Introduction

For the couple having trouble to achieve pregnancy, the options and opportunities for assistance have never been brighter than nowadays. Options such as controlled ovarian hyperstimulation, in vitro fertilization and intracytopasmic sperm injection have been developed over the past five decades and provided hope for couples who previously would have been considered infertile. In vitro fertilization and intracytoplasmic sperm injection represent a coalescence of advances in physiology, endocrinology, pharmacology, technology and clinical care. In vitro fertilization has assisted well over one million couples in their efforts to start or build a family, and the demand for such services continues to increase ***(Feinberg et al., 2005).***

Despite the fact that top-quality embryos may be available for transfer during in-vitro fertilization (IVF) intracytoplasmic sperm (ICSI) cycles, only a maximum of one third of the embryos transferred finally implant ***(Jarvela et al., 2005).***

Many factors contribute to obtaining a successful pregnancy. Understanding these factors can help in counseling patients regarding their chances of success and offering interventions for improvements and endometrial differentiation factors for both embryologic development and endometrial differentiation. The possible predictors of pregnancy that have been evaluated are endometrial thickness endometrial pattern and endometrial blood flow ***(McWilliams and Frattavelli, 2007).***

The endometrium thickens during the proliferative phase of the menstrual cycle in response to estrogen secretion by mature follicles. The endometrium provides a site for attachment and is the source of nourishment for an implanting embryo during its first few weeks until the placenta develops. While there is little doubt that a physiologically thickened endometrium is critical to a successful implantation and pregnancy, controversy exists regarding the clinical significance of variation in endometrial thickness observed among patients undergoing assisted reproduction ***(Richer et al., 2007).***

Endometrial thickness has been evaluated as a possible predictor of pregnancy in multiple studies ***(Rashidi et al., 2007).***

Several studies suggested a correlation between endometrial thickness and receptivity, reporting significantly greater endometrial thickness for pregnancy cycle versus non-pregnancy cycles either autologous or donor-oocyte IVF-ET, or significantly higher pregnancy rates with thicker versus thinner endometrial linings in autologous ***(Zhang et al., 2005)*** or donor-occyte IVF-ET cycles.

A good blood supply toward the endometrium is usually considered as an essential requirement for implantation. Three-dimensional (3D) ultrasound has become an available technology any desired plane through an organ can be obtained with 3D ultrasound, a volume at a region of interest can be acquired and stored. This volume can be further analyzed in several ways, such as navigation, multiplanar display or volume calculation. This

technique also allows a whole assessment of the endometrial and subendometrial visualization ***(Alcazar, 2006).***

Raine-Fenning et al. (2004) proposed that the degree of change in endometrial perfusion from the late follicular phase through to the early luteal phase is a more important determinant of endometrial receptivity. Three dimensional power Doppler ultrasound is useful for evaluating endometrial and subendometrial neovascularization in intrauterine insemination cycles.

Merce et al. (2008) concluded that 3D power doppler indices are statistically significant in predicting the pregnancy outcome (a clinical study). However, ***Ernest et al. (2006)*** concluded that endometrial and subendometrial blood flows measured by 3D power doppler ultrasound were not a good predictor of pregnancy (prospective observational study). Owing to the great clinical significance of this subject, we decided to study the role of endometrial thickness and endometrial and subendometrial blood flow by 3D power Doppler ultrasound to predict IVF outcomes.

Aim of the Work

This is a case study designed to evaluate the relation between endometrial thickness and vascularity measured by 3D ultrasound, Doppler and pregnancy outcome in IVF cycles.

The Art Of Ivf

Definition and types of ART:

Assisted reproductive technology (ART) refers to all techniques involving direct retrieval of oocytes from the ovary. The first procedure is in-vitro fertilization, but there is an ever-increasing list of technologies ***(Bing and Ouellette, 2009) (Table 1).***

Table (1): Types of ART procedures:

IVF	In-vitro fertilization: Extraction of oocytes, fertilization laboratory and trans-cervical transfer of embryos into the uterus.
GIFT	Gamete intra-Fallopian Transfer: The placement of oocytes and sperm into the fallopian tube.
ZIFT	Zygote intra-Fallopian: The placement of fertilized oocytes into the Fallopian tube.
TET	Tubal embryo transfer: The placement of cleaving embryos into the Fallopian tube.
POST	Peritoneal oocyte and sperm transfer: The placement of oocytes and sperm into the pelvic cavity.
In addition, techniques of sperm retrieval and sperm injection are now part of the assisted reproductive technology	
ICSI	Intracytoplasmic sperm injection (of a single spermatozoon)
TESE	Testicular sperm extraction.
MESA	Microsurgical epididymal sperm aspiration

(Gosden et al., 2003)

Intracytoplasmic Sperm Injection (ICSI):

Intracytoplasmic sperm injection (ICSI) is a technique in which a single spermatozoon is inserted directly into the oocyte using micromanipulation. This approach bypasses the requisite penetration steps, including capacitation followed by the acrosome reaction, where in the sperm enters the egg during natural fertilization ***(Bavister, 2002)***.

Extremely low sperm counts, impaired motility, and abnormal morphology represent the main causes of failed fertilization in conventional IVF. Today, ICSI is the ultimate option to treat these cases of severe male-factor infertility. One single viable spermatozoon, preferably of good morphology, is selected by the embryologist and injected in each oocyte available. ICSI is based on micromanipulation of oocytes and spermatozoa. Initially, partial zona dissection (PZD) was established to facilitate sperm penetration ***(Cohen et al., 1991).*** The barrier to fertilization represented by the zona pellucid was disrupted mechanically so that the inseminated sperm cells obtained direct access to sub zonal insemination (SUZI) represented the next step in micromanipulation techniques. SUZI enabled the immediate delivery of several motile sperm cells into the perivitelline space by means of an injection pipette. ICSI is even more invasive because a single spermatozoon is directly injected into the ooplasma, thereby crossing not only the zona pellucida but also the oolemma ***(Palermo et al., 1992)***.

ICSI had been first used successfully to obtain live offspring in rabbits and cattle ***(Iritani, 1991)***, and a preclinical evaluation was reported by the Norfolk group ***(Lanzendorf, 1988)***. The first human pregnancies and births resulting from this novel assisted-fertilization procedure were reported in 1992 ***(Palermo et al., 1992)***. Thereafter, ICSI was revealed to be superior to SUZI in terms of oocyte fertilization rate, number of embryos produced, and embryo implantation rate ***(Palermo et al., 1993)***. As a result, ICSI has been used successfully worldwide to treat infertility due to severe oligo-astheno-teratozoospermia, or azoospermia caused by impaired testicular function or obstructed excretory ducts ***(Anderson et al., 2005).***

Since the first publication describing the ICSI procedure, minor modifications contributed to reduced rates of oocyte degeneration, oocyte activation (one-pronuclear), and abnormal fertilization (three-pronuclear). Hyaluronidase may be responsible for oocyte activation; therefore, the concentration used during oocyte denudation and the exposure time of oocytes to the enzyme have been reduced ***(Van de Velde et al., 1998)***. The moment of denudation relative to oocyte pick-up (immediately or four hours later) does not influence the ICSI results ***(Van de Velde et al., 1998)***. The orientation of the polar body during injection does, however, influence embryo quality ***(Nagy et al., 1995)***. Motile sperm cells are selected and immobilized prior to injection ***(Palermo et al., 1996)***. Cytoplasm aspiration to ensure oolemma rupture is critical to the success of the ICSI procedure because the method of

rupture has been correlated with oocyte degeneration ***(Nagy et al., 1995)***. Furthermore, the morphology of the injected spermatozoon is related to the fertilization outcome of the procedure as well as to the pregnancy outcome ***(De Vos et al., 2003) (Figure 1).***

