Review Article

# The Role of Ultrasonography in In-vitro Fertilization And Embryo Transfer (IVF-ET)

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**ABSTRACT**

**BACKGROUND:** Although ultrasonography was introduced into Obstetrics and Gynaecology by a British Gynaecologist over five decades ago, the requirement for formal training in its use by gynecologists in Nigeria is just beginning to catch on, despite its indispensible role in various aspects of our clinical practice.

**OBJECTIVE**: To describe the role of ultrasonography in in-vitro fertilization and the indispensability of the instrument to reproductive medicine.

**METHOD**: A review of literature written in English language on the use and application of ultrasonography in in-vitro fertilization was done. The review covered articles published between 1980-2012.

**RESULTS**: Ultrasound is the most versatile method for pre-treatment assessment in IVF being the dominant instrument for assessing ovarian reserve, pelvic pathologies and for assessing the uterine cavity. The ability of ultrasonography to measure endometrial thickness in addition to detecting uterine masses gives it an edge over laparoscopy/hysteroscopy as a diagnostic procedure in uterine cavity assessment, although hysteroscopy has the advantage of therapeutic potential. Similarly, ultrasonography is superior to biochemical methods for follicular monitoring because of its ability to demonstrate the number and sizes of follicles, and guide preparations for oocyte retrieval. The relative ease of ultrasound guided oocyte retrieval; its less technical demands and the possibility of conducting the procedure under local anaesthesia have made ultrasound guided oocyte retrieval more popular across the world. Randomized controlled trials show that ultrasound-guided transfer techniques have better outcomes than the clinical touch technique in terms of on-going pregnancies and clinical pregnancies. Ultrasonography is now the key instrument for diagnosing and monitoring pregnancy following embryo transfer, biochemical methods being complimentary.

**CONCLUSION**: Ultrasonography is now the single most important instrument in in-vitro fertilization programmes and gynaecologists with interest in reproductive medicine need necessarily to obtain a formal training in its use.

**KEY WORDS:** ultrasonography, in-vitro fertilization, infertility, assisted reproduction technology

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## INTRODUCTION

### Context

There is concern over the development of ultrasound practices in Obstetrics and Gynaecology in developing countries especially in Nigeria because of its increasing availability and the need to promote high standards in its practice. There is no doubt that radiologists and radiographers with special training in obstetric and gynaecological ultrasonography could be important personnel in an ultrasound practice within Obstetrics and Gynaecology departments, exactly how much responsibility should be devolved by gynaecologists to these groups has not been defined. To underline the need for formal training in ultrasonography by obstetrician/gynaecologists, the Royal College of Obstetricians and Gynaecologists (RCOG) recently liaised with the Society and College of Radiographers (SCoR) of the UK and the British Medical Ultrasound Society (BMUS) to institute a new competency-based ultrasound training programme that forms a compulsory part of the curriculum for doctors undergoing speciality training in obstetrics and gynaecology.[1] One subspecialty where ultrasound training is particularly required is in assisted reproduction and in-vitro fertilization.

## CONCEPTS

### Infertility

Infertility is defined as the inability of a couple to achieve a pregnancy after one year of unprotected well-timed sexual intercourse [2]. Worldwide infertility affects 10-14 % of couples and one in seven couples in the UK has infertility [3, 4]. Infertility is described as primary when a woman has never achieved a pregnancy before or secondary when the woman had achieved a pregnancy before irrespective of the outcome of the pregnancy. The causes of infertility can be traceable to the woman in about 40-50% of cases, the man in 35%-40 of cases, both in 15-20% and in 10% of cases, the cause of the infertility remains unknown after all investigations [3]. Compared to developed countries, developing countries are thought to have higher prevalences of infertility due to higher prevalences of genital infections including sexually transmitted pelvic inflammatory disease, postpartum infections and post abortal infections in the developing world [5]. However, according to The European Society for Human Reproduction and Embryology, it is thought that more couples in developed countries are developing infertility due to

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factors such as obesity, smoking and delaying of first pregnancy resulting in ovarian ageing [6]. The treatment of infertility can take any form from simple processes such as ovulation induction or antibiotic therapy to more complex and invasive procedures like tubal surgery and assisted reproductive technology (ART).

