An Overview on In-Vitro Fertilization and Intracytoplasmic Sperm Injection

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Abstract:

The increasing focus on developing new tools to more accurately diagnose and select individual sperm before intracytoplasmic sperm injection will allow us to counsel and treat couples with greater confidence and efficiency. Current sperm selection techniques are based on the premise that if an ejaculated spermatozoon has cleared spermatogenesis with the correct morphology and/or membrane properties then it is most likely normal. Techniques that are designed to prepare a clean "normal" sperm population or that assist in selecting an individual "normal" spermatozoon are currently being investigated. The use of techniques, including density-gradient preparation, electrophoretic separation, microfluidics, high-magnification sperm morphology selection, and hyaluronic acid binding, is discussed. The research evidence that supports the interrelated developmental and genetic integrity of the selected sperm, particularly sperm DNA damage and clinical outcome evidence are presented.

Keywords: In-Vitro Fertilization, Intracytoplasmic Sperm Injection, Embryo Transfer.

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Introduction:

Edwards and Steptoe first described the technique for in-vitro fertilization (IVF) and embryo transfer (ET) in 1976 and the subsequent births of two normal babies in 1978 **(1).** Since then, the success rate of the system has been improved (to 30%) by the use of fertility drugs to provide more oocytes and prematuration to mature the oocytes before fertilization**.** The techniques are now used in 53 countries throughout the world. In 1993, the results of 492 units from all over the world were collected from national surveys and registers. Since 1985, more than 53 635 women had been treated and 34 316 babies had been born from 224473 treatment cycles, following more than 160518 transfer cycles. Only about 65-75% of all resulting pregnancies attained live births. The remainder ended with spontaneous abortions (26%), or ectopic pregnancies (5.54%). The multiple pregnancy rate (22%) was higher than the normal population and contributed to higher rates of preterm deliveries and perinatal mortality. No increased incidence of chromosomal alterations and malformations were noted during the years (2.25%) **(2).**

Since the birth of the first IVF baby, tremendous developments have occurred regarding the indications for assisted reproductive technology (ART). For example, the dramatic

development concerning male infertility which initially was considered to involve a small fraction of patients benefiting from IVF, now with the development of intracytoplasmic sperm injection (ICSI), involves up to 35% of started cycles **(3).**

However, a substantial group of patients still remain unhelped. There is a reduction in success rates in women between the ages of 35 and 39 years, and a further reduction in women older than 40 years of age. There is also doubt about the cost-benefit of ART versus conventional therapy for subfertility. In women with unexplained infertility, hMG super ovulation therapy for subfertility. In women with unexplained infertility, hMG super ovulation treatment is as successful, less expensive, and carries a smaller risk than the surgical approach of ART. However, IVF is more expensive than three cycles of hMG and intrauterine insemination. The indications for treatment were broadened over the years and the procedure became a final step for diagnosis and treatment of unexplained infertility **(3).**

Intracytoplasmic sperm injection (ICSI) has emerged as the most popular insemination technique globally nowadays. In fact, 66.5% of assisted reproductive techniques (ART) employ ICSI as the preferred technique **(4).**

ICSI use has dramatically increased due to its dependability in achieving fertilization in cases of severe male factor infertility; it is now frequently used for patients of advanced maternal age (AMA), low oocyte maturity, cryopreserved oocytes and in conjunction with preimplantation genetic testing (PGT) **(5).**

Intracytoplasmic sperm injection (ICSI) is the process of injecting a single mature immobilized normal spermatozoon into the cytoplasm of a mature metaphase II egg. ICSI has completely changed the way that male factor infertility is treated and it helped couples for whom donation or adoption were the only options for treatment to achieve adequate pregnancy and implantation rates. Patients whose oocytes had not gotten fertilized following insemination with motile spermatozoa were the first to benefit from the successful use of ICSI **(6).**

