SURGERY AND ISCHEMIA IN UTERUS TRANSPLANTATION

STUDIES IN VARIOUS EXPERIMENTAL MODELS

Caiza Almén Wranning

2007

Department of Obstetrics and Gynecology
Institute of Clinical Science
The Sahlgrenska Academy at Göteborg University
Göteborg, Sweden

ISBN 978-91-628-7237-3

Till Joel och Tova

"It's a magical world, Hobbes, ol' buddy, ol' pal…..
Let's go exploring!"

Last line of the final *Calvin and Hobbes* strip
by Bill Watterson, 1995

POPULÄRVETENSKAPLIG SAMMANFATTNING

Det senaste decenniet har intresset för att använda transplantation som behandling av tillstånd som inte är livshotande men som har avgörande betydelse för individens livskvalitet ökat och detta intresse innefattar även transplantation av fortplantningsorgan. Livmodertransplantation har föreslagits som bot mot sterilitet hos kvinnor som av olika skäl saknar livmoder och därför inte kan bli gravida. För att undersöka om livmodertransplantation är möjligt att genomföra och för att utveckla säkra metoder för detta krävs omfattande studier i lämpliga experimentmodeller. Denna avhandling undersöker hur livmodern påverkas av den syrebrist som uppstår när ett organ tas ut ur donatorn och innan blodflödet genom organet etablerats i mottagaren. Studierna har genomförts i olika experimentmodeller och även dessa modeller presenteras och diskuteras i avhandlingen.

När blodcirkulationen återställs i ett organ som tidigare varit utsatt för syrebrist startar en rad processer som kan leda till inflammation och i värsta fall organdöd. Denna inflammation kan även förvärra avstötningsreaktionen och därför är olika åtgärder för att minska inflammation vid transplantation ett viktigt forskningsområde. Kyla minskar hastigheten för alla biologiska processer och används för att förlänga den tid som ett organ kan vara utan blodcirkulation. Det finns även en rad olika skyddande lösningar som används för att ersätta blodet i organet under kylförvaring och som bidrar till att ytterligare dämpa utvecklingen av skador. Det har visat sig att olika organ har olika tålighet för syrebrist och kylförvaring.

Eftersom livmodertransplantation är ett relativt nytt forskningsområde är det vikigt att etablera grundläggande kunskap om livmoderns specifika egenskaper på detta område så att säkra metoder för transplantation av livmoder kan utvecklas. De fem delstudier som ingår i denna avhandling har genomförts utifrån olika frågeställningar kring kylförvaring av livmodern och i olika experimentmodeller.

Transplantation mellan genetiskt lika möss genomfördes efter att livmodern förvarats i en skyddande lösning i 24 timmar. Efter att mössen läkt från

operationen infördes befruktade ägg i den transplanterade livmodern och avkomman visade sig vara normal. Undersökningar av livmodervävnad som donerats av kvinnor som låtit ta bort sin livmoder pga blödnings besvär eller muskelknutor i livmodern visade också på bibehållen muskelfunktion och biokemisk aktivitet efter 24 timmars kylförvaring i en skyddande förvaringslösning.

Livmodertransplantation utfördes även i får och gris på så sätt att samma djur agerade som både donator och mottagare. Här undersöktes hur allvarliga störningar en kort tids kylförvaring kan orsaka och om de skyddande egenskaperna hos en förvaringslösning har någon mätbar positiv effekt. Det visade sig att livmodern endast uppvisar små skador efter kort tids kylförvaring och att en skyddande förvaringslösning ytterligare kan dämpa dessa skador.

Transplantation mellan genetiskt lika råttor genomfördes efter 24 timmars kylförvaring av livmodern. Studien undersökte om förbehandling av både donator och mottagare med det kvinnliga könshormonet progesteron kan minska de skador som uppstår efter syrebrist. Resultaten visar att progesteron kan ha en inflammationsdämpande effekt liknande den som fås vid kortisonbehandling. Fortsatta studier av progesteron i livmodertransplantation är av intresse då både positiva och negativa effekter av detta hormon koncentreras främst till livmodern, till skillnad från kortisonpreparat som har en mer spridd verkan i kroppen.

Sammanfattningsvis visar denna avhandling att livmodern har en relativt hög tolerans mot skador som orsakas av syrebrist och att experimentmodeller i mus, råtta och får är användbara i fortsatta studier som syftar till att ta fram säkra metoder för livmodertransplantation i behandling mot ofrivillig barnlöshet som orsakas av avsaknad av en funktionsduglig livmoder.

Göteborg, juli 2007

ABSTRACT

The use of transplantation to enhance quality of life is a growing clinical field that also includes transplantation of reproductive organs. Transplantation of the uterus has been suggested as future method to treat uterus factor infertility. To investigate the feasibility of uterine transplantation and to develop safe methods, research must be performed in appropriate animal and *in vitro* models. This thesis investigates the effects of ischemia and reperfusion on functional, morphological and biochemical parameters in the uterus in several experimental models and also describes these models.

The tolerance of the uterus to cold ischemia was evaluated in a previously developed mouse model for uterus transplantation. Cold ischemia for 24 h did not impair the ability of the mouse uterus to implant embryos and produce normal offspring if University of Wisconsin preservation buffer (UW) was used during ischemia. Also, in vitro studies on human myometrium showed that myometrial contractions, protein synthesis and energy production were well preserved after cold ischemia in UW or the preservation solution Perfadex for 6 h.

A pig model for auto-transplantation of the uterus was developed and found to be of less value since the number of successful transplantations was low (21%) due to surgical difficulties related to the anatomy of the pig. In the development of a sheep model other surgical strategies could be used and the success rate was considerably higher (71%). Evaluation of biochemical and morphological parameters during reperfusion after of short time cold ischemia showed recovered metabolism and only a slight inflammatory response that was further reduced by the use of the preservation solution Perfadex during cold ischemia.

A rat model was also developed to complement to the mouse as a small animal model for uterine transplantation research. Morphological signs of post-ischemic inflammation in rat uteri transplanted after 24 h of cold ischemia were reduced by pre-treatment of the donor and the recipient with progesterone or prednisolone.

In summary, it was found that the mouse, the rat and the sheep can serve as appropriate model animals for studies of various aspects of uterus transplantation. It was also found that in these non-rejecting models the uterus is fairly resistant to injuries induced by surgery and cold ischemia and can tolerate cold ischemic storage for at least 24. However, in a possible future human application, organ injuries induced by ischemia should be reduced. Potential strategies to achieve this could include the use of short ischemic times, appropriate preservation solutions and anti-inflammatory pre-treatment of donor and recipient. These studies have moved the research front in uterine transplantation forward and it is predicted that uterine transplantation can reach the clinical setting within 5 years.

Göteborg, July 2007

LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to by their Roman numerals:

I. Pregnancy in transplanted mouse uterus after long-term cold ischaemic preservation.

Racho El-Akouri R., Wranning C.A., Mölne J., Kurlberg G., Brännström M.

Hum Reprod. 2003 Oct;18(10):2024-30.

II. Short-term ischaemic storage of human uterine myometrium--basic studies towards uterine transplantation.

Wranning C.A., Mölne J. El-Akouri R.R., Kurlberg G., Brännström M.

Hum Reprod. 2005 Oct;20(10):2736-44.

III. Auto-transplantation of the uterus in the domestic pig (Sus scrofa): Surgical technique and early reperfusion events.

Wranning C.A., El-Akouri R.R., Lundmark C., Dahm-Kähler P., Mölne J., Enskog A., Brännström M.

J Obstet Gynaecol Res. 2006 Aug;32(4):358-67.

IV. Transplantation of the uterus in the sheep – oxidative stress and reperfusion injury after short time cold storage.

Wranning C.A., Dahm-Kähler P., Mölne J., Nilsson U.A., Enskog A., Brännström M.

Fertility and Sterility,2007. In press

V. Effects of progesterone and prednisolone on post-operative inflammation after cold ischemia in a rat model for uterus transplantation.

Wranning C.A., Mölne J., Kurlberg G., Brännström M.

In manuscript

CONTENTS

ABBREVIATIONS

ABTS - azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)

ANOVA – analysis of variance

ART – assisted reproductive technique

ATP – adenosine trisphosphate

AUC – area under the curve

DNA – deoxyribonucleic acid

FSH – follicle stimulating hormone

GnRH – gonadotropin releasing hormone

GSH – reduced glutathione

GSSG – oxidised glutathione

HES - hydroxy ethyl starch

ICSI – intra-cytoplasmic sperm injection

IVF – *in vitro* fertilization

LH – luteinising hormone

MRKH-syndrome – Mayer-Rokitansky-Kuster-Hauser syndrome

NADPH – nicotinamide adenine dinucleotide phosphate

PER – Perfadex preservation solution

RIN – Ringer acetate solution

RNS – reactive nitrogen species/substances

ROS – reactive oxygen species/substances

TBARS – thiobarbituric acid reactive species/substances

UW – University of Wisconsin preservation solution

INTRODUCTION

To reproduce is a central component in the fulfilment of normal life and infertility can have profound negative psychological and social consequences for those affected (Cousineau and Domar 2007). When the first successful *in vitro* fertilisation (IVF) with a live born baby was reported nearly thirty years ago (Steptoe and Edwards 1978; Edwards *et al.* 1980) the first reliable method to treat the main type of female infertility – tubal infertility - was introduced. Since then new and improved treatments such as intra cytoplasmic sperm injection (ICSI) (Palermo *et al.* 1992) have been established and today we can circumvent many of the causes of both male and female infertility. Also, cryopreservation of ovarian tissue followed by auto-transplantation to rescue fertility in female cancer patients have been reported with successful outcome (Donnez *et al.* 2004). The development of these assisted reproductive techniques (ARTs) have led to a changed view on infertility so that we now consider infertility as a disease that should be cured, rather than a tragic condition that must be accepted and this is a strong motivator for further research in the area.

Transplantation of organs that are not life supporting but of great importance for general wellbeing has also become a growing area of interest. Advancements in immunological research, improved surgical methods and more effective immunosuppressive regimens have considerably reduced the risks connected with transplantation and opened up for an expansion of the types of organs that can be transplanted. Just recently there have been reports on successful transplantations of complex, composite tissue such as the face (Kanitakis *et al.* 2006), abdominal wall (Levi *et al.* 2003) and hand/forearm (Lanzetta *et al.* 2005).

Uterus factor infertility is today largely untreatable and the growing significance of transplantation as a means to enhance the quality of life has also made transplantation of the uterus for fertility purposes interesting. The past seven years, research on uterus transplantation has emerged and developed and continues to attract interest from researchers and potential patients.

In development of uterine transplantation as a treatment for infertility it is of importance to study many aspect of possible relevance in order to minimize the risks involved for the donor, the mother and the prospective child. Such issues would include surgical methods, the impact of cold ischemic preservation as well as suitable immunosuppressive regimens to avoid rejection and their impact on pregnancy and pre- and post-natal development. Many lessons can be drawn from previous research in several related areas in transplantation research. However, little is known about the uterus as a transplant and since the purpose of the transplantation is to generate life, new considerations have to be made and much research has to be done in models specific for uterus transplantation, both *in vitro* and *in vivo* in suitable animal models.

This thesis deals with the effects of ischemia and reperfusion injury in several experimental models. The significance of appropriate animal models for studies on different aspects of uterus transplantation cannot be underestimated and different aspects of the models used in uterine transplantation research are also addressed.

The uterus

The uterus is a relatively simple organ regarding its anatomy but it exhibits remarkable physiological features. It has an inherent capacity of growth and shrinkage, undergoes constant cyclic changes of tissue modulation and responsiveness to various stimuli and it can accept and allow the growth of a semi-allogeneic or fully allogeneic conceptus.

Anatomy

In the non-gravid state most of the uterine mass is made up of smooth muscle (myometrium) surrounding a cavity lined with mucosal tissue (endometrium). The uterine cavity has its connection with the vagina through the cervix and to the abdominal cavity through the two Fallopian tubes on each side of the upper part of the uterus (the corpus). The cervix is the downward extension of the uterus

with its lowermost part exposed into the vagina and it consists mainly of connective tissue.

The myometrium is made up of three somewhat blended layers of smooth muscle cells. These muscle cells are interconnected through gap-junctions to make up a functional syncytium (MacKenzie and Garfield 1985) and the myometrium exhibits a high degree of spontaneous contractility (Wray *et al.* 2001). The innermost layer of the uterus is the endometrium, that consists of a monolayer of cuboidal epithelial cells facing the cavity and beneath that is the stroma. Endometrial glands embedded in the stroma extend their ducts to the epithelial surface and spiral arterioles supply the endometrium with oxygenated blood. The endometrium is constantly broken down and regenerated in the non-gravid uterus, following the cyclic changes of sex steroids.

The endometrium lining the uterine cavity is the site of implantation of the fertilized egg. The implanted embryo grows within the decidual endometrium where the placenta develops from the outer cell mass of the blastocyst to ensure exchange between foetal and maternal blood throughout pregnancy.

The uterus is vascularised by the bilateral uterine and ovarian arteries. The uterine arteries arise from the anterior divisions of the internal iliac arteries and run in a cranial direction along the lateral sides of uterine body from the isthmus. The uterine artery branches off into the arcuate arteries that encircle the uterus and at intervals the arcuate arteries give off radial arteries that penetrate directly inward to the endometrium. At the level of the round ligaments, the uterine artery anastomoses with the ovarian artery in a continuum and the supply of blood to the uterus comes at least partly from the ovarian side. The uterine and ovarian veins are multiple and follow the arteries.

Even though the basic anatomy of the uterus is similar in most mammals there are certain anatomical differences that have to be accounted for in the development of surgical approaches for transplantation. One major difference is the relative length of the uterine horns that reflects the number of offspring normal for the specific species. For example, animals like rodents and pigs, that carry a large

number of foeti, have bicornuate uteri with long uterine horns, while sheep that carry only one to three lambs has a bicornuate uterus with shorter uterine horns. Primates, which usually cary a singleton pregnancy, have uteri with a common cavity after the Müllerian ducts have fused during foetal life. The vascular anatomy is also slightly different between species and reflects the functional requirements determined by number of offspring normally carried in a pregnancy. For example, in the rodent and pig the uterine vessels ramify to create a network of vessels outside the uterine tissue that extends over the length of the uterine horns. These vessels are surrounded and supported by ligaments (corresponding to the broad ligaments in the human) that allow for flexibility of the rather long uterine horns. In the sheep and human uterus there are few or no ramifications of the uterine vessels outside the uterine body and the uterine vessels run on the surface of the uterine body.

