Section A -Research paper

Intracytoplasmic sperm injection: Review article

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Abstract:

Among infertile couples, 25% involve both male and female factors, while male factor alone accounts for another 25% due to oligo-, astheno-, teratozoospermia, a combination of the three, or even a complete absence of sperm cells in the ejaculate and can lead to a poor prognosis even with the help of assisted reproductive technology (ART). Intracytoplasmic sperm injection (ICSI) has been with us now for a quarter of a century and in spite of the controversy generated since its inception, it remains in the forefront of the techniques utilized in ART. The development of ICSI in 1992 has drastically decreased the impact of male factor, resulting in millions of pregnancies worldwide for couples who, without ICSI, would have had little chance of having their own biological child. This review focuses on the state of the art of ICSI regarding utility of bioassays that evaluate male factor infertility beyond the standard semen analysis and describes the current application and advances in regard to ICSI, particularly the genetic and epigenetic characteristics of spermatozoa and their impact on reproductive outcome.

Keywords: Intracytoplasmic sperm injection, assisted reproductive techniques, ovarian stimulation.

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Introduction:

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 **(1).**

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) (**2).**

The number of annual ART cycles in the United States was recorded 112,988 by the Society for Assisted Reproductive Technology (SART) in 2003. This number increased significantly to 248,086 cycles by 2017. In addition to this increase, patients are choosing to undergo additional ART procedures to improve their chances of becoming pregnant. These procedures include PGT, which determines the genetic profile of embryos and increases the likelihood of implantation **(3).**

Many patients are also opting for oocyte and embryo cryopreservation aiming for fertility preservation for many causes, given that the total incidence rate of cancer patients among young adults (aged 15–39) in the United States is rising by 0.9% annually. However, despite its widespread use, there is disagreement over whether ICSI is superior to conventional in vitro fertilization (IVF) **(4).**

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 **(5).**

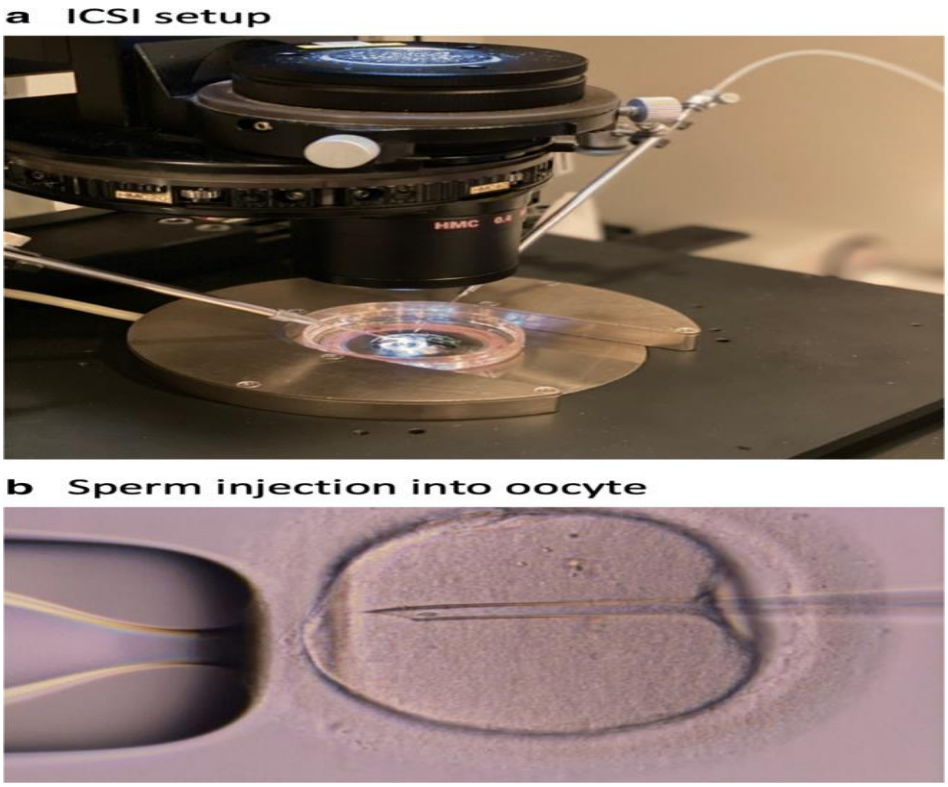

Figure 1: Intracytoplasmic sperm injection (ICSI) procedure. (a) The injection (right) and holding (left) pipettes are placed into micromanipulation tool holders on an inverted microscope and positioned using hydraulic joysticks. The ICSI dish is placed on the microscope stage. (b) The injecting pipette enters from the 3 o'clock portion of the oocyte and deposits the spermatozoon at the 9 o'clock portion of the ooplasm **(6).**