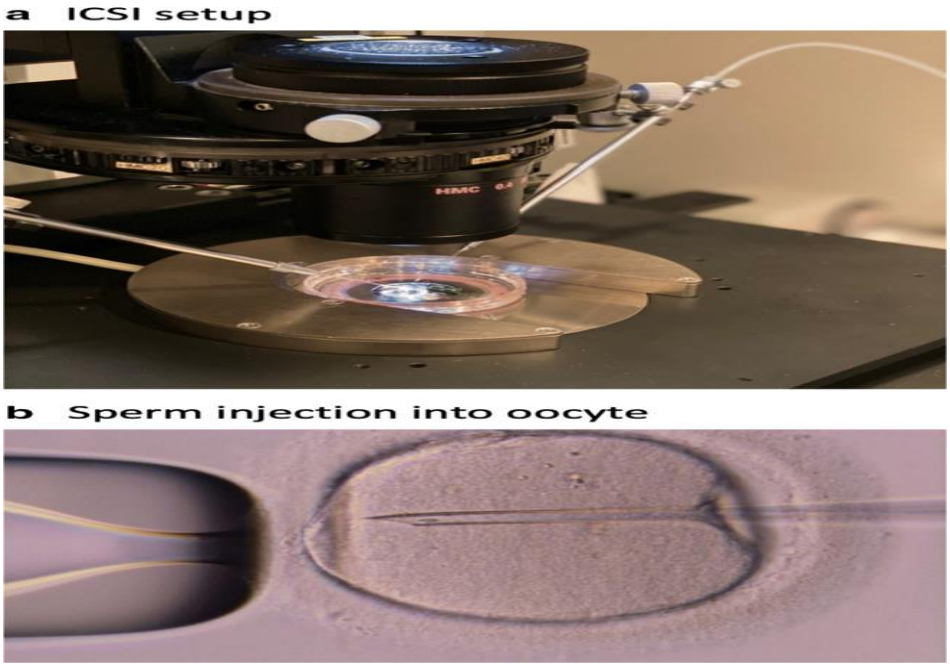

Figure (1): Intracytoplasmic sperm injection (ICSI) procedure. **(**a**)** The injection (*right*) and holding (*left*) pipettes are placed into micromanipulation tool holders on an inverted microscope and positioned using hydraulic joysticks. The ICSI dish is placed on the microscope stage. (b) The injecting pipette enters from the 3 o'clock portion of the oocyte and deposits the spermatozoon at the 9 o'clock portion of the ooplasm. **(7)**

Comparing IVF and ICSI fertilization processes:

The fertilization process happens "naturally" in conventional IVF and micromanipulation is not used. As in spontaneous conception, the fertilizing spermatozoon passes through the zona pellucida (ZP) to fertilize the oocyte. In contrast, one spermatozoon selected by a biologist is fully micro-injected inside the ooplasm during ICSI, avoiding the ZP binding/selection and gametes-fusion processes **(8).**

ICSI assists in vitro fertilization by enabling the creation of embryos (and offspring) in couples where it would not have been technically or biologically possible otherwise. Examples of these couples include those with globozoospermia, azoospermia patients due to congenital bilateral absence of vas deferens (CBAVD), or when using gametes with a lower fertilizing power, like frozen-thawed oocytes. **(9).**

Although the initial goal of in vitro fertilization (IVF) was to treat gynecological infertility, severe male factor and total fertilization failure (TFF) were the main reasons for male-based intracytoplasmic sperm injections (ICSIs). The percentage of ICSI on conventional IVF has continued to rise from the first clinical applications in 1992 and has stabilized at 70% of new in vitro cycles since 2013 **(10).**

With surgically extracted spermatozoa and frozen-thawed oocytes, as well as in the event of preimplantation genetic testing (PGT), ICSI is the preferred procedure. Both immature and frozen-thawed spermatozoa can be used for ICSI **(11).**

There are a number of variations including physiological intracytoplasmic sperm injection (PICSI) which is said to be a successful insemination technique for fragile oocytes, and intracytoplasmic morphologically selected spermatozoa injection (IMSI), in which spermatozoa are observed at 6600× (400× in conventional ICSI) and are thus selected based on organelle morphology **(12).**

Indications for ART:

Tubal infertility:

Tubal infertility is defined as persistent bilateral tubal obstruction, absence of tubes or tubal damage which has resulted in a period of infertility of more than 12 months' duration. Possible therapy of tubal infertility includes hysterosalpingogram, chromatography at the time of laparoscopy, selective transcervical tubal cannulation and falloposcopy. Each of their effects is restricted to the mechanical defect that may involve the tubal pathology **(3).**

Hydrosalpinx is dilation of the fallopian tube in the presence of distal tubal obstruction, which may result from a number of causes **(13)**. In women undergoing IVF, the presence of hydrosalpinx is associated with early pregnancy loss and poor implantation and pregnancy rates probably due to alteration in endometrial receptivity **(3).**

Cibula et al. (14) showed that tubal surgery such as laparoscopic salpingectomy significantly increased live birth rate and pregnancy rate in women with hydrosalpinges before IVF when compared with no treatment. There were no significant differences in the odds of ectopic pregnancy, miscarriage, treatment complication or implantation.

