Introduction

ICSI revolutionized assisted reproductive technology. It has provided solutions for male factor infertility and for female factor infertility and has improved fertilization rates with oocytes that have undergone in vitro maturation ***(Goldberg et al., 2007).***

The visualization of the antral follicles which are the early Graafian follicles that are 2-10mm in diameter, round to oval, echo lucent fluid filled structures, easily imaged by ultrasound, waiting for further recruitment and stimulation by gonadotropins ***(Gregory et al., 2004),*** by transvaginal ultrasound has attracted the interest as a test of ovarian reserve. ***(Van Rooij et al., 2005).*** The transvaginal ultrasound allows the visualization of the pelvic structures, including assessment of their size and morphology. Also, it is used for assessing the ovarian function including the ovarian volume and antral follicular count ***(Zaidi et al., 2007).***

Many of the systematic reviews give the superiority of the antral follicular count over the basal FSH level in the prediction of the poor ovarian response, and the counting of the antral follicles by the high resolution ultrasound has shown to be predictive of ovarian response ***(Hendricks et al., 2005).***

A gradual decrease in the sonographically detected antral follicles has been found with advancing age. There is relation between the antral follicle count and the ovarian response

(Kwee et al., 2007). It is clear that with chronological aging, both oocyte quantity and quality decline, but it isn't clear, that these three parameters: chronological age, oocyte number, and oocyte health, decline in parallel ***(Rosen et al., 2011).***

The Antral Follicullar Count (AFC) should not be used to exclude women from IVF treatment. However, it is a very useful instrument for patient counseling and determining appropriate treatment protocols and gonadotropin dosing in IVF/ICSI cycles ***(Maseelall et al., 2009),*** and predicts ovarian response and the risk of cycle cancelation ***(Styer and Toth, 2011).*** Antral follicular count predicts ovarian response, not embryo or pregnancy quality ***(Hsu et al., 2011).***

Ovarian volume increases from 0.7ml at 10 years to 5.8ml at 17 years of age. It has been suggested that, there are no major changes in the ovarian volume during the reproductive life till the premenopausal period. There is dramatic drop in ovarian volume, in women over 40 years old, which is not related to the parity ***(Kwee et al., 2007).***

Many studies have been carried out to understand the effect of **endometrial thickness** on the reproductive outcome while the factors affecting the pattern itself are still unknown, the mean endometrial thickness was 7.2±1.8 mm. The endometrium was thinner in older patients compared with younger ones. There is a possibility of higher chance of pregnancy in endometrial thickness ($6 < \text{Endometrial Thickness} \leq 10$ mm) regardless the age of the patient ***(Habibzadeh et al., 2011).***

Several studies have been published, investigating the endometrial thickness in the luteal phase of the conception cycle, and found that the endometrial thickness vary according to patients' parity, and status of pregnancy. The Endometrium seems to be thicker in conception compared to non-conception cycles, and in normal pregnancy compared to abnormal pregnancy ***(Dmitrovic and Simunic, 2009).***

Uterine volume could be affected by adenomyosis or fibroid, but the ICSI outcome could not be affected, as adenomyosis had no adverse effect on IVF/ICSI outcome in infertile women with proven endometriosis who were pretreated with long-term GnRH-agonist and the studies don't support the pre-IVF myomectomy in patient with small to moderate uterine fibroids, regardless their site ***(Mijatovic et al., 2010) (Vimercati et al., 2007).***

Transvaginal ultrasound will detect an intrauterine pregnancy, as a visible gestational sac, as early as four weeks and 3-4 days after the last menstrual period ***(Dmitrovic and Simunic, 2009).*** There are no differences observed between those who miscarried and those who did not in gestational age, endometrial thickness ***(Zahov et al., 2007).***

Aim of the Work

This work aims to assess the accuracy of the ultrasonographic parameters namely

Antral follicular count, ovarian volume in addition to endometrial thickness as

Predictors of the occurrence of clinical pregnancy in Patients undergoing ICSI.

