of the CD4/CD8 ratio in peripheral blood by fluorescence activated cell sorter. Immunologically, the trial was controlled - an episode of acute rejection was treated on the ninth day with anti-thymocytic globulin. The transplanted uterus responded well to combined estrogen-progesterone therapy. It caused endometrial proliferation up to 18mm. The patient had two episodes of withdrawal bleeding upon cessation of the hormonal therapy. The main issue centred around the uterine arteries and veins. They were extended using reversed segments of the great saphenous vein, then connected to the external iliac arteries and veins, respectively. The double anastomosis as well as inadequate uterine structure support unfortunately led to acute vascular thrombosis 99 days after transplantation secondary to probable tension, torsion, or kinking of the connected vascular uterine grafts. The necrosed uterus was removed via hysterectomy. Macro- and microscopic histopathological examination revealed acute thrombosis in the vessels of the uterine body, with resulting infarction. Both Fallopian tubes remained viable, however, with no evidence of rejection.[61]

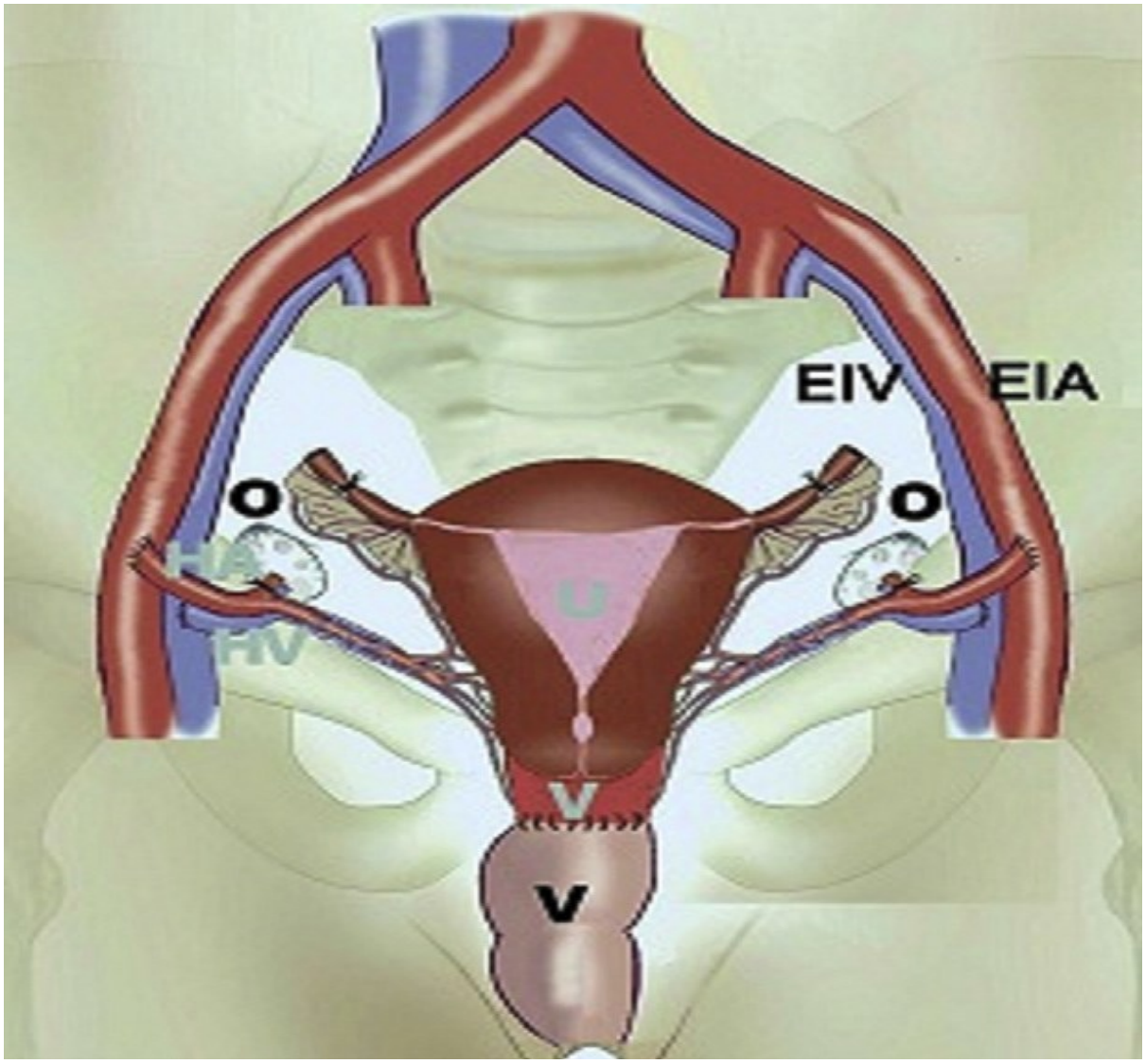
At the time of writing of this thesis, the second human UTx had been carried out unexpectedly.[261] Unexpectedly as it was by a team of plastic surgeons in Turkey led by Professor Omer Ozkan with no

previous record or publications on the topic of UTx. However it is an exciting and well planned study, promising a first real breakthrough in the human application of UTx since the Saudi case. *Ozkan et al* describe the first-year results of the first human uterus transplantation case from a multi-organ donor (and second human case historically). After approval from the local transplantation committee and the institutional review board, three candidates out of 10 patients with MRKH syndrome who had previously undergone vaginal reconstruction surgery were initially selected over a two year period.[261]

The AUFI patient was a 21-year-old woman with complete Mullerian agenesis who had been previously operated on for vaginal reconstruction. The patient underwent two IVF cycles, which yielded eight grade-1 embryos. The deceased donor was a nullipara, 22-year-old, braindead woman who had incurred cerebral trauma in a traffic accident. Uterus and proximal vagina retrieval with preservation of the bilateral ovarian and common iliac artery and vein tracts was achieved with wide excision of the broad ligament, round ligament, and vesicouterine and sacrouterine peritoneal sheets. The graft was perfused with University of Wisconsin (UW) solution at 4◦C to ensure removal of all blood cells and fibrin and to induce central core cooling for tissue preservation until revascularization. She underwent the UTx procedure consisting of orthotopic replacement and fixation of the retrieved uterus, revascularization, and end-to-site anastomoses of bilateral hypogastric arteries and veins to bilateral external iliac arteries and veins (**Figure 1**).[261]

The authors described their immunosupression regimen in detail. Immunosuppression induction with a 2.0 mg/dL daily dose of Thymoglobulin was administered intra-operatively, adjusted based on the CD3 levels, and stopped on day 10. An initial intraoperative dose of 1000mg of prednisolone was administered and then reduced to 20mg at 1 week after surgery. Tacrolimus (0.2 mg/kg) was not started simultaneously but rather on day 7 to decrease the rate of complication s related to immunosuppressive therapy. Maintenance immunosuppression therapy consisted of tacrolimus (0.2 mg/kg with plasma levels 15–20 ng/mL), mycophenolate mofetil (2 g/day), and prednisolone (20 mg/day). Cotrimoxazole (2.5 mg/kg per day orally) was administered for the first 6 months. Subcutaneous heparin was administered for antithrombotic prophylaxis.[261]

Figure 1 Schematic representation of the surgical procedure (Courtesy of *Ozkan et al*) [261]



Key: EIA, recipient external iliac artery; **EIV,** recipient external iliac vein; **HA,** donor hypogastric artery; **HV,** donor hypogastric vein; **O,** recipient ovary; **U,** donor uterus; **V/V,** recipient and donor vaginas. Note bilateral salpingeal tubes were ligated.

The patient recovered well following the surgery and did not experience any complications. She was discharged at the end of the first month. Her menstrual cycles resumed, with menarche 20 days after transplant surgery. Her menstruation was irregular for the next two menstrual cycles after the first menstrual cycle, and her endometrial thickness reached 21mm. She has had 12 menstrual cycles since the operation. Vaginal biopsy samples were taken every two weeks for the first three months, whereas endometrial biopsy were obtained every three months. The mucosa of the transplanted vagina at

month six demonstrated a few lymphocytes around the capillaries, but there was no fibrinoid necrosis. The endometrial biopsy at 6 months revealed vacuolization of the glandular epithelium and oedematous stroma.

Significantly this is the longest-lived transplanted human uterus to date with acquirement of menstrual cycles. The authors performed a planned and well-thought out experiment, with the necessary attention paid to all aspects - surgery, immunology, IVF and post-operative assessment. The authors provide detailed information on the first 12 months of the longest-lived transplanted human uterus to date, which subsequently achieved menstrual cycles. They also add a caveat of their awareness that the actual success will be the delivery of a single healthy fetus from the uterus transplant.[261]

UTx in the United Kingdom

The completion of this PhD will signal the commencement of the UK team's efforts to perform the first UK case series of five human uterine transplants in 2015. Therefore, future work will entail:

(a) Application and approval from the necessary national and local ethics committees for the commencement of the first five human uterine transplants;
(b) Raising of the necessary funds;
(c) Drawing up of scientific protocols (four will be required: surgical, immunological, fertility, obstetric)

The scientific protocols will be based on information derived from the first two human cases and all animal experiments to date. The UTx team will consist of gynaecological and transplant surgeons, immunologists, obstetricians and fertility specialists. As stated by *Brannstrom et al*, the introduction of human UTx should be performed and assessed under the newly formed IDEAL guidelines for a science-based approach to the application of surgical innovations.[292] They are based on five principles: Innovation, Development, Exploration, Assessment, and Long-term study, which were

developed by an international group of surgeons, researchers, journal editors, methodologists, statisticians, and other people who are committed to producing, disseminating, and evaluating quality research in surgery.

Table 1 describes the UTx process once the five patients are selected in the first half of 2014, using the selection criteria describe above. We predict that, provided there is either no failure of the graft or miscarriage following the first embryo transfer, it may require 2-2.5 years until the birth of the first baby, and 5 years until the birth of the second. This does not differ from the duration involved with normal conception, surrogacy or adoption. Depending on the patient's wishes as to the timing, the uterus will be removed following a completion Caesarean hysterectomy. An elective Caesarean section should be the mode of delivery in order to prevent any damage to the uterine graft fixation points which may occur with a natural delivery. IVF treatment should be performed prior to UTx. This is so that the couple's fertility may be confirmed and also that the embryos may be stored for future transfer around 9-12 months post-transplantation as postulated by the international recommendations for transplant patients.

