INTRODUCTION

Definitions of infertility differ, with demographers tending to define infertility as childlessness in a population of women of reproductive age, while the epidemiological definition is based on "trying for" or "time to" a pregnancy, generally in a population of women exposed to a probability of conception **(Gurunath et al., 2011)**.

A woman under 35 has not conceived after 12 months of contraceptive-free intercourse. Twelve months is the lower reference limit for *Time to Pregnancy* (TTP) by the World Health Organization. A woman over 35 has not conceived after 6 months of contraceptive-free sexual intercourse **(Cooper et al., 2010)**.

ICSI is most commonly used to overcome male infertility problems, such as low sperm counts (oligospermia), low sperm motility (asthenospermia), or abnormally-shaped sperm (teratospermia). Additional conditions that may require ICSI include problems with sperm binding to and penetrating the egg, prior or repeated failure with conventional IVF methods, and obstruction of the reproductive tract. It can be used in teratozoospermia, because once the egg is fertilized, abnormal sperm morphology does not appear to influence

blastocystdevelopment or blastocyst morphology. Even with severe teratozoospermia, microscopy can still detect the few sperm cells that have a "normal" morphology, allowing for optimal success rate **(French et al., 2010)**.

IVF involves a sequence of events which begin with different stimulation protocols with controlled ovarian hyperstimulation with exogenous gonadotrophin. Many techniques are developed to obtain a lot of follicles. During the past decade, an important increase has occurred in the use of ovulation induction regimens, mainly those using gonadotropins and GnRH analogues. COH causes to achieve multiple oocytes, but this condition results in supraphysiologic E_2levels and might affect endometrial implantation. High E_2 levels on the day of hCG administration might cause better IVF-ICSI outcome or a decreased outcome, caused by disrupted endometrial receptivity **(Kara et al., 2012)**.

Oocyte retrieval is done from the ovaries under the guidance of ultrasonography, fertilization in the laboratory, and transcervical transfer of embryos into the uterus. Since the last two decades, ART has been used in treatment of infertile couples but the low implantation, pregnancy rate and high cost of treatment leads to the need to assess the predictors of success in IVF- ET cycles. Prognostic factors of IVF success are basal

FSH, serum estradiol, endometrial receptivity and embryo grading. Endometrial receptivity is one of the important factors of IVF outcome. It has been estimated that uterine receptivity accounts for about 31–64% of implantation. Implantation of blastocyst in the endometrium takes place only during short duration called the window of implantation which exists for about 48 hours, beginning 6–10 days after the LH surge in a spontaneous cycle. Embryo implantation is regulated by a multitude of factors. This endometrial receptivity can be assessed by ultrasound markers.Serum estradiol plays a crucial role in the reproductive system, being involved in cervical mucus, in endometrial proliferation for embryo implantation, and in induction of midcycle LH surge in natural cycle. E_2 synthesis is merely associated with dominant follicle development and plasma E_2 concentration is an index to assess follicle maturity. During ART cycle E_2 synthesis is directly related to follicle size and the contribution of the mature follicle to E_2 outcome may be estimated about 200pg/ml. For many years focus of research interest has been on the possible association between serum estradiol level and the IVF outcome on the day of HCG trigger. If E_2 levels are high they might have better effect on IVF/ICSI outcome or might have an adverse effect cause by a decreased endometrial receptivity **(Gahlot,2014)**.

Supraphysiological serum estradiol E_2 levels associated with controlled ovarian hyperstimulation (COH) is suspected to be correlated (either negatively or positively) with IVF outcome and therefore, thoroughly investigated for many years. However, such an association could be neither demonstrated nor denied. Furthermore, the mechanism (s) underlying a probable association, such as endometrial receptivity, embryo viability and adhesion capacity, or both, also couldn't have been clarified. As controlled ovarian stimulation aiming high oocyte yield is associated with supraphysiological E_2 levels, E_2 effect on pregnancy rates (PR) in IVF/ICSI cycles is a major concern for long years. High E_2 levels reached in follicular phases of stimulated cycles are thought to accentuate proliferative phase causing endometrial advancement in 90 % of the cycles **(Taskin et al., 2014)**.