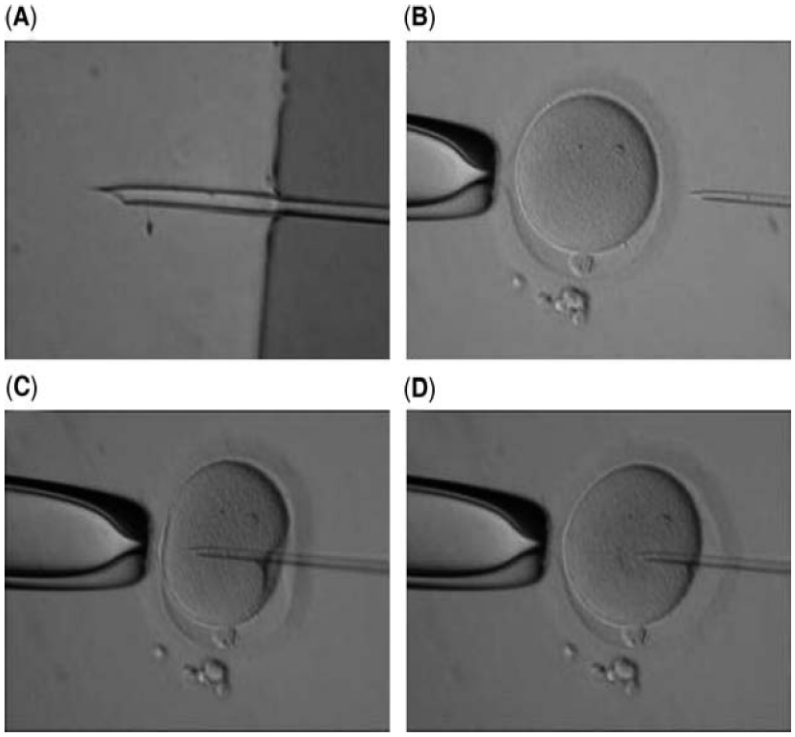

Figure (1): Intracytoplasmic sperm injection procedure. (A) A single motile spermatozoon is selected and immobilized by pressing its tail between the micro needle and the bottom of the dish. The sperm cell is then aspirated tail-first into the injection pipette. (B) Using the holding pipette, the mature oocyte is fixed with the polar body at the 6 o0clock position. The sperm cell is brought to the tip of the injection pipette. (C) The injection pipette is introduced at the 3 o0clock position and rupture of the oolemma is ascertained by slight suction. Then the sperm cell is delivered into the oocyte with a minimal volume of medium; afterwards, the pipette can be carefully withdrawn. (D) A single sperm cell can be appreciated in the center of the ooplasma ***(Cohen et al., 2007).***

Indications for ICSI:

Before the era of ICSI, attempts were made to modify and refine conventional IVF to achieve increased rates of conception in cases of male-factor infertility. Today, ICSI has clearly overshadowed the use of modified IVF procedures (including high insemination concentration) for the treatment of severe malefactor infertility. ICSI requires only one spermatozoon with a functional genome and centrosome for the fertilization of each oocyte ***(Cohen et al., 2007).***

Indications for ICSI are not restricted to impaired morphology of the spermatozoa, but also include low sperm counts and impaired kinetic quality of the sperm cells. ICSI can also be used with spermatozoa from the epididymis or testis when there is an obstruction in the excretory ducts. Azoospermia caused by testicular failure can be treated by ICSI if enough spermatozoa can be retrieved in testicular tissue samples. **Table 2** gives an overview of the current indications for ICSI.

Table (2): Current Indications for Intracytoplasmic Sperm Injection ***(Cohen et al., 2007)***

- **Ejaculated spermatozoa**
- **Oligozoospermia**
- **Asthenozoospermia (caveat for 100% immotile spermatozoa)**
- **Teratozoospermia (4% normal morphology using strict criteria caveat for globozoospermia)**
- **High titers of antisperm antibodies**
- **Repeated fertilization failure after conventional IVF.**
- **Autoconsered frozen sperm from cancer patients in remission.**
- **Ejaculatory disorders (e.g., electroejaculation, retrograde ejaculation)**
- **Epididymal spermatozoa**
- **Congenital bilateral absence of the vas deferens.**
- **Young syndrome**
- **Failed vasovasostomy**
- **Obstruction of both ejaculatory ducts.**
- **Testicular spermatozoa**
- **All indications for epididymal sperm**
- **Failure of epididymal sperm recovery because of fibroses**
- **Azoospermia caused by testicular failure (maturation arrest, germ-cell aplasia)**
- **Necrozoospermia.**

Highlight on technological advances shaping the field of ART:

A) Hormonal management of IVF cycles and oocyte retrieval:

1-Controlled ovarian stimulation:

At the time of the birth of Louise Brown, IVF attempts were primarily performed using monitored natural ovulatory cycles ***(Brinsden, 2009).*** Gonadotropins had been used in the context of infertility treatment for conditions such as

hypogonadotropic hypogonadism since the 1930s, when the medications had been extracted from animal sources ***(Bassett and Driebergen, 2005).*** Gonadotropins from the human pituitary and the urine of menopausal women had been established as a safer source by the middle of the 20th century ***(Cha et al., 1991).*** The advent of controlled ovarian hyper stimulation (COH) using urinary derived gonadotropins was pioneered first by Howard and Georgeanna Jones in the United States and then by Alan Trounson in Australia ***(Brinsden, 2009).***At the same time others achieved success in ovarian follicular stimulation using clomiphine citrate ***(Hoult et al., 1981).***

Many of today's IVF protocols utilize inject able gonadotropins, containing follicle stimulation hormone (FSH) and luteinizing hormone (LH), followed by an injection of human chorionic gonadotropin (hCG). These protocols expose the oocytes housed within the ovary to supraphysiologic levels of hormones that promote follicular development and oocyte maturation. This development made it possible to time oocyte retrieval more predictably and to obtain multiple oocytes (often 10–20) from a single IVF cycle, rather than one or two oocytes, as occurs in a natural cycle. Retrieval of multiple oocytes enhances the patient's ability to achieve pregnancy in fewer cycles and in less time ***(Jones, 2008).*** Furthermore, excess embryos not selected for uterine transfer may be cryopreserved, thawed, and transferred in subsequent cycles, increasing the overall pregnancy rate associated with any given IVF cycle ***(Macklon et al., 2006)***. Therefore, gonadotropin therapy

usered in a new era for the field. The following years would witness modifications of stimulation protocols ***(Brinsden, 2009).*** Ovarian stimulation still remains a fundamental tool in managing IVF treatment cycles, now with a variety of regimen options to choose from.

2-Ovulation induction:

Despite the ability to induce the simultaneous development of multiple follicles successfully, significant challenges remained. Ovulation occurs 10 h, plus or minus 5 hours, after the time of the LH surge peak ***(Garcia et al., 1981)***. Testart et al. in France developed a plasma luteinizing hormone LH assay which could detect the initial LH rise, allowing accurate prediction of the ideal time for the retrieval of oocytes ***(Testart et al., 1981).***

Early IVF cycles relied on determining the LH surge to time surgical retrieval of oocytes ***(Jones, 2003).*** This approach required evaluation of LH hormone levels up to four times daily; surgical staff and facilities needed to be available around the clock ***(Trounson et al., 1981).*** Needless to say, this protocol required significant resources and also resulted in considerable inconveniences for IVF patients.

The gonadotropin-releasing hormone agonist (GnRHa) is a small decapeptide whose continuous administration results in an initial increase but then a precipitous fall in endogenous sex steroid levels. In the 1980s, GnRH agonists were first used to suppress the endogenous LH surge in IVF cycles ***(Fleming et***

al., 1982). This technique allowed physicians to recruit more follicles, with more aggressive controlled ovarian stimulation protocols, to perform oocyte retrieval procedures at more predictable times, and to minimize the number of follicles lost due to premature ovulation. More recently, GnRH antagonists have been used to inhibit the LH surge in IVF cycles. GnRH antagonists took almost 30 years of research and development to produce a clinically efficacious and safe medication. Unlike GnRH agonists, GnRH antagonists have an immediate suppressive affect on the hypothalamus. There is not a difference in pregnancy rates, birth rates, and neonatal outcomes between the use of GnRH agonists and antagonists in IVF cycles ***(Macklon et al., 2006).***

3-Ultrasound guided oocyte retrieval

Another limiting factor in early IVF procedures was the reliance on laparoscopy to obtain follicles ***(Jones, 2003).*** In the early 1970s the concept that follicular growth could be followed via ultrasound monitoring was introduced ***(Hackeloer, 1977).*** Lenz and Lauritsen described trans-abdominal oocyte aspiration using an ultrasound-guided needle ***(Lenz and Lauritsen, 1982).*** In the early 1980s, the practice of retrieving oocytes vaginally from ovarian follicles under ultrasound guidance was described ***(Cohen et al., 2005).*** This minimally invasive approach to retrieving oocyte has become the gold standard for performing IVF procedures **(Figure2)**.