The donor graft can come from two potential two sources, either a live-related or brain-stem dead heart-beating donor. This debate is currently still on-going, with the UK and USA teams planning to use the latter as they feel that the optimum graft is obtained from a multi-organ donor, with the application of the usual criteria for all organ donations. The Swedish team feel that a living donor would suit them better. The advantages of using a brain-stem dead, heart-beating organ donor are as follows. First, there is no surgical risk imposed on the donor as would be with a live donor. Also, with a live donor, she would have to be in good general health to minimize the surgical risk at hysterectomy, which is not at all a factor of importance with a deceased donor. Second, long-term survival and graft function is more likely with our chosen method. This is because as concluded following the first human UTx case in 2000, the uterus necessitates a tension-free and large diameter vascular anastomosis only possible when retrieving the larger pelvic vessels. Such a procedure, whereby the vascular tree may be more extensive, can only be performed in a deceased donor where

there is no future need for these vessels. This would allow recovery of the larger pelvic arteries and veins, thus ensuring easier anastomosis at transplantation. Joining transplantation retrieval teams is a good pointer as to where this theory is in fact applicable in practice. *Del Priore et al* participated in a local organ donor network retrieval team for over six months in order to determine if a uterus can be retrieved for reproductive organ transplantation and to describe the surgical technique.[293] 1800 heart-beating, brain-dead multi-organ donors were identified through an existing donor network following routine protocols, with multi-organ procurement surgery taking place in approximately 150 of these. Nine were specifically consented for uterine retrieval following institutional review board and organ donor network approval of the UTx project. This occurred in eight donors, with uterine retrievals performed by experienced gynaecologic surgeons. All were parous females, aged 30-45 years, with one to three deliveries of healthy children. Additional operating time ranged from 30 to 100 minutes, with the actual time decreasing down to 15 minutes eventually. Estimated blood loss was 10-20ml; this does not include one case which had a 250ml loss due to a lacerated internal iliac vein. The complete and bilateral internal, external and common iliac arteries and veins were recovered in two grafts, and the vascular pedicle included the vessels up to the anterior portions of the iliacs in five grafts. However unilateral loss of uterine vessels was demonstrated in two out of these. After retrieval, serial histology sections throughout the period of cold ischaemia, taken every 15-30 minutes, showed no signs of morphological change in the myometrium and endometrium over 12 hours of cold ischaemia. This was a useful experiment for its main finding that the human uterus can be obtained from local organ donor networks using existing protocols and minimal impact on the life-saving organ retrievals. Furthermore, it also demonstrated that separate flushing is necessary for the uterus, with femoral arties preferred for flush cannulation because they do not interfere with existing retrieval protocols.[293] *Brannstrom et al* also report that in their collaborative group, transplant surgeons have recovered uteri from seven multi-organ donors.[294] In these trials vascular pedicles including the complete uterine vessels, internal iliac vessels, common iliac vessels, and lower part of the aorta and vena cava were recovered.[275,294]

Table 1 UK Uterine Transplantation process

Stage	Cumulative Time
1st Clinic Appointment	Day 0
Medical and Psychological Evaluation	Day 0 - Month 3
Embryo Harvest	Month 3
Embryo Storage **Donor Location**	Months 3-6
UTx	Month 6
Embryo Transfer	Month 15-18
Delivery via Caesarean Section	Month 24-27

The main disadvantage with use of an organ from a deceased donor is the lack of time that can be spent investigating the donor because of the two/three day window prior to organ harvest. This 'investigation' is necessary to obtain an accurate and complete medical, surgical and gynaecological history regarding the patient, to rule out several uterine-associated pathological conditions before transplantation, to diagnose any gynaecological pathology such as neoplasia and leiomyomas and rule out any microbiological or oncological pathology. The uterus donated by a live donor would also be investigated pre-operatively, as a result of this extra time, by magnetic resonance imaging to diagnose vascular anomalies or atherosclerosis of uterine vessels. In addition, the negative effect of the major systemic inflammatory changes on graft survival.[275,295] This negative effect is directly related to the time interval between brain death and organ recovery. Finally, by using a live donor, one can opt for a relative as the donor i.e. an older sister, the mother, or an aunt because the chance of matching blood/tissue type would be high. *Johannesson et al* performed a study assessing the practicality of

uterine retrieval from a live donor for the purpose of subsequent transplantation.[294] The main aim was to see whether the uterine graft had long and wide enough vascular pedicles for direct vascular anastomosis bilaterally to the external iliacs. This would mean that extensions with saphenous grafts would not be required. The experiment involved vascular dissection of the uterine arteries and veins at radical hysterectomy in 19 patients diagnosed with cervical cancer. The uterine arteries and veins were dissected separately from the anterior divisions of the internal iliacs. The lengths (median) of the free portions of the left uterine artery and vein were 68 mm and 55 mm, and the right uterine artery and vein were 65 mm and 50 mm, respectively. The inter-external iliac artery distance (median) was 90 mm. The inter-external iliac artery distance, corresponding to distance between proposed bilateral anastomosis sites, was 90mm. All these measurements demonstrate precisely what would be sufficient for direct bilateral anastomosis to the external iliacs. Peri-operative and postoperative outcomes were compared with 76 patients (control group) undergoing standard radical hysterectomy without particular uterine vessel dissection, with no statistical difference between those outcomes. In conclusion, the study demonstrates two points. First, that long vascular pedicles can be obtained after selective dissections of the uterine arteries and veins from a live donor and second, that post-operative recovery of a live donor is not compromised.[294]

Thus the decision on which kind of donor to use would be left to the individual teams to decide. Other donor as well as recipient criteria is more certain. The recipient should be a genetic female of an age between 18 and 38 to ensure an appropriate ovarian reserve. The reserve should be evaluated by ultrasound and by measuring a patient's levels of follicle-stimulating hormone, oestradiol, and anti-Mullerian hormone, as well as the patient's antral follicle count and karyotype (normal female 46,XX). She should also be in good medical and psychological health, with no significant medical, surgical or gynaecological history and a BMI<25 kg/m^2. In particular, a cancer-free patient is desired; if she had been diagnosed with cancer previously, five years at least should pass after cancer surgery to ensure that there is no risk for recurrence of the disease. Finally, the recipient must have a long-term partner and a good support network involving family and friends.

With regards to the donor, the following are paramount: she or her family have signed an advanced directive for post-mortem organ-donation; negative human papilloma virus status; normal endometrial cavity on ultrasound (no leiomyomas or endometrial polyps); normal Pap smear history (no cervical dysplasia); and age between 18 and 50 years. Above the age of 50, the donor is likely to be peri-/post-menopausal with a risk of atherosclerosis of the vessels required for anastomosis. Ideally, the uterus should come from a donor with proven fertility, with no previous gynaecological history. Finally, a blood type and tissue match has to be performed with the latter being of less importance, according to modern transplantation standards.[296]

Also in need of consideration by the UK team is what may be the best method to fix the uterine graft into the pelvis. With respect the first human UTx case, uterine prolapse did occur and this must be prevented with future cases. The vaginal rim of the graft will naturally be anastomosed to the vaginal vault of the recipient. The lengthy round ligaments recovered during retrieval should fixed to the pelvic sidewalls and the uterosacral ligaments, which are nearly always preserved in women diagnosed with AUFI, should be fixed to the lower posterior part of the uterus. They would also support the cervix and ensure prevention of displacement and prolapse, especially of a gravid uterus. *Brannstrom et al* also believe that part of the bladder peritoneum should be sutured on top of the uterus as extra fixation.[4,275]

Finally, with respect to the immunosuppression protocol, the UK team will use a standard regimen protocol, involving induction and maintenance therapy that is used for kidney transplantation at Hammersmith Hospital. Induction therapy would involve a steroid, monoclonal antibody and possibly an antithymocyte globulin to lower the numbers of circulating T cells. Maintenance therapy would be the standard triple immunosuppression (tacrolimus/CsA, corticosteroids, anti-proliferative agent). Interestingly, the above proposed immunosuppression protocol has led to 100% graft survival of highly immunogenic composite tissues such as the hand and the face.[275,297]

Applications, Ethics and Originality

From the on-going research work over the last 10 years, one can conclude that in terms of progress, UTx is close to moving to the human setting. The aim is to apply future results achieved from the above research in order to allow the 'perfect' setting for the commencement of human transplantation. With regards to our UK-based work, over a hundred women have submitted written requests to our department wishing to undergo a uterine transplant. FIGO Committee for the Ethical Aspects of Human Reproduction and Women's Health recently brought forward such ethical considerations which touch on fundamental issues concerning perception of procreation and parenthood as well as those of medical advancements.[276] These perceptions differ globally and as described by *Brannstrom et al*, this diversity is moral, religious and philosophical. Values in dissimilar societies will most likely render UTx acceptable in some societies and unacceptable in others.[65,177]

It is important to distinguish between the ethics of UTx research and the ethics of the UTx procedure. That carrying out UTx research over the past 50 years has been ethical is confirmed by the permission granted by various Animal Research bodies to perform the animal experiments. Furthermore, the greater technical precision gained from a better understanding of pelvic anatomy advanced our existing knowledge of operative gynaecological oncology procedures. There have already been a number of 'spin-offs' directly related to gynaecological practice which have arisen from our previous research. They include the first case of fertility sparing surgery for a giant adenomatoid tumour of the uterus[226], a tumour previously managed by hysterectomy and a successful cessation of haemorrhage in a patient with a ruptured cornual ectopic pregnancy without recourse to hysterectomy.[175] Also, some of the techniques have proved useful in the surgical management of Gestational Trophoblastic Disease, [156,174,298] as well as the treatment of uterine arterio-venous malformation [227] where selective temporary ligation of the uterine and ovarian vessels was applied. Finally, the idea of ART, as described previously, has demonstrated that the uterus is capable of sustaining a term pregnancy with only one of its redundant vascular pedicles intact. Similarly, knowledge gained from the

immunological aspects of UTx can be shared with other transplant fields. Benefits may also come from the ability to study perfusion in a smooth muscle organ transplant, how a transplanted organ adapts to the change in its vascular environment during pregnancy, and the proliferation of functional muscle mass.

With respect to the ethics of the actual UTx procedure, the main debate focuses on whether a radical procedure such as UTx is needed. And if so, whether the benefits of it outweigh the intra-operative and post-operative risks to the mother and fetus. Need is a difficult topic to judge. If looking at numbers, around 100,000 women between the ages of 16-42 suffer from AUFI, whereas in America that number is most likely close to seven million. The figures are staggering and that is simply when looking at two countries in the Western hemisphere. This is not a life-saving transplant yet neither is kidney, and more recently, hand, larynx and face which are now offered in selected centres. The area of transplantation has been expanding over the recent years and does now include qualify-of-life improving transplants. UTx is most certainly offered as a quality-of-life improving procedure i.e. to improve the situation of a woman and couple who are unable to have their own genetic child by any other method. The importance of infertility to a couple is not for anyone else to determine - to some it may appear irrelevant yet to others it is of a vital importance. When IVF was first introduced it also had to face similar criticisms and challenges, both religious and scientific. Yet if UTx remains the only option for a couple to who are unable to conceive a genetic child (and surrogacy and adoption are not possible as a result of legal, ethical or moral reasons), then surely the decision on whether to carry out such a procedure lies with the feet of the patient and the medical team. For most of the world, including much of western Europe, surrogacy is considered illegal for these reasons.