**Assisted Reproductive Technology**

The international committee for monitoring Assisted Reproductive Technology and the World Health Organization (WHO) define ART as" all treatments and procedures that include the in-vitro handling of both human oocytes and sperm, or embryos, for the purpose of establishing a pregnancy" and state that ART does not include artificial insemination [7]. The WHO estimates, that about 10% of infertile couples in any nation would need assisted reproduction procedure [8]. For the purpose of this review, we adopt the WHO definition of ART. Accordingly, types of assisted reproductive techniques will include in-vitro fertilization and embryo transfer (IVF-ET), gamete intra-fallopian transfer (GIFT), zygote intra-fallopian transfer (ZIFT), intracytoplasmic sperm injection and pre-implantation genetic diagnosis (PGD) [7].

The indications for assisted reproductive techniques have expanded over time since the first attempts of ART for tubal disease and now include conditions such as endometriosis, severe oligozoospermia, azoospermia, unexplained infertility, previous bilateral salpingectomy, bilateral vasectomy etc [9]. However, irrespective of the indication, in-vitro fertilization and embryo transfer can be done to overcome the problem of infertility [9].

**In-vitro Fertilization and Embryo Transfer**

IVF-ET is a sequential procedure. The key steps involve preliminary assessment of the uterine cavity, superovulation induction by hormonal manipulation, aspiration of the mature follicle, sperm washing and treatment (capacitation), incubation of the ova and sperm in a special medium, transfer of fertilized ova into the endometrial cavity for implantation to occur, and monitoring the developing pregnancy. Factors associated with success in IVF-ET include the age of the woman, the duration of infertility and the technique of transfer [10]. Current practices and guidelines on IVF-ET aim at maintaining a high standard of professional and ethical practice at every step of the process [5, 6]. Although guidelines are in agreement as to what steps need to be taken during IVF-ET, some steps in IVF-ET can be undertaken through more than one technique. With advancement in medical technology, refinements in various alternative procedures continue to emerge. The basis for choosing one method in preference to the other should be determined by the availability of evidence of superiority. Alternative methods are available at each of the major steps of IVF-ET such as pre-treatment uterine cavity assessment (hysteroscopy versus Ultrasonography), egg retrieval (laparoscopic guidance versus ultrasound guidance) and embryo transfer (ultrasound guidance versus clinical touch technique [5, 6].

**SOURCES OF LITERATURE**

The information contained in this review were obtained through electronic literature search conducted in major data bases including PubMed, Medline, EMBASE, Scopus, Google scholar, Cochrane database and central register of controlled trials using the following search terms individually and in combination*: infertility, assisted reproduction technology, in-vitro fertilization, embryo transfer, ultrasonography, uterine cavity assessment, follicular monitoring, superovulation induction, follicular tracking, oocyte retrieval, early pregnancy monitoring, ultrasonography in IVF-ET*. All relevant peer-reviewed English language articles and publications were identified, retrieved and reviewed. We also obtained further articles by reviewing the bibliographies of the relevant published documents obtained in the primary search of databases. In addition to these, we consulted the website of the International Federation of Gynaecology and Obstetrics (FIGO) and the European Society for Human Reproduction and Embryology (ESHRE).

**HISTORY OF IVF AND ULTRASONOGRAPHY**

There are many parallel reports of the historical development of in-vitro fertilization in different parts of the world, especially the United Kingdom, the United States and Australia. The first human IVF attempt was reported by Rock and Menkin in the United States in 1944, but the attempt ended with no convincing evidence of fertilization [11]. In 1963, R. G. Edwards began his research on the human egg in the United Kingdom and in 1965 published his first paper on in-vitro maturation of human ovarian oocytes and was able to calculate the moment of human ovulation necessary to plan retrieval of the eggs in a timely fashion [11]. The first pregnancy achieved through in-vitro fertilization of a human oocyte was reported in The Lancet by the Monash University team in 1973, although it lasted only a few days and was essentially a biochemical pregnancy. An ectopic pregnancy following IVF-ET was reported by Steptoe and Edwards in 1976 [12]. These earlier studies preceded the landmark achievement of 1978 by Steptoe and Edwards of first live birth by in-vitro fertilization [12]. It must be said that the development of ART coincided with the development of ultrasonography and ultrasound was to be deployed as a tool for the conduct of key steps in in-vitro fertilization from its early years.