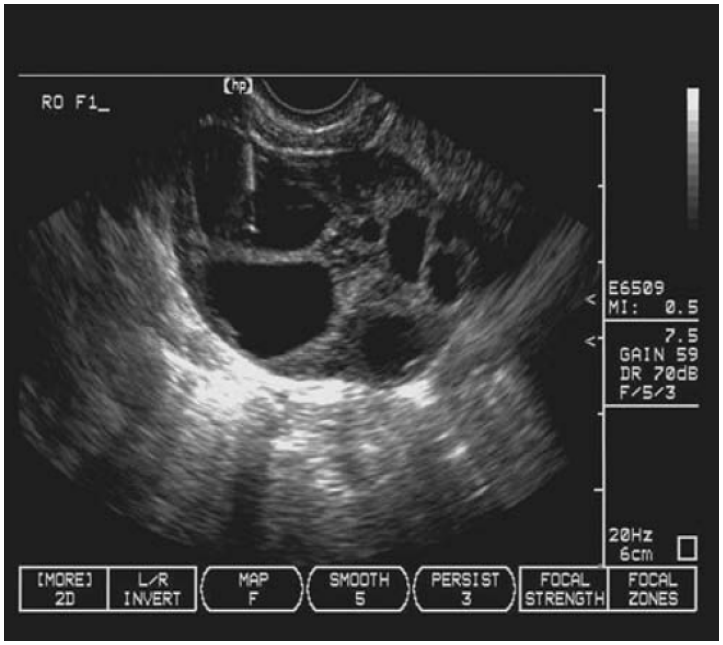

Figure (2): Ultrasound image taken during oocyte retrieval. The highly echogenic band around the distal end of the needle and the tip of the follicle aspiration needle are visualized in the superior-most follicle. The needle is maneuvered within the ovary to aspirate all follicles ***(Abington Reproductive Medicine, Gladstone, New Hersey, 2002).***

B) Embryo cryopreservation:

The term cryopreservation refers to the storage of viable cells at low temperature, normally at 196 °C in liquid nitrogen. Cryopreservation in assisted reproduction is primarily used for preserving surplus embryos after IVF, gamete or embryo donation, postponement of fertility or pregnancy, and fertility preservation ***(Porcu et al., 2008).*** Successful cryopreservation of human reproductive tissues was initially achieved in 1954 with spermatozoa ***(Bunge et al., 1954),*** and then with embryos ***(Trounson and Mohr, 1983)*** and with oocytes in 1986 ***(Chen, 1986).*** Cryopreservation of human sperm and embryos has now become an integral part of IVF treatment procedures.

Techniques used for cryopreservation of human reproductive tissues must ensure high survival and viability after thawing. However, the process of cryopreservation, through the formation of intracellular ice crystal spikes and other mechanisms, may damage embryos ***(Granne et al., 2008).*** The two major technologies currently used to accomplish cryopreservation are slow-freezing and vitrification protocols. Slow-freezing protocols involve a programmed cooling process coupled with low concentrations of cryopretectants aimed at preventing ice crystal formation ***(Loutradi et al., 2008)***. Although the technique has been shown to have inconsistent results, slow-freezing has been the most widely used method for freezing human gametes and embryos for years ***(Kolibianakis et al., 2009).*** Vitrification is accomplished using high concentrations of cryoprotectants and extremely rapid cooling rates (15,000– 30,000 °C per minute), resulting in the solidification of water into a glass-like state without ice crystal formation. Recent data suggest that vitrification may result in a better clinical outcome compared to slow-freezing protocols. However, concerns about toxicity and the risk of contamination need to be addressed in the future ***(Loutradi et al., 2008).***

Additionally, several recent trials have described successful pregnancies following cryopreservation, through vitrification, of retrieved non-fertilized oocytes ***(Noyes et al., 2010).*** The ability to freeze oocytes successfully in this manner has significant implications for the application of this technology in the future.