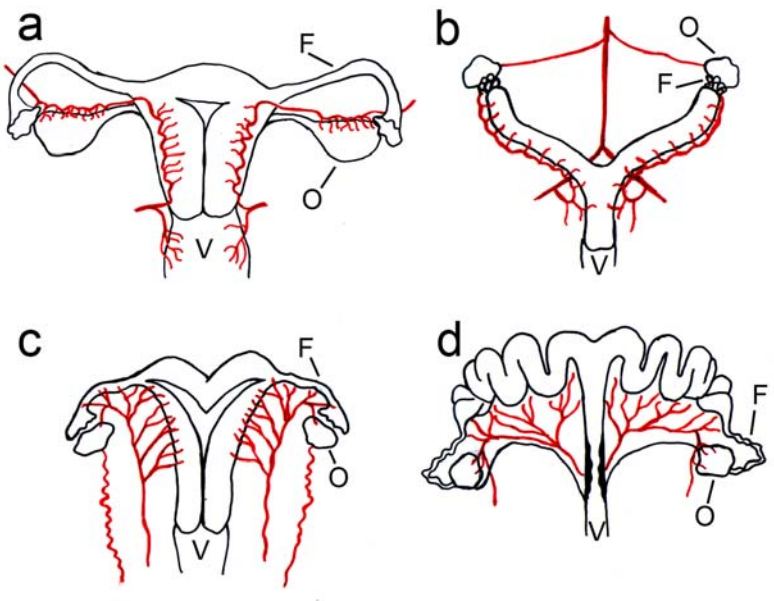


Figure 1 *Schematic illustration of the gross anatomy of the uterus in the human (a), the mouse and rat (b), the sheep (c) and the pig (d). Red lines represent the arterial vascular supply to the uterus. O=ovary, V=vagina, F=Fallopian tube*

Hormonal regulation

All placental mammals share a similar reproductive system; the regulatory hypothalamic system releases gonadotrophin releasing hormone (GnRH) in pulses that stimulates the release of the pituitary gonadotropins, follicle stimulating hormone (FSH) and luteinising hormone (LH). In females FSH and LH tightly regulate the production of the major ovarian sex steroids estradiol and progesterone and these exert profound influences on uterine function. All sex-steroids as well as corticosteroids are produced from cholesterol via pregnenolone by a series of enzymatic steps. Estradiol is produced primarily by developing follicles and the main source of progesterone is the corpus luteum. During pregnancy the placenta produces both hormones.

The structure of estradiol and progesterone and their respective receptors are highly conserved through evolution (Thornton *et al.* 2003) and the serum levels of estradiol and progesterone as well as uterine receptor density and responsiveness vary in a cyclic manner that is similar in all species. In non-primates these cyclic changes are referred to as the estrus cycle and in primates it is called the menstrual cycle. Animals exhibiting an estrus cycles reabsorb the endometrium if conception does not occur during a cycle, while animals that have menstrual cycles shed the endometrium through menstruation.

The phases of the estrus and menstrual cycle correlate and can be divided into four phases. Estradiol levels begin to rise at the start of the follicular phase (proestrus) and peak at late follicular phase (estrus), just prior to ovulation. After ovulation, during the early luteal phase (metestrus), estradiol levels drop and the corpus luteum is formed, producing progesterone. Progesterone levels that are low during the follicular phase, peak at mid luteal phase and influence the differentiation of the endometrium and endometrial glands. In late luteal phase (diestrus) the corpus luteum regresses during a non-conception cycle, progesterone levels drop and the endometrium is reabsorbed/shed. If implantation and pregnancy occur, the developing placenta releases chorionic gonadotrophin that rescues the corpus luteum so that the secretion of estradiol and progesterone is maintained.

Varying levels of estradiol and progesterone also modulate the myometrial sensitivity to contractile agents, with estradiol as stimulatory and progesterone as inhibitory mediators. For example, the high levels of progesterone during pregnancy maintain uterine quiescence and a drop in progesterone in relation to estradiol levels at term sensitizes the uterus to contractile agents. The main stimulatory agents for myometrial contractions are oxytocin and prostaglandins and the sensitivity (i.e. density and affinity of receptors) of myometrium to their action is partly regulated via sex steroids (Wathes *et al.* 1996; Myatt and Lye 2004).

Innervation

The uterus is innervated by sympathetic fibres from the thoracolumbar segments and parasympathetic nerves from the spinal segments. In the non pregnant human uterus most of the nerve terminals are cholinergic (>50%) or adrenergic (~30%) (Morizaki *et al.* 1989). The importance of neuronal influence on the uterus is not entirely clear. During pregnancy the innervation gradually degenerates due to reduced production of functional nerve growth factor beta (NGFβ) (Lobos *et al.* 2005) and the denervation is almost complete at term (Chavez-Genaro *et al.* 2006). Studies have shown that the neuronal stimulatory effect is weak also in non-pregnant myometrium, indicating that neuronal influence on myometrial contractility can be modulatory but not dominant (Morizaki *et al.* 1989; Houdeau *et al.* 2003).

Lymph drainage

The anatomy of the lymphatic structures in the uterus has not been investigated by many. A few studies in the human, rat and sheep show that the lymph vessels approximately follow the venous route outside the uterus and that the abundance and morphology of lymph vessels in the endometrium vary with hormonal status (Abdel Rahim and Bland 1985; Uchino *et al.* 1987).

Immunology

Since the conceptus expresses cell surface molecules of both maternal and paternal origin it is partly foreign to the mother and could be regarded as a semi-

allogeneic transplant. However, during a normal pregnancy an intense cross-talk between maternal and foetal tissue induces changes in the uterine environment that allows for maternal tolerance of the foetus (Vigano *et al.* 2003). For example, regulatory T cells that are commonly associated with maintenance of tolerance to self-antigens have been shown to be involved in the suppression of maternal allo-responses (Tafuri *et al.* 1995). During the non-pregnant hormonal cycle there are variations in uterine immune cell density (Kaushic *et al.* 1998; Kaeoket *et al.* 2001) and phenotypes (Keenihan and Robertson 2004; Sentman *et al.* 2004) as well as alterations in expression of stromal and epithelial cell surface markers (Wira and Sullivan 1981) and inflammatory factors (Hasty *et al.* 1994; Ramhorst *et al.* 2006). These changes are influenced by the ovarian steroids and participate in regulation of endometrial receptivity.

Uterus factor infertility

If transplantation of the uterus can be developed in to an acceptably safe method with reasonable chances of achieving successful pregnancies, women that today are infertile due to uterus factor infertility could carry their own pregnancies.

Etiology

Uterus factor infertility refers to infertility due to lack of a functioning uterus. This type of infertility can be caused by a complete absence or malformation of the uterus or adherences in the uterine cavity that hinder a normal pregnancy. The cause of a complete absence of the uterus can be congenital or a consequence of surgical removal of the uterus as treatment for disease. Malformations can also be congenital or caused by large myomas that distort the uterine cavity and adherences of the endometrium in the uterine cavity are usually caused by infections (Sharma *et al.* 2007) or curettage (March 1995). Congenital malformations, myomas and adherences have varying effects on fertility depending on the severity of the condition (Sanders 2006). It is estimated that only in the UK, around 15 000 women or around 3% of infertile women are infertile due to uterus factor (Sieunarine *et al.* 2005).

Congenital absence of the uterus and vagina, or the Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome, has an estimated incidence of approximately 1 in 4000 to 10 000 newborn girls (Evans *et al.* 1981; Cheroki *et al.* 2006) and is caused by a failure of the Müllerian ducts to develop during embryonic life. This causes an absence of the uterus and the upper part of the vagina in a woman that usually has normal ovaries and hormone production (Fraser *et al.* 1973; Karam *et al.* 1977). The MRKH syndrome can be associated with other disorders (Oppelt *et al.* 2007) and even though the condition possibly has a genetic component, this is yet unidentified (Griffin *et al.* 1976; Cheroki *et al.* 2006). In a follow up of 17 daughters to MRKH mothers born after gestational surrogacy, no congenital malformations of the uterus were seen that would indicate dominant inheritance of the condition (Petrozza *et al.* 1997).

Large, symptomatic leiomyomas are often treated with hysterectomy that naturally renders the patient infertile. Leiomyomas are benign tumors derived from smooth muscle cells in the uterine wall and if large they can cause pressure, bleeding and pain (Buttram 1986). The incidence of leiomyomas among pre-menopausal women increases with age and an estimate of 1% of all women between 30 and 34 years and 2.5 % of those between 35 and 39 will undergo hysterectomy as treatment for myomas (Marshall *et al.* 1997). In a patient of childbearing age who has not yet formed a family, myomectomy (i.e. removal of the myomas alone) can be an alternative to hysterectomy as an attempt to preserve her fertility (Lefebvre *et al.* 2003). The result of myomectomy is however largely dependent on the size and position of the myomas and especially at removal of sub-endometrial myomas with associated intramural myomas, fertility is at risk (Casini *et al.* 2006).

Cervical cancer is a gynecological malignancy which affects also younger women of childbearing age. The age-related incidence is fairly constant from the age of 20 and most of these cancers are diagnosed at an early stage, when it has not spread from the cervix. The standard treatment for this early stage cervical cancer is a radical hysterectomy with preservation of the ovaries. Early stage endometrial cancer can also be considered for a surgical procedure involving hysterectomy

and sparing of ovaries. However, this malignancy is fairly rare in younger age groups.

Asherman's syndrome is a collective name for adhesions in the uterine cavity. Adhesions can occur after intrauterine infections secondary to surgical abortions or after genital tuberculosis (Schenker 1996). These adhesions can be few and cured with corrective surgery while more severe adhesions that obstruct the uterine cavity completely are difficult to treat so that fertility is restored (Zikopoulos *et al.* 2004).

Family building options for women with untreatable uterus factor infertility

For a woman with untreatable uterus factor infertility who wishes to form a family, adoption is the most obvious alternative. Every year around 2000 international and national adoptions are approved in Sweden (Socialstyrelsen 2007) and it can be assumed that several of these are approved to infertile couples. However, for infertile couples where the genetic connection to the child is vital or in countries where international adoption is not socially accepted, adoption is not an option.

Since most of the women of the above mentioned patient groups have functioning ovaries with normal hormone production and ovulation, gestational surrogacy can be an alternative to adoption. Gestational surrogacy (or IVF surrogacy) is an arrangement where a third party (the surrogate mother) carries the genetic (or commissioning) parents' child throughout gestation and the child is then adopted by the commissioning couple at birth. By the use of IVF treatment, an embryo is generated from the commissioning parents' gametes and placed in the uterus of the surrogate. This arrangement is allowed in a few countries in Europe, in some states in the US and Australia and it is practiced in several countries where no legislation concerning surrogacy currently exists (Cohen and Jones 2001). However, because of the complicated ethical and legal nature of gestational surrogacy (Ber 2000), most countries in Europe as well as Catholic and Muslim leaders have prohibited the arrangement (deBlois and O'Rourke 1995; Husain 2000). In Sweden the law concerning parenthood was recently adjusted to allow

for egg donation and at the same time surrogacy became outlawed (Svensk Författningssamling, Föräldrabalk 2002:252) and in Finland surrogacy was practiced without legislation up until this year, when a new prohibitory law was installed (P.O. Jansson, personal communication).

Research on uterus transplantation

Historical perspectives on uterus transplantation

The idea to use transplantation to restore fertility is far from new. As early as 1903 two cases of ovarian tissue transplantation from live, fertile donors to women who had been subjected to oophorectomy was reported with "promising results" (Martin 1903). The earliest reports of uterine transplantation in the scientific literature is from 1918 (Hesselberg *et al.* 1918) reporting scantily described methodology and results in the guinea-pig. After the first successful kidney transplantation (Merrill *et al.* 1956) the research area of transplantation grew immensely and through the 1960s and the 1970s several reports on experimental uterine transplantation were published. This research primarily involved replantation (auto-transplantation), where the uterus with its appendages was isolated from the animal, taken out for a short period of ischemia and then reintroduced into the same animal. Two principally different modes of securing blood flow to the transplanted uterus were tested; vascular anastomosis, to achieve immediate reperfusion, and attachment of the uterus to an abdominal surface, to acquire a gradual revascularization through outgrowth of new blood vessels.

Vascular anastomosis was first used in *en bloc* autotransplantations of the uterus, oviducts and ovaries in the dog (Eraslan *et al.* 1966). Pregnancies were reported in a minority of the transplanted animals. By means of a comparable surgical method, non-pregnant and pregnant uteri of dogs were transplanted to both female and male recipients that were immunosuppressed by azathioprine (Yonemoto *et al.* 1969). Most of the uteri were necrotic, as a sign of rejection, at autopsy several weeks later but a small number were reported to be viable. The variable course of

these allogeneic transplants may be explained by differences in histocompatibility disparity between individual donor-recipient pairs of animals.

Revascularization by omental wrapping (omentopexy) and vascular anastomosis were first compared in a dog model for autotransplantation (Barzilai *et al.* 1973; Paldi *et al.* 1975), demonstrating necrosis after omentopexy and survival of most uteri after vascular anastomosis. One study on uterus transplantation in a primate species (rhesus monkey) showed that omentopexy was sufficient to reinitiate blood flow that was enough for resumed regular menstruations but pregnancies were not achieved (Scott *et al.* 1971). Viable uterine transplants were also reported in the rabbit (Confino *et al.* 1986) and in the guinea-pig (Bland 1972) after re-implantation of the uterus within the broad ligament or to the abdominal wall, respectively.

Collectively, these studies pointed towards that vascular anastomosis, with an immediate blood flow to the transplanted organ, is needed when transplanting a uterus of a relatively larger size, such as the uterus of the dog, but not when transplanting a uterus of smaller size, such as that of the rhesus monkey, guinea-pig and rabbit.

At the time when most of these studies were performed the mechanisms underlying the allogeneic reaction were poorly understood and maternal immunological tolerance of the semi-allogeneic foetus during pregnancy had led to the suspicion that the uterus itself is an immunoprivileged organ. However, the few early reports on allogeneic transplantation of the uterus or uterine tissue (Yonemoto *et al.* 1969; Mattingly *et al.* 1970; Scott *et al.* 1970) indicated that the uterus is rejected in a similar manner as other vascularised organs. Immunosuppressant regimens to control rejection of an allogeneic transplant at this time usually consisted of azathioprine and corticosteroids (Yonemoto *et al.* 1969; Scott *et al.* 1970) and the survival rate of allo-transplants, both in experimental and clinical transplantation, was therefore quite low (Finkelstein *et al.* 1975; Schroeder *et al.* 1976). It was not until the introduction of cyclosporine A in the late seventies (Calne *et al.* 1978; Starzl *et al.* 1982) that rejection could

be more successfully controlled. With the introduction of IVF as treatment for the main cause of female infertility - tubal infertility - the interest for uterus transplantation declined and only a few studies were reported between the years 1980 and 2000 (Confino *et al.* 1986; Lee *et al.* 1995).

Current research on uterus transplantation

In the year 2000 a transplantation of a uterus in woman was performed in Saudi Arabia. A 26 year old woman who had lost her uterus at an emergency postpartum hysterectomy received a uterus from a 46-year woman operated for benign ovarian disease (Fageeh *et al.* 2002). The transplanted patient was treated with standard immunosuppressant drugs (cyclosporine A, azathioprine and prednisolone) and the uterus survived for 99 days, when it had to be removed due to signs of massive necrosis. The cause of necrosis was reported to be vascular thrombosis, possibly due to torsion of the vessels of the inadequately fixed uterus. This first attempt to transplant a uterus in a woman was preceded by a few studies of surgical techniques in goats and baboons and the demise of the transplant points out the necessity of detailed studies of all aspects of a new treatment in appropriate animal models before further human trials are performed.

Today, a handful of research groups are studying uterus transplantation in different experimental models. Between 2002 and today (july 2007) 13 original studies and 7 reviews concerning uterine transplantation can be identified by PubMed. The original work includes development of research models in the mouse (Racho El-Akouri *et al.* 2002), the rat (Jiga *et al.* 2003) and the pig (Sieunarine *et al.* 2006), studies of reproductive performance after embryo transfer in transplanted mice (Racho El-Akouri *et al.* 2003), effects of immunosuppressive therapy in the mouse (Wranning *et al.* 2007) and surgical retrieval of uterine grafts in multi organ donors (Del Priore *et al.* 2007). The above mentioned 13 original papers also include the published papers in the current thesis concerning the effects of cold ischemia and these will be discussed thoroughly in later sections. Our group has also developed a sheep model for uterus transplantation (*paper IV* in the current thesis).