AIM OF THE WORK

The aim of this work was to assess the relation between serum E2 level on the day of HCG administration and ICSI outcome.

INTRACYTOPLASMIC SPERM INJECTION

I) Definition:

Intracytoplasmic sperm injection (ICSI) involves micromanipulation techniques to inject a viable sperm into the ooplasm of the egg, after cumulus and corona cell removal (denudation), oocytes are used for microinjection**(Jayakrishnan and Aby, 2012).**

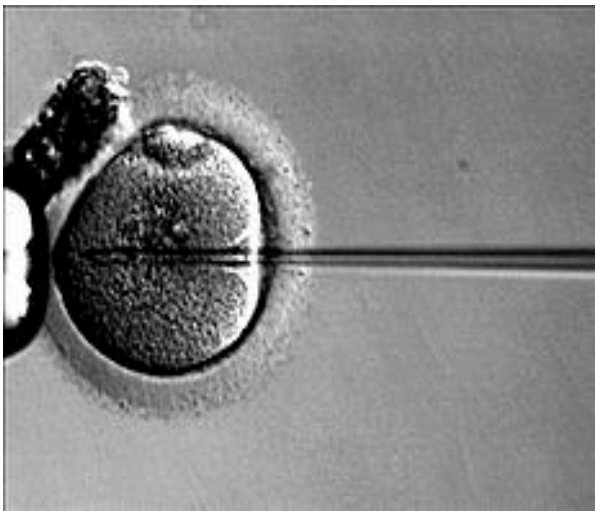

Fig. (1): Oocyte is injected during ICSI. (The pipette pierces the oolemma and the sperm is deposited in the cytoplasm of the oocyte) **(Meniru, 2004a).**

II) Indications:

ICSI is indicated in any infertile couple in whom there is a significant chance of failed or very poor fertilization following treatment with conventional IVF**.**A group of patients who will benefit from having ICSI at the

outset rather than conventional IVF are those who have a marked depression of their semen parameters. This includes patients with moderate to severe oligo-, astheno-, and/or teratozoospermia. It may be prudent to offer ICSI if the concentration of sperm in the ejaculate is less than 5 million/ml or the progressive motility is less than 10%. Some authorities will not consider ICSI unless the total motile sperm count after sperm preparation is less than one million. Patients with azoospermia are increasingly being offered ICSI as it is possible to recover sperm, by surgical means, from the genital tract or testes of these men if the azoospermia is obstructive in origin. Even in patients with non-obstructive azoospermia, it may be possible to recover sperm from the ejaculate in up to 35% of cases, after long periods of painstaking search of the pellet obtained by centrifuging the semen sample at high speeds for up to 30 minutes, ***so indications of (ICSI) in males as follow:***

1- Previous failed in vitro fertilization.

2- Marked depression of semen parameters:

- Sperm concentration < 5 million/ml
- Progressive motility < 10%
- Normal morphology < 4%

- Total motile sperm count after sperm preparation < 1 million

3-Borderline semen parameters

4- Specific patients groups:

- Retrograde ejaculation
- Impotence necessitating electroejaculation
- Severe seminal antisperm antibody problem
- Frozen poor quality sperm (e.g. from testicular cancer patients)

5- Sperm retrieved from patients with initial diagnosis of azoospermia

(Ron-El et al., 1997andMeniru et al., 1998)

Since the birth of Louise Brown in July 1978, IVF has proven to be an efficient treatment to alleviate female factor infertility, especially tubal infertility **(Edwards et al., 1980).** In subsequent years IVF was also successfully applied in couples with unexplained infertility, with male infertility as well as endometriosis **(Mahadevan et al., 1983).** Since the second half of 1992, only ICSI has been applied in centers when assisted fertilization was indicated **(Van Steirteghem et al., 1993b).**