The important ethical principle of dominance of benefits over risks should thoroughly be analyzed where four subjects may be involved; the donor (living or deceased) with immediate family, the recipient, the partner of the recipient who is also the prospective father, and the future child. The ethics of the last subject, i.e. the offspring, as a fetus, neonate and independent person, are the most controversial. Maternal immunosuppression does not seem to increase perinatal risk [152] but long-term

effects of fetal exposure to immunosuppressants may exist.[299,300] In addition, how does one assess the right of an unborn child to choose treatment i.e. immunosuppressants that may result in a higher incidence of preterm deliveries, low birth weights, intra-uterine growth restrictions yet it is that specific treatment that allows it to be born? [141-143] Although non-existent in the UK, a concept termed 'wrongful birth' has been used in the US justice system whereby a person can sue his/her parents or the medical profession for allowing their birth and ensuing (difficult) life experience. All this must be considered yet the most significant rule of obstetrics remains, `that the life of the mother is a priority and should be treated as such if the post-UTx pregnancy takes an unexpected turn.

Interestingly, the concept of UTx covers all aspects of human ethics: *primum non nocere* (non-maleficence), autonomy, beneficence, justice and dignity. UTx offers all of the last four qualities to a patient suffering from AUFI. Further research proposed earlier will allow us to meet the criteria for ethical analysis of a surgical innovation, as proposed by Moore:[278] sound research foundation, adequate combination of knowledge and expertise from all fields related to the procedure, and internationally recognised proficiency in the institution in which the procedure is performed.[3] It is only the application of such a thorough approach which will lead to compliance with the first and most important pillar of medical ethics: *primum non nocere.*[65,278] And most importantly, that ever-elusive life goal of *eudaimonia* (human flourishing), an Ancient Greek concept of the highest form of human goodness and happiness, can be attained by couples for whom the concept of family was always an impossibility.

With regards to our group of women, they are mostly non-cancer patients. We believe that cancer patients are inappropriate at this initial stage of human UTx until anti-rejection protocols are fully worked out. Instead, preferred patients for this initial step are women who have been born without a uterus or those who have had a hysterectomy, but not as part of a curative cancer treatment. In addition, 50-70 women per year undergo surrogacy in the UK.

Research into UTx has flourished over the past 15 years, with the procedure performed in small and large animal models, primates, autogeneic and allogeneic models, and most importantly with pregnancies now being demonstrated. A point is now being reached, whereby human UTx is attempted secondary to various ethics bodies around the globe realising that performing more animal research as opposed to attempting human transplants would not be advancing the field further. No animal model can ever predict the actual clinical experience. Therefore, the ultimate decision will be influenced by the judgment of the UTx team, the IRB of the participating institution, and most importantly, the patient to whom the transplant will be offered.[3]

Appendix F: Summary of Permission for Third Party Copyright Works

Page Number	Type of work	Source work	Copyright holder & year	Work out of copyright	Permission to re-use	Permission requested	Permission refused	Orphan Work
Page 31	Figure 2.1	J Obstet Gynaecol. 2013; 33:232-8.	© 2013 Informa Healthcare		✓ Licence Number: 3437890595530			
Page 47	Figure 2.2	Reprod Sci. 2012; 19:123-34.	©2012 Society for Gynaecologic Investigation	✓		✓		
Page 53-67	whole paper (Chapter 3)	BJOG. 2012; 119:187-93	© 2012 RCOG		✓ Licence Number: 3437870282335			
Page 69	Figure 4.1	Aust N Z J Obstet Gynaecol. 2011; 51:199-203.	© 2011 The Royal Australian and New Zealand College of Obstetricians and Gynaecologists		✓ Licence Number: 3437880119428			
Page 71	Figure 4.2	Acta obstetricia et gynecologica Scandinavica 2008; 87:1239-47.	© 2008 Acta Obstet Gynecol Scand		✓ Licence Number: 3437871095283			
Page 161	Figure 5.1	J Obstet Gynaecol Res 2008;34:784-93.	© 2008 Japan Society of Obstetrics and Gynaecology		✓ Licence Number: 3437880577131			

Page 167	**Figure 5.2**	**Hum Reprod 2011; 26:3028-36.**	**© 2008 Oxford University Press**		✓ Licence Number: 3437880888825			
Page 338-344	**Chapter 10: Epilogue**	**Hum Reprod 2013; 28:288-91.**	**© 2008 Oxford University Press**		✓ Licence Number: 3437890271735			
Spread out throughout thesis	**whole paper**	**Reprod Sci. 2012; 19:123-34.**	**©2012 Society for Gynaecologic Investigation**	✓				

References

1. Salguero PC. The Buddhist medicine king in literary context: reconsidering an early medieval example of Indian influence on Chinese medicine and surgery. History of Religions 2009;48:183-210.
2. Saso S, Ghaem-Maghami S, Chatterjee J, et al. Immunology of Uterine Transplantation: A Review. Reproductive sciences (Thousand Oaks, Calif) 2011.
3. Nair A, Stega J, Smith JR, Del Priore G. Uterus transplant: evidence and ethics. Annals of the New York Academy of Sciences 2008;1127:83-91.
4. Brannstrom M, Wranning CA, Altchek A. Experimental uterus transplantation. Human reproduction update 2010;16:329-45.
5. Keith LG, Del Priore G. Uterine transplantation in humans: a new frontier. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics 2002;76:243-4.
6. Altchek A. Uterus transplantation. The Mount Sinai journal of medicine, New York 2003;70:154-62.
7. Grimbizis GF, Camus M, Tarlatzis BC, Bontis JN, Devroey P. Clinical implications of uterine malformations and hysteroscopic treatment results. Human reproduction update 2001;7:161-74.
8. Hauser GA, Schreiner WE. [Mayer-Rokitansky-Kuester syndrome. Rudimentary solid bipartite uterus with solid vagina]. Schweizerische medizinische Wochenschrift 1961;91:381-4.
9. Griffin JE, Edwards C, Madden JD, Harrod MJ, Wilson JD. Congenital absence of the vagina. The Mayer-Rokitansky-Kuster-Hauser syndrome. Annals of internal medicine 1976;85:224-36.
10. Folch M, Pigem I, Konje JC. Mullerian agenesis: etiology, diagnosis, and management. Obstetrical & gynecological survey 2000;55:644-9.
11. Edmonds DK, Rose GL, Lipton MG, Quek J. Mayer-Rokitansky-Kuster-Hauser syndrome: a review of 245 consecutive cases managed by a multidisciplinary approach with vaginal dilators. Fertility and sterility 2012;97:686-90.
12. Carson SA, Simpson JL, Malinak LR, et al. Heritable aspects of uterine anomalies. II. Genetic analysis of Mullerian aplasia. Fertility and sterility 1983;40:86-90.
13. Davies MC, Creighton SM. Vaginoplasty. Current opinion in urology 2007;17:415-8.
14. Petrozza JC, Gray MR, Davis AJ, Reindollar RH. Congenital absence of the uterus and vagina is not commonly transmitted as a dominant genetic trait: outcomes of surrogate pregnancies. Fertility and sterility 1997;67:387-9.
15. Farquhar CM, Steiner CA. Hysterectomy rates in the United States 1990-1997. Obstetrics and gynecology 2002;99:229-34.
16. Goldfarb JM, Austin C, Peskin B, Lisbona H, Desai N, de Mola JR. Fifteen years experience with an in-vitro fertilization surrogate gestational pregnancy programme. Human reproduction (Oxford, England) 2000;15:1075-8.
17. Kwee A, Bots ML, Visser GH, Bruinse HW. Emergency peripartum hysterectomy: A prospective study in The Netherlands. European journal of obstetrics, gynecology, and reproductive biology 2006;124:187-92.
18. Porreco RP, Stettler RW. Surgical remedies for postpartum hemorrhage. Clinical obstetrics and gynecology 2010;53:182-95.
19. Cengiz H, Yasar L, Ekin M, Kaya C, Karakas S. Management of intractable postpartum haemorrhage in a tertiary center: A 5-year experience. Nigerian medical journal : journal of the Nigeria Medical Association 2012;53:85-8.
20. You W, Dainty LA, Rose GS, et al. Gynecologic malignancies in women aged less than 25 years. Obstetrics and gynecology 2005;105:1405-9.
21. Creasman WT, Odicino F, Maisonneuve P, et al. Carcinoma of the corpus uteri. FIGO 26th Annual Report on the Results of Treatment in Gynecological Cancer. International journal of

gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics 2006;95 Suppl 1:S105-43.
22. Sonoda Y, Abu-Rustum NR, Gemignani ML, et al. A fertility-sparing alternative to radical hysterectomy: how many patients may be eligible? Gynecologic oncology 2004;95:534-8.
23. Al-Inany H. Intrauterine adhesions. An update. Acta obstetricia et gynecologica Scandinavica 2001;80:986-93.
24. Lonnroth K, Raviglione M. Global epidemiology of tuberculosis: prospects for control. Seminars in respiratory and critical care medicine 2008;29:481-91.
25. Bukulmez O, Yarali H, Gurgan T. Total corporal synechiae due to tuberculosis carry a very poor prognosis following hysteroscopic synechialysis. Human reproduction (Oxford, England) 1999;14:1960-1.
26. Friedler S, Margalioth EJ, Kafka I, Yaffe H. Incidence of post-abortion intra-uterine adhesions evaluated by hysteroscopy--a prospective study. Human reproduction (Oxford, England) 1993;8:442-4.
27. Schenker JG, Margalioth EJ. Intrauterine adhesions: an updated appraisal. Fertility and sterility 1982;37:593-610.
28. Fernandez H, Al-Najjar F, Chauveaud-Lambling A, Frydman R, Gervaise A. Fertility after treatment of Asherman's syndrome stage 3 and 4. Journal of minimally invasive gynecology 2006;13:398-402.
29. Heinonen PK, Saarikoski S, Pystynen P. Reproductive performance of women with uterine anomalies. An evaluation of 182 cases. Acta obstetricia et gynecologica Scandinavica 1982;61:157-62.
30. Saravelos SH, Cocksedge KA, Li TC. Prevalence and diagnosis of congenital uterine anomalies in women with reproductive failure: a critical appraisal. Human reproduction update 2008;14:415-29.
31. Buttram VC, Jr. Mullerian anomalies and their management. Fertility and sterility 1983;40:159-63.
32. Homer HA, Li TC, Cooke ID. The septate uterus: a review of management and reproductive outcome. Fertility and sterility 2000;73:1-14.
33. Papp Z, Mezei G, Gavai M, Hupuczi P, Urbancsek J. Reproductive performance after transabdominal metroplasty: a review of 157 consecutive cases. The Journal of reproductive medicine 2006;51:544-52.
34. Mertens AC, Yasui Y, Neglia JP, et al. Late mortality experience in five-year survivors of childhood and adolescent cancer: the Childhood Cancer Survivor Study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2001;19:3163-72.
35. Larsen EC, Schmiegelow K, Rechnitzer C, Loft A, Muller J, Andersen AN. Radiotherapy at a young age reduces uterine volume of childhood cancer survivors. Acta obstetricia et gynecologica Scandinavica 2004;83:96-102.
36. Bath LE, Critchley HO, Chambers SE, Anderson RA, Kelnar CJ, Wallace WH. Ovarian and uterine characteristics after total body irradiation in childhood and adolescence: response to sex steroid replacement. British journal of obstetrics and gynaecology 1999;106:1265-72.
37. Holm K, Nysom K, Brocks V, Hertz H, Jacobsen N, Muller J. Ultrasound B-mode changes in the uterus and ovaries and Doppler changes in the uterus after total body irradiation and allogeneic bone marrow transplantation in childhood. Bone marrow transplantation 1999;23:259-63.
38. Wallace WH, Thomson AB, Saran F, Kelsey TW. Predicting age of ovarian failure after radiation to a field that includes the ovaries. International journal of radiation oncology, biology, physics 2005;62:738-44.
39. Marshall LM, Spiegelman D, Barbieri RL, et al. Variation in the incidence of uterine leiomyoma among premenopausal women by age and race. Obstetrics and gynecology 1997;90:967-73.

40. Chen CR, Buck GM, Courey NG, Perez KM, Wactawski-Wende J. Risk factors for uterine fibroids among women undergoing tubal sterilization. American journal of epidemiology 2001;153:20-6.
41. Marino JL, Eskenazi B, Warner M, et al. Uterine leiomyoma and menstrual cycle characteristics in a population-based cohort study. Human reproduction (Oxford, England) 2004;19:2350-5.
42. Farhi J, Ashkenazi J, Feldberg D, Dicker D, Orvieto R, Ben Rafael Z. Effect of uterine leiomyomata on the results of in-vitro fertilization treatment. Human reproduction (Oxford, England) 1995;10:2576-8.
43. Oliveira FG, Abdelmassih VG, Diamond MP, Dozortsev D, Melo NR, Abdelmassih R. Impact of subserosal and intramural uterine fibroids that do not distort the endometrial cavity on the outcome of in vitro fertilization-intracytoplasmic sperm injection. Fertility and sterility 2004;81:582-7.
44. Connolly G, Doyle M, Barrett T, Byrne P, De Mello M, Harrison RF. Fertility after abdominal myomectomy. Journal of obstetrics and gynaecology : the journal of the Institute of Obstetrics and Gynaecology 2000;20:418-20.
45. Fernandez H, Sefrioui O, Virelizier C, Gervaise A, Gomel V, Frydman R. Hysteroscopic resection of submucosal myomas in patients with infertility. Human reproduction (Oxford, England) 2001;16:1489-92.
46. Chang MC. Fertilization of rabbit ova in vitro. Nature 1959;184(Suppl 7):466-7.
47. Steptoe PC, Edwards RG. Reimplantation of a human embryo with subsequent tubal pregnancy. Lancet 1976;1:880-2.
48. Steptoe PC, Edwards RG. Birth after the reimplantation of a human embryo. Lancet 1978;2:366.
49. Lopata A, Johnston IW, Hoult IJ, Speirs AI. Pregnancy following intrauterine implantation of an embryo obtained by in vitro fertilization of a preovulatory egg. Fertility and sterility 1980;33:117-20.
50. Jones HW, Jr., Jones GS, Andrews MC, et al. The program for in vitro fertilization at Norfolk. Fertility and sterility 1982;38:14-21.
51. Zhordania IF, Gotsiridze OA. AUTOTRANSPLANTATION OF THE UTERUS INTO THE OMENTUM. International journal of fertility 1963;8:849-57.
52. O'Leary JA, Feldman M, Gaensslen DM. Uterine and tubal transplantation. Fertility and sterility 1969;20:757-60.
53. Scott JR, Anderson WR, Kling TG, Yannone ME. Uterine transplantation in dogs. Gynecologic investigation 1970;1:140-8.
54. Barzilai A, Paldi E, Gal D, Hampel N. Autotransplantation of the uterus and ovaries in dogs. Israel journal of medical sciences 1973;9:49-52.
55. Paldi E, Gal D, Barzilai A, Hampel N, Malberger E. Genital organs. Auto and homotransplantation in forty dogs. International journal of fertility 1975;20:5-12.
56. Eraslan S, Hamernik RJ, Hardy JD. Replantation of uterus and ovaries in dogs, with successful pregnancy. Archives of surgery (Chicago, Ill : 1960) 1966;92:9-12.
57. Mattingly RF, Clark DO, Lutsky, II, Huang WY, Stafl A, Maddison FE. Ovarian function in uteroovarian homotransplantation. American journal of obstetrics and gynecology 1970;108:773-94.
58. Wingate MB, Karasewich E, Wingate L, Lauchian S, Ray M. Experimental uterotubovarian homotransplantation in the dog. American journal of obstetrics and gynecology 1970;106:1171-6.
59. Yonemoto RH, Du Sold WD, Deliman RM. Homotransplantation of uterus and ovaries in dogs. A preliminary report. American journal of obstetrics and gynecology 1969;104:1143-51.
60. Smith JR, Boyle DC, Corless DJ, et al. Abdominal radical trachelectomy: a new surgical technique for the conservative management of cervical carcinoma. British journal of obstetrics and gynaecology 1997;104:1196-200.

61. Fageeh W, Raffa H, Jabbad H, Marzouki A. Transplantation of the human uterus. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics 2002;76:245-51.
62. Kandela P. Uterine transplantation failure causes Saudi Arabian government clampdown. Lancet 2000;356:838.
63. Fageeh W. Apology to Dr Wafa Mohammed Khalil Fageeh, obstetrician and gynaecologist and assistant professor at King Abdilaziz University, and her medical team. Lancet 2001;358:1076.
64. Brannstrom M, Diaz-Garcia C. Transplantation of female genital organs. The journal of obstetrics and gynaecology research 2011;37:271-91.
65. Brannstrom M, Racho El-Akouri R, Wranning CA. Uterine transplantation. European journal of obstetrics, gynecology, and reproductive biology 2003;109:121-3.
66. Merrill RM, Capocaccia R, Feuer EJ, Mariotto A. Cancer prevalence estimates based on tumour registry data in the Surveillance, Epidemiology, and End Results (SEER) Program. International journal of epidemiology 2000;29:197-207.
67. Scott JR, Pitkin RM, Yannone ME. Transplantation of the primate uterus. Surgery, gynecology & obstetrics 1971;133:414-8.
68. Wranning CA, Akhi SN, Kurlberg G, Brannstrom M. Uterus transplantation in the rat: model development, surgical learning and morphological evaluation of healing. Acta obstetricia et gynecologica Scandinavica 2008;87:1239-47.
69. Racho El-Akouri R, Kurlberg G, Dindelegan G, Molne J, Wallin A, Brannstrom M. Heterotopic uterine transplantation by vascular anastomosis in the mouse. The Journal of endocrinology 2002;174:157-66.
70. Lee S, Mao L, Wang Y, et al. Transplantation of reproductive organs. Microsurgery 1995;16:191-8.
71. Jiga LP, Lupu CM, Zoica BS, Ionac M. Experimental model of heterotopic uterus transplantation in the laboratory rat. Microsurgery 2003;23:246-50.
72. Ramirez ER, Ramirez DK, Pillari VT, Vasquez H, Ramirez HA. Modified uterine transplant procedure in the sheep model. Journal of minimally invasive gynecology 2008;15:311-4.
73. Makikallio K, Tekay A, Jouppila P. Effects of bleeding on uteroplacental, umbilicoplacental and yolk-sac hemodynamics in early pregnancy. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 2001;18:352-6.
74. Menger MD, Rucker M, Vollmar B. Capillary dysfunction in striated muscle ischemia/reperfusion: on the mechanisms of capillary "no-reflow". Shock (Augusta, Ga) 1997;8:2-7.
75. Menger MD, Vollmar B. Role of microcirculation in transplantation. Microcirculation (New York, NY : 1994) 2000;7:291-306.
76. Quiroga I, McShane P, Koo DD, et al. Major effects of delayed graft function and cold ischaemia time on renal allograft survival. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 2006;21:1689-96.
77. Schramm R, Menger MD, Kirsch S, et al. The subepicardial microcirculation in heterotopically transplanted mouse hearts: an intravital multifluorescence microscopy study. The Journal of thoracic and cardiovascular surgery 2007;134:210-7, 7 e1.
78. Schwarz A, Mengel M, Gwinner W, et al. Risk factors for chronic allograft nephropathy after renal transplantation: a protocol biopsy study. Kidney international 2005;67:341-8.
79. Howard TK, Klintmalm GB, Cofer JB, Husberg BS, Goldstein RM, Gonwa TA. The influence of preservation injury on rejection in the hepatic transplant recipient. Transplantation 1990;49:103-7.
80. Hanafy A, Diaz-Garcia C, Olausson M, Brannstrom M. Uterine transplantation: one human case followed by a decade of experimental research in animal models. The Australian & New Zealand journal of obstetrics & gynaecology 2011;51:199-203.
81. Totsuka E, Fung JJ, Hakamada K, et al. Synergistic effect of cold and warm ischemia time on postoperative graft function and outcome in human liver transplantation. Transplantation proceedings 2004;36:1955-8.