The knowledge gained from both older and current studies shows that vascular anastomosis is the mode of revascularization to prefer and that when properly revascularized and devoid of allogeneic challenge the transplanted uterus can regain its function and harbour normal pregnancies. It is also apparent that the knowledge regarding the rejection pattern and modes of immunosuppression in uterus transplantation needs to be expanded and that much of that research can be performed in existing animal models.

Ischemia and reperfusion in transplantation

The transplantation process consists of a series of events that all pose potential harm to the organ. From procurement of the transplant in the donor until reperfusion in the recipient, the organ will be subjected to ischemia and mechanical stress of varying degrees that cause metabolic impairment and induction of pro-inflammatory factors. These changes can compromise the outcome of the transplantation. It has also been acknowledged that tolerance to ischemia is organ specific. For example, in clinical transplantation the maximum cold ischemic time practiced is around 8 h for the heart and around 36 h for the kidney and the pancreas. This variation in tolerance to cold ischemia between organs is determined by metabolic demands, parenchymal cell function, resident immunecell populations and intrinsic antioxidant capacity of each specific organ.

Metabolic changes during ischemia

Ischemia is the lack of perfusion of blood in tissue and the direct consequence of ischemia is a stall in transportation of oxygen and nutrients to and carbon dioxide and products of metabolism and catabolism from the ischemic tissue. This has a time dependent impact on many organisation levels. On the cellular level the lack of oxygen as terminal electron receptor in mitochondrial electron transport will halt the production of adenosine triphosphate (ATP) through oxidative phosphorylation and the cell has to rely on the less efficient process of glycolysis for energy production. ATP is the main source of cellular energy and required in virtually all energy demanding processes in a cell like maintenance of membrane potential mainly via the Na^+/K^+ ATPase (Skou and Esmann 1992) and other ionic

transporters (Gerencser and Zhang 2003; Floyd and Wray 2007), protein processing (Hartl and Hayer-Hartl 2002) and transportation of nutrients and waste products over cell membranes. To compensate for the lack of oxygen during ischemia, glycolysis is intensified in order to meet with cellular energy demands. Due to the lack of perfusion, this will lead to a deprivation of available glucose and an accumulation of lactate accompanied with an increase in $[H^+]$ (Robergs *et al.* 2004; Tejchman *et al.* 2006). Cells use Na^+/H^+ exchange to eliminate excess protons, but in the process accumulate excess Na+ which cannot be exported via the Na^+/K^+ ATPase due to ATP deficiency. As a consequence, excess sodium is exported via the Na^+/Ca^{+2}-exchanger, loading cells with Ca^{+2}. The entry of Ca^{+2} into mitochondria can open the mitochondria permeability transition pore (Halestrap 2006), resulting in mitochondrial collapse and necrotic cell death (Kim *et al.* 2003).

Oxidative stress at reperfusion

With the reintroduction of oxygen at reperfusion, former ischemic tissue is challenged with oxidative stress, mainly from reactive oxygen and nitrogen species (ROS and RNS) produced by various sources. During ischemia, endogenous xanthine dehydrogenase is converted to xanthine oxidase (Kayyali *et al.* 2001; Berry and Hare 2004). At the same time ATP and other purine nucleotides are catabolised to hypoxanthine. Xanthine oxidase converts hypoxanthine to superoxide in the presence of oxygen and since both xanthine oxidase and hypoxanthine accumulate during ischemia, large amounts of highly reactive superoxide radicals can be formed upon reperfusion when oxygen is reintroduced (McCord and Roy 1982). Infiltrating granulocyte neutrophils are the first immune cells to respond to pro-inflammatory signals from the former ischemic tissue (Vinten-Johansen 2004). Activated neutrophils produce superoxide, which can be dismutated into hydrogen peroxide and further to hypochlorous acid by myeloperoxidase. Hypochlorous acid reacting with superoxide can in turn produce hydroxyl radicals.

ROS and RNS cause DNA damage and oxidise lipids in the cell membranes and amino acids in proteins, thus inactivating enzymes and causing damage to

structural proteins. These deleterious effects by radicals can be counteracted by antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, other proteins like albumin, ferritin, ceruloplasmin as well as numerous smaller molecules like reduced glutathione, α-tocopherol, β-carotene, bilirubin and uric acid (Halliwell and Gutterige 1999).

Inflammatory responses to ischemia and reperfusion

Even a short course of ischemia can initiate an inflammatory response at reperfusion. During reperfusion after ischemia, alteration in gene expression of several inflammatory factors is initiated, resulting in increased production of cytokines, chemokines and adhesion molecules (Gu *et al.* 2004). Also, mitochondrial and cytoplasmic contents released by dead or dying cells act as danger signals activating several components in the inflammatory cascade such as the complement system (Schmidt *et al.* 2004). The above mentioned ROS produced during early reperfusion can also act as pro-inflammatory signals (Asehnoune *et al.* 2004).

The inflammation proceeds and propagates after these initial events, with infiltration of neutrophils and macrophages, oedema and stasis, thrombotic formations and fibrin deposits. This can in severe cases result in the so called "no-reflow" phenomenon and delayed function or death of the organ. However, the main harm by post-ischemic inflammation in transplantation is the influence of the initial innate response on the adaptive immune response. The post-ischemic inflammatory response induces the maturation of immature dendritic cells in the transplant to become potent initiators of the adaptive immune response in the recipient (Land 2005; Munz *et al.* 2005).

Preservation strategies in transplantation

To minimize the damage caused by ischemia during the transplantation process and to allow for prolongation of the ischemic time so that organs can be transported between transplantation centres, much effort has been put into developing strategies for organ preservation. Cold ischemic storage is the most commonly used method. Hypothermia reduces the rate of all biological processes

and cooling of the organ delays the onset of harmful effects of energy depletion and accumulation of toxic waste products. To avoid blood clotting within the tissue and reduce the harmful influence of donor cells released into the recipient blood stream at reperfusion, the vascular system of the transplant is flushed with a buffer replacing the donor blood. The preservation solutions used for flushing and cold storage are designed to provide physiological conditions like blood in terms of pH and osmotic pressure. They usually also contain buffering molecules, metabolic precursors and antioxidants to further protect the tissue. Ionic composition (ie sodium and potassium) can be either intra- or extracellular like, depending the rational employed and what organ the solution is mainly designed to be used for (Muhlbacher *et al.* 1999).

Since no preservation solution can abolish the ischemic injuries in cold ischemic organ preservation and since the shortage of organs for transplantation has increased the use of organs retrieved from older and more marginal donors, there is an increasing interest in alternative preservation strategies like continuous machine perfusion (Maathuis *et al.* 2007). Also, a treatment to achieve immunological allograft tolerance in the recipient is a long searched goal that would reduce the impact of ischemia in transplantation (Steinman 2006; Girlanda and Kirk 2007).

AIM

The general aim of this thesis was to study the tolerability of the uterus to surgical trauma and ischemia and to investigate some methods to reduce injuries induced by these events. To better address the many aspects involved, different experimental models were developed and used for this purpose.

The specific aims were:

- To determine the time frame for preservation of functionality (i.e. ability to harbour a pregnancy) of the uterus after extended cold storage and reperfusion in a mouse model for syngeneic uterus transplantation (*paper I*).
- To assess the effect of cold ischemic storage alone on functional, morphological and biochemical parameters in human uterine tissue and to compare the effect of three different buffers on these parameters (*paper II*).
- To study the effect of surgical trauma and short term cold ischemic storage on early reperfusion events after auto-transplantation of the uterus in two larger animal models (*papers III and IV*) and to compare the effect of two different buffers on these parameters (*paper IV*).
- To compare the effect of donor and recipient pre-treatment with progesterone to that of prednisolone on ischemia induced histological changes after extended cold ischemia in a rat model for syngeneic uterus transplantation (*paper V*).

METHODS

Below is a brief summary of the methods used in the studies of the current thesis. More elaborate descriptions can be found in the individual papers.

Surgery

Animal models for uterus transplantation (papers I, III, IV and V)

To study the effects of surgical trauma and ischemia alone and isolate these events from the immunological reactions resulting from tissue incompatibility between donor and recipient, non-rejecting animal models for uterus transplantation were used. In the rodent models (*paper I and paper* V) transplantation was performed between genetically identical animals (syngeneic transplantation) and in the pig (*paper* III) and sheep (*paper IV*) the same animal served as both donor and recipient (auto-transplantation) to avoid rejection reactions.

All animals were purchased from accredited suppliers. The studies followed the rules and guidelines issued by the Animal Care Agency (Djurskyddsmyndigheten) in Sweden and were approved by the Animal Ethics Committee in Göteborg, Sweden.

The general method for transplantation of the uterus in the different animal models was the same; the uterus, cervix and feeding and draining uterine vessels were isolated from surrounding tissue as well as from the ovary and fallopian tubes in the donor. The vascular system of the transplant was flushed with a cold, buffering solution *in situ* and the transplant was then removed from the donor and kept in cold buffer for a number of hours. In the recipient, circulation of blood through the transplant was established by connecting the vessels of the transplant to suitable vessels in the recipient. Evaluation of the effect of the cold ischemic treatment was done after different time intervals and by different methods.

In the rodent models (*paper I and paper V*) and the sheep (*paper* IV) one uterine horn, the common uterine cavity, the cervix and a few millimetres of the vaginal

rim was isolated from surrounding tissue by the use of gentle dissection, ligation and diathermy. A vascular pedicle long enough to allow end-to side anastomosis to the aorta and vena cava in the recipient was created. In the recipient the aorta and vena cava were mobilized, clamped and a slit was cut to allow end-to-side anastomosis of the vessel ends of the transplant. In the mouse (*paper I)* the vascular pedicle of the transplant consisted of the uterine vessels, all minor vessels connecting to the common lilac vessels plus the common iliac vessels and the aorta and vena cava up to a level just caudal of the ovarian vessels. In the rat the uterine and common iliac vessels up to the bifurcation of the aorta and vena cava were used to create a sufficiently long vascular pedicle for anastomosis. In the rodent models the uterus of the recipient (the native uterus) was left intact and the cervix of the transplant was exteriorized as a stoma on the abdomen.

In the pig (*paper III*) the whole uterus, cervix and uterine vessels were isolated. Since the pelvic anatomy did not allow harvesting of vascular pedicles outside the mesometrium, the uterine vessels intended for anastomosis were procured in such a way that anastomosis of the vessels of the transplant could be achieved by end-to-end technique to the original feeding and draining vessels on both sides. The cervix was sutured back to the vagina.

In the sheep (*paper IV*) the uterine artery and the utero-ovarian vein down to the bifurcation from the internal iliac vessels comprised the vascular pedicle used for anastomosis and in the recipient these were anastomosed end-to-side to the external iliac vessels. Here also, the cervix was sutured to its original position.

Human uterine tissue (paper II)

Human uterine tissue was used to analyse functional, biochemical and histological effects of cold ischemic preservation without reperfusion. The study was approved by the Ethics Committee in Göteborg, Sweden and potential donors were selected on criteria concerning reproductive age (pre-menopausal), diagnosis (benign; bleeding disorders and/or myomas) and mode of surgery chosen (laparotomic or vaginal hysterectomy). On the day of admission the patient was informed of the study and if consent was given, enrolled. Immediately

after the uterus had been removed it was brought to the lab, cannulated through the uterine arteries and flushed with Ringer Acetate. Biopsies were then taken from the outer and the inner muscle layers and the endometrium for cold storage and analysis.

Ischemia and preservation

Preservation times

The length of ischemia and preservation was determined by the aim of the different studies as well as by the experimental situation. In the rodent models (*paper I and V*) long preservation times were feasible and cold ischemia for 24 h and more were used. In the pig (*paper III*) and the sheep (*paper IV*) short cold ischemic times for between one to two hours were used. In the study on human tissue (*paper* II) the cold ischemic time extended up to 24 h.

Preservation solutions

In all studies one to three different preservation solutions were used, depending on the aim of the study. Where preservation solutions were compared, saline or Ringer Acetate (RIN) were used as controls and tested against one or two preservation solutions; University of Wisconsin solution (UW) and Perfadex solution (PER). In contrast to saline and RIN, UW and PER are specifically designed to protect tissue during cold ischemic preservation. In *paper I*, saline was used as comparison to UW, in *paper II*, human tissue was preserved in RIN, UW or PER, *paper III* used only RIN, *paper IV* compared RIN to PER and in *paper V*, PER was used for preservation. The composition of the different solutions is listed in Table 1.

Reperfusion

The time frame of interest during reperfusion in each study was determined by the aim and experimental conditions.

To assess for how long the uterus can be cold stored without loss of functionality, the mouse model (*paper I*) was used. Analysis of contractility and histology was performed after 24, 48 and 72 h of cold ischemia alone. After 24 h of cold

ischemia and subsequent transplantation the animal was left to recover for 14 days and then analyzed or further tested. In the rat (*paper V*) histological signs of the post-operative/ischemic inflammatory response at day one after transplantation were used as reference points for comparison of different anti-inflammatory treatments. In the pig (*paper III*) and the sheep (*paper IV*) the very early events during the first 3 h of reperfusion after a short course of cold ischemia was studied.

In the study on human tissue (*paper II*) true blood reperfusion was not feasible but the physical conditions during the functional test of contractility somewhat resembles reperfusion in terms of temperature, pH and ionic composition of the buffer used.

Table 1. *Composition and properties of the different solutions used for flushing and cold storage of uteri.*

Component		**RIN**	**UW**	**PER**
K^+	(mmol/L)	4	140	6
Na^+	(mmol/L)	130	20	138
Mg^{2+}	(mmol/L)	1	5	0.8
Cl^-	(mmol/L)	110		142
Ca^{2+}	(mmol/L)	2		
Ac^-	(mmol/L)	30		
$H_2PO_4^-$ + HPO_4^{2-}	(mmol/L)		25	0.8
HES	(g/L)		50	
Dextran 40	(g/L)			50
Glucose	(mmol/L)			5
Raffinose	(mmol/L)		30	
Lactobionate	(mmol/L)		100	
Adenosine	(mmol/L)		5	
Allopurinol	(mmol/L)		1	
Glutathione	(mmol/L)		3	
Osmolarity	(mOsm/kg)	270	320	320
pH		5.5	7.4	7.4

RIN = Ringer Acetate, UW = University of Wisconsin solution, PER = Perfadex solution

Methods for analysis of post-operative and post-ischemic injury

To analyse the effects of surgical trauma and ischemia on different organisation levels and at different stages of the temporal development of ischemia induced injury, samples taken at different time points were analysed for several factors.

Functionality (paper I and II)

In the mouse model (*paper* I) and the study on human tissue (*paper II*) myometrial tissue was tested for ability to generate spontaneous contractions and respond to prostaglandin stimulation after cold ischemic storage alone. Contractions were recorded and analysed in terms of start of spontaneous contractions, amplitude, frequency and quality of the shape of the contraction curve as well as the area under the curve (AUC) for the 10 min measured, both before and after stimulation.

After healing was confirmed in mice transplanted after cold ischemic storage of the uterus, embryo transfer was performed. The pups were delivered by caesarean section at term, counted, measured and followed to maturity at 8 weeks.