So indications of (ICSI) in females as follow:

1) <u>Tubal factor infertility:</u>

Tubal factor infertility is responsible for approximately 30% of infertility cases **(Yildizhan et al., 2009).**There are multiple etiologic factors responsible for the involvement of the fallopian tube in infertility, which include tubal damage from pelvic inflammatory disease (PID), the use of intrauterine devices, a history of a perforated appendicitis, ectopic pregnancy, and septic abortion.Tubal adhesions and tubal obstruction can also be due to endometriosis and previous surgical trauma**(Kupesic and Plavsic, 2007).**Tubal occlusion, peritubal and periovarian adhesions are factors responsible for inhibition of ovum pickup and transport. In developed countries the major cause of tubal infertility is pelvic inflammatory disease usually as a consequence of an ascending infection by chlamydia orgonorrhea **(Haider et al., 2010).**

Treatment of fallopian tube obstruction has traditionally been treated with surgery (tuboplasty) with a goal of restoring patency to the tubes.While IVF/ICSI therapy has largely replaced tubal surgery in the treatment of infertility; the presence of hydrosalpinx is a detriment to IVF/ICSI success because fluid in hydrosalpinx may have a direct

embryotoxic effect as it contains inflammatory cytokines and may also inhibit fertilization **(Strandell et al., 2004).**It has been recommended that prior to IVF/ICSI, laparoscopic surgery should be done to either block or remove hydrosalpinges**(Johnson et al., 2010).**

2) Anovulation and Polycystic ovary syndrome (PCOS):

Chronic anovulation is probably the major cause of human infertility and is essentially associated with four distinct endocrine conditions; hyperprolactinemia anovulation,hypogonadotrophic anovulation, Normogonadotrophic anovulation and hypergonadotrophic anovulation. Patients with normogonadotrophic anovulation are likely to have polycystic ovary **(Crosignani et al., 1999).**

The polycystic ovarian disease is one of the most common endocrine disorders in female (5-10 %) and constitutes one of the most common causes of female infertility **(Boomsma et al., 2008)**. The Rotterdam ESHRE/ASRAM sponsored consensus workshop group 2004, has defined the PCOS as a presence of the 2 criteria out of three criteria; oligomenorrhea or anovulation, an evidence of hyperandrogenism whether clinical or laboratory, picture of PCO with U/S according Adams criteria **(Azziz, 2006)**.

The main lines of treatment of PCOS are weight reduction, insulin sensitizing agent, ovulation induction, laparoscopic ovarian drilling, and finally assisted reproduction in the form of IVF and ICSI if the previous measures have been filed. The main problems during superovulation in PCOSis the higher incidence of ovarian hyper stimulation syndrome which occur in about 8- 23 % of cases**(Brinsden et al., 1995).**

Ovulation induction with gonadotrophins in obese PCOS women requires higher doses than in lean PCOS women, the rate of ovulatory cycles is lower, and the rate of multifollicular development and incidence of miscarriage is also higher in obese women **(Fridström et al., 1997).**

3) <u>Endometriosis:</u>

Endometriosis is defined as the presence of endometrial-like tissue outside the uterus, which induces a chronic, inflammatory reaction. The condition is predominantly found in women of reproductive age, it has been estimated that endometriosis occurs in roughly 4–10% of women**(Bulletti et al., 2010).**

The possible mechanisms by which endometriosis may cause infertility include:

- Anatomical distortions and adhesions.