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 (**7).**

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. (**8).**

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 (**9).**

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 (**4).**

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 (IMSI), in which spermatozoa are observed at 6600× (400× in conventional ICSI) and are thus selected based on organelle morphology (**10).**

Regular IVF mimics the normal fertilization process, even if it is carried out in vitro. Motile spermatozoa around the oocyte-cumulus-corona radiata complex release hyaluronidase enzymes through acrosomal processes, which allow the enzymes to pass through the cumulus-corona radiata complex and reach the zona pellucida (ZP). After that, spermatozoa continue to travel via the ZP to the perivitelline area after the spermatozoa head attaches to the ZP3 glycoprotein (**11).**

One spermatozoon then makes contact with the metaphase II oocyte membrane. When the sperm head comes into contact with the oocyte microvilli, the two gametes fuse together to form a continuous plasma membrane. Calcium ions in the oocyte's submembrane vesicles are released immediately during gamete fusion and prevent other sperm from penetrating through the depolarization of the cytoplasmic membrane (**5).**

The release of Ca2+ not only inhibits polyspermy but also increases oxidative metabolism and intercellular pH, which in turn boosts oocyte respiration and metabolism. Following gametes fusion, the extremely densely packed nuclear chromatin in spermatozoa begins to decondense **(7).**

Histone proteins take the place of the protamine proteins that are complexed with sperm DNA. The chromatin travels in close proximity to the oocyte nuclear material as it extends out into the nucleus, which is now known as the pronucleus. While the female genome retains methylation, the male genome is demethylated. Anaphase II and telophase II quickly follow one another as the metaphase II egg completes the second meiotic division **(12).**

The perivitelline space is the release point for the second polar body. As the pronuclei get closer to one another, one chromosome splits into two chromatids during DNA replication. After

then, chromosomes mix together and the pronuclei membranes degrade. Now, it is the embryo one-cell stage. Around the mitotic spindle formed by the duplicated sperm centrosome, maternal and paternal chromosomes rapidly assemble **(13).**

Because the fertilizing spermatozoon in ICSI is micro-injected directly into the ooplasm after immobilization, it does not pass via the ZP or the cumulus-corona radiata complex to fuse with the metaphase II oocyte. According to **(6),** immobilization is a crucial micromanipulation that makes the sperm membrane permeable and permits the release of "sperm cytosolic factors," which is required for oocyte activation.

It is seen that the oocyte membrane depolarizes following sperm micro-injection as a result of calcium release from cortical vesicles. IVF-fertilized oocytes and ICSI-fertilized oocytes have similar in vitro embryo development, with both pronuclei appearing and fading at the zygote stage **(14).**

Indications for ICSI

Since Louise Brown was born in 1978, conventional IVF has been effectively employed, but when sperm quantity or quality is low the results are lower. ICSI is now the ideal treatment for couples with severe male factor infertility and is also used for a variety of non-male factor causes. This is because a single spermatozoon with a functioning centrosome and genome may fertilize an egg and yield a viable embryo (**15**).

- **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. **(2)**

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 (**3).**

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 (**18).**

- **Oligoasthenoteratozoospermia (OAT)**:

OAT comprises three conditions: poor sperm motility (asthenozoospermia), abnormal sperm shape (teratozoospermia) and low sperm count (oligozoospermia) (**19).**

- **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% (**8).**

- **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) **(20).**

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 (**21).**

- **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 **(22).**

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 (**23).**

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 **(24).**

- **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 **(25).**

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 **(1)**.

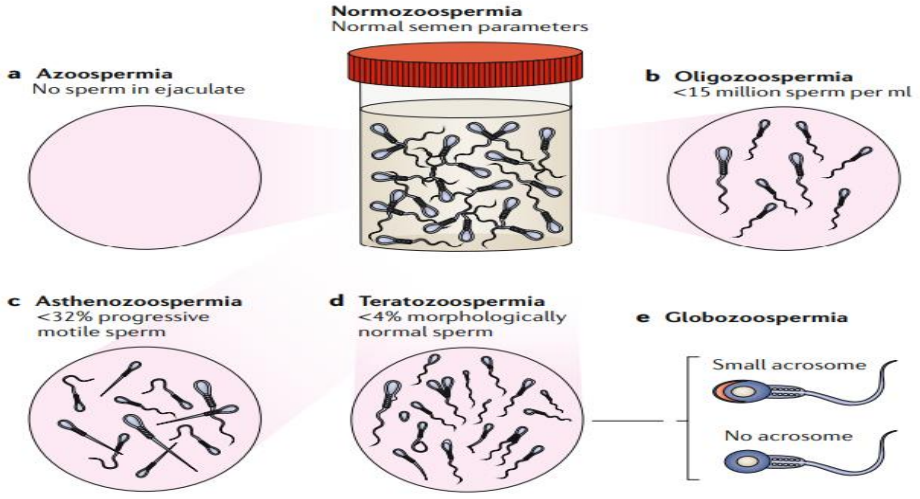

Figure 2: Seminal alterations associated with male infertility**(1)**

- **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 IUI treatment **(26)**.