Tubal ligation:

A surgical method known as tubal ligation is used to permanently block, clip or remove the fallopian tubes in order to sterilize females. This stops sperm from fertilizing eggs, which stops fertilized eggs from implanting. Tubal ligation is regarded as a permanent form of sterilization and reproductive control **(15).**

Male infertility:

Male infertility has become as common as tubal factors as an indication for IVF/ET. And with the establishment of ICSI for couples with male infertility, most causes of male infertility can now be treated. The majority of infertile males have oligospermia and/or low sperm motility and are subfertile rather than sterile. In men with azoospermia, sperm cells obtained from the epididymis or by testicular biopsy may prove satisfactory for the ICSI technique **(3).**

Indications for ICSI:

Azoospermia:

About 1% of men and 10% to 15% of infertile men have azoospermia, or the total lack of spermatozoa in the ejaculate. The two main types of azoospermia are non-obstructive (NOA) and obstructive azoospermia (OA). Histopathological testing verifies complete spermatogenesis in obstructive azoospermia, while germ cell aplasia (Sertoli-cell-only (SCO) syndrome), maturation arrest or hypo spermatogenesis are the causes of non-obstructive azoospermia **(16).**

Obstructive and non-obstructive azoospermia:

NOA is caused by spermatogenesis dysfunction, whereas OA is caused by obstruction of the genital and testicular ductular systems. One in 100 men are thought to be affected by NOA. The etiology is either pre-testicular or testicular. **(5)**

Pre-testicular NOA, also known as secondary hypogonadism, results from an imbalance in hormones that prevents a physically normal testis from being adequately stimulated to create sperm. This hormone aberration is typically caused by hypothalamic-pituitary diseases. A natural abnormality in the testicles that impairs spermatogenesis is the cause of testicular azoospermia, also known as primary hypogonadism **(16).**

Hypo-spermatogenesis, maturation arrest, and Sertoli-cell only (SCO) are used to categorize the histological characteristics of NOA. All phases of germ cells are present in hypo-spermatogenesis, however they are relatively few in number **(17).**

Spermatogenesis stops at the primary or secondary spermatocyte (early) or spermatid (late) stages when there is a maturation arrest. Mature spermatozoa are therefore typically lacking. Germinal epithelium is completely lost in patients with SCO syndrome. It is important to recognize that men with NOA frequently have heterogeneous histology patterns **(18).**

Percutaneous acquisition and open surgery, which can be carried out with or without the assistance of microsurgery, are the two techniques most frequently utilized to harvest sperm in men with obstructive and non-obstructive azoospermia **(19).**

Oligoasthenoteratozoospermia (OAT):

OAT comprises three conditions: poor sperm motility (asthenozoospermia), abnormal sperm shape (teratozoospermia) and low sperm count (oligozoospermia) **(20).**

Isolated teratozoospermia:

The results of sperm morphology have been routinely used to choose ICSI candidates. The first to suggest using criteria to categorize sperm abnormalities and to suggest ICSI in cases where the percentage of normal sperm in each ejaculate was low was Kruger et al. in 1986. According to these authors, after traditional IVF the fertilization rate was less than 8% for patients with less than 4% normal sperm morphology and more than 60% for those with sperm morphology between 4% and 14% **(9).**

Absolute asthenozoospermia:

When there are few motile spermatozoa in the ejaculate or absolute asthenozoospermia (100 % immotile spermatozoa in the ejaculate), ICSI has been advised. While the latter is occasionally observed in freeze-thawed specimens, the former is primarily linked to ultrastructural anomalies of the sperm tail or full necrozoospermia (no live spermatozoa in the ejaculate) **(21).**