Research Hypothesis:

- **Research Question:** Will the measurement of the ultra-sonographic parameters namely antral follicular count, ovarian volume in addition to endometrial thickness and provide us with better predictive value for ICSI outcome?
- **Research Alternative Hypothesis:** There is a high chance of pregnancy with thick endometrium rather than thin endometrium during the menstrual cycle. The antral follicular count is superior to the FSH level in prediction of the ovarian response.
- **Objectives:** To assess accuracy of the ultrasonographic parameters namely antral follicular count, ovarian volume in addition to endometrial thickness as better predictors of the occurrence of clinical pregnancy in patients coming for ICSI.
- **Medical application:** If we are able to prove the accuracy and the sensitivity of the ultrasonographic parameters in combination as better predictors for ICSI outcome, we can detect the infertile patients coming for ICSI with high chances of pregnancy without costing them a lot of investigations and invasive procedures.

Chapter (1):

ICSI is The Solution

Infertility is defined as a lack of conception in a couple that has been having unprotected intercourse, one to three times per week, for one year. About 25 percent of American couples experience an episode of infertility at some point during their reproductive lives ***(Fridley et al., 2003).***

Infertility is an increasing problem in the U.S. Lack of conception is related to female factor in 50% of the cases and to male factor in 30%, and a combined male/female factor or unknown causes in 20% of cases ***(Karpman et al., 2005).***

Infertility and sterility will be the third most serious disease worldwide in this century, estimated by The World Health Organization ***(Shan Juan, 2010).***

Female factors include, ovulatory problems; one of the most basic requirement for pregnancy to occur is a healthy egg, which may not be present in case of hypothyroidism,polycystic ovary syndrome (PCOS), high prolactin levels, obesity, and having a very low body weight. Fallopian Tube blockage either due to pelvic adhesions or infection. Uterine cavity abnormalities**,** such as fibroids or polyp which may interfere with the implantation ***(Rosedale et al., 2003).***

Male factor includes; the presence of small amount of sperms or large amount of abnormal forms, which may interfere with fertilization ***(Blaine et al., 2003).***

The first successful in-vitro fertilization(IVF) and embryo transfer(ET) treatment described by Edwards and Steptoe in 1978**,** this led to much new clinical and research oriented work in the field of endocrine infertility ***(Healy et al., 1994).***

Several procedures of assisted fertilization based on micromanipulation of oocytes and spermatozoa have been established after IVF. The evolution of these techniques started with partial zonal dissection (PZD), followed by sub-zonal insemination (SUZI) and finally led to the procedure of intra-cytoplasmic sperm injection (ICSI). ICSI represents the injection of a single spermatozoon directly into the ooplasm, thereby crossing not only the zona pellucida but also the oolemma ***(De Vos, 2000).***

A revolutionary technique known as intra-cytoplasmic sperm injection (ICSI) was first described and has been widely available in most IVF clinics since the mid 1990s. ICSI allows couples with even severe male and female factor infertility to have offspring and improves fertilization rates with oocytes that have undergone in vitro maturation ***(Hudson, 2003) (Goldberg et al., 2007).***

The first pregnancy resulting from the technique of intra-cytoplasmic sperm injection (ICSI), was reported by the group

of the Dutch-speaking Free University of Belgium in Brussels. ICSI is very successful and is now exclusively used by most assisted reproduction programmes ***(Palermo et al., 1992).***

Intra-cytoplasmic sperm injection (ICSI) could be used in; severe male factor as very low number of motile sperms, poor quality eggs with thickened zona, anti-sperm antibodies, and repeated fertilization failure with standard IVF, surgical retrieved sperm from the testis or the epididymis ***(Hudson, 2003).***

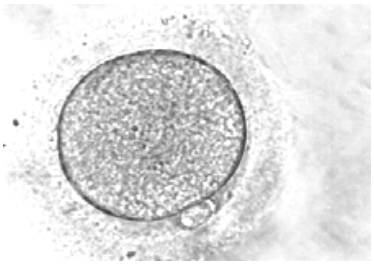
In normal fertilization and conventional IVF more than a single sperm is required for an egg to be fertilized. In IVF, each egg is usually exposed to approximately 100,000 sperm to ensure normal fertilization. In ICSI, a single sperm is injected into the egg, and therefore, theoretically, only one viable sperm from a semen sample is required for each egg ***(De Vos, 2000) (Hudson, 2003).***