Morphology (paper I, II, III, IV and V)

In the animal models (*papers I, III, IV and V*) gross morphology of the transplant such as colour changes from white to red and from darker to brighter red that indicate adequate perfusion and release of stasis were noted at immediate reperfusion. Biopsies were also taken at different time points for light microscopy analysis. In the rodent models these biopsies were taken from the transplant and native uterus of the recipient at the end of the experiment; 14 days after surgery in the mouse model (*paper I*) and on day one post-transplantation in the rat model (*paper V*). In the pig (*paper III*) and sheep (*paper* IV) one biopsy was taken as soon as possible at the beginning of surgery (control) and one after 3 h of reperfusion, at the end of the experiment. In the study on human tissue (*paper II*), biopsies stored for different times and in different solutions were compared to those taken at sampling. All biopsies were fixed, sectioned and stained and analyzed by light microscopy. Appropriate histology markers of injury such as celldeath, cytoplasmic vacuolisation, stasis and oedema, loss of cell-to-cell

contacts and infiltration of leucocytes were graded. Human endometrial biopsies were also analysed for hydropic changes and degeneration of chromatin by electron microscopy (*paper II*).

Biochemical parameters (paper II, III and IV)

To asses the quality of the immediate reperfusion, markers of cell respiration such as pH, lactate, carbon dioxide and oxygen pressure in uterine venous blood was analyzed during the first 2 to 3 h of reperfusion in the pig and sheep models (*papers III and IV*). Metabolic disturbances after cold ischemia alone were assessed by analysis of ATP and protein concentration in human tissue (*paper II*).

Oxidative stress and lipid peroxidation during early reperfusion were assessed in the sheep model (*paper* IV) by analysis of ascorbyl radicals and thiobarbituric acid reactive species (TBARS) in venous plasma. Antioxidant defence was assessed after cold ischemia alone by measurement of reduced and oxidized glutathione in human tissue (*paper II*) and at reperfusion in the sheep (*paper IV*) by measurement of total antioxidant capacity in uterine venous plasma.

Statistics

For analysis of contractility (*papers I and II*) the difference between $\log(AUC)_{dose}$ and $\log(AUC)_{spont}$, was related to log dose using an orthogonal linear regression within each specimen. The difference in dose-response between groups was evaluated using Wilcoxon test of the slope and intercept parameters from the regression models.

In *paper II,* concentration of total GSH and GSSG/GSH as well as concentrations of ATP and protein in tissue biopsies from the different cold ischemic groups was compared by the use of Kruskal Wallis ANOVA by ranks test and where significant differences were found, comparisons between groups were done by Wilcoxon matched pairs test.

In *paper IV,* the time taken for different stages of surgery and control values for the tested blood and plasma parameters was compared between groups by the Mann-Whitney U-test. Pairwise comparisons within groups of each time point to

control for pH, lactate, pCO2 to pO2, total antioxidant capacity, ascorbyl radical intensity and TBARS were done by Wilcoxon sign ranks test. Comparison of number of infiltrating neutrophils in endometrium between the RIN and PER groups was done by using the Mann-Whitney U-test and comparison between number of neutrophils in control and reperfused samples within groups was done by Wilcoxon sign ranks test.

RESULTS AND DISCUSSION

Organ injuries induced by ischemia and reperfusion can have a significant impact on transplantation outcome since these injuries are implicated in impaired post-transplantation perfusion (Schramm *et al.* 2007), delayed graft function (Quiroga *et al.* 2006), acute rejection events (Howard *et al.* 1990; Totsuka *et al.* 2004) and chronic rejection (Schwarz *et al.* 2005). The innate and adaptive immune components involved in post-operative and post-ischemic injuries in transplantation are interconnected (Land 2005) and to isolate the impact of surgical trauma, cold ischemia and reperfusion from the allogeneic response, syngeneic and autologous transplantation models as well as *in vitro* analysis were used. In the following sections, the results from the studies included in this thesis as well as the experimental the models used will be presented and discussed.

Animal models for uterus transplantation (*papers I, III, IV and V*)

Small animal models (papers I and V)

The mouse (*Mus musculus*) and the rat (*Rattus norvegicus*) are the most widely used species in medical research today. These are small animals with relatively short life cycles and large number of offspring per pregnancy. The accumulated knowledge concerning reproductive and general physiology, immunology and genetics of rats and mice is vast and there are inbred strains of both species that reduce variation between individuals. For these reasons rats and mice make good experimental models for studies of many aspects concerning uterus transplantation such as reproductive performance after transplantation and the effect of immunosuppressive treatment on transplant rejection as well as on gestation and the offspring.

A mouse model for uterus transplantation was developed five years ago in our lab and has been thoroughly characterized in a previous thesis by Randa Racho El-Akouri (Racho El-Akouri 2003). Transplantation by vascular anastomosis with heterotopic placement of the graft and the recipient native uterus left *in situ* was employed. In this model the transplant consists of one uterine horn with the

common uterine cavity and cervix with a vascular pedicle including the uterine vessels, iliac vessels and abdominal aorta and vena cava (Racho El-Akouri *et al.* 2002). This mouse model has proven to be an excellent tool in studies of functional aspects of the transplanted uterus (Racho El-Akouri *et al.* 2003) and has a large potential as a model for other studies concerning uterine physiology and immunology by the use of gene modified mice. Also, the large reproductive capacity of the mouse, the relative ease by which superovulation is achieved and the high yield of pregnancies after embryo transfer (Chin and Wang 2001) make the mouse a suitable animal model in reproductive studies. However, the small size of the mouse makes the surgical technique of vascular anastomosis difficult to master. Also, the mouse is fairly resistant to the effects of immunosuppressive drugs (Wranning *et al.* 2007) and is therefore less appropriate as a general model for studies of immunosuppression and rejection in uterus transplantation.

The rat (*Rattus norvegicus*) shares many of the features of the mouse with its well known physiology, short gestation time and large litters. The relatively larger size of the rat makes microsurgery somewhat easier and there are also techniques for genetic modification in the rat and several transgenic strains (Popova *et al.* 2005). In studies of rejection and effects of immunosuppressive drugs in uterus transplantation, the rat might be a more appropriate experimental animal than the mouse since dose requirements for suppression of allo-reactions are lower in the rat (Huang *et al.* 2003; Hullett *et al.* 2005) compared to the mouse (Wang *et al.* 2003; Tanaka *et al.* 2005). The larger size of the rat also allows for consecutive blood-sampling and larger amounts of tissue for analysis. The rat is however more resistant to induction of superovulation (Corbin and McCabe 2002) and implantation rate after embryo transfer in the rat is considerably lower when compared to the mouse (Byers *et al.* 2006), making studies of reproductive performance by the use of embryo transfer more elaborate in the rat.

In development of the rat model in our lab, a surgical approach similar to that in the mouse was employed, with only minor adjustments. The transplant consisted of one uterine horn with the common uterine body and cervix and a vascular pedicle with the uterine vessels and the common iliac vessels up to the level of

the bifurcation from the aorta and vena cava. During the dissection of the aorta and vena cava in the recipient to allow space for clamping during anastomosis, less vascular disturbance could be achieved in the rat due to the larger size of these vessels. Also, in the mouse the aorta and vena cava have to be clamped simultaneously during anastomosis, because of the small size of the vessels. In the rat, the aorta and vena cava can be clamped individually during anastomosis, resulting in an approximate 50% reduction of the ischemic time of the lower part of the body as compared to the mouse.

Two research groups have reported previous experience of rat models for utero-ovarian transplantation (Lee *et al.* 1995; Jiga *et al.* 2003). In these models ortothopic transplantation was performed, preceded by hysterectomy in the recipient. The graft consisted of the whole bicornuate uterus with ovaries and the upper part of the vagina, with a vascular pedicle with the uterine and iliac vessels plus the abdominal aorta and vena cava, with or without the ovarian vessels. Success rates were similar to those achieved in our mouse model. Long term outcome after syngeneic transplantation (Lee *et al.* 1995) and rejection pattern after allogeneic transplantation (Lee *et al.* 1995; Jiga *et al.* 2003) were also similar to those in the mouse model (Racho El-Akouri *et al.* 2003; El-Akouri *et al.* 2006).

Performance of vascular surgery in small animals as mice and rats requires the aid of a proper microscope, fine instruments of high quality and thin ligatures and sutures. The learning curve for achievement of satisfactory success rates is long. First, sufficient technical skill have to be achieved to allow for proper graft harvesting and anastomosis technique which is a requirement for satisfactory reperfusion in the recipient. From my personal experience in development of the rat model and having no previous surgical training, about 40 operations (most of them being graft harvesting) were needed to gain proper instrumentation skills. After survival of recipient and graft could be achieved, around 40 transplantations were needed to reduce the time for the different stages of surgery and increase the success rate in terms of recipient and graft survival to satisfactory levels. This learning phase also included finer adjustments of peri- and post-operative

conditions, such as anaesthesia, analgesia and fluid therapy that are important for good post-operative recovery of the animal. At present, after a total of about 90 full transplantations the time taken for graft harvest is around 55 minutes, for anastomosis around 50 minutes, the recipient and graft survival rates of 90% and 80%, respectively. The main cause of recipient death is bleeding from the anastomosis sites and the main cause of graft loss is thrombosis at the arterial anastomosis site. It is possible that additional technical refinements can further increase the success rate. In the much smaller mouse, the learning curve was similar for another researcher (Racho El-Akouri *et al.* 2002). There is no doubt that the use of proper equipment and individual qualities of the surgeon such as motor function and stamina determine the slope of the learning curve when trying to achieve sufficient skills in microsurgery in small animals.

Larger animal models (papers III and IV)

Even though much can be learned from rodent models, the small size and the evolutionary distance to man makes it difficult to extrapolate knowledge gained in a rodent model directly to humans. Especially when considering development of surgical techniques and effects of transplantation on whole animal physiology, larger animal models with organ size and metabolic rates similar to humans are more appropriate. Furthermore, the longer gestation times in larger animals make them more suitable for of studies of pregnancy physiology and effects of immunosuppressants at uterine transplantation.

The domestic pig (*Sus scrofa*) is commonly used for surgical training and development of surgical methods because of its relatively good correlations to human anatomy and physiology (Hammer 1997). Because of the economical importance of the pig in meat production, its reproductive and general physiology is well mapped. Inbred strains for experimental purposes exist among miniature swine but not in the large domestic pigs. However, breeding strategies have reduced anatomical and physiological variability within different breeds. The reproductive capacity is high with large litters and several estrus cycles per year. For these reasons, the domestic pig would be a suitable animal model for uterus transplantation studies, providing a suitable surgical method can be employed.

However, there are certain anatomical and physiological drawbacks in using the pig as a model for uterine transplantation. The uterine horns are of great length (up to one meter) and with large individual variation in vascular anatomy within the extensive mesometrium. Also, the narrow pelvis reduces the accessibility of the larger internal and external iliac vessels. In our experience this made the preparation of the uterus for procurement and flushing lengthily and difficult and the surgical approach which was required for vascular anastomosis was quite different from what would be feasible in the human.

In the auto-transplantation experiments in the pig the graft consisted of the two uterine horns, the small common uterine cavity and half of the cervix. The vascular pedicle consisted of the uterine vessels down to approximately halfway between the ramifications in the mesometrium and their origin at the internal iliac vessels. With this method, end-to-end anastomosis of the uterine vessels of the graft to their counterpart on the recipient side had to be employed. The surgery was performed by experienced gynaecological surgeons and the learning curve was quite steep in terms of reduction of the time taken for the different stages of surgery. However, the success rate in this model was low with only 4 out of 19 auto-transplantations (25%) leading to adequate reperfusion of the transplant. This indicates a readily formation of micro-thrombosis in the specimen or that the anastomosis sites in many cases did not allow large enough blood flow to the specimen. The blood flow to the uterus had already been reduced by the severance of the uterine branch of the ovarian artery during the early preparation of the specimen and the circulation from the uterine branch of the ovarian arteries seem to contribute substantially to the circulation of the pig uterus (Stefanczyk-Krzymowska and Krzymowski 2002).

The sheep (*Ovis aries*) is also a popular animal in research, especially in reproductive studies. As in the pig, the reproductive physiology is well mapped and breeding strategies have produced breeds with high reproductive capacity. The sheep is a seasonal breeder with mating season during the dark months. However, hormonal stimulation can induce estrus cycling outside the breeding season. The uterus and pelvic vessels of the sheep are of approximately the same

size as in the human and they normally carry one to three lambs per pregnancy. The smaller body size of the animal and a wider pelvis, together with the smaller size of the sheep uterus as compared to the pig uterus, allowed for a surgical approach with end-to-side anastomosis to the external iliac vessels, similar to what would be feasible in the human.

In our experiments, auto-transplantation of one uterine horn without the ovaries plus the cervix and vaginal rim was used. The vessels used for the graft consisted of the ipsilateral uterine artery down to and including the distal end of the internal iliac artery and the utero-ovarian vein down to its branching from the the internal iliac vein. The graft vessels were connected to the external iliac vessels by end-to-side anastomosis. The success rate in terms of well perfused uteri during 3 h after reperfusion was high (10 out of 14 animals, ~70%) in comparison to the pig. Since the transplantations were performed by the same team of surgeons as in the pig, the higher success rate was probably a result from accumulated experience as well as the more favourable conditions with a smaller uterus, larger vessels and better access to the pelvis in the sheep.

Methodological consideration

The impact of surgical trauma on adverse post-operative events has been studied from several different standpoints. Especially in major abdominal surgery there is a risk for systemic inflammation without infection with induction of pro-inflammatory mediators being released into the systemic circulation (Malik *et al.* 2001). Agents used for anaesthesia might also enhance endothelial cell susceptibility for oxidative damage (Shayevitz *et al.* 1991) but in the perspective of induction of harmful changes within the organ itself, mechanical stress induced by handling of the organ and the duration of ischemia during procurement, storage and anastomosis must be considered as the main contributing factors.

During surgery in the rodent models, there is very little manipulation of the uterus itself and care is taken not to stretch the graft vessels during anastomosis. However, flushing of the uterus is performed via a syringe and even if this is done gently, there are no means to closely control the pressure and this is a potential

source of endothelial damage and activation. In the pig and sheep it is necessary to handle the uterus to gain access for dissection and anastomosis. Flushing is however performed with a controlled pressure of 100 ml Hg, approximating normal systolic pressure in the pig and the sheep. Thus, in the larger animals the potential injuries induced during surgery itself would mainly derive from mechanical manipulation.

None of the studies in the present thesis directly study the impact of surgery on the post-operative inflammatory response, but it was noted at histology analysis of uterine biopsies taken halfway through donor surgery in the sheep (*paper IV*), that neutrophils were attached to endothelia. This indicates a surgery and/or anaesthesia induced activation of endothelial cells that most likely also is time dependent.

One contributing factor to the poor outcome in the pig model might be that no anti-thrombotic treatment was used in that study. In experimental auto-transplantation, where the same animal serves as both donor and recipient, it is not clear when to administer heparin. The risk of uncontrollable bleeding to occur during preparation and at the anastomosis site at early reperfusion was in the pig study considered to out-weigh the benefits of heparinization before the uterus would be taken out. However, with increasing surgical experience the bleeding risk would have decreased and in retrospect, heparin might have been beneficial in some of the later experiments in the pig. In the sheep heparin was given after dissection and just before circulation to the transplant was interruped. In the rodent models heparin or low molecular weight heparin (LMWH) was administered to both donor and recipient prior to surgery.