Assessing sperm viability is crucial in extreme asthenozoospermia because injecting unidentified immotile sperm is linked to lower likelihood of fertilization and pregnancy. A number of laboratory techniques have been suggested to enhance ICSI, such as exposing sperm to theophylline or pentoxifylline to increase motility, as well as the hypo-osmotic swelling test and laser use to enhance viable sperm selection. In the event that none of these methods work, testicular sperm can be extracted from the testis and injected **(22).**

Globozoospermia:

Globozoospermia is an uncommon morphological anomaly of sperm that is characterized by a coiled tail and a round-headed spermatozoon as a result of missing acrosomes. The rate of fertilization is lower than with non-globozoospermic sperm **(23).**

It is distinguished by a round-headed sperm, an abnormal nuclear membrane, midpiece abnormalities and the total lack of the acrosomal vesicle. About 0.1% of infertile males have globozoospermia, an uncommon genetic disorder that is incurable. Men with globozoospermia have spermatozoa that are unable to naturally fertilize the egg, even when their sperm count and motility are normal **(24).**

In these situations, because the sperm is less able to stimulate the egg and initiate zygote formation and embryo development, fertilization and pregnancy rates remain low **(25).**

Antisperm antibodies:

Seminal antisperm antibodies (ASAs) are typically associated with a male reproductive system blockage or a breach of the blood–testis barrier. Three to twelve percent of males

receiving examination for infertility have elevated amounts of ASAs in their semen. Testicular torsion, surgery, vasectomy, epididymo-orchitis, testicular cancer, cryptorchidism, and HIV infection have all been linked to this syndrome **(26).**

Because of their effects on sperm motility, sperm capacitation, acrosomal reaction and sperm–oocyte binding, ASAs may reduce fertility. The discharge of cytokines by antibodies against sperm can potentially have an adverse effect on sperm function **(4).**

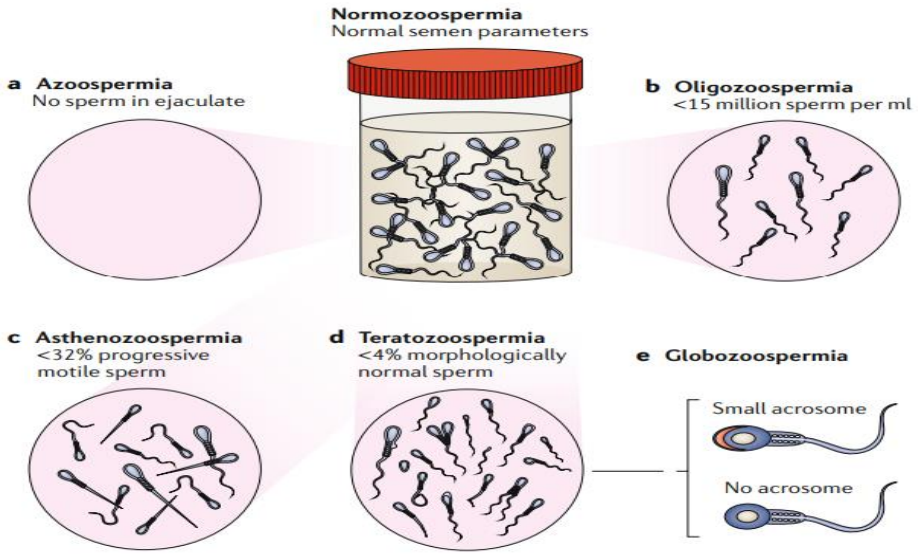

Figure (2): Seminal alterations associated with male infertility **(4)**

Preimplantation genetic testing:

Preimplantation genetic testing (PGT) has been used in conjunction with assisted reproductive technology (ART) for the past 30 years to study embryonic DNA (blastocyst or cleavage stage) to identify genetic abnormalities **(27).**

Sero-discordant couples:

When semen washing is done before IUI, IVF or ICSI vertical transmission of HIV is very unusual in sero-discordant couples with a seropositive male partner. Because ICSI only employs one spermatozoon, some studies contend that technique is less risky of transmitting HIV than IUI and traditional IVF **(9).**

Endometriosis:

The etiology of infertility in endometriosis is unclear, and a multitude of factors are involved. In advanced (Stage III and IV) endometriosis, endometriosis and pelvic adhesions distort pelvic anatomy and mechanically interfere with the reproductive process. In stage I and II disease, the mechanism of infertility is less clear. These effects are relative and individually variable, and some women with endometriosis have no infertility problems **(3).**

- A variety of mechanisms through which limited endometriosis may lower fertility have been postulated:

- Ovulatory dysfunction, the prevalence of which would seem to be similar in endometriosis and in the infertile population in general.
- Alterations in gamete/embryo transfer.
- Anti-fertility effects of the peritoneal fluid, which may affect sperm motility/survival, fertilization, gamete interaction and early embryo development.
- Peritoneal macrophages causing increased sperm phagocytosis, decreased motility and impaired ability to penetrate the egg.
- An anti-implantation effect related to evidence for antigen-antibody reaction in the uterine cavity of women with endometriosis which may be the mechanism of infertility and recurrent miscarriages. **(3)**

Most of the mechanisms implicated in endometriosis should be corrected by IVF/ET **(3)**.

Ovulatory disorders:

Disorders of ovulation resistant to treatment by ovulation induction with either clomiphene citrate or urinary gonadotropins may respond to IVF/ET. The most common disorder of ovulation which may prove resistant is PCOS **(2).**

Unexplained infertility:

Unexplained infertility is idiopathic infertility, meaning that the cause is still unknown even after a work-up for infertility, which typically entails a semen analysis for men and an evaluation of the woman's ovulation and fallopian tubes. Accordingly, the authors proposed dividing the pool of oocytes between IVF and ICSI for infertile couples who had previously tried and failed with intra-uterine insemination (IUI) treatment **(28)**.

ICSI procedure:

With the exception of fertilization, every step of the ICSI procedure is identical to that of conventional IVF. With ICSI, a single healthy and cleansed sperm is chosen, picked up and injected into a mature egg as opposed to allowing several sperm to mix with an egg in a lab dish. Infertility difficulties that are severe or for couples who have not been able to conceive with traditional IVF can be avoided by employing a direct injection, which also increases the possibility of successful fertilization. It also prevents issues with sperm decreased motility, cervix issues, etc **(29)**.

1. Starting phase:

The female will first need to go through a group of tests related to fertility, such as an examination of her uterus and fallopian tubes, hormonal assessment and any additional tests that could be necessary. The male will also have a semen analysis performed **(25)**.

At this point, the patient will also receive all the information she requires regarding the course of therapy, such as instructions on how to perform the medications injections every day at home and any other medications that may be recommended for ovarian stimulation **(25)**.

2. Phase of ovarian stimulation:

Following an evaluation and consultation regarding fertility, education about the ICSI cycle and consent to the ICSI treatment, the Ovarian Stimulation Phase will be commenced. Hormonal medications will be given daily for 9–12 days beginning on day 2 or 3 of the menstrual cycle to encourage the ovaries to create as many follicles as possible during this critical stage **(30)**.

Every ovarian follicle on the ovaries contains a single oocyte or immature egg. Many of these follicles will begin to grow and develop throughout a typical menstrual cycle, but often only one will go on to generate a mature egg. The objective is for every growing follicle to mature into an egg that can be harvested with the best possibility of success **(30)**.

The doctor will keep an eye on the ovarian response to the hormonal drugs throughout this time. During the nine to twelve days of stimulation, the patient will be required to visit the clinic every few days for ultrasounds and blood testing. The doctor may alter the doses and treatment strategy based on these findings. When the test results indicate that the eggs are fully mature, a procedure known as a "trigger injection" to prepare them for ovulation and release them from the follicle wall, enabling to perform the egg retrieval procedure **(30)**.

3. Semen collection and egg retrieval:

Aspirating follicles with a modest surgical operation will yield eggs ±36 hours after the trigger. To extract or recover eggs, the physician will utilize ultrasonography to guide a thin needle through the pelvic cavity. To provide a comfortable and painless surgery, general anesthesia will be used during this process. Typically, the entire procedure takes about fifteen to twenty minutes **(31)**.

The partner's semen will also be collected on the same day as the oocytes are being recovered. The doctor usually prefers a male partner to collect his semen directly from the patient at the clinic; but, if he is unable to do so on that particular day, he may obtain a sample of semen one day before the egg is removed and have the sperm frozen **(31)**.