**Ian Donald** is widely credited with the introduction of ultrasonography into Obstetrics and Gynaecology, although ultrasound had been in use for several decades

before his foray into experiments on the use of ultrasonic metal flaw detector to distinguish between fibroid masses and ovarian cysts excised during surgery in 1955 [13]. He later published his results in The Lancet of 7 June 1958 under the title 'Investigation of Abdominal Masses by Pulsed Ultrasound' [13] . Ultrasound in Gynaecology started as a diagnostic tool in the differentiation and assessment of solid, cystic or solid masses in the pelvis and one of the earliest uses of ultrasonography in Gynaecology was in monitoring the development of ovarian follicles during superovulation in in-vitro fertilization [14]. In 1976, Hackloer and Hansmann reported a successful monitoring of ovarian follicular size and number in patients undergoing ovulation induction, using a full bladder as a sonic window [13]. Ultrasound monitoring was formally introduced into ovulation induction programmes in 1979 and by 1980-1982, a number of important reports had attested to the usefulness of abdominal ultrasound in the assessment of follicular development and ovulation [13]. Colm O'Herlihy and Australian co-workers published a paper on important follicular size criterion and protocols for ovulation induction [13].

Transvaginal scans were introduced in the mid-1980s and the addition of endometrial evaluation using transvaginal scan enhanced diagnostic accuracies in the management of ovulation cycles [13]. Interventional sonography in Gynaecology dates back to the early 1970s when Hans Henrik Holm described percutaneous puncture of ovarian tumors in 1972 [15]. In 1982, David Graham and Roger Sanders revisited the idea of transvaginal aspiration of pelvic masses under trans abdominal ultrasound guidance [16]. There was necessity to develop similar techniques for the retrieval of ovarian follicles in in-vitro fertilization programmes which had hitherto been achieved only through laparoscopy. Susan Lenz and JG Lauritsen described percutaneous trans abdominal- transvesical aspiration of ovarian follicles in 1982 and 1983 which showed for the first time that ovum retrieval could be performed as an ultrasound guided outpatient procedure [17, 18]. Transvaginal ovum retrieval under trans abdominal guidance was described by Gleicher in Chicago in1982 and in 1984 by Dellenbach and co-workers in France [19-21]. The advantage of this technique is that ovaries are more accessible and the procedure is safer and relatively pain-free. More importantly, the procedure is repeatable on an outpatient basis and it dramatically cut down the cost of IVF procedures. In 1985, Kemeter and Feichtinger described the use of transvaginal aspiration of follicles under transvaginal ultrasound guidance [21-23]. Stuart Campbell's group at Kings College London was among one of the earliest pioneering groups to have set up an out-patient oocyte retrieval service in IVF [13]. Color Doppler was introduced in the mid-1980s about the same time as transvaginal probe. Tom Bourne and colleagues were among the first to document the usefulness of peri-ovulatory blood flow in ovarian and uterine arteries in the management of assisted reproductive cycles [13]. With improvements in ultrasonic and computer technology, work on three-dimensional visualization began to appear in the early 1980's. In gynecological applications, Davor Jurkovic at Kings College London convincingly demonstrated in 1995 the usefulness of 3-D ultrasound in accurately differentiating uterine anomalies such as bicornuate uterus and septate uteri [13]. Similarly the assessment of the endometrial cavity with 3-D sonohysterography and characterization of endometrial masses, adhesions, tubo-ovarian masses, hydrosalpinges, ovarian cysts, small intraovarian tumours and mullerian anomalies have all been quickly and convincingly demonstrated [13].