- The release of factors from endometriotic cysts which are detrimental to gametes or embryos. An endometriotic cyst contains free iron, reactive oxygen species,proteolytic enzymes and inflammatory molecules**(Sanchez et al., 2013).**

IVF/ICSI is appropriate treatment especially if tubal function is compromised, if there is also male factor infertility, and/or other treatments have failed**(Barnhart et al., 2002).**

Laparoscopic ovarian cystectomy is recommended if an ovarian endometrioma ≥ 4 cm in diameter is present to confirm the diagnosis histologically; reduce the risk of infection; improve access to follicles and possibly improve ovarian response. Laparoscopic ovarian cystectomy in patients with unilateral endometriomas between 3 and 6 cm in diameter before IVF/ICSI can decrease ovarian response without improving cycle outcome **(Demirol et al., 2006).**

4) <u>Unexplained infertility:</u>

Unexplained infertility is defined asthe inability of a couple to conceive after 3 years when the infertility evaluation of the couple is normal and there no any detectable cause. It is around 15-20% and it doesn't mean that there is no reason for infertility; only the reason is not identified at that time.

Requirement of diagnosis of unexplained infertility include normal semen analysis, ultrasound evidence of ovulation, normality of uterus, documentation of fallopian tube patency by hysterosalpingography or laparoscopy and endocrine evaluation as thyroid, prolactin, LH, FSH to be normal. In couples more than 35 years, treatment has to be more aggressive in terms of IVF/ICSI, with long standing unexplained infertility with repeated IUI failures, patient can be directly selected for IVF/ICSI **(Sushma, 2007).**

III) Protocols:

1- Controlled ovarian hyperstimulation:

The dose of gonadotrophin injection administered to any particular patientdepends on factors such as age, build, dose used in previous treatment cycles, whether the ovaries are polycystic, history of ovarian hyperstimulation syndrome(OHSS) in a previous treatment cycle and ovarian or periovarian surgery. The dose may have to be increased or decreased depending on her response to the administereddrugs. Some patients may receive 100–150 IU a day while others will receive 225 IU or more. A successful treatment outcome becomes less common when more than 450 IU are required each day especially when there is evidence of incipient ovarian failure in the pre-treatment

period. Gonadotrophin injections are continued until many of the developing follicles attain the diameter of 18–22 mm. Theinjections are usually given for a total of 12–16 days but administration can be extended to 21 days for poor responders or when there is a very cautious approachto superovulation, for example, in those who had a previous OHSS or are known to have PCOS **(Meniru, 2004b).**

Gonadotrophin releasing hormone agonists (GnRHa) are analogues of gonadotrophin releasing hormone (GnRH) but are several times more powerful. Their administration causes a high output of FSH and LH from the pituitary gland but this is relatively short lasting. Continued administration of GnRHa depletes the pituitary stores of these hormones and their output falls to very low levels. This is called pituitary downregulation which can be verified by measuring the endometrial lining which should be 5 mm or lower, serum E_2 (estradiol) is usually <200 pmol/l, vaginal ultrasound examination is also needed to confirm the absence of an ovarian cyst and the lack of response of the pituitary gland to further doses of GnRHa is said to be due to pituitary desensitization. These events are usually completed within 10–14 days of starting chronic GnRHa administration. Gonadotrophin releasing hormone antagonists have now been developed and just entered clinical practice. These antagonists

act by immediately suppressing pituitary output of FSH and LH without causing the initial flare up of the production of these hormones unlike GnRHa **(Lin et al., 1999)**.

Most units now use a combination of GnRHa and gonadotrophin injections. Various brands of gonadotrophins are available. Some contain mainly FSH while others contain a mixture of LH and FSH. Initially these gonadotrophin preparations were produced by extraction of FSH and LH from the urine of postmenopausal women. However, in recent years two preparations (Gonal F and Puregon – Follistim in the USA) have come into clinical use and are produced using recombinant DNA technology **(Weissman, 1999).**

(A) GnRH–agonists:

1- The long (desensitization) protocol:

The agonist is commenced from either the midluteal phase (Day 21) of the menstrual cycle preceding the treatment cycle or from the early follicular phase (Day 2) of the treatment cycle itself. The GnRHa is administered for 10–14 days before commencing the injection of gonadotrophins and continued until the administration of the ovulation trigger. Stimulation with gonadotropins is started when pituitary and ovarian suppression has been achieved. Comparing different initiation moments of GnRH agonists, it was found that the initiation of GnRH agonist