- **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)**.

- **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 **(28)**.

- **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 **(8)**.

Sperm selection methods

A: Density gradient centrifugation: Liquefied semen is placed on top of two colloidal gradients in a sequence that were centrifuged and had densities of 45% and 90%. According to **Ku et al. (29),** the highly active spermatozoa are abundant in the soft pellet at the bottom and migrate towards the sedimentation gradient.

B: Swim-up: Sperm medium is layered on top of liquefied semen. Motile sperm move from the underlayer sperm suspension to the upper layer throughout the incubation phase. Spermatozoa that are actively motile are present in the supernatant (**29).**

C: Sorting sperm cells via magnetic activation: The non-apoptotic healthy sperm cells can pass through the selection column whereas the apoptotic sperm attached to micromagnetic beads coated with annexin V are retained by the activated magnetic field (**29).**

D: The binding of hyaluronic acid: According to **Ku et al. (29),** immature sperm do not bind to hyaluronic acid, which is a major component of cumulus cells, while mature sperm do.

E: Analysis of motile sperm organelle morphology: Spermatozoa images are obtained with a digitally improved light microscope equipped with Nomarski optics, which have a minimum magnification of ×6,000. Following morphological assessment, spermatozoa are sorted and graded according to the size and form of their nuclear vacuoles **(29).**