## THE ROLE OF ULTRASONOGRAPHY PRE-TREATMENTASSESSMENT

Pre-treatment assessment of women prior to IVF involves assessment of ovarian reserve, identification of adnexal pathology and uterine pathology especially uterine cavity with a view to identifying any factor that can impair the success of IVF. Ovarian reserve is defined as the number and quality of the remaining ovarian follicular pool at any given time [24, 25]. The aim of assessing ovarian reserve is to identify those women likely to respond poorly to ovulation induction, those who have a low chance of success, those who are more likely to have their treatment cycle cancelled and those prone to ovarian hyperstimulation, which is associated with significant morbidity and even mortality [25]. The assessment of ovarian reserve is often made indirectly through serum measurement of follicle-stimulating hormone (FSH) during the early follicular phase of the cycle or the endocrine factors produced by the developing follicles, including estradiol, inhibin B, and anti-Mullerian hormone (AMH) [25-27]. Ovarian reserve may also be assessed directly using ultrasound, which can be used to quantify the total number of antral follicles, mean ovarian volume and ovarian vascularity [28-31]. All of these tests have different sensitivities, specificities and predictive values, but there is a lack of consensus as to the best single test or combination of tests [32]. Although, basal FSH is the most widely used marker of ovarian reserve worldwide, ultrasound assessment strongly complements this by directly demonstrating the availability of antral follicles [33].

Ultrasound is the very useful for identifying pelvic pathologies associated with aberrant and compromised outcomes during IVF, including polycystic ovarian disease, moderate and severe degrees of endometriosis (often characterised by rectovaginal disease and the presence of ovarian endometrioma), fibroids, endometrial polyps and tubal disease [34-40]. Functional cysts can interfere with treatment and can enlarge

following down regulation treatment or secrete oestrogen and progesterone, which can prevent or delay successful pituitary desensitisation [34]. Pre-treatment ultrasound may also reveal complex multiloculated cysts containing thick-walled septae suggestive of malignancy, which require removal [34]. The diagnosis of hydrosalpinx can generally be made using transvaginal ultrasound with a high degree of confidence [35].

Pre-treatment uterine cavity assessment is based on the belief that a healthy endometrium is a pre-requisite for implantation to occur following natural fertilization or artificial fertilization and embryo transfer [34]. Diseases and conditions interfering with endometrial integrity such as submucous fibroids, endometrial polyps, hormonal imbalance, endometritis and endometrial fibrosis with synechia can all impair implantation [41-47].

The methods applied for uterine cavity assessment include ultrasonography, hysterocontrastsonography (HyCoSy), saline hysterosonography, hysterosalpingography and hysteroscopy. Hysterosalpingography has poor reproducibility, is expensive, involves ionizing radiation and can be quite painful and is therefore not considered first choice for assessing the uterine cavity [48]. Whereas hysteroscopy allows inspection of the uterine cavity under direct vision, it is thought to be invasive and expensive and may necessitate the use of anaesthesia [49]. Hysteroscopy however has the advantage of being both diagnostic and potentially therapeutic as lesions such as polyps, adhesive bands and submucous fibroids can be excised under direct vision [49]. In addition, hysteroscopy is thought to have a better sensitivity, specificity, negative and positive predictive values in distinguishing between normal and abnormal endometrium [14]. Transvaginal ultrasonography is less invasive and allows for measurement of endometrial thickness which is a valuable index for estimating the degree of luteinization of the endometrium [50-52]. Saline hysterosonography is a modification of transvaginal scanning in which saline solution is instilled into the uterine cavity during transvaginal ultrasound scanning to help delineate lesions such as polyps. It allows for easier diagnosis of polyps and submucous fibroids than plain ultrasound scan [14].

It would therefore appear that with respect to pretreatment uterine cavity assessment, there appears to be little clarity with respect to the preferable technique [42], [53]. No randomized controlled studies comparing hysteroscopy versus ultrasonography is available. Current practice appears to favour a choice between ultrasound based assessments (transvaginal scan, saline hysterosonography and hysterocontrastsonography) and hysteroscopy [34].