ICSI involves many steps similar to a couple going through a traditional IVF treatment cycle. Hormonal stimulation and egg retrieval are the same. Production of the semen sample and subsequent processing of the sperm are the same (except in those few cases where surgical sperm retrieval is required). After ICSI, culture and handling of the embryos is exactly the same, and the embryos are also transferred back to the woman in exactly the same manner as those generated through IVF. The embryos generated by ICSI look exactly like those obtained by IVF. The differences between ICSI and IVF are the method of fertilization (the sperm injection procedure)

and the preparation of the eggs for this injection. When the eggs are removed from the ovary they are surrounded by a "cloud" of cells, this complete structure is referred to as the oocyte-cumulus-corona complex ***(De Vos, 2000) (Hudson, 2003).***

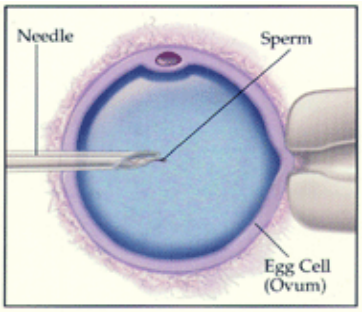
The preparation of the egg is done by the removal of these "cumulus" and "corona" cells (oocyte –cumulus-corona complex). This is achieved by exposing the egg to an enzyme called hyaluronidase. This enzyme is found normally in the heads of sperm cells and, in normal fertilization, allows the sperm to "digest" their way through the cloud of cells to the egg. After the removal of oocyte-cumulus-corona complex has been done, the eggs are assessed for maturity. A mature egg, suitable for sperm injection, has a polar body present.

This egg is ready for sperm injection ***(De Vos, 2000).***



Each mature egg is then injected with one sperm. A Tiny glass needle called an "injection pipette" is used to isolate a single sperm from a small sample of prepared sperm. This sperm will be assessed for for normal shape and motility**.** The sperm is immobilized by striking the tail. This cause breaking not separating the tail of the sperm, and the aim is to prevent the swimming of the sperm in the egg and prevent the damage of the internal egg structure ***(De Vos, 2000) (Hudson, 2003).***

The egg is held in place and secured by glass needle called a holding pipette and the injection pipette is then advanced to pierce the outer coating of the egg called the zona pellucida, followed by the deposition of the sperm into the egg cell ***(De Vos, 2000) (Hudson, 2003).***

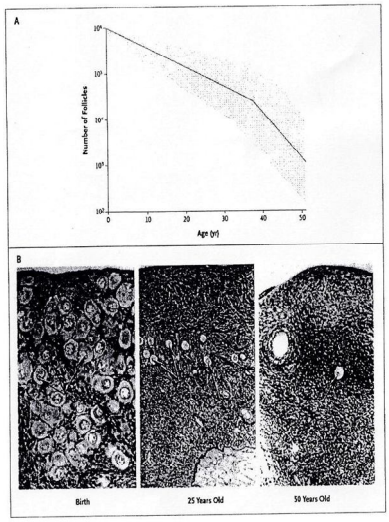


After a further 24 h in vitro, the cleavage characteristics of the fertilized oocytes are evaluated: numbers and sizes of blastomeres and the presence of anucleate cytoplasmic fragments are recorded. About two thirds of the 2pronuclear oocytes after ICSI develop into cleaved embryos which are suitable for transfer. Within an ICSI programme, the embryo transfer policy is similar than for conventional IVF ***(De Vos, 2000).***

ICSI does not increase the incidence of multiple gestations as compared to standard IVF. Because ICSI is a relatively new technique, first performed in 1992, long-term data concerning future health and fertility of children conceived with ICSI is not available. Follow-up of almost 2000 children born after ICSI did not show a higher incidence of malformations in comparison with standard IVF. Despite these concerns, ICSI is a major advance in the treatment of severe infertility ***(Bonduelle et al., 1999).***