Summary of the animal models

The rat, the mouse and the sheep are appropriate model animals for uterus transplantation while the pig is less suitable due to pelvic anatomy. Transplantation of the uterus requires elaborate surgery, but with proper training adequate success rates can be achieved.

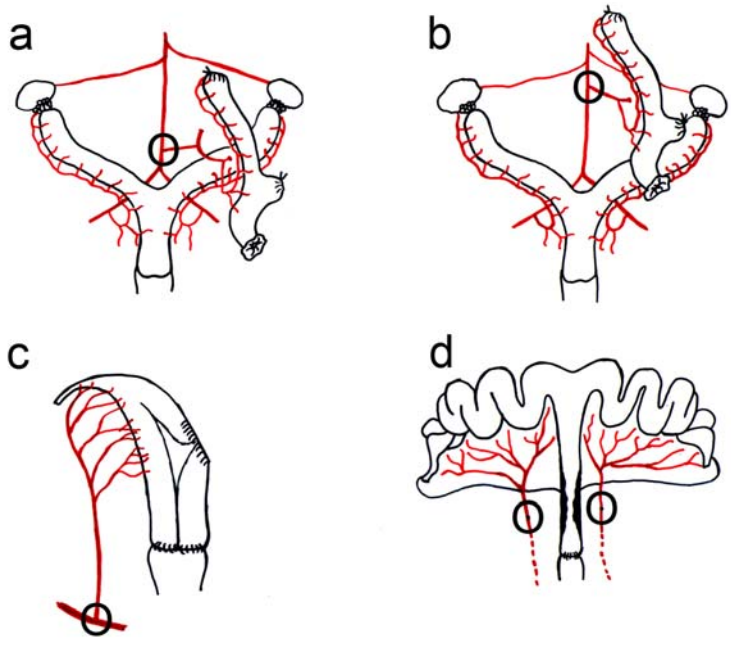


Figure 2
Schematic drawing representing the different models for uterus transplantation in the mouse (a), the rat (b), the sheep (c) and the pig (d). The anastomosis sites are encircled. The methodology is described in detail in papers I, III, IV and V.

Sampling of human uterine tissue (*paper II*)

Apart from using animal models for studies on uterus transplantation, some issues can be addressed by *in vitro* studies of human tissue. In *paper II* myometrium and endometrium from women who underwent hysterectomy for benign reasons was obtained and cold stored in three different solutions for 6 and 24 hours. Since no blood reperfusion was possible in this setting, the analyses used were aimed mainly at detecting parameters known to change during severe ischemia. Because of the operation time at hysterectomy (around 1 to 2 hours) plus the time taken for cannulation, flushing and sampling of tissue from the discarded uterus, all samples had already been subjected to a substantial amount of warm ischemia. To correct for this, control samples taken at the same time as samples for cold

storage constituted the baseline. The results in *paper II* are presented and discussed in the following section.

Cold ischemia alone (*papers I and II*)

In *paper II*, human uterine tissue was subjected to cold ischemic storage for different times and in different buffers and then analysed for signs of degenerative changes. Some of these parameters were also studied in the mouse uterus after cold ischemia alone (*paper I*).

Metabolic parameters

Concentrations of ATP and protein was analysed in human myometrium (*paper II*) as indicators of metabolic disturbances during cold ischemia. Extended cold storage leads to a reduction of protein synthesis and the degree of reduction differs between organs (Fisher *et al.* 1993; Vreugdenhil *et al.* 1999; Bull *et al.* 2000). In the tissue samples of human myometrium, protein concentrations had not changed after 6 and 24 h storage in any of the solutions. Surprisingly, specimens stored in UW or PER for 6 or 24 h contained higher concentrations of ATP than control specimens. This is of course contradictory to theoretical assumptions and experimental findings by others (Wedenberg *et al.* 1991; Wedenberg *et al.* 1995). The rise of ATP concentrations found in *paper II* can most likely be explained by the fact that the control values constitutes a base line which is the sum of the events after 40 minutes of warm ischemia and by the following period of 100 minutes of cold ischemia and that the rise in [ATP] is a recovery from a much lower level than would be immediately after disruption of circulation. Smooth muscle have a large intrinsic capacity to utilize glycolysis in the absence of oxygen (Wendt 1989) and it is probable that myometrium, when given the protective environment of UW or PER, can utilize glycolysis and/or the oxygen dissolved in the storage solution to produce enough ATP to sustain cellular homeostasis when the metabolic rate is suppressed by hypothermia.

Antioxidant defense

Glutathione (GSH) is one of the more abundant redox molecules in tissue and has an important role in the cellular antioxidant defence (Halliwell and Gutterige 1999). Glutathione is also a supplement in UW solution. However, glutathione is readily oxidised in solution and must be reduced by glutathione reductase with the expense of NADPH to regain its antioxidant property. Therefore it is uncertain if the addition of GSH to a preservation buffer is beneficial if most of it has to be reduced at reperfusion. Also, the uptake of GSH from blood to tissue has been shown to be limited (Hahn *et al.* 1978) so that the capacity to reduce ROS resides mainly in the extracellular space. However, in human myometrial biopsies (*paper II*), about a third of the excess of total GSH in UW-preserved samples that would be derived from the preservation solution, was in its reduced form and might be useful in protection against ROS at reperfusion.

Contractility

The non-pregnant uterus exhibits distinct patterns of spontaneous contractions throughout the menstrual cycle that serves the purpose of downward transportation, towards the vagina of the shed endometrium and blood during menstruation (Martinez-Gaudio *et al.* 1973; de Vries *et al.* 1990) and upward transportation (from the vagina to the distal end of the fallopian tube) of sperm during the late follicular phase (Kunz *et al.* 1996). During the early luteal phase the contractions are minimal to allow for implantation of a conceptus. These cycling pattern of spontaneous contractions are influenced by varying concentrations of prostaglandins in the uterus (Wiqvist *et al.* 1983) and the receptivity of the myometrium to prostaglandin stimulation is regulated by sex steroids (Kelly and Verhage 1981). In the non-pregnant uterus prostaglandin F2α (PGF2α), I2 (PGI2 or prostacyclin) and E2 (PGE2) are synthesized in vascular epithelia, endometrial gland epithelial cells and myometrial smooth muscle cells (Kam and See 2000). In non-pregnant uteri, PGF2α stimulates contractions, prostacyclin inhibits contractions and PGE2 constricts at lower doses and inhibits contractions at high doses (Wilhelmsson 1981). The contractile response by human myometrial strips to PGF2α in similar experiments and at the same doses

used in *papers I and II* was shown to be independent of hormonal phase (Wiqvist *et al.* 1983). Therefore, PGF2α was used as the contractile agent in *papers I and II*.

In *paper I and II* the ability of uterine muscle tissue to contract spontaneously and as a response to PGF2α stimulation was used as a functional test of viability and as an indicator of the ionic integrity of the muscle cells as well as the severity of degeneration of structural proteins and enzymes. In the mouse (*paper I*) the contractile ability was surprisingly intact after 24 h cold storage in UW while more pronounced disturbances were seen after 24 h in saline and 48 h in both saline and UW media. In human tissue (*paper II*) more severe disturbances were seen after 24 h in any of the preservation solutions and this can probably be explained by the warm ischemic time the human tissue was exposed to during surgery and preparation. The results from the contractility tests in *paper I* and *paper II* demonstrates that myometrial cells under proper storage conditions can retain their integrity and thus the ability to contract and relax *in vitro* for at least up to 24 h.

Morphology by light and electron microscopy

The signs of morphologic degeneration of the preserved mouse uteri (*paper I*) after cold ischemia alone for 48 and 72 h expressed essentially the same pattern as described for other organs after prolonged cold ischemia, with typical vacuolization, nuclear pycnosis, endothelial detachment and finally necrosis (Momii and Koga 1990). After 24 h storage little or no changes were seen on the light microscopy level. Also, in human tissue (*paper II*) little or no changes were seen by light microscopy after 24 h cold storage. However, electron microscopy of human myometrium revealed hydropic changes and coarse chromatin as early indicators of loss of cellular integrity in specimens stored in RIN for 24 h. Biopsies stored in UW and PER showed little changes compared to control. This is line with the results from the contractility tests, where RIN preserved specimens showed defects in spontaneous contractility and little or no response to PGF2α. A general conclusion from these observations is that light microscopy can not be used as a reliable method to detect morphological disturbances within

preserved tissue. Thus, the recent findings on retrieved and preserved whole uteri from human multiorgan donors (Del Priore *et al.* 2007), with examinations only by light microscopy should be interpreted with caution.

Early reperfusion (*papers III and IV*)

Metabolic markers at early reperfusion

Due to the lack of oxygen as a terminal electron acceptor for cellular respiration during ischemia, cells switch to intense glycolysis to meet up with persistent cellular energy demands, producing lactate. Since protons produced by ATP hydrolysis cannot be effectively used for oxidative phosphorylation in the absence of oxygen, H+ will also accumulate. When circulation is restored and the accumulated extracellular fluid is washed out, there will be a decline in pH and an elevation of lactate in venous blood. This decline will only be restricted to a relatively short time if the circulation carries enough oxygen. Furthermore, with the reintroduction of oxygen, hypoxic cells will respire more intensely to restore the high energy phosphate pool that has been reduced during ischemia and this will be seen as an initial rise in pCO_2 in venous blood. If reperfusion is adequate and aerobic metabolism is restored, the levels of pH, lactate and pCO_2 in blood will eventually return to normal. In this perspective, these parameters are valuable diagnostic factors in judging the patency of vascular anastomoses as well as the severity of tissue damage by ischemia.

Of the three animals analysed in *paper III*, levels of pH, lactate and pCO_2/pO_2 in uterine venous blood returned towards control values in two and remained altered in one uterus. This result correlated to histological findings in biopsies taken at the end of the experiment, where the one uterus with indications of disturbed perfusion showed increased infiltration of leukocytes. In *paper IV* the disturbances in pH, lactate and pCO_2/pO_2 values were less prominent in the PER stored uteri than in the RIN group, that also correlated to histological findings. These results points toward the necessity to use a proper storage solution also for shorter periods of ischemic storage.

In both papers the disturbances in glucose metabolism and cellular respiration were judged to be mild when compared to what has been found by others in a similar experiments in pig liver but with longer ischemic times (Gillispie *et al.* 2007).

Oxidative stress and anti-oxidant defence at early reperfusion

During reperfusion and oxidative stress phospholipids in cell membranes are peroxidised and at decomposition of these lipid radicals, thiobarbituric acid reactive species (TBARS) are formed that are released into the circulation. Elevated concentrations of TBARS in plasma has been shown to correlate with histological markers of reperfusion injury (Davenport *et al.* 1995; Hauet *et al.* 2000). Also, oxidation of circulating ascorbate by ROS produces ascorbyl radicals that - if found to be elevated in plasma - indicates oxidative stress (Bagenholm *et al.* 1997). Total antioxidant capacity in plasma or tissue homogenates can be measured by the ABTS assay (Miller *et al.* 1993) and serve as an additional marker of oxidative stress since antioxidant enzyme activity is depressed during reperfusion (Dobashi *et al.* 2000).

In the studies of early reperfusion events in the sheep (*paper IV*) levels of TBARS and ascorbyl radicals in uterine venous plasma were found to be slightly elevated during the course of 3 h of reperfusion, while total antioxidant capacity was unchanged. The changes in TBARS and ascorbyl radical levels were more pronounced in the RIN group compared to the PER group. TBARS in uterine venous plasma were also analysed in samples from three pigs in *paper III*, but no changes could be detected during 2 h of reperfusion. It might be that changes would have appeared later if reperfusion had commenced, since the elevation of TBARS found in *paper IV* did not appear until after 2 h. The collective results of these analyses in *paper IV* suggests that one to two hours of cold ischemia induces only mild oxidative stress and that the use of PER during cold storage further reduces these changes.

Histological markers of ischemia/reperfusion injury

Severe ischemia/reperfusion injury leads to histological patterns of necrotic and apoptotic cell death, oedema, stasis and detachment of epithelial cells (Garcia-Poblete *et al.* 1997). The results from the histology analysis in *papers III* and *IV*, with an elevation of infiltrating neutrophils as the main indicator of an existing inflammatory response suggest that the endometrium might be more susceptible to reperfusion damage and inflammatory changes than the myometrium. However, the endometrium has the capacity to regenerate from endometrial stem cells (Gargett *et al.* 2007), a phenomenon occurring during each hormonal cycle, and this would promote regained structure and function of the endometrium.

Post-ischemic inflammation and healing (*papers I and V*)

Reproductive performance in the mouse after cold ischemia/reperfusion

In *paper I*, it was shown that uteri stored in UW solution for 24 h regained the ability to implant embryos and harbour pregnancies to term. 14 days after transplantation embryos were transferred to the transplanted as well as the native uterus in 6 animals. Five of these became pregnant in the transplanted uterus and showed similar implantation and pregnancy rate as in the native uterus. Body weight at birth and growth trajectory up to maturity was no different in pups from the grafted or native uterus. It may well be that the lack of allogeneic stimulus as well as absence of functional challenge during the healing process were beneficial for creation of conditions for full recovery of the transplanted uterus. In allogeneic transplantation of the uterus, it is possible that the recovery will be delayed even with proper immunosuppression and anti-inflammatory treatment. However, since full function like hormone induced tissue cyclicity and pregnancy will not be immediately demanded, a period of functional rest at the new site might allow the graft to recover from the transplantation trauma. It is known from kidney and pancreas transplantations that the use of dialysis or insulin therapy is necessary during the initial post-transplantation period and that it may be a prerequisite for recovery of initially non-functioning grafts (Hansen and Birkeland 1985; Sutherland *et al.* 1998).

These results also indirectly show that the at least initial absence of nerv connections and lymph drainage from the uterus has no effect on function in this non-rejecting model. Neo-angiogenesis of lymph vessels has been shown to occur early after kidney transplantation and defective lymph drainage seems to negatively affect one year graft function (Stuht *et al.* 2007). It remains to be elucidated if neo-angiogenesis of lymph vessels occur in the uterine graft and what possible implication lymph drainage has in leukocyte trafficking and rejection in allogeneic uterus transplantation.

Anti-inflammatory pre-operative treatment of donor and recipient

Progesterone and prednisolone (a corticsteroid with predominantly glucocorticosteroid features) both bind to their separate and specific intracellular nuclear receptors that upon ligand binding act as transcription factors, regulating the expression of other genes. Corticosteroids also exhibit non-genomic anti-inflammatory actions (Liu *et al.* 2005). The actions of progesterone and prednisolone are diverse and somewhat overlapping, with anti-inflammatory and immunosuppressive effects as one prominent common feature. In transplantation prednisolone is often used as the steroid component in combination treatments against allograft rejection. All mechanisms behind corticosteroid and progesterone anti-inflammatory and immunosuppressive effects are not entirely clear, but control of pro-inflammatory cytokines and prostaglandin production via nuclear factor kappa B (NF-kappaB) is one common mechanism (Kagoshima *et al.* 2003; Hardy *et al.* 2006). Progesterone has also been shown to promote maturation of immature dendritic cells towards a tolerogeneic phenotype (Yang *et al.* 2006).

Progesterone receptors are expressed mainly in female reproductive organs and the brain while glucocorticoid receptors are more widely expressed throughout the body. The reasoning behind considering treatment with progesterone alone or in combination with glucocorticosteroids is that the effects, both positive and adverse, would be concentrated to the uterus.