## FOLLICULAR MONITORING

Monitoring follicular development during controlled ovarian stimulation can be done biochemically by assay of serum estrogen or by ultrasound examination of increasing sizes of developing follicles- a process often referred to as follicular tracking [9, 41]. The monitoring process is intended to enable the physician to choose the most suitable protocol, to obtain best possible outcome, and to avoid complications [9, 34]. Monitoring therefore allows for the determination of the optimal time for the administration of human chorionic gonadotropin to mimic the luteinizing hormone surge of natural cycles in order to minimize the incidence and severity of ovarian hyperstimulation syndrome (OHSS) [34]. The first blood test and ultrasound scan are usually done approximately 8 days after starting the FSH injections and the combined results are used to decide the ideal time for egg retrieval [9]. Administration of human chorionic gonadotrophin for follicular maturation is done at ovarian follicular diameter of 18-20mm and endometrial thickness of 8-10mm [9]. Aspiration of oocytes is done about 37 hours following the administration of the human chorionic gonadotropin [9].

A recent Cochrane review found only two randomized controlled trials to determine the effect of monitoring stimulated cycles with estrogen assay plus ultrasonography versus ultrasonography alone [54, 55]. The review concluded that there was no evidence that monitoring with estrogen and transvaginal ultrasound was superior to ultrasound alone in terms of pregnancy rates and live births but recommended that cycle monitoring by transvaginal ultrasound plus serum estradiol may need to be retained as a precautionary good practice point. However, the current practice is tilting towards the use of ultrasound scan alone in follicular and endometrial thickness tracking as against its combination with serum estradiol monitoring [56, 57].

## OOCYTE RETRIEVAL

With respect to oocytes retrieval, no recent studies have been done and evidence available dates back to the 1980s.There are no recent randomized controlled studies comparing laparoscopy with ultrasound guided aspiration. A number of comparative observational studies done in the late eighties showed that there was no significant differences between the outcomes of IVF-ET with laparoscopic oocytes retrieval compared to oocytes aspiration under ultrasound guidance either transvaginally, trans urethrally or transvesically. Lewin and colleagues early in 1986 randomized 120 women to ultrasound guided aspiration and laparoscopy and found similar clinical pregnancy rates [58]. In a study in Norfolk, England, Flood and co-workers also showed no statistically significant differences with respect to total number of follicles aspirated per cycle, preovulatory oocytes aspirated per cycle, number of concepti of

preovulatory origin transferred per cycle between laparoscopic and ultrasound-guided procedures [59]. They concluded that ultrasound guided aspiration was comparable to laparoscopy and should be the method of choice. In a study in Yugoslavia, Tomazevic and colleagues compared 105 cycles of ultrasound guided retrieval with 218 cycles of laparoscopic retrieval and also concluded that ultrasound retrieval was simpler, less painful and should be preferred [60]. Several comparative studies done in the late 1980s reached similar conclusions [61-65].

Despite the lack of evidence from randomized controlled studies, there is sufficient evidence from comparative non-randomized studies to show that ultrasound guided oocyte retrieval is easier, less technically demanding and cheaper than laparoscopic approach [34]. The possibility of conducting the procedure under local anaesthesia has made ultrasound guided retrieval more popular among IVF centres across the world.

**EMBRYO TRANSFER**

During embryo transfer, embryos are replaced into the uterine cavity, through a trans-cervical transfer catheter, at the 4-8-cell stage (between days 2 to 5) of development [9]. Traditionally, the "clinical touch" method has been used to guide placement of the transfer catheter to within 10 mm from the uterine fundus prior to injection of the embryos [9]. This method is essentially "blind" and relies on the clinician's tactile senses to judge when the transfer catheter is in the correct position [10]. Some clinicians transfer the embryos at a fixed distance from the external os (6cm), however this may not take into account variation in cervical length or uterine size, or position [9,34].