Chapter (2):
Assessment of Ovarian Outcome

The ovarian assessment is combination between the ultrasound measurement of the number of small follicles present in the ovary (the antral follicle count: AFC), the total ovarian volume, and the concentration of the hormones in the blood including, the follicular stimulating hormone (FSH), Estradiol (E2), inhibin B, anti-mullerian hormone (AMH). Using this information we can predict the response to the treatment program, and adapt this treatment according to the indicated best approach ***(Glasgow et al., 2011).***



The Ovarian outcome may describe the functioning ovary, and reflects the number and the quality of the oocyte in it ***(Macklon and Fauser, 2005).*** It is manifested by the number of the follicles and oocytes collected with the peak of oestradiol concentration ***(Ravhon et al., 2000).***

The human ovary contains a fixed pool of primordial follicles, the maximal number at five months of intrauterine life, and the number at the time of birth around 700,000. At the time of menarche, the pool reduces to 250,000 to 300,000 and

declines with aging. The follicular depletion accelerates, and menopause is estimated at a mean of 50-51 years. This age may vary in different population ***(Wallace and Kelsey, 2004).*** Follicular depletion occurs largely due to atresia, and is accompanied by reduction in the ovarian volume, which is also related to the age ***(Santoro et al., 2003).***

I. Ultrasound Assessment For Ovarian Outcome:

The ultrasound scan of the ovaries is used to examine the presence of cysts caused by endometriosis or cystic structures associated with the Fallopian tubes (hydrosalpinges), all of which can interfere with fertility, and influence the outcome of ICSI. The detailed examination of the ovaries allows us to count the number of small follicles present (AFC), assess the ovarian volume, and check if there are indication of polycystic ovary syndrome ***(Glasgow et al., 2011).***

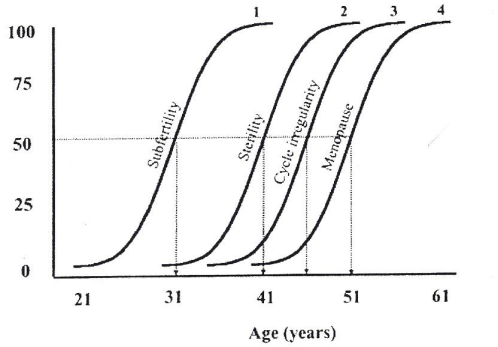
The transvaginal ultrasound allows the visualization of the pelvic structures, including assessment of their size and morphology. Also, it is used for assessing the ovarian function including the ovarian volume and antral follicular count ***(Zaidi et al., 2007).***

1. Ovarian Volume:

The volume of each ovary is calculated by measuring the three perpendicular diameters (longitudinal, transverse and antroposterior).The ovarian volume is determined by applying the formula for ellipsoid (D1×D2×D3×0.523), where D1,D2

and D3 are the dimensions of the selected ovary for maximal longitudinal, transverse and antroposterior diameters. Mean ovarian volume is the mean volume calculated for both ovaries in the same individual ***(Lass et al., 1997).***

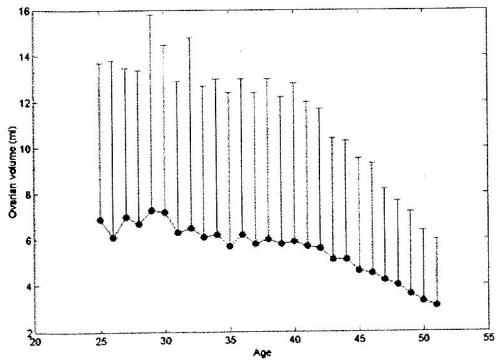
The ovarian volume increases from 0.7ml at 10 years to 5.8ml at 17 years of age. It has been suggested that, there are no major changes in the ovarian volume during the reproductive life till the premenopausal period ***(Kwee et al., 2007).***



Variations in age at the occurrence of specific stages of ovarian ageing. (Broekman et al., 2006)