In *paper V* a rat model for uterus transplantation is presented and used to compare the anti-inflammatory effect of progesterone and prednisolone. Donor and recipient rats were pre-treated with relatively high does of progesterone and prednisolone for two days before 24 h of cold ischemia of the graft, followed by transplantation. Analysis of histological signs of post-ischemic inflammation of the graft showed an equal reduction of infiltrating neutrophils in the two treated groups and no further detectable signs of inflammation were found when grafts were compared to the native uterus. In the un-treated control group, thrombi were more readily formed in the graft as compared to the treated groups. Infiltration of neutrophils was also slightly elevated when compared to the native uterus. Since there was no detectable histological distinction between the two treatments, more extensive studies are needed to reveal possible differences. These studies would include examination of expression levels of several factors related to tissue damage.

Clinical perspectives of uterus transplantation

To use transplantation as a method to cure infertility in an otherwise healthy woman is controversial for many reasons. It raises fundamental questions about our view on infertility and the medical concerns are many. However, under the conditions that an acceptable level of safety for donor, mother and prospective child can be reached, the potential benefits for certain patients might outweigh the risks and costs involved.

Some of the medical concerns

The concerns regarding the safety of the mother focus mainly on surgical methods and immunosuppressive treatment. The failure of the only attempt to transplant a uterus in the human (Fageeh *et al.* 2002) was stated to be related to poor support of the uterus that led to torsion of the anastomosed vessels and thrombosis and thus obstruction of blood flow to the uterus. Such issues must be solved. In this human case it can not be excluded that rejection caused thrombosis formation in the transplant. The patient had one episode of acute rejection that was stated to be resolved by medication, but no biopsies were taken from the uterus to confirm

that. The rejection pattern of the uterus and suitable immunosuppression regimens to hinder rejection are thus issues that have to be extensively studied in various experimental models. It should be emphasized that extensive animal studies ought to be performed before a new human trial in order to optimize the conditions.

Regarding the prospective child, the effects of immunosuppression on foetal and post natal development constitute the main concern. In animal studies it has been shown that immunosuppression with cyclosporin A reduces implantation rate and foetal survival.(Fein *et al.* 1989) and induces chronic nephropathy (Tendron-Franzin *et al.* 2004). There are also concerns regarding the effect of immunosuppressive treatment on the development of the immune system during foetal life (Classen 1998). Experience from pregnancies in transplanted women under immunosuppression shows an increase in pregnancy associated hypertension, spontaneous abortion and preterm delivery of babies that are small for gestational age (Sibanda *et al.* 2007). In children born to transplanted mothers some show abnormalities of the urinary tract (Willis *et al.* 2000) but no elevated incidence of other malformations has been reported (Bar Oz *et al.* 2001). Even though these reports provide knowledge that is useful when considering uterus transplantation, extensive studies in specific animal models for uterus transplantation should be performed to further elucidate these issues. Only a few studies exists regarding the long term effects of immunosuppression on offspring development, immunology and reproductive performance and these aspects are crucial in uterus transplantation.

Since the use of immunosuppressants is a main concern in uterus transplantation, additional means to subdue the allo-reaction should be used so that dose requirements of immunosuppressants can be reduced. Besides poor blood-group and tissue compatibility between donor and recipient, advanced donor age and initial poor status of the graft (Naumovic *et al.* 2005; Nunes *et al.* 2006; Parzanese *et al.* 2006), the main influences on frequency of acute rejection events and progression of chronic rejection are related to ischemia/reperfusion injuries (Grinyo 2001). The “holy grail” in transplantation immunology is the induction of immunological tolerance and when a clinical method to achieve this has been

developed, the issue of immunosuppressant side effects can be put aside. However, no such method exists today but there are still strategies that can be employed to reduce the impact of the above mentioned factors. Live donation by a well matched donor with a healthy uterus, minimized warm and cold ischemic times and the use of an adequate flushing solution are some means to reduce the post-operative and post-ischemic inflammation at uterus transplantation. Also, hormonal and/or anti-inflammatory pre-treatment of the donor to further subdue the induction of innate immune responses in the graft could be considered. This must however be carefully weighed against the safety and autonomy of the donor.

A few ethical considerations

When uterus transplantation is discussed from an ethical point of view, it should be compared to existing alternatives to achieve parenthood for women with uterus factor infertility: adoption and gestational surrogacy. The concept of adoption is widely accepted in the Nordic countries where we tend to emphasize the social component in parenthood. The best interest of the child is put in front, but when no acceptable family solution can be found in the child's close environment, adoption can be considered. In those situations, the possible negative impact of adoption for the adoptee's sense of identity and connection to its genetic, ethnic and very early background is considered to be outweighed by the better prospects for good health and economical and social stability provided by the adoption. The arrangement is then of mutual benefit for the infertile couple and the child. This perception is however based on culture and in many other communities, adoption is not always a socially accepted solution for infertile couples to achieve parenthood.

The other alternative to achieve parenthood for the patients who also would benefit from uterus transplantation is gestational surrogacy. As previously stated, surrogacy is prohibited in the Nordic and most other western societies and only allowed in a few countries in the EU and some states in the US and Australia. The arguments against surrogacy are mainly that it poses a threat for the autonomy of the surrogate, commissioning couple and child, that too much emphasis is put on the genetic connection rather than gestational and social bond between parents

and child and that it opens up for exploitation of economically disadvantaged groups of women (Dodds and Jones 1989; Van Zyl 2002). The main arguments for surrogacy are that individuals autonomy includes the right to enter a contract as in surrogacy arrangements and that it is possible to protect all parties involved with proper selection criteria, counselling and practice (Wilkinson 2003; Goold 2004) As in adoption, gestational surrogacy can be regarded partly as a gesture of altruism and also an arrangement of mutual benefit for the parties involved. Here again, culture is a major determinant for how we perceive these arrangements.

In comparison to adoption, transplantation might seem an extreme means to take to achieve parenthood and the ethical issues that arise when the two arrangements are compared touches the fundamental questions regarding our right to have a biological child versus the moral obligation to care for already existing children. When uterus transplantation is compared to gestational surrogacy, the ethical concerns are related to the identity of the risk-taker (the surrogate, the donor, the genetic mother or the child), the autonomy of the parties involved (organ donor, surrogate, parents and child) and the risk of female reproduction becoming a commodity. What ever the future situation will be regarding uterus transplantation these concepts will most certainly continue to be debated.

CONCLUSIONS

The results from the studies included in the current thesis show that:

- suitable animal models for uterus transplantation research have been created in the mouse, the rat and the sheep
- the uterus is comparably resistant to cold ischemia and reperfusion injuries under non-allogeneic conditions
- the use of a proper preservation solution is beneficial even when the cold ischemic time is short and post-ischemic injuries are mild
- anti-inflammatory pre-treatment of donor and recipient reduces the post-ischemic inflammation and that progesterone might be an agent to investigate further

ACKNOWLEDGEMENTS

I would like to thank all of You who have helped, encouraged and pushed me along the way:

Professor **Mats Brännström**, my supervisor, for so generously providing a solid platform from which I can work and develop, for Your faith in my abilities that spurs me on and for Your tremendous support, great enthusiasm and friendship.

Professor **Ian Milsom**, chairman of the Institute of Clinical Science and professor **Henrik Hagberg**, chairman of the Department of Obstetrics and Gynecology for providing me with the opportunity to achieve my goals and for Your warm support.

My three associate supervisors; **Randa Akouri**, for Your warm friendship and for guiding me into the world of rodents - good luck with Your studies! **Johan Mölne** for warmly sharing Your expertise and guiding me in microscopy analysis. **Göran Kurlberg** for generously sharing Your invaluable insights in microsurgery.

Emeritus professor **P-O Jansson,** for always being warm, welcoming and supportive.

Associate Professor **Ulf Nilsson** at the department of Nephrology for sharing Your expertise in the area of oxidative stress and for analysis and valuable input as co-author.

Ann Wallin at SpecLab for Your invaluable help in the lab and with artwork and also for Your warm friendship that extends outside the lab!

Cecilia Lundmark – You are an unusually warm and generous friend as well as a solid rock in the operating theatre! I deeply appreciate You welcoming me into Your extended family at Änggården.

Anders Enskog for sharing Your insights in such diverse areas as physiology, intensive care, coffee, music and human relations, for Your great support and guidance, regardless of topic and for Your warm friendship.

Yihong Zhu for being such a wonderful roommate and friend, for sharing Your thoughts with me and listening to mine! I truly appreciate all our chats on everything from science to "dagis", as well as our hours of silence when we both work intensely, sensing but not disturbing each other.

All past and present colleagues at SpecLab and especially; **Birgitta Weidegård** for sharing Your knowledge, for helping me in the lab and for Your friendship. **Gun Abrahamsson** for keeping track of all things, all people and me as well, **RoseMarie Svensson** for help with ordering things and for taking care of the dishes when I forget, **Anita Sjögren** for always being warm and friendly, **David Hallberg** for Your friendship and support and for making sure I eat sometimes! **Hanna, Ximena, Tina, Helene, Karin W, Karin J, Tajana** and **everyone at the IVF-lab** for tasty cakes and interesting discussions on everything from immunohistochemistry to horses, litterature and food recipes.

The Sheep Team: **Nadia Karlsson –** Your commitment and all hours You've put in has been invaluable and so is Your friendship! **Pernilla Dahm-Kähler -** Your warm friendship and wonderful ability to pass on Your positive attitude definitely brightens even the dullest day! **Klaus Groth** – our interesting discussions, Your friendship and Your help during the sheep-night shift has been invaluable. **Janusz Marcickiewicz**, **Åsa Eli-Johansson** and **Mia Lidemyr –** it is always a pleasure working with You!

Lillebil Hagberg, Ewa Crusner, Eva Oscarsson, Sara Persson, Susanne Exing, Lars Ewaldsson, Rolf Werme, Jeanette Ryding and all others at EBM - thank You for all help and guidance with the animals and for supporting me with coffee and chats!

Anja Andersson and **Ingrid Prim-Ritzopolus –** many thanks for helping me with all those tedious things regarding administrative work! The world would run less smoothly without You!

I also would like to thank:

My friends in the world outside medical research - by being the grand people You are You provide me with all those things that make a difference and make sure I keep at least some perspective

My sister **Malin Almén** and **Mats Öhr** – for loving support, always being there on the phone and sometimes also closer, for lending Your ears to whatever is on my mind, cheering me up and always, always standing by me.

My mother **Margareta Wiman** and her husband and my "extra-dad" **Folke Wiman** - for all Your love and encouragement, picking up the kids, economic support and dinners I am eternally thankful! I could not have done this without You!

Per Wranning, father of my children, for being the best co-parent I could wish for, for supporting me and being my friend all the way through. Now it's Your turn!!!

Och sist men inte minst – mina älskade barn **Joel** and **Tova**. Ni är de vikigaste personerna i min värld och det är ett stort äventyr att få vara er mamma!

REFERENCES

Swedish Government, 2002, Svensk Författningssamling, Föräldrabalk 2002:252, Socialdepartementet

Abdel Rahim, S. E. and Bland, K. P. (1985) The lymphatic drainage of the cranial part of the sheep's uterus and its possible functional significance. J Anat 140 (Pt 4), 705-9

Asehnoune, K., Strassheim, D., Mitra, S., Kim, J. Y. and Abraham, E. (2004) Involvement of reactive oxygen species in Toll-like receptor 4-dependent activation of NF-kappa B. J Immunol 172:4, 2522-9

Bagenholm, R., Nilsson, U. A. and Kjellmer, I. (1997) Formation of free radicals in hypoxic ischemic brain damage in the neonatal rat, assessed by an endogenous spin trap and lipid peroxidation. Brain Res 773:1-2, 132-8

Bar Oz, B., Hackman, R., Einarson, T. and Koren, G. (2001) Pregnancy outcome after cyclosporine therapy during pregnancy: a meta-analysis. Transplantation 71:8, 1051-5

Barzilai, A., Paldi, E., Gal, D. and Hampel, N. (1973) Autotransplantation of the uterus and ovaries in dogs. Isr J Med Sci 9:1, 49-52

Ber, R. (2000) Ethical issues in gestational surrogacy. Theor Med Bioeth 21:2, 153-69

Berry, C. E. and Hare, J. M. (2004) Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications. J Physiol 555:Pt 3, 589-606

Bland, K. P. (1972) Implantation and early pregnancy in the autotransplanted uterus of the guinea-pig. J Reprod Fertil 28:1, 103-4

Bull, D. A., Connors, R. C., Reid, B. B., Albanil, A., Stringham, J. C. and Karwande, S. V. (2000) Improved biochemical preservation of lung slices during cold storage. J Surg Res 90:2, 144-8

Buttram, V. C., Jr. (1986) Uterine leiomyomata--aetiology, symptomatology and management. Prog Clin Biol Res 225, 275-96

Byers, S. L., Payson, S. J. and Taft, R. A. (2006) Performance of ten inbred mouse strains following assisted reproductive technologies (ARTs). Theriogenology 65:9, 1716-26

Calne, R. Y., White, D. J., Thiru, S., Evans, D. B., McMaster, P., Dunn, D. C., Craddock, G. N., Pentlow, B. D. and Rolles, K. (1978) Cyclosporin A in patients receiving renal allografts from cadaver donors. Lancet 2:8104-5, 1323-7

Casini, M. L., Rossi, F., Agostini, R. and Unfer, V. (2006) Effects of the position of fibroids on fertility. Gynecol Endocrinol 22:2, 106-9

Chavez-Genaro, R., Lombide, P. and Anesetti, G. (2006) A quantitative study of rat uterine sympathetic innervation during pregnancy and post partum. Reprod Fertil Dev 18:5, 525-31

Cheroki, C., Krepischi-Santos, A. C., Rosenberg, C., Jehee, F. S., Mingroni-Netto, R. C., Pavanello Filho, I., Zanforlin Filho, S., Kim, C. A., Bagnoli, V. R., Mendonca, B. B., Szuhai, K. and Otto, P. A. (2006) Report of a del22q11 in a patient with Mayer-Rokitansky-Kuster-Hauser (MRKH) anomaly and exclusion of WNT-4, RAR-gamma, and RXR-alpha as major genes determining MRKH anomaly in a study of 25 affected women. Am J Med Genet A 140:12, 1339-42

Chin, H. J. and Wang, C. K. (2001) Utero-tubal transfer of mouse embryos. Genesis 30:2, 77-81

Classen, J. B. (1998) Cyclosporine induced autoimmunity in newborns prevented by early immunization. Autoimmunity 27:3, 135-9

Cohen, J. and Jones, H. W. (2001) Worldwide legislation in Textbook of Assisted Reproductive Techniques: Laboratory and Clinical Perspectives. D. K. Gardner, A. Weissman and C. M. Howles. London, Martin Dunitz Ltd: 731-751

Confino, E., Vermesh, M., Thomas, W., Jr. and Gleicher, N. (1986) Non-vascular transplantation of the rabbit uterus. Int J Gynaecol Obstet 24:4, 321-5

Corbin, T. J. and McCabe, J. G. (2002) Strain variation of immature female rats in response to various superovulatory hormone preparations and routes of administration. Contemp Top Lab Anim Sci 41:2, 18-23

Cousineau, T. M. and Domar, A. D. (2007) Psychological impact of infertility. Best Pract Res Clin Obstet Gynaecol 21:2, 293-308