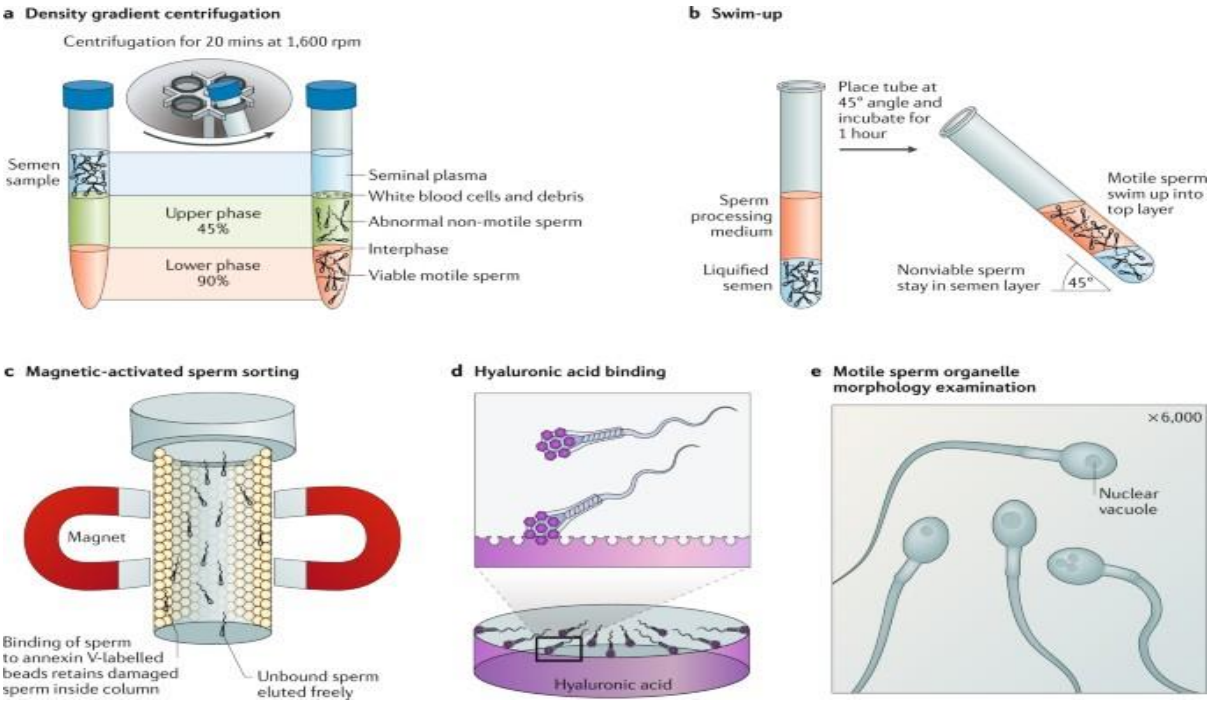

Figure 3: Sperm selection methods **(29).**

sperm extraction

The two most popular techniques for collecting epididymal sperm are Micro-surgical Epididymal Sperm Aspiration (MESA) and Percutaneous Epididymal Sperm Aspiration (PESA). In contrast, the techniques utilized to harvest testicular sperm are testicular sperm aspiration (TESA) and open testicular sperm extraction, either with or without the assistance of microsurgery (micro-TESE and TESE, respectively). These operations can be carried out as outpatient procedures in conjunction with oocyte retrieval and immediate sperm injection or they can be done with the goal of cryopreserving sperm for later use. Spermatogenesis is normal in males with obstructive azoospermia, and sperm can be readily extracted from the testis or epididymis. Results from ICSI appear to be unaffected by the method used to retrieve the sperm as well as the underlying cause of obstructive azoospermia (**30).**

MESA is suggested to produce more motile sperm than PESA, which means that more sperm can be cryopreserved and potentially used in numerous ICSI cycles without requiring additional sperm retrieval. However, compared to percutaneous retrieval techniques, MESA requires greater technical expertise **(7)**.