82. Boom H, Mallat MJ, de Fijter JW, Zwinderman AH, Paul LC. Delayed graft function influences renal function, but not survival. Kidney international 2000;58:859-66.
83. Nakagawa K, Koo DD, Davies DR, et al. Lecithinized superoxide dismutase reduces cold ischemia-induced chronic allograft dysfunction. Kidney international 2002;61:1160-9.
84. Racho El-Akouri R, Wranning CA, Molne J, Kurlberg G, Brannstrom M. Pregnancy in transplanted mouse uterus after long-term cold ischaemic preservation. Human reproduction (Oxford, England) 2003;18:2024-30.
85. Feuillu B, Cormier L, Frimat L, et al. Kidney warming during transplantation. Transplant international : official journal of the European Society for Organ Transplantation 2003;16:307-12.
86. Kayyali US, Donaldson C, Huang H, Abdelnour R, Hassoun PM. Phosphorylation of xanthine dehydrogenase/oxidase in hypoxia. The Journal of biological chemistry 2001;276:14359-65.
87. Zhang W, Wang M, Xie HY, et al. Role of reactive oxygen species in mediating hepatic ischemia-reperfusion injury and its therapeutic applications in liver transplantation. Transplantation proceedings 2007;39:1332-7.
88. Schmidt A, Tomasdottir H, Bengtsson A. Influence of cold ischemia time on complement activation, neopterin, and cytokine release in liver transplantation. Transplantation proceedings 2004;36:2796-8.
89. Gu XP, Qiu YD, Xu FT, Jiang Y, Ding YT. In vivo suppressive effect of nuclear factor-kappaB inhibitor on neutrophilic inflammation of grafts after orthotopic liver transplantation in rats. World journal of gastroenterology : WJG 2004;10:3654-8.
90. Jaeschke H, Lemasters JJ. Apoptosis versus oncotic necrosis in hepatic ischemia/reperfusion injury. Gastroenterology 2003;125:1246-57.
91. Kim JS, Qian T, Lemasters JJ. Mitochondrial permeability transition in the switch from necrotic to apoptotic cell death in ischemic rat hepatocytes. Gastroenterology 2003;124:494-503.
92. Muhlbacher F, Langer F, Mittermayer C. Preservation solutions for transplantation. Transplantation proceedings 1999;31:2069-70.
93. Huddleston CB, Mendeloff EN. Heart and lung preservation for transplantation. Journal of cardiac surgery 2000;15:108-21.
94. Nankivell BJ, Alexander SI. Rejection of the kidney allograft. The New England journal of medicine 2010;363:1451-62.
95. Colvin RB. Antibody-mediated renal allograft rejection: diagnosis and pathogenesis. Journal of the American Society of Nephrology : JASN 2007;18:1046-56.
96. Issa F, Schiopu A, Wood KJ. Role of T cells in graft rejection and transplantation tolerance. Expert review of clinical immunology 2010;6:155-69.
97. Briscoe DM, Alexander SI, Lichtman AH. Interactions between T lymphocytes and endothelial cells in allograft rejection. Current opinion in immunology 1998;10:525-31.
98. Csencsits KL, Bishop DK. Contrasting alloreactive CD4+ and CD8+ T cells: there's more to it than MHC restriction. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2003;3:107-15.
99. Barry M, Bleackley RC. Cytotoxic T lymphocytes: all roads lead to death. Nature reviews Immunology 2002;2:401-9.
100. Halloran PF, Kung L, Noujaim J. Calcineurin and the biological effect of cyclosporine and tacrolimus. Transplantation proceedings 1998;30:2167-70.
101. Baksh S, Burakoff SJ. The role of calcineurin in lymphocyte activation. Seminars in immunology 2000;12:405-15.
102. Sieunarine K, Doumplis D, Kuzmin E, et al. Uterine allotranspiantation in the rabbit model using a macrovascular patch technique. International surgery 2008;93:288-94.
103. Wranning CA, Akhi SN, Diaz-Garcia C, Brannstrom M. Pregnancy after syngeneic uterus transplantation and spontaneous mating in the rat. Human reproduction (Oxford, England) 2011;26:553-8.

104. Diaz-Garcia C, Akhi SN, Wallin A, Pellicer A, Brannstrom M. First report on fertility after allogeneic uterus transplantation. Acta obstetricia et gynecologica Scandinavica 2010;89:1491-4.
105. Racho El-Akouri R, Kurlberg G, Brannstrom M. Successful uterine transplantation in the mouse: pregnancy and post-natal development of offspring. Human reproduction (Oxford, England) 2003;18:2018-23.
106. Confino E, Vermesh M, Thomas W, Jr., Gleicher N. Non-vascular transplantation of the rabbit uterus. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics 1986;24:321-5.
107. Sieunarine K, Hakim NS, Corless DJ, et al. Is it feasible to use a large vessel patch with a uterine allograft en bloc for uterine transplantation? International surgery 2005;90:257-61.
108. Saso S, Hurst S, Chatterjee J, et al. Test of long-term uterine survival after allogeneic transplantation in rabbits. The journal of obstetrics and gynaecology research 2013.
109. Diaz-Garcia C, Akhi SN, Martinez-Varea A, Brannstrom M. The effect of warm ischemia at uterus transplantation in a rat model. Acta obstetricia et gynecologica Scandinavica 2013;92:152-9.
110. Truta E, Pop I, Popa D, Ionescu M, Truta F. Experimental re- and transplantation of the internal female genital organs. Romanian medical review 1969;13:53-8.
111. Stoyanov D YG-Z. Soft tissue deformation for robotic assisted minimally invasive surgery. Annual International Conference of the IEEE Engineering in Medicine and Biology Society. Minneapolis, MN, USA2009:254-7.
112. Damber JE, Lindahl O, Selstam G, Tenland T. Testicular blood flow measured with a laser Doppler flowmeter: acute effects of catecholamines. Acta physiologica Scandinavica 1982;115:209-15.
113. Motoc A, Jiga L, Ionac M, Raica M, Motoc M, Chiovschi S. Preliminary results of orthotopic en bloc uterus and ovary transplantation in the laboratory rat. Clinical and experimental obstetrics & gynecology 2003;30:239-42.
114. Zhang Z, Zhu L, Quan D, et al. Pattern of liver, kidney, heart, and intestine allograft rejection in different mouse strain combinations. Transplantation 1996;62:1267-72.
115. Riederer I, Silva-Barbosa SD, Rodrigues ML, Savino W. Local antilaminin antibody treatment alters the rejection pattern of murine cardiac allografts: correlation between cellular infiltration and extracellular matrix. Transplantation 2002;74:1515-22.
116. El-Akouri RR, Molne J, Groth K, Kurlberg G, Brannstrom M. Rejection patterns in allogeneic uterus transplantation in the mouse. Human reproduction (Oxford, England) 2006;21:436-42.
117. Wranning CA, El-Akouri RR, Groth K, Molne J, Parra AK, Brannstrom M. Rejection of the transplanted uterus is suppressed by cyclosporine A in a semi-allogeneic mouse model. Human reproduction (Oxford, England) 2007;22:372-9.
118. Rusnak F, Mertz P. Calcineurin: form and function. Physiological reviews 2000;80:1483-521.
119. Cebecauer M, Guillaume P, Hozak P, et al. Soluble MHC-peptide complexes induce rapid death of CD8+ CTL. Journal of immunology (Baltimore, Md : 1950) 2005;174:6809-19.
120. Groth K, Akouri R, Wranning CA, Molne J, Brannstrom M. Rejection of allogenic uterus transplant in the mouse: time-dependent and site-specific infiltration of leukocyte subtypes. Human reproduction (Oxford, England) 2009;24:2746-54.
121. Klein J, Figueroa F, David CS. H-2 haplotypes, genes and antigens: second listing. II. The H-2 complex. Immunogenetics 1983;17:553-96.
122. Groth K, Akhi SN, Molne J, Wranning CA, Brannstrom M. Effects of immunosuppression by cyclosporine A on allogenic uterine transplant in the rat. European journal of obstetrics, gynecology, and reproductive biology 2012;163:97-103.
123. Akhi SN, Diaz-Garcia C, El-Akouri RR, Wranning CA, Molne J, Brannstrom M. Uterine rejection after allogeneic uterus transplantation in the rat is effectively suppressed by tacrolimus. Fertility and sterility 2012.

124. Groth K, Brannstrom M, Molne J, Wranning CA. Cyclosporine A exposure during pregnancy in mice: effects on reproductive performance in mothers and offspring. Human reproduction (Oxford, England) 2010;25:697-704.
125. Mason RJ, Thomson AW, Whiting PH, et al. Cyclosporine-induced fetotoxicity in the rat. Transplantation 1985;39:9-12.
126. Fein A, Vechoropoulos M, Nebel L. Cyclosporin-induced embryotoxicity in mice. Biology of the neonate 1989;56:165-73.
127. Esquifino AI, Moreno ML, Agrasal C, Villanua MA. Effects of cyclosporine on ovarian function in sham-operated and pituitary-grafted young female rats. Proceedings of the Society for Experimental Biology and Medicine Society for Experimental Biology and Medicine (New York, NY) 1995;208:397-403.
128. Gore-Langton RE. Cyclosporine differentially affects estrogen and progestin synthesis by rat granulosa cells in vitro. Molecular and cellular endocrinology 1988;57:187-98.
129. Robertson SA. Immune regulation of conception and embryo implantation-all about quality control? Journal of reproductive immunology 2010;85:51-7.
130. Kaminski P, Bobrowska K, Pietrzak B, Bablok L, Wielgos M. Gynecological issues after organ transplantation. Neuro endocrinology letters 2008;29:852-6.
131. Lessan-Pezeshki M, Ghazizadeh S, Khatami MR, et al. Fertility and contraceptive issues after kidney transplantation in women. Transplantation proceedings 2004;36:1405-6.
132. Parolin MB, Rabinovitch I, Urbanetz AA, Scheidemantel C, Cat ML, Coelho JC. Impact of successful liver transplantation on reproductive function and sexuality in women with advanced liver disease. Transplantation proceedings 2004;36:943-4.
133. Phocas I, Sarandakou A, Kassanos D, Rizos D, Tserkezis G, Koutsikos D. Hormonal and ultrasound characteristics of menstrual function during chronic hemodialysis and after successful renal transplantation. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics 1992;37:19-28.
134. Hogan B BRCF. Manipulating the Mouse Embryo. In: Press CSHL, ed. New York, USA1994.
135. Pijnenborg R, Robertson WB, Brosens I, Dixon G. Review article: trophoblast invasion and the establishment of haemochorial placentation in man and laboratory animals. Placenta 1981;2:71-91.
136. Alonso A, Fernandez C, Villaverde P, et al. Kidney-pancreas transplants: is it so difficult to start a program? Transplantation proceedings 2005;37:1455-6.
137. Meregalli E, Biggioggero M, Borghi O, Meroni P, Cimaz R. In vivo effects of maternal immunosuppression during pregnancy on the immune function of newborn infants. Arhiv za higijenu rada i toksikologiju 2005;56:151-6.
138. Bedaiwy MA, Shahin AY, Falcone T. Reproductive organ transplantation: advances and controversies. Fertility and sterility 2008;90:2031-55.
139. Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? Immunology today 1993;14:353-6.
140. Dei Malatesta MF, Rossi M, Rocca B, et al. Pregnancy after liver transplantation: report of 8 new cases and review of the literature. Transplant immunology 2006;15:297-302.
141. McKay DB, Josephson MA. Pregnancy in recipients of solid organs--effects on mother and child. The New England journal of medicine 2006;354:1281-93.
142. Framarino di Malatesta ML, Poli L, Pierucci F, et al. Pregnancy and kidney transplantation: clinical problems and experience. Transplantation proceedings 1993;25:2188-9.
143. Armenti VT, Radomski JS, Moritz MJ, et al. Report from the National Transplantation Pregnancy Registry (NTPR): outcomes of pregnancy after transplantation. Clinical transplants 2004:103-14.
144. Framarino Dei Malatesta M, Rossi M, Rocca B, et al. Fertility following solid organ transplantation. Transplantation proceedings 2007;39:2001-4.