Davenport, A., Hopton, M. and Bolton, C. (1995) Measurement of malondialdehyde as a marker of oxygen free radical production during renal allograft transplantation and the effect on early graft function. Clin Transplant 9:3 Pt 1, 171-5

de Vries, K., Lyons, E. A., Ballard, G., Levi, C. S. and Lindsay, D. J. (1990) Contractions of the inner third of the myometrium. Am J Obstet Gynecol 162:3, 679-82

deBlois, J. and O'Rourke, K. D. (1995) Care for the beginning of life. The revised Ethical and Religious Directives discuss abortion, contraception, and assisted reproduction. Health Prog 76:7, 36-40

Del Priore, G., Stega, J., Sieunarine, K., Ungar, L. and Smith, J. R. (2007) Human uterus retrieval from a multi-organ donor. Obstet Gynecol 109:1, 101-4

Dobashi, K., Ghosh, B., Orak, J. K., Singh, I. and Singh, A. K. (2000) Kidney ischemia-reperfusion: modulation of antioxidant defenses. Mol Cell Biochem 205:1-2, 1-11

Dodds, S. and Jones, K. (1989) Surrogacy and autonomy. Bioethics 3:1, 1-17

Donnez, J., Dolmans, M. M., Demylle, D., Jadoul, P., Pirard, C., Squifflet, J., Martinez-Madrid, B. and van Langendonckt, A. (2004) Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. Lancet 364:9443, 1405-10

Edwards, R. G., Steptoe, P. C. and Purdy, J. M. (1980) Establishing full-term human pregnancies using cleaving embryos grown in vitro. Br J Obstet Gynaecol 87:9, 737-56

El-Akouri, R. R., Molne, J., Groth, K., Kurlberg, G. and Brannstrom, M. (2006) Rejection patterns in allogeneic uterus transplantation in the mouse. Hum Reprod 21:2, 436-42

Eraslan, S., Hamernik, R. J. and Hardy, J. D. (1966) Replantation of uterus and ovaries in dogs, with successful pregnancy. Arch Surg 92:1, 9-12

Evans, T. N., Poland, M. L. and Boving, R. L. (1981) Vaginal malformations. Am J Obstet Gynecol 141:8, 910-20

Fageeh, W., Raffa, H., Jabbad, H. and Marzouki, A. (2002) Transplantation of the human uterus. Int J Gynaecol Obstet 76:3, 245-51

Fein, A., Vechoropoulos, M. and Nebel, L. (1989) Cyclosporin-induced embryotoxicity in mice. Biol Neonate 56:3, 165-73

Finkelstein, F. O., Lytton, B., Schiff, M. and Black, H. R. (1975) Rejection episodes and patient and graft survival after renal transplantation. Clin Nephrol 3:6, 217-9

Fisher, R. L., Hasal, S. J., Sanuik, J. T., Scott, K. S., Gandolfi, A. J. and Brendel, K. (1993) Cold- and cryopreservation of human liver and kidney slices. Cryobiology 30:3, 250-61

Floyd, R. and Wray, S. (2007) Calcium transporters and signalling in smooth muscles. Cell Calcium

Fraser, I. S., Baird, D. T., Hobson, B. M., Michie, E. A. and Hunter, W. (1973) Cyclical ovarian function in women with congenital absence of the uterus and vagina. J Clin Endocrinol Metab 36:4, 634-7

Garcia-Poblete, E., Fernandez, H., Alvarez, L., Torralba, A. and Escudero, C. (1997) Structural and ultrastructural study of the myocardium after 24-hour preservation in University of Wisconsin solution. Histol Histopathol 12:2, 375-82

Gargett, C. E., Chan, R. W. and Schwab, K. E. (2007) Endometrial stem cells. Curr Opin Obstet Gynecol 19:4, 377-383

Gerencser, G. A. and Zhang, J. (2003) Existence and nature of the chloride pump. Biochim Biophys Acta 1618:2, 133-9

Gillispie, A., Rooyackers, O., Wernerman, J. and Nowak, G. (2007) Effect of extended cold ischemia time on glucose metabolism in liver grafts: experimental study in pigs. J Hepatobiliary Pancreat Surg 14:2, 183-8

Girlanda, R. and Kirk, A. D. (2007) Frontiers in nephrology: immune tolerance to allografts in humans. J Am Soc Nephrol 18:8, 2242-51

Goold, I. (2004) Surrogacy: is there a case for legal prohibition? J Law Med 12:2, 205-16

Griffin, J. E., Edwards, C., Madden, J. D., Harrod, M. J. and Wilson, J. D. (1976) Congenital absence of the vagina. The Mayer-Rokitansky-Kuster-Hauser syndrome. Ann Intern Med 85:2, 224-36

Grinyo, J. M. (2001) Role of ischemia-reperfusion injury in the development of chronic renal allograft damage. Transplant Proc 33:7-8, 3741-2

Gu, X. P., Qiu, Y. D., Xu, F. T., Jiang, Y. and Ding, Y. T. (2004) In vivo suppressive effect of nuclear factor-kappaB inhibitor on neutrophilic inflammation of grafts after orthotopic liver transplantation in rats. World J Gastroenterol 10:24, 3654-8

Hahn, R., Wendel, A. and Flohe, L. (1978) The fate of extracellular glutathione in the rat. Biochim Biophys Acta 539:3, 324-37

Halestrap, A. P. (2006) Calcium, mitochondria and reperfusion injury: a pore way to die. Biochem Soc Trans 34:Pt 2, 232-7

Halliwell, B. and Gutterige, J. M. C. (1999) Antioxidant defences in Free radicals in biology and medicine. Oxford, Oxford University Press: 105-245

Hammer, C. (1997) Evolution: its complexity and impact on xenotransplantation. in Xeno-transplantation. The transplantation of organs and tissues between species. D. K. C. Cooper, E. Kemp, J. L. Platt and D. J. G. White. Berlin, Springer-Verlag: 665-82

Hansen, B. L. and Birkeland, S. A. (1985) Recovery of kidney function after cessation of graft function or prolonged dialysis treatment. Scand J Urol Nephrol 19:3, 217-9

Hardy, D. B., Janowski, B. A., Corey, D. R. and Mendelson, C. R. (2006) Progesterone receptor plays a major antiinflammatory role in human myometrial cells by antagonism of nuclear factor-kappaB activation of cyclooxygenase 2 expression. Mol Endocrinol 20:11, 2724-33

Hartl, F. U. and Hayer-Hartl, M. (2002) Molecular chaperones in the cytosol: from nascent chain to folded protein. Science 295:5561, 1852-8

Hasty, L. A., Lambris, J. D., Lessey, B. A., Pruksananonda, K. and Lyttle, C. R. (1994) Hormonal regulation of complement components and receptors throughout the menstrual cycle. Am J Obstet Gynecol 170:1 Pt 1, 168-75

Hauet, T., Baumert, H., Amor, I. B., Goujon, J. M., Gibelin, H., Godart, C., Vandewalle, A., Carretier, M. and Eugene, M. (2000) Protection of autotransplanted pig kidneys from ischemia-reperfusion injury by polyethylene glycol. Transplantation 70:11, 1569-75

Hesselberg, C., Kerwin, W. and Loeb, L. (1918) Auto and homio-transplantation of the uterus in the guinea-pig. J Med res 38, 11

Houdeau, E., Rossano, B. and Prud'homme, M. J. (2003) Regional and muscle layer variations in cholinergic nerve control of the rat myometrium during the oestrous cycle. Auton Neurosci 104:1, 1-9

Howard, T. K., Klintmalm, G. B., Cofer, J. B., Husberg, B. S., Goldstein, R. M. and Gonwa, T. A. (1990) The influence of preservation injury on rejection in the hepatic transplant recipient. Transplantation 49:1, 103-7

Huang, W. H., Yan, Y., De Boer, B., Bishop, G. A. and House, A. K. (2003) A short course of cyclosporine immunosuppression inhibits rejection but not tolerance of rat liver allografts. Transplantation 75:3, 368-74

Hullett, D. A., Laeseke, P. F., Malin, G., Nessel, R., Sollinger, H. W. and Becker, B. N. (2005) Prevention of chronic allograft nephropathy with vitamin D. Transpl Int 18:10, 1175-86

Husain, F. A. (2000) Reproductive issues from the Islamic perspective. Hum Fertil (Camb) 3:2, 124-128

Jiga, L. P., Lupu, C. M., Zoica, B. S. and Ionac, M. (2003) Experimental model of heterotopic uterus transplantation in the laboratory rat. Microsurgery 23:3, 246-50

Kaeoket, K., Dalin, A. M., Magnusson, U. and Persson, E. (2001) The sow endometrium at different stages of the oestrous cycle: studies on the distribution of CD2, CD4, CD8 and MHC class II expressing cells. Anim Reprod Sci 68:1-2, 99-109

Kagoshima, M., Ito, K., Cosio, B. and Adcock, I. M. (2003) Glucocorticoid suppression of nuclear factor-kappa B: a role for histone modifications. Biochem Soc Trans 31:Pt 1, 60-5

Kam, P. C. and See, A. U. (2000) Cyclo-oxygenase isoenzymes: physiological and pharmacological role. Anaesthesia 55:5, 442-9

Kanitakis, J., Badet, L., Petruzzo, P., Beziat, J. L., Morelon, E., Lefrancois, N., Frances, C., Claudy, A., Martin, X., Lengele, B., Testelin, S., Devauchelle, B. and Dubernard, J. M. (2006) Clinicopathologic monitoring of the skin and oral mucosa of the first human face allograft: Report on the first eight months. Transplantation 82:12, 1610-5

Karam, K. S., Salti, I. and Hajj, S. N. (1977) Congenital absence of the uterus. Clinicopathologic and endocrine findings. Obstet Gynecol 50:5, 531-5

Kaushic, C., Frauendorf, E., Rossoll, R. M., Richardson, J. M. and Wira, C. R. (1998) Influence of the estrous cycle on the presence and distribution of immune cells in the rat reproductive tract. Am J Reprod Immunol 39:3, 209-16

Kayyali, U. S., Donaldson, C., Huang, H., Abdelnour, R. and Hassoun, P. M. (2001) Phosphorylation of xanthine dehydrogenase/oxidase in hypoxia. J Biol Chem 276:17, 14359-65

Keenihan, S. N. and Robertson, S. A. (2004) Diversity in phenotype and steroid hormone dependence in dendritic cells and macrophages in the mouse uterus. Biol Reprod 70:6, 1562-72

Kelly, R. E. and Verhage, H. G. (1981) Hormonal effects on the contractile apparatus of the myometrium. Am J Anat 161:4, 375-82

Kim, J. S., Qian, T. and Lemasters, J. J. (2003) Mitochondrial permeability transition in the switch from necrotic to apoptotic cell death in ischemic rat hepatocytes. Gastroenterology 124:2, 494-503

Kunz, G., Beil, D., Deininger, H., Wildt, L. and Leyendecker, G. (1996) The dynamics of rapid sperm transport through the female genital tract: evidence from vaginal sonography of uterine peristalsis and hysterosalpingoscintigraphy. Hum Reprod 11:3, 627-32

Land, W. G. (2005) The role of postischemic reperfusion injury and other nonantigen-dependent inflammatory pathways in transplantation. Transplantation 79:5, 505-14

Lanzetta, M., Pozzo, M., Bottin, A., Merletti, R. and Farina, D. (2005) Reinnervation of motor units in intrinsic muscles of a transplanted hand. Neurosci Lett 373:2, 138-43

Lee, S., Mao, L., Wang, Y., D'Silva, M., Yoo, C. H., Wolf, P., Chung, W. S., Takahashi, E., Chung, D. Y. and Gittes, R. F. (1995) Transplantation of reproductive organs. Microsurgery 16:4, 191-8

Lefebvre, G., Vilos, G., Allaire, C., Jeffrey, J., Arneja, J., Birch, C., Fortier, M. and Wagner, M. S. (2003) The management of uterine leiomyomas. J Obstet Gynaecol Can 25:5, 396-418; quiz 419-22

Levi, D. M., Tzakis, A. G., Kato, T., Madariaga, J., Mittal, N. K., Nery, J., Nishida, S. and Ruiz, P. (2003) Transplantation of the abdominal wall. Lancet 361:9376, 2173-6

Liu, L., Wang, Y. X., Zhou, J., Long, F., Sun, H. W., Liu, Y., Chen, Y. Z. and Jiang, C. L. (2005) Rapid non-genomic inhibitory effects of glucocorticoids on human neutrophil degranulation. Inflamm Res 54:1, 37-41

Lobos, E., Gebhardt, C., Kluge, A. and Spanel-Borowski, K. (2005) Expression of nerve growth factor (NGF) isoforms in the rat uterus during pregnancy: accumulation of precursor proNGF. Endocrinology 146:4, 1922-9

Maathuis, M. H., Leuvenink, H. G. and Ploeg, R. J. (2007) Perspectives in organ preservation. Transplantation 83:10, 1289-98

MacKenzie, L. W. and Garfield, R. E. (1985) Hormonal control of gap junctions in the myometrium. Am J Physiol 248:3 Pt 1, C296-308

Malik, E., Buchweitz, O., Muller-Steinhardt, M., Kressin, P., Meyhofer-Malik, A. and Diedrich, K. (2001) Prospective evaluation of the systemic immune response following abdominal, vaginal, and laparoscopically assisted vaginal hysterectomy. Surg Endosc 15:5, 463-6

March, C. M. (1995) Intrauterine adhesions. Obstet Gynecol Clin North Am 22:3, 491-505

Marshall, L. M., Spiegelman, D., Barbieri, R. L., Goldman, M. B., Manson, J. E., Colditz, G. A., Willett, W. C. and Hunter, D. J. (1997) Variation in the incidence of uterine leiomyoma among premenopausal women by age and race. Obstet Gynecol 90:6, 967-73

Martin, F. H. (1903) Ovarian transplantation and reconstruction of fallopian tubes: with report of two cases and review of literature. Chic Med Rec 25, 1-12

Martinez-Gaudio, M., Yoshida, T. and Bengtsson, L. P. (1973) Propagated and nonpropagated myometrial contractions in normal menstrual cycles. Am J Obstet Gynecol 115:1, 107-11

Mattingly, R. F., Clark, D. O., Lutsky, II, Huang, W. Y., Stafl, A. and Maddison, F. E. (1970) Ovarian function in uteroovarian homotransplantation. Am J Obstet Gynecol 108:5, 773-94

McCord, J. M. and Roy, R. S. (1982) The pathophysiology of superoxide: roles in inflammation and ischemia. Can J Physiol Pharmacol 60:11, 1346-52

Merrill, J. P., Murray, J. E., Harrison, J. H. and Guild, W. R. (1956) Successful homotransplantation of the human kidney between identical twins. J Am Med Assoc 160:4, 277-82

Miller, N. J., Rice-Evans, C., Davies, M. J., Gopinathan, V. and Milner, A. (1993) A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin Sci (Lond) 84:4, 407-12

Momii, S. and Koga, A. (1990) Time-related morphological changes in cold-stored rat livers. A comparison of Euro-Collins solution with UW solution. Transplantation 50:5, 745-50

Morizaki, N., Morizaki, J., Hayashi, R. H. and Garfield, R. E. (1989) A functional and structural study of the innervation of the human uterus. Am J Obstet Gynecol 160:1, 218-28

Muhlbacher, F., Langer, F. and Mittermayer, C. (1999) Preservation solutions for transplantation. Transplant Proc 31:5, 2069-70