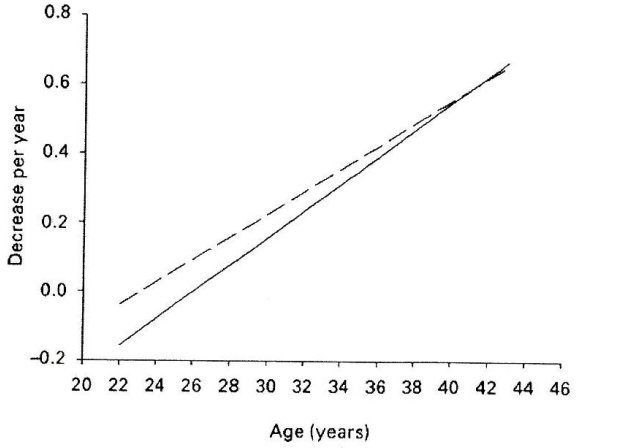
The mean ovarian volume in 140 patients varied from 0.5 to 18.9cm3 and it was concluded that there was a strong association between ovarian volume and ovarian outcome ***(Lass et al., 1997).***

Total ovarian volume was a predictor of cycle cancellation. Large ovarian volumes are associated with good IVF/ICSI outcome whereas small ovarian volumes are associated with poor outcome. Beyond maternal age, total ovarian volume is a significant predictor of the success of assisted reproductive techniques ***(Syrop et al., 1995).***



Mean ovarian volume and 95% upper confidence limit related to age for ages 25–51 years. *(Wallace and Kelsey, 2004)*

It is well known that the ovarian volume decreases with the female age leading to senile ovarian atrophy. So, clinical studies suggest that a small ovarian volume and reduced stromal area indicate poor response to ovarian stimulation ***(Wu et al., 1998).***



Decline rate in the OV (ml per year) and AF count (n per year) with age. The dashed line indicates the decline rate for the AF count. *(Tufan et al., 2004)*

There is dramatic drop in ovarian volume, in women over 40 years old, which is not related to the parity ***(Kwee et al., 2007).***

In women with small ovaries (<3 cm3), the cancellation rate of ICSI is higher ***(Sharara et al., 1999)***. Low ovarian volume has also been found to correlate with the number of growing follicles, but not with the number of oocytes retrieved ***(Tomas et al., 1997).*** A correlation was found between ovarian volume and reproductive success in IVF/ICSI cycles; however, the likelihood ratio of a positive pregnancy test was 1.0–1.4, suggesting that its value is limited ***(Syrop et al., 1995) (Lass et al., 1997).*** Moreover, there is a wide range in the definition of normal ovarian volume in the reproductive age group ***(Maheshwari et al., 2006).***

Total volume of the ovaries detected by transvaginal ultrasound is correlated with the outcome parameters but not better than the antral follicular count. Its performance was slightly too moderately less than that of antral follicular count (AFC), for both poor and high response ***(Broekmans et al., 2006).***

In some review, the ovarian volume as a marker for assessment of ovarian outcome has little clinical applicability for the prediction of a poor pregnancy response ***(Broekmans et al., 2006)***. So, because of its easy execution, the measurement of the ovarian volume could be included in the preparatory protocols, adding information to the patient's medical records ***(De Carvalho et al., 2008).***

2. Antral Follicular Count(AFC):

Antral follicles are the early Graafian follicles that are 2-10mm in diameter, round to oval, echo lucent fluid filled structures, easily imaged by ultrasound, waiting for further recruitment and stimulation by gonadotropins ***(Gregory et al., 2004).***

Antral Follicles are the name given to the small ovarian cysts that contain eggs. At the beginning of a menstrual cycle, we can visualize and even measure these follicles in the ovary using the ultrasound. A normal follicle will be less than 9 or 10 mm in diameter ***(Tsafrir et al., 2009).***

The follicles measuring 2–5 mm are present very early in the follicular phase of the cycle. These follicles are in an early antral phase, and are easily detected by transvaginal ultrasound, as they contain a small amount of antral fluid. The number of small follicles at the beginning of the cycle may well represent the actual functional ovarian outcome. So, the numbers of small antral follicles are clearly related to age and could well reflect the size of the remaining primordial pool in women with proven natural fertility ***(Kwee et al., 2007).***

A gradual decrease in the sono-graphically detected antral follicles has been found with advancing age. There is relation between the antral follicle count and the ovarian response ***(Kwee et al., 2007).*** An age related decline in the AFC has been observed ***(Ruess et al., 1996) (Ng et al., 2003) (van Rooij et al., 2005).***