145. Scantlebury V, Gordon R, Tzakis A, et al. Childbearing after liver transplantation. Transplantation 1990;49:317-21.
146. Radomski JS, Ahlswede BA, Jarrell BE, et al. Outcomes of 500 pregnancies in 335 female kidney, liver, and heart transplant recipients. Transplantation proceedings 1995;27:1089-90.
147. Armenti VT, Moritz MJ, Cardonick EH, Davison JM. Immunosuppression in pregnancy: choices for infant and maternal health. Drugs 2002;62:2361-75.
148. Armenti VT, Radomski JS, Moritz MJ, et al. Report from the National Transplantation Pregnancy Registry (NTPR): outcomes of pregnancy after transplantation. Clinical transplants 2002:121-30.
149. Jain A, Venkataramanan R, Fung JJ, et al. Pregnancy after liver transplantation under tacrolimus. Transplantation 1997;64:559-65.
150. Chaouat G, Ledee-Bataille N, Dubanchet S. Immune cells in uteroplacental tissues throughout pregnancy: a brief review. Reproductive biomedicine online 2007;14:256-66.
151. Opelz G, Dohler B, Ruhenstroth A. Cytomegalovirus prophylaxis and graft outcome in solid organ transplantation: a collaborative transplant study report. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2004;4:928-36.
152. Bar J, Stahl B, Hod M, Wittenberg C, Pardo J, Merlob P. Is immunosuppression therapy in renal allograft recipients teratogenic? A single-center experience. American journal of medical genetics Part A 2003;116A:31-6.
153. Kallen B, Westgren M, Aberg A, Olausson PO. Pregnancy outcome after maternal organ transplantation in Sweden. BJOG : an international journal of obstetrics and gynaecology 2005;112:904-9.
154. Sieunarine K, Boyle DC, Corless DJ, et al. Pelvic vascular prospects for uterine transplantation. International surgery 2006;91:217-22.
155. Sieunarine K, Zakaria FB, Boyle DC, et al. Possibilities for fertility restoration: a new surgical technique. International surgery 2005;90:249-56.
156. Wranning CA, El-Akouri RR, Lundmark C, et al. Auto-transplantation of the uterus in the domestic pig (Sus scrofa): Surgical technique and early reperfusion events. The journal of obstetrics and gynaecology research 2006;32:358-67.
157. Avison DL, DeFaria W, Tryphonopoulos P, et al. Heterotopic uterus transplantation in a swine model. Transplantation 2009;88:465-9.
158. McCracken JA, Baird DT, Goding JR. Factors affecting the secretion of steroids from the transplanted ovary in the sheep. Recent progress in hormone research 1971;27:537-82 passim.
159. Baird DT, Land RB, Scaramuzzi RJ, Wheeler AG. Functional assessment of the autotransplanted uterus and ovary in the ewe. Proceedings of the Royal Society of London Series B, Containing papers of a Biological character Royal Society (Great Britain) 1976;192:463-74.
160. Wranning CA, Dahm-Kahler P, Molne J, Nilsson UA, Enskog A, Brannstrom M. Transplantation of the uterus in the sheep: oxidative stress and reperfusion injury after short-time cold storage. Fertility and sterility 2008;90:817-26.
161. Dahm-Kahler P, Wranning C, Lundmark C, et al. Transplantation of the uterus in sheep: methodology and early reperfusion events. The journal of obstetrics and gynaecology research 2008;34:784-93.
162. Wranning CA, Marcickiewicz J, Enskog A, Dahm-Kahler P, Hanafy A, Brannstrom M. Fertility after autologous ovine uterine-tubal-ovarian transplantation by vascular anastomosis to the external iliac vessels. Human reproduction (Oxford, England) 2010;25:1973-9.
163. Ramirez ER, Ramirez Nessetti DK, Nessetti MB, et al. Pregnancy and outcome of uterine allotransplantation and assisted reproduction in sheep. Journal of minimally invasive gynecology 2011;18:238-45.
164. Gauthier T, Bertin F, Fourcade L, et al. Uterine allotransplantation in ewes using an aortocava patch. Human reproduction (Oxford, England) 2011;26:3028-36.

165. Friedland S, Benaron D, Coogan S, Sze DY, Soetikno R. Diagnosis of chronic mesenteric ischemia by visible light spectroscopy during endoscopy. Gastrointestinal endoscopy 2007;65:294-300.
166. Yoo H, Kim JW, Shishkov M, et al. Intra-arterial catheter for simultaneous microstructural and molecular imaging in vivo. Nature medicine 2011;17:1680-4.
167. Kalab P, Soderholm J. The design of Forster (fluorescence) resonance energy transfer (FRET)-based molecular sensors for Ran GTPase. Methods (San Diego, Calif) 2010;51:220-32.
168. LeRoux PD, Winter TC, Berger MS, Mack LA, Wang K, Elliott JP. A comparison between preoperative magnetic resonance and intraoperative ultrasound tumor volumes and margins. Journal of clinical ultrasound : JCU 1994;22:29-36.
169. Karliczek A, Benaron DA, Zeebregts CJ, Wiggers T, van Dam GM. Intraoperative ischemia of the distal end of colon anastomoses as detected with visible light spectroscopy causes reduction of anastomotic strength. The Journal of surgical research 2009;152:288-95.
170. Sauvage V. Fluorescence and Diffuse Reflectance Spectroscopy and Endoscopy for Tissue Analysis. Imperial College London, UK: Imperial College London; 2012.
171. Sieunarine K, Moxey P, Boyle DC, et al. Selective vessel ligation in the pelvis: an invaluable tool in certain surgical procedures. International journal of gynecological cancer : official journal of the International Gynecological Cancer Society 2005;15:967-73.
172. Ungar L, Palfalvi L, Hogg R, et al. Abdominal radical trachelectomy: a fertility-preserving option for women with early cervical cancer. BJOG : an international journal of obstetrics and gynaecology 2005;112:366-9.
173. Enskog A, Johannesson L, Chai DC, et al. Uterus transplantation in the baboon: methodology and long-term function after auto-transplantation. Human reproduction (Oxford, England) 2010;25:1980-7.
174. Saso S, Haddad J, Ellis P, et al. Placental site trophoblastic tumours and the concept of fertility preservation. BJOG : an international journal of obstetrics and gynaecology 2012;119:369-74.
175. Savvidou MD, Setchell TE, Sieunarine K, Smith JR. Conservative surgical management of ruptured interstitial pregnancy. Acta obstetricia et gynecologica Scandinavica 2006;85:629-31.
176. Moxey P, Sieunarine K, Cox J, Lawson AD, Ungar L, Smith JR. Pulse oximetry and perfusion index measurement to assess uterine perfusion and viability. International surgery 2006;91:223-7.
177. Zuzak KJ, Schaeberle MD, Lewis EN, Levin IW. Visible reflectance hyperspectral imaging: characterization of a noninvasive, in vivo system for determining tissue perfusion. Analytical chemistry 2002;74:2021-8.
178. Tracy CR, Terrell JD, Francis RP, et al. Characterization of renal ischemia using DLP hyperspectral imaging: a pilot study comparing artery-only occlusion versus artery and vein occlusion. Journal of endourology / Endourological Society 2010;24:321-5.
179. Zakian C, Pretty I, Ellwood R, Hamlin D. In vivo quantification of gingival inflammation using spectral imaging. Journal of biomedical optics 2008;13:054045.
180. Gebhart SC, Thompson RC, Mahadevan-Jansen A. Liquid-crystal tunable filter spectral imaging for brain tumor demarcation. Applied optics 2007;46:1896-910.
181. Styles IB. Selection of optimal filters for multispectral imaging. Applied optics 2008;47:5585-91.
182. Frykman PK, Lindsley EH, Gaon M, Farkas DL. Spectral imaging for precise surgical intervention in Hirschsprung's disease. Journal of biophotonics 2008;1:97-103.
183. Bae Y, Nelson JS, Jung B. Multimodal facial color imaging modality for objective analysis of skin lesions. Journal of biomedical optics 2008;13:064007.
184. PN P. Introduction to Biophotonics: John Wiley & Sons; 2003.
185. Briers JD, Richards G, He XW. Capillary Blood Flow Monitoring Using Laser Speckle Contrast Analysis (LASCA). Journal of biomedical optics 1999;4:164-75.
186. Song L. Endoscopic Laser Speckle Contrast Analysis for Tissue Perfusion. Imperial College London; 2013:1-176.