Munz, C., Steinman, R. M. and Fujii, S. (2005) Dendritic cell maturation by innate lymphocytes: coordinated stimulation of innate and adaptive immunity. J Exp Med 202:2, 203-7

Myatt, L. and Lye, S. J. (2004) Expression, localization and function of prostaglandin receptors in myometrium. Prostaglandins Leukot Essent Fatty Acids 70:2, 137-48

Naumovic, R., Djukanovic, L., Marinkovic, J. and Lezaic, V. (2005) Effect of donor age on the outcome of living-related kidney transplantation. Transpl Int 18:11, 1266-74

Nunes, P., Parada, B., Pratas, J., Roseiro, A., Figueiredo, A., Macario, F., Rolo, F. and Mota, A. (2006) Cadaveric donor factor variations during a 12-year period: influence on kidney transplant outcomes. Transplant Proc 38:6, 1867-9

Oppelt, P., von Have, M., Paulsen, M., Strissel, P. L., Strick, R., Brucker, S., Wallwiener, D. and Beckmann, M. W. (2007) Female genital malformations and their associated abnormalities. Fertil Steril 87:2, 335-42

Paldi, E., Gal, D., Barzilai, A., Hampel, N. and Malberger, E. (1975) Genital organs. Auto and homotransplantation in forty dogs. Int J Fertil 20:1, 5-12

Palermo, G., Joris, H., Devroey, P. and Van Steirteghem, A. C. (1992) Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 340:8810, 17-8

Parzanese, I., Maccarone, D., Caniglia, L., Pisani, F., Mazzotta, C., Rizza, V. and Famulari, A. (2006) Risk factors that can influence kidney transplant outcome. Transplant Proc 38:4, 1022-3

Petrozza, J. C., Gray, M. R., Davis, A. J. and Reindollar, R. H. (1997) Congenital absence of the uterus and vagina is not commonly transmitted as a dominant genetic trait: outcomes of surrogate pregnancies. Fertil Steril 67:2, 387-9

Popova, E., Bader, M. and Krivokharchenko, A. (2005) Strain differences in superovulatory response, embryo development and efficiency of transgenic rat production. Transgenic Res 14:5, 729-38

Quiroga, I., McShane, P., Koo, D. D., Gray, D., Friend, P. J., Fuggle, S. and Darby, C. (2006) Major effects of delayed graft function and cold ischaemia time on renal allograft survival. Nephrol Dial Transplant 21:6, 1689-96

Racho El-Akouri, R., Kurlberg, G., Dindelegan, G., Molne, J., Wallin, A. and Brannstrom, M. (2002) Heterotopic uterine transplantation by vascular anastomosis in the mouse. J Endocrinol 174:2, 157-66

Racho El-Akouri, R. (2003) "Uterus transplantation: An experimental study in the mouse" Doctoral Thesis, Sahlgrenska Academy, Göteborg University, Sweden, ISBN: 91-628-5842-4

Racho El-Akouri, R., Kurlberg, G. and Brannstrom, M. (2003) Successful uterine transplantation in the mouse: pregnancy and post-natal development of offspring. Hum Reprod 18:10, 2018-23

Ramhorst, R., Patel, R., Corigliano, A., Etchepareborda, J. J., Fainboim, L. and Schust, D. (2006) Induction of maternal tolerance to fetal alloantigens by RANTES production. Am J Reprod Immunol 56:5-6, 302-11

Robergs, R. A., Ghiasvand, F. and Parker, D. (2004) Biochemistry of exercise-induced metabolic acidosis. Am J Physiol Regul Integr Comp Physiol 287:3, R502-16

Sanders, B. (2006) Uterine factors and infertility. J Reprod Med 51:3, 169-76

Schenker, J. G. (1996) Etiology of and therapeutic approach to synechia uteri. Eur J Obstet Gynecol Reprod Biol 65:1, 109-13

Schmidt, A., Tomasdottir, H. and Bengtsson, A. (2004) Influence of cold ischemia time on complement activation, neopterin, and cytokine release in liver transplantation. Transplant Proc 36:9, 2796-8

Schramm, R., Menger, M. D., Kirsch, S., Langer, F., Harder, Y., Hamacher, J. and Schafers, H. J. (2007) The subepicardial microcirculation in heterotopically transplanted mouse hearts: an intravital multifluorescence microscopy study. J Thorac Cardiovasc Surg 134:1, 210-7, 217 e1

Schroeder, J. S., Rider, A. K., Stinson, E. B. and Shumway, N. E. (1976) Cardiac transplantation: review of seven years' experience. Transplant Proc 8:1, 5-8

Schwarz, A., Mengel, M., Gwinner, W., Radermacher, J., Hiss, M., Kreipe, H. and Haller, H. (2005) Risk factors for chronic allograft nephropathy after renal transplantation: a protocol biopsy study. Kidney Int 67:1, 341-8

Scott, J. R., Anderson, W. R., Kling, T. G. and Yannone, M. E. (1970) Uterine transplantation in dogs. Gynecol Invest 1:3, 140-8

Scott, J. R., Pitkin, R. M. and Yannone, M. E. (1971) Transplantation of the primate uterus. Surg Gynecol Obstet 133:3, 414-8

Sentman, C. L., Meadows, S. K., Wira, C. R. and Eriksson, M. (2004) Recruitment of uterine NK cells: induction of CXC chemokine ligands 10 and 11 in human endometrium by estradiol and progesterone. J Immunol 173:11, 6760-6

Sharma, J. B., Roy, K. K., Pushparaj, M., Gupta, N., Jain, S. K., Malhotra, N. and Mittal, S. (2007) Genital tuberculosis: an important cause of Asherman's syndrome in India. Arch Gynecol Obstet

Shayevitz, J. R., Varani, J., Ward, P. A. and Knight, P. R. (1991) Halothane and isoflurane increase pulmonary artery endothelial cell sensitivity to oxidant-mediated injury. Anesthesiology 74:6, 1067-77

Sibanda, N., Briggs, J. D., Davison, J. M., Johnson, R. J. and Rudge, C. J. (2007) Pregnancy after organ transplantation: a report from the UK Transplant pregnancy registry. Transplantation 83:10, 1301-7

Sieunarine, K., Zakaria, F. B., Boyle, D. C., Corless, D. J., Noakes, D. E., Lindsay, I., Lawson, A., Ungar, L., Del Priores, G. and Smith, J. R. (2005) Possibilities for fertility restoration: a new surgical technique. Int Surg 90:5, 249-56

Sieunarine, K., Boyle, D. C., Corless, D. J., Noakes, D. E., Ungar, L., Marr, C. E., Lindsay, I., Del Priore, G. and Smith, J. R. (2006) Pelvic vascular prospects for uterine transplantation. Int Surg 91:4, 217-22

Skou, J. C. and Esmann, M. (1992) The Na,K-ATPase. J Bioenerg Biomembr 24:3, 249-61

Socialstyrelsen, 2007, Statistics - Social Welfare: Family Law 2006, D. o. F. L. National Board of Haelth and Welfare

Starzl, T. E., Iwatsuki, S., Malatack, J. J., Zitelli, B. J., Gartner, J. C., Jr., Hakala, T. R., Rosenthal, J. T. and Shaw, B. W., Jr. (1982) Liver and kidney transplantation in children receiving cyclosporin A and steroids. J Pediatr 100:5, 681-6

Stefanczyk-Krzymowska, S. and Krzymowski, T. (2002) Local adjustment of blood and lymph circulation in the hormonal regulation of reproduction in female pigs--facts, conclusions and suggestions for future research. Reprod Biol 2:2, 115-32

Steinman, R. M. (2006) Linking innate to adaptive immunity through dendritic cells. Novartis Found Symp 279, 101-9; discussion 109-13, 216-9

Steptoe, P. C. and Edwards, R. G. (1978) Birth after the reimplantation of a human embryo. Lancet 2:8085, 366

Stuht, S., Gwinner, W., Franz, I., Schwarz, A., Jonigk, D., Kreipe, H., Kerjaschki, D., Haller, H. and Mengel, M. (2007) Lymphatic neoangiogenesis in human renal allografts: results from sequential protocol biopsies. Am J Transplant 7:2, 377-84

Sutherland, D. E., Gruessner, A. C. and Gruessner, R. W. (1998) Pancreas transplantation: a review. Transplant Proc 30:5, 1940-3

Tafuri, A., Alferink, J., Moller, P., Hammerling, G. J. and Arnold, B. (1995) T cell awareness of paternal alloantigens during pregnancy. Science 270:5236, 630-3

Tanaka, M., Mokhtari, G. K., Balsam, L. B., Cooke, D. T., Kofidis, T., Zwierzchonievska, M. and Robbins, R. C. (2005) Cyclosporine mitigates graft coronary artery disease in murine cardiac allografts: description and validation of a novel fully allogeneic model. J Heart Lung Transplant 24:4, 446-53

Tejchman, K., Domanski, L., Sienko, J., Sulikowski, T., Kaminski, M., Romanowski, M., Pabisiak, K., Ostrowski, M. and Ciechanowski, K. (2006) Early acid-base balance disorders during kidney transplantation. Transplant Proc 38:1, 123-6

Tendron-Franzin, A., Gouyon, J. B., Guignard, J. P., Decramer, S., Justrabo, E., Gilbert, T. and Semama, D. S. (2004) Long-term effects of in utero exposure to cyclosporin A on renal function in the rabbit. J Am Soc Nephrol 15:10, 2687-93

Thornton, J. W., Need, E. and Crews, D. (2003) Resurrecting the ancestral steroid receptor: ancient origin of estrogen signaling. Science 301:5640, 1714-7

Totsuka, E., Fung, J. J., Hakamada, K., Ohashi, M., Takahashi, K., Nakai, M., Morohashi, S., Morohashi, H., Kimura, N., Nishimura, A., Ishizawa, Y., Ono, H., Narumi, S. and Sasaki, M. (2004) Synergistic effect of cold and warm ischemia time on postoperative graft function and outcome in human liver transplantation. Transplant Proc 36:7, 1955-8

Uchino, S., Ichikawa, S., Okubo, M., Nakamura, Y. and Iimura, A. (1987) Methods of detection of lymphatics and their changes with oestrous cycle. Int Angiol 6:3, 271-8

Van Zyl, L. (2002) Intentional parenthood: responsibilities in surrogate motherhood. Health Care Anal 10:2, 165-75

Wang, H., Hosiawa, K. A., Min, W., Yang, J., Zhang, X., Garcia, B., Ichim, T. E., Zhou, D., Lian, D., Kelvin, D. J. and Zhong, R. (2003) Cytokines regulate the pattern of rejection and susceptibility to cyclosporine therapy in different mouse recipient strains after cardiac allografting. J Immunol 171:7, 3823-36

Wathes, D. C., Mann, G. E., Payne, J. H., Riley, P. R., Stevenson, K. R. and Lamming, G. E. (1996) Regulation of oxytocin, oestradiol and progesterone receptor concentrations in different uterine regions by oestradiol, progesterone and oxytocin in ovariectomized ewes. J Endocrinol 151:3, 375-93

Wedenberg, K., Ronquist, G., Waldenstrom, A. and Ulmsten, U. (1991) Regional differences in energy charge of the pregnant human uterus regardless of functional status in comparison with the non-pregnant uterus. Biochim Biophys Acta 1058:2, 147-51

Wedenberg, K., Ronquist, G., Ulmsten, U. and Waldenstrom, A. (1995) Energy economy of human uterine muscle strips under different in vitro conditions and its dependence on tissue redox potential. Eur J Obstet Gynecol Reprod Biol 62:1, 115-9

Wendt, I. R. (1989) Effects of substrate and hypoxia on smooth muscle metabolism and contraction. Am J Physiol 256:4 Pt 1, C719-27

Vigano, P., Mangioni, S., Pompei, F. and Chiodo, I. (2003) Maternal-conceptus cross talk--a review. Placenta 24 Suppl B, S56-61

Wilhelmsson, L. (1981) "Biological actions of prostaglandins on different tissues within the nonpregnant human uterus" Thesis, Department of obstetrics and gynecology, University of Göteborg, Sweden, Göteborg, ISBN:

Wilkinson, S. (2003) The exploitation argument against commercial surrogacy. Bioethics 17:2, 169-87

Willis, F. R., Findlay, C. A., Gorrie, M. J., Watson, M. A., Wilkinson, A. G. and Beattie, T. J. (2000) Children of renal transplant recipient mothers. J Paediatr Child Health 36:3, 230-5

Vinten-Johansen, J. (2004) Involvement of neutrophils in the pathogenesis of lethal myocardial reperfusion injury. Cardiovasc Res 61:3, 481-97

Wiqvist, N., Lindblom, B., Wikland, M. and Wilhelmsson, L. (1983) Prostaglandins and uterine contractility. Acta Obstet Gynecol Scand Suppl 113, 23-9

Wira, C. R. and Sullivan, D. A. (1981) Effect of estradiol and progesterone on the secretory immune system in the female genital tract. Adv Exp Med Biol 138, 99-111

Wranning, C. A., El-Akouri, R. R., Groth, K., Molne, J., Parra, A. K. and Brannstrom, M. (2007) Rejection of the transplanted uterus is suppressed by cyclosporine A in a semi-allogeneic mouse model. Hum Reprod 22:2, 372-9

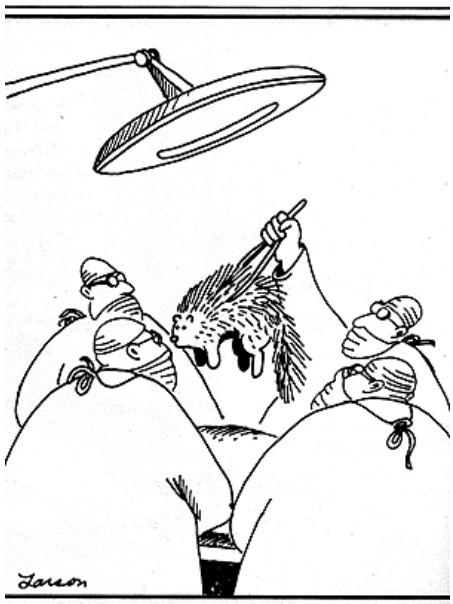
Wray, S., Kupittayanant, S., Shmygol, A., Smith, R. D. and Burdyga, T. (2001) The physiological basis of uterine contractility: a short review. Exp Physiol 86:2, 239-46

Vreugdenhil, P. K., Ametani, M. S., Haworth, R. A. and Southard, J. H. (1999) Biphasic mechanism for hypothermic induced loss of protein synthesis in hepatocytes. Transplantation 67:11, 1468-73

Yang, L., Li, X., Zhao, J. and Hou, Y. (2006) Progesterone is involved in the maturation of murine spleen CD11c-positive dendritic cells. Steroids 71:10, 922-9

Yonemoto, R. H., Du Sold, W. D. and Deliman, R. M. (1969) Homotransplantation of uterus and ovaries in dogs. A preliminary report. Am J Obstet Gynecol 104:8, 1143-51

Zikopoulos, K. A., Kolibianakis, E. M., Platteau, P., de Munck, L., Tournaye, H., Devroey, P. and Camus, M. (2004) Live delivery rates in subfertile women with Asherman's syndrome after hysteroscopic adhesiolysis using the resectoscope or the Versapoint system. Reprod Biomed Online 8:6, 720-5



"Well, I guess that explains the abdominal pains."