It is advised to gather semen by masturbating in order to prevent coming into contact with any bodily fluids (such as vaginal fluids) from either the male or the female partner. These liquids could include microorganisms that infect the culture or fertilization media. A specialist waiting in the Andrology Laboratory, which is adjacent to the collection room, will receive the sample that the male partner returns through a window once he has collected it **(31)**.

After being collected, the semen will be allowed to liquefy for around half an hour. Subsequently, it will be properly cleaned to eliminate any debris, immobile sperm and any other substances present in the semen. This process is known as semen analysis. **(31)**.

The significance of this semen analysis is in its ability to assist doctors in determining whether to use advanced fertilization ICSI or standard fertilization IVF to fertilize eggs and partner sperm. The usual IVF method is selected if the semen sample is normal. However, if the results of the semen analysis are below average, the doctor might suggest using the second alternative ICSI technique for fertilization, since it enhances the likelihood that the fertilization will succeed **(31)**.

In the ICSI procedure, a single healthy sperm is specifically chosen and injected directly into a mature egg to accomplish fertilization, as opposed to the IVF technique, which mixes eggs and sperm and lets them fertilize on their own in a laboratory dish. The fertilized eggs are regarded as embryos once fertilization has taken place **(31)**.

4. ICSI fertilization:

Using a narrow micropipette, the embryologist will carefully choose a single, healthy and fast-moving sperm and inject it straight into a chosen mature egg. This process will be carried out for every single developed egg. The embryologist will look for evidence of healthy fertilization the next morning. Embryos are any eggs that have been fertilized naturally **(5)**.

5. Embryo culture:

Following fertilization, embryos are then cultivated in a special incubator for five to six days until they reach the blastocyst stage, at which point they are prepared for implantation into the uterine cavity. Embryos that are unable to reach the blastocyst stage will not be used because they are deemed weak and incompetent. In order to maintain a stable environment in the laboratory where the embryos can grow and develop properly, the embryo culture process is extremely sensitive and requires embryologists who are well-trained and experienced to manage environmental conditions and operate advanced technical equipment **(32)**.

Utilizing the newest time lapse incubator technology available, the Geri-Time-Lapse Incubator allows for detailed tracking of embryo development without requiring the plate or the embryos to move. Each culture chamber is equipped with a single, high-quality microscope camera system. With autonomous management and monitoring, every chamber offers unique and ideal culture conditions. Because they provide more stable settings for embryos to develop, mini-incubators have been linked to higher pregnancy rates **(32)**.

6. Embryo transfer:

Ultimately, one or more blastocysts will be chosen by the physician and embryologist and placed back into the uterus to develop into a baby. The skilled embryologist will load the chosen blastocyst(s) into a small tube called a catheter during this procedure, which is guided by ultrasound. The doctor will then insert the catheter through the cervix and into the uterus, releasing the blastocyst into the cavity of the uterus so it can implant into the wall of the uterus and start to develop and grow **(12)**.

Luteal phase support: Cycles with GnRH agonist protocol use luteal phase support with hCG or progesterone is necessary to optimize pregnancy rate. The use of hCG may increase the risk of OHSS, so its better to be avoided, and luteal support by progesterone suppository or ampoules. If pregnancy occurs, progesterone should be continued until 10 week pregnancy **(33)**.

Confirmation of the pregnancy: Confirmation of pregnancy is performed by assessment of serum B-unit HCG, 14-16 days after embryo transfer. If pregnancy test is positive, progesterone as luteal phase support is given for next 10 weeks of pregnancy. Ultrasound scan will be arranged at 6-8 week gestations if it shows ongoing pregnancy (clinical pregnancy), obstetrical follow up for prenatal and obstetric care should be arranged **(34)**.

7. Freezing of embryos:

Any healthy embryos that are surplus to transfer needs can be stored using a method known as vitrification, which involves freezing the embryos extremely quickly to prevent the water molecules from crystallizing, and then storing them in a deep freezer. If kept in a high-quality and well-maintained laboratory with regular and constant quality control and monitoring of the volume of liquid nitrogen and the integrity of the equipment, this leads in the long-lasting and high-quality preservation of embryos for an endless period of time **(35)**.

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