187. Dainty J. Laser speckle and related phenomena: Springer-Verlag; 1975.
188. Stern MD. In vivo evaluation of microcirculation by coherent light scattering. Nature 1975;254:56-8.
189. Briers JD, Fercher AF. Retinal blood-flow visualization by means of laser speckle photography. Investigative ophthalmology & visual science 1982;22:255-9.
190. Duncan DD, Kirkpatrick SJ. Can laser speckle flowmetry be made a quantitative tool? Journal of the Optical Society of America A, Optics, image science, and vision 2008;25:2088-94.
191. Luo Z, Yuan Z, Pan Y, Du C. Simultaneous imaging of cortical hemodynamics and blood oxygenation change during cerebral ischemia using dual-wavelength laser speckle contrast imaging. Optics letters 2009;34:1480-2.
192. Bezemer R, Legrand M, Klijn E, et al. Real-time assessment of renal cortical microvascular perfusion heterogeneities using near-infrared laser speckle imaging. Optics express 2010;18:15054-61.
193. Forrester KR, Stewart C, Leonard C, Tulip J, Bray RC. Endoscopic laser imaging of tissue perfusion: new instrumentation and technique. Lasers in surgery and medicine 2003;33:151-7.
194. Zimnyakov DA, Sviridov AP, Kuznetsova LV, Baranov SA, Ignatieva NY. Monitoring of tissue thermal modification with a bundle-based full-field speckle analyzer. Applied optics 2006;45:4480-90.
195. Wilkins KM, Warnock JK, Serrano E. Depressive symptoms related to infertility and infertility treatments. The Psychiatric clinics of North America 2010;33:309-21.
196. Laggari V, Diareme S, Christogiorgos S, et al. Anxiety and depression in adolescents with polycystic ovary syndrome and Mayer-Rokitansky-Kuster-Hauser syndrome. Journal of psychosomatic obstetrics and gynaecology 2009;30:83-8.
197. LeRoy S. Vaginal reconstruction in adolescent females with Mayer-Rokitansky-Kuster-Hauser syndrome. Plastic surgical nursing : official journal of the American Society of Plastic and Reconstructive Surgical Nurses 2001;21:23-7, 39.
198. Langer M, Grunberger W, Ringler M. Vaginal agenesis and congenital adrenal hyperplasia. Psychosocial sequelae of diagnosis and neovagina formation. Acta obstetricia et gynecologica Scandinavica 1990;69:343-9.
199. Heller-Boersma JG, Edmonds DK, Schmidt UH. A cognitive behavioural model and therapy for utero-vaginal agenesis (Mayer-Rokitansky-Kuster-Hauser syndrome: MRKH). Behavioural and cognitive psychotherapy 2009;37:449-67.
200. Heller-Boersma JG, Schmidt UH, Edmonds DK. Psychological distress in women with uterovaginal agenesis (Mayer-Rokitansky-Kuster-Hauser Syndrome, MRKH). Psychosomatics 2009;50:277-81.
201. El Kissi Y, Romdhane AB, Hidar S, et al. General psychopathology, anxiety, depression and self-esteem in couples undergoing infertility treatment: a comparative study between men and women. European journal of obstetrics, gynecology, and reproductive biology 2013.
202. Psaros C, Kagan L, Auba E, Alert M, Park ER. A brief report of depressive symptoms and health promoting behaviors among women with infertility attending a clinical mind-body program. Journal of psychosomatic obstetrics and gynaecology 2012;33:32-6.
203. Heller-Boersma JG, Schmidt UH, Edmonds DK. A randomized controlled trial of a cognitive-behavioural group intervention versus waiting-list control for women with uterovaginal agenesis (Mayer-Rokitansky-Kuster-Hauser syndrome: MRKH). Human reproduction (Oxford, England) 2007;22:2296-301.
204. Rosenberger EM, Dew MA, Crone C, Dimartini AF. Psychiatric disorders as risk factors for adverse medical outcomes after solid organ transplantation. Current opinion in organ transplantation 2012.
205. Morris PJ, Bradley JA, Doyal L, et al. Facial transplantation: a working party report from the Royal College of Surgeons of England. Transplantation 2004;77:330-8.

206. Morris P, Bradley A, Doyal L, et al. Face transplantation: a review of the technical, immunological, psychological and clinical issues with recommendations for good practice. Transplantation 2007;83:109-28.
207. Clarke A, Butler PE. The psychological management of facial transplantation. Expert review of neurotherapeutics 2009;9:1087-100.
208. Del Priore G GJ, Zhang JJ. Exploring a cancer patient's reproductive options. Contemp Ob/Gyn 2002:53-66.
209. White E. Projected changes in breast cancer incidence due to the trend toward delayed childbearing. American journal of public health 1987;77:495-7.
210. Dargent D BJ, Roy M, et al. La trachelectomie elargie (TE) une alternative a l'hysterectomie radicale dans le traitement des cancers infiltrants developes sur la face externe du col uterin. . Journal d'Obstetrique et Gynecologie 1994;2:285-92.
211. Renaud MC, Plante M, Roy M. Combined laparoscopic and vaginal radical surgery in cervical cancer. Gynecologic oncology 2000;79:59-63.
212. Girardi F, Lichtenegger W, Tamussino K, Haas J. The importance of parametrial lymph nodes in the treatment of cervical cancer. Gynecologic oncology 1989;34:206-11.
213. Plante M. Vaginal radical trachelectomy: an update. Gynecologic oncology 2008;111:S105-10.
214. Covens A, Shaw P, Murphy J, et al. Is radical trachelectomy a safe alternative to radical hysterectomy for patients with stage IA-B carcinoma of the cervix? Cancer 1999;86:2273-9.
215. Beiner ME, Hauspy J, Rosen B, et al. Radical vaginal trachelectomy vs. radical hysterectomy for small early stage cervical cancer: a matched case-control study. Gynecologic oncology 2008;110:168-71.
216. Ungar L, Smith JR, Palfalvi L, Del Priore G. Abdominal radical trachelectomy during pregnancy to preserve pregnancy and fertility. Obstetrics and gynecology 2006;108:811-4.
217. Mandic A, Novakovic P, Nincic D, Zivaljevic M, Rajovic J. Radical abdominal trachelectomy in the 19th gestation week in patients with early invasive cervical carcinoma: case study and overview of literature. American journal of obstetrics and gynecology 2009;201:e6-8.
218. Abu-Rustum NR, Tal MN, DeLair D, Shih K, Sonoda Y. Radical abdominal trachelectomy for stage IB1 cervical cancer at 15-week gestation. Gynecologic oncology 2010;116:151-2.
219. Boyle DCM UL, Del Priore G, Smith JR. . An Atlas of Gynecologic Oncology, Investigation and Surgery. 1st ed. London, UK: Martin Dunitz Publishing; 2001.
220. Saso S, Ghaem-Maghami S, Chatterjee J, et al. Abdominal radical trachelectomy in West London. BJOG : an international journal of obstetrics and gynaecology 2012;119:187-93.
221. Del Priore G, Ungar L, Smith JR. Complications after fertility-preserving radical trachelectomy. Fertility and sterility 2006;85:227.
222. Rob L, Skapa P, Robova H. Fertility-sparing surgery in patients with cervical cancer. The lancet oncology 2011;12:192-200.
223. Morice P, Narducci F, Mathevet P, Marret H, Darai E, Querleu D. French recommendations on the management of invasive cervical cancer during pregnancy. International journal of gynecological cancer : official journal of the International Gynecological Cancer Society 2009;19:1638-41.
224. Saso S, Chatterjee J, Smith JR. Radical trachelectomy: need for a randomized controlled trial? Acta obstetricia et gynecologica Scandinavica 2012;91:758.
225. Diaz JP, Sonoda Y, Leitao MM, et al. Oncologic outcome of fertility-sparing radical trachelectomy versus radical hysterectomy for stage IB1 cervical carcinoma. Gynecologic oncology 2008;111:255-60.
226. Sieunarine K, Cowie AS, Bartlett JD, Lindsay I, Smith JR. A novel approach in the management of a recurrent adenomatoid tumor of the uterus utilizing a Strassman technique. International journal of gynecological cancer : official journal of the International Gynecological Cancer Society 2005;15:671-5.

227. Milingos D, Doumplis D, Sieunarine K, Savage P, Lawson AD, Smith JR. Uterine arteriovenous malformation: fertility-sparing surgery using unilateral ligation of uterine artery and ovarian ligament. International journal of gynecological cancer : official journal of the International Gynecological Cancer Society 2007;17:735-7.
228. Mehaisen GM. Embryo cryopreservation program in a rabbit line selected by growth rate: Superovulation and in vivo embryo recovery protocols: Valencia; 2005.
229. Mehaisen GM, Vicente JS, Lavara R, Viudes-de-Castro MP. Effect of eCG dose and ovulation induction treatments on embryo recovery and in vitro development post-vitrification in two selected lines of rabbit does. Animal reproduction science 2005;90:175-84.
230. Besenfelder U, Strouhal C, Brem G. A method for endoscopic embryo collection and transfer in the rabbit. Zentralblatt fur Veterinarmedizin Reihe A 1998;45:577-9.
231. Mehaisen GM, Vicente JS, Lavara R. In vivo embryo recovery rate by laparoscopic technique from rabbit does selected for growth rate. Reproduction in domestic animals = Zuchthygiene 2004;39:347-51.
232. Vicente JS, Viudes-de-Castro MP, Garcia ML. In vivo survival rate of rabbit morulae after vitrification in a medium without serum protein. Reproduction, nutrition, development 1999;39:657-62.
233. Chavatte-Palmer P, Laigre P, Simonoff E, Chesne P, Challah-Jacques M, Renard JP. In utero characterisation of fetal growth by ultrasound scanning in the rabbit. Theriogenology 2008;69:859-69.
234. Hewitt CD, Innes DJ, Savory J, Wills MR. Normal biochemical and hematological values in New Zealand white rabbits. Clinical chemistry 1989;35:1777-9.
235. Sieunarine K, Lindsay I, Ungar L, Del Priore G, Smith JR. Cold ischaemic preservation of human uterine tissue. International surgery 2008;93:366-72.
236. Laskowski I, Pratschke J, Wilhelm MJ, Gasser M, Tilney NL. Molecular and cellular events associated with ischemia/reperfusion injury. Annals of transplantation : quarterly of the Polish Transplantation Society 2000;5:29-35.
237. Opelz G, Dohler B. Multicenter analysis of kidney preservation. Transplantation 2007;83:247-53.
238. Massberg S, Gonzalez AP, Leiderer R, Menger MD, Messmer K. In vivo assessment of the influence of cold preservation time on microvascular reperfusion injury after experimental small bowel transplantation. The British journal of surgery 1998;85:127-33.
239. Wehner S, Behrendt FF, Lyutenski BN, et al. Inhibition of macrophage function prevents intestinal inflammation and postoperative ileus in rodents. Gut 2007;56:176-85.
240. Turler A, Moore BA, Pezzone MA, Overhaus M, Kalff JC, Bauer AJ. Colonic postoperative inflammatory ileus in the rat. Annals of surgery 2002;236:56-66.
241. Giessler GA, Gades NM, Friedrich PF, Bishop AT. Severe tacrolimus toxicity in rabbits. Experimental and clinical transplantation : official journal of the Middle East Society for Organ Transplantation 2007;5:590-5.
242. Saso S, Logan K, Abdallah Y, et al. Use of cyclosporine in uterine transplantation. Journal of transplantation 2012;2012:134936.
243. Haynes RA, 2nd, Ware E, Premanandan C, et al. Cyclosporine-induced immune suppression alters establishment of HTLV-1 infection in a rabbit model. Blood 2010;115:815-23.
244. Gratwohl A, Riederer I, Graf E, Speck B. Cyclosporine toxicity in rabbits. Laboratory animals 1986;20:213-20.
245. Trowsdale J, Betz AG. Mother's little helpers: mechanisms of maternal-fetal tolerance. Nature immunology 2006;7:241-6.
246. Croy BA, He H, Esadeg S, et al. Uterine natural killer cells: insights into their cellular and molecular biology from mouse modelling. Reproduction (Cambridge, England) 2003;126:149-60.
247. Davison JM, Bailey DJ. Pregnancy following renal transplantation. The journal of obstetrics and gynaecology research 2003;29:227-33.