Numerous methods to improve the technique of embryo transfer have been studied to try and improve pregnancy rates, including changing the type of catheter used, performing a trial or "mock" transfer before the actual procedure, encouraging bed rest following embryo transfer [66,67].

Embryo transfer has been the subject of many randomized controlled trials and at least four systematic reviews between 2003 and 2012 including two Cochrane reviews in 2007 and 2010 [68-78]. Results have been conflicting with respect to the relative advantage of ultrasound-guided transfer method over the clinical touch method of embryo transfer. In the most recent Cochrane Systematic Review update in which 17 randomized trials were selected out of potential 52, the authors found that "although clinical pregnancies and ongoing pregnancies were increased for the ultrasound guided group compared with the clinical touch group; there was no evidence of a difference between ultrasound guided embryo transfer and clinical touch for the outcome of live birth. The risks of harm using ultrasound guided transfer, including miscarriage, ectopic pregnancies and multiple pregnancies, are no different to when clinical judgment is used."

**EARLY PREGNANCY MONITORING**

Following a successful embryo transfer, the developing pregnancy needs to be monitored to determine its viability and eventual location [9]. Two methods are used for this monitoring. These are serum B-hCG and ultrasonography [9]. Biochemical pregnancy can be detected by a positive pregnancy test as early as 4 weeks following implantation, although a false positive test at this stage may result from the gonadotropin administered as luteal support prior to follicular aspiration [9]. With respect to biochemical testing, serum pregnancy test is preferred because it is more sensitive than urine pregnancy test. However, a negative serum pregnancy test at 2 weeks after embryo transfer does not completely exclude a pregnancy. Bjercke and co-workers posited that single serum β hCG on 12$^{th}$ day after embryo transfer has a comparable result with serial serum β hCG on the diagnosis of pregnancy [79].

Ultrasonography can detect a beating embryonic heart as early as 5weeks plus 2 days [9]. The detection of a visible heart beat on ultrasound is an absolute sign of an ongoing pregnancy. With the advent of transvaginal ultrasonography (TVUS), the diagnosis of pregnancy can be made even earlier than is feasible with trans-abdominal ultrasonography (TAUS) [9]. Mid-luteal endometrial thickness of = 11 mm was found to be a good prognostic factor for detecting early pregnancy by Sholan and colleagues [58]. TVUS is preferred to TAUS during early pregnancy for reasons including the fact that it can detect signs of intrauterine pregnancy approximately 1 week earlier than TAUS; patients do not require to have a full bladder and are not required to endure uncomfortable pressure on the abdominal wall from the abdominal probe; pelvic organs are far better imaged by TVUS in obese patients [9, 80]. The major disadvantage of TVUS is that some patients are anxious about the transvaginal probe and may object to its insertion [80].

The earliest structure identified with TVUS is the gestational sac (GS) at 4-5 weeks gestation and the GS grows at a rate of 1 mm/d in early gestation [80]. By 5.5-6 weeks' gestation, a double-decidual sign can be seen, which is the Gestational Sac surrounded by the thickened decidua [80]. The ultrasound scan is also used to detect the number of gestation and calculate age of pregnancy [80]. The foetal cardiac motion is detected at 5-6 weeks [80]. All these facts make ultrasonography unarguably the most useful and precise tool for early pregnancy detection and subsequent monitoring.

**SUMMARY**

Ultrasonography is a key instrument in any in-vitro fertilization programme. It is the most versatile single instrument for pre-treatment assessment. Its ability to measure endometrial thickness in addition to detecting uterine masses gives it an edge over laparoscopy/hysteroscopy, although the ability of the later to offer curative procedures suggest that facilities for both ultrasonography and hysteroscopy are needed for the initial assessment. Ultrasonography is the method of choice for monitoring the growth and development of ovarian follicles during controlled ovarian stimulation. Ultrasound has also become the key instrument for egg retrieval. Randomized controlled trials show that ultrasound-guided transfer techniques result in higher on-going and clinical pregnancy rates compared to the clinical touch technique**.** Unquestionably, ultrasonography is the key instrument for diagnosing and monitoring pregnancy following embryo transfer.

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