248. Ekberg H, Tedesco-Silva H, Demirbas A, et al. Reduced exposure to calcineurin inhibitors in renal transplantation. The New England journal of medicine 2007;357:2562-75.
249. Kino T, Inamura N, Sakai F, et al. Effect of FK-506 on human mixed lymphocyte reaction in vitro. Transplantation proceedings 1987;19:36-9.
250. Becker T, Foltys D, Bilbao I, et al. Patient outcomes in two steroid-free regimens using tacrolimus monotherapy after daclizumab induction and tacrolimus with mycophenolate mofetil in liver transplantation. Transplantation 2008;86:1689-94.
251. Jain AB, Shapiro R, Scantlebury VP, et al. Pregnancy after kidney and kidney-pancreas transplantation under tacrolimus: a single center's experience. Transplantation 2004;77:897-902.
252. Wieers G, Gras J, Bourdeaux C, Truong DQ, Latinne D, Reding R. Monitoring tolerance after human liver transplantation. Transplant immunology 2007;17:83-93.
253. Fritzsching E, Kunz P, Maurer B, Poschl J, Fritzsching B. Regulatory T cells and tolerance induction. Clinical transplantation 2009;23 Suppl 21:10-4.
254. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Pillars article: immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J. Immunol. 1995. Journal of immunology (Baltimore, Md : 1950) 2011;186:3808-21.
255. Kang SM, Tang Q, Bluestone JA. CD4+CD25+ regulatory T cells in transplantation: progress, challenges and prospects. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2007;7:1457-63.
256. Golshayan D, Jiang S, Tsang J, Garin MI, Mottet C, Lechler RI. In vitro-expanded donor alloantigen-specific CD4+CD25+ regulatory T cells promote experimental transplantation tolerance. Blood 2007;109:827-35.
257. Roncarolo MG, Battaglia M. Regulatory T-cell immunotherapy for tolerance to self antigens and alloantigens in humans. Nature reviews Immunology 2007;7:585-98.
258. Sasaki Y, Sakai M, Miyazaki S, Higuma S, Shiozaki A, Saito S. Decidual and peripheral blood CD4+CD25+ regulatory T cells in early pregnancy subjects and spontaneous abortion cases. Molecular human reproduction 2004;10:347-53.
259. Waid TH, Thompson JS, Siemionow M, Brown SA. T10B9 monoclonal antibody: a short-acting nonstimulating monoclonal antibody that spares gammadelta T-cells and treats and prevents cellular rejection. Drug design, development and therapy 2009;3:205-12.
260. Kawai T, Cosimi AB, Spitzer TR, et al. HLA-mismatched renal transplantation without maintenance immunosuppression. The New England journal of medicine 2008;358:353-61.
261. Ozkan O, Akar ME, Erdogan O, et al. Preliminary results of the first human uterus transplantation from a multiorgan donor. Fertility and sterility 2013;99:470-6.
262. Grace RF. Pulse oximetry. Gold standard or false sense of security? The Medical journal of Australia 1994;160:638-44.
263. Middleton PM, Henry JA. Pulse oximetry: evolution and directions. International journal of clinical practice 2000;54:438-44.
264. Zackrisson U, Mikuni M, Peterson MC, Nilsson B, Janson PO, Brannstrom M. Evidence for the involvement of blood flow-related mechanisms in the ovulatory process of the rat. Human reproduction (Oxford, England) 2000;15:264-72.
265. Clancy N. Multispectral Imaging of Organ Viability during Uterine Transplantation Surgery. Proc SPIE 2014;8935.
266. Kohl M, Lindauer U, Royl G, et al. Physical model for the spectroscopic analysis of cortical intrinsic optical signals. Physics in medicine and biology 2000;45:3749-64.
267. Pilet J LV, Fua P. . Real-time non-rigid surface detection. Proceedings of the IEEE Computer Society Conference on Computer Vision and Pattern Recognition. San Diego, CA, USA2005:822-8.
268. Webster J. Medical Instrumentation - Application and Design2010.
269. Kanitakis J, Jullien D, Petruzzo P, et al. Clinicopathologic features of graft rejection of the first human hand allograft. Transplantation 2003;76:688-93.

270. Nevins TE, Matas AJ. Medication noncompliance: another iceberg's tip. Transplantation 2004;77:776-8.
271. Clarke A, Murphy F, White P, Brough V, Renshaw A, Butler PE. Transplant professionals' attitudes toward facial transplantation in the United Kingdom. Progress in transplantation (Aliso Viejo, Calif) 2007;17:228-33.
272. Clarke A, Simmons J, White P, Withey S, Butler PE. Attitudes to face transplantation: results of a public engagement exercise at the Royal Society Summer Science Exhibition. Journal of burn care & research : official publication of the American Burn Association 2006;27:394-8.
273. Vianna R, Kubal C, Mangus R, Fridell J, Tector J. Intestinal and multivisceral transplantation at Indiana University: 6 years' experience with 100 cases. Clinical transplants 2009:219-28.
274. Pomahac B, Pribaz J, Eriksson E, et al. Three patients with full facial transplantation. The New England journal of medicine 2012;366:715-22.
275. Brannstrom M, Diaz-Garcia C, Hanafy A, Olausson M, Tzakis A. Uterus transplantation: animal research and human possibilities. Fertility and sterility 2012;97:1269-76.
276. Milliez J. Uterine transplantation FIGO Committee for the Ethical Aspects of Human Reproduction and Women's Health. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics 2009;106:270.
277. Lefkowitz A, Edwards M, Balayla J. The Montreal Criteria for the Ethical Feasibility of Uterine Transplantation. Transplant international : official journal of the European Society for Organ Transplantation 2012;25:439-47.
278. Moore FD. Ethical problems special to surgery: surgical teaching, surgical innovation, and the surgeon in managed care. Archives of surgery (Chicago, Ill : 1960) 2000;135:14-6.
279. Mihara M, Kisu I, Hara H, et al. Uterine autotransplantation in cynomolgus macaques: the first case of pregnancy and delivery. Human reproduction (Oxford, England) 2012;27:2332-40.
280. Johannesson L, Kvarnstrom N, Molne J, et al. Uterus transplantation trial: 1-year outcome. Fertility and sterility 2015;103:199-204.
281. Brannstrom M, Johannesson L, Bokstrom H, et al. Livebirth after uterus transplantation. Lancet 2014.
282. Kisu I, Banno K, Mihara M, Suganuma N, Aoki D. Current status of uterus transplantation in primates and issues for clinical application. Fertility and sterility 2013.
283. Del Priore G, Schlatt S, Wagner R, Reynoso E, Malanowska-Stega J. Uterus transplantation: on the edge. Seminars in reproductive medicine 2011;29:55-60.
284. Del Priore G, Saso S, Meslin EM, et al. Uterine transplantation--a real possibility? The Indianapolis consensus. Human reproduction (Oxford, England) 2013;28:288-91.
285. Todo S, Ueda Y, Demetris JA, et al. Immunosuppression of canine, monkey, and baboon allografts by FK 506: with special reference to synergism with other drugs and to tolerance induction. Surgery 1988;104:239-49.
286. Siemionow M. Analysis of historical outcomes of composite tissue allograft transplants in nonhuman primates. Transplantation 2005;80:1374-5.
287. Cendales LC, Xu H, Bacher J, Eckhaus MA, Kleiner DE, Kirk AD. Composite tissue allotransplantation: development of a preclinical model in nonhuman primates. Transplantation 2005;80:1447-54.
288. Stevens VC. Some reproductive studies in the baboon. Human reproduction update 1997;3:533-40.
289. Johannesson L, Enskog A, Dahm-Kahler P, et al. Uterus transplantation in a non-human primate: long-term follow-up after autologous transplantation. Human reproduction (Oxford, England) 2012;27:1640-8.
290. Mihara M, Kisu I, Hara H, et al. Uterus autotransplantation in cynomolgus macaques: intraoperative evaluation of uterine blood flow using indocyanine green. Human reproduction (Oxford, England) 2011;26:3019-27.

291. Johannesson L, Enskog A, Molne J, et al. Preclinical report on allogeneic uterus transplantation in non-human primates. Human reproduction (Oxford, England) 2013;28:189-98.
292. McCulloch P, Altman DG, Campbell WB, et al. No surgical innovation without evaluation: the IDEAL recommendations. Lancet 2009;374:1105-12.
293. Del Priore G, Stega J, Sieunarine K, Ungar L, Smith JR. Human uterus retrieval from a multi-organ donor. Obstetrics and gynecology 2007;109:101-4.
294. Johannesson L, Diaz-Garcia C, Leonhardt H, et al. Vascular pedicle lengths after hysterectomy: toward future human uterus transplantation. Obstetrics and gynecology 2012;119:1219-25.
295. Segev DL, Gentry SE, Warren DS, Reeb B, Montgomery RA. Kidney paired donation and optimizing the use of live donor organs. JAMA : the journal of the American Medical Association 2005;293:1883-90.
296. Fabrizii V, Kovarik J, Bodingbauer M, Kramar R, Horl WH, Winkelmayer WC. Long-term patient and graft survival in the eurotransplant senior program: a single-center experience. Transplantation 2005;80:582-9.
297. Petruzzo P, Lanzetta M, Dubernard JM, et al. The International Registry on Hand and Composite Tissue Transplantation. Transplantation 2010;90:1590-4.
298. Doumplis D, Al-Khatib K, Sieunarine K, et al. A review of the management by hysterectomy of 25 cases of gestational trophoblastic tumours from March 1993 to January 2006. BJOG : an international journal of obstetrics and gynaecology 2007;114:1168-71.
299. Scott JR, Branch DW, Holman J. Autoimmune and pregnancy complications in the daughter of a kidney transplant patient. Transplantation 2002;73:815-6.
300. Padgett EL, Seelig LL, Jr. Effects on T-cell maturation and proliferation induced by lactational transfer of cyclosporine to nursing pups. Transplantation 2002;73:867-74.