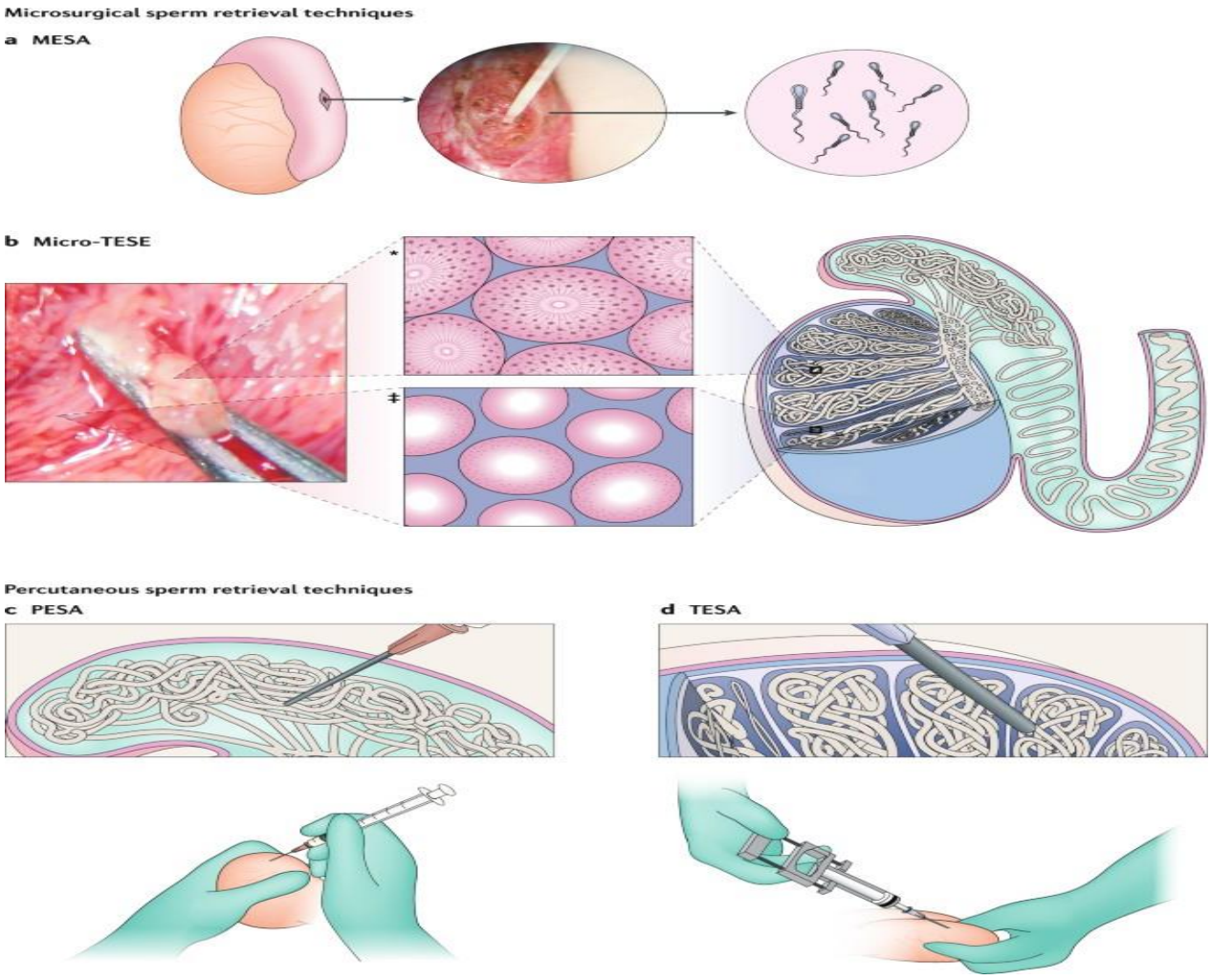

Figure 4*: Sperm retrieval methods* **(30)**

A: Microsurgical epididymal sperm aspiration (MESA): A dilated The epididymal tubule is split open. The fluid is aspirated, transported to the lab for analysis, and diluted with sperm medium.

B: Microsurgical extraction of testicular sperm (micro-TESE): The seminiferous tubules are exposed following the exteriorization of the testicle through a single, broad incision made in an avascular region of the albuginea. Using microforceps, the dilated tubules are located and extracted. The figure displays histological cross sections of a narrow tubule with germ cell aplasia and a dilated seminiferous tubule with active spermatogenesis.

C: PESA stands for percutaneous epididymal sperm aspiration: The forefinger, thumb, and index finger stabilize the epididymis. Via the scrotal skin, a 23 G needle fitted with a tuberculin syringe is introduced into the epididymis to aspirate fluid.

D: Aspiration of testicular sperm (TESA): The testis is percutaneously punctured using a 13 G needle that is attached to a 20 ml syringe that fits into the Cameco holder. To sample different locations and disrupt the seminiferous tubules, negative pressure is applied and the needle tip is moved within the testis. Aspiration is performed on the testicular parenchyma (**30).**

The 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 **(31).** It also prevents issues with sperm decreased motility, cervix issues, etc.

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 (**24).**

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 (**24).**

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 (**32).**

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 **(32).**

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 **(32).**

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 **(33).**

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 (**33).**

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 **(33).**

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. (**33).**

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 (**33).**

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 (**33).**

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 **(2).**

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 **(12).**

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 **(12).**

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 (**10).**

A blood pregnancy test can confirm the pregnancy approximately 7–10 days following the embryo transfer procedure. Approximately two more weeks after conception, an ultrasound can be performed to confirm pregnancy **(10).**

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. **(30).**

References:

1. Shibahara, H., Wakimoto, Y., Fukui, A. & Hasegawa, A. 2021. Anti-sperm antibodies and reproductive failures. Am J Reprod Immunol, 85, 1-3.
2. Darmishonnejad, Z., Zarei-Kheirabadi, F., Tavalaee, M., Zarei-Kheirabadi, M., Zohrabi, D. & Nasr-Esfahani, M. H. 2020. Relationship between sperm telomere length and sperm quality in infertile men. Andrologia, 52, 45-76.
3. Peña, V. N., Kohn, T. P. & Herati, A. S. 2020. Genetic mutations contributing to non-obstructive azoospermia. Best Pract Res Clin Endocrinol Metab, 34, 1-6.
4. Bosch, E., Broer, S., Griesinger, G., Grynberg, M., Humaidan, P., et al. 2020. ESHRE guideline: ovarian stimulation for IVF/ICSI. Hum Reprod Open, 2020, 104-8.
5. Nie, H., Tang, Y. & Qin, W. 2020. Beyond acephalic spermatozoa: the complexity of intracytoplasmic sperm injection outcomes. Biomed Res Int, 2020, 45-55.
6. Haddad, M., Stewart, J., Xie, P., Cheung, S., Trout, A., Keating, D., et al. 2021. Thoughts on the popularity of ICSI. J Assist Reprod Genet, 38, 101-23.
7. Arshad, M. A., Majzoub, A. & Esteves, S. C. 2020. Predictors of surgical sperm retrieval in non-obstructive azoospermia: summary of current literature. Int Urol Nephrol, 52, 2015-38.
8. Sciorio, R. & Esteves, S. C. 2022. Contemporary use of ICSI and epigenetic risks to future generations. J Clin Med, 11, 2135-55.

9. Ayele, W. M., Tegegne, T. B., Damtie, Y., Chanie, M. G. & Mekonen, A. M. 2021. Prevalence of consistent condom use and associated factors among serodiscordant couples in Ethiopia, 2020: a mixed-method study. Biomed Res Int, 2021, 2-5.
10. Esteves, S. C., Roque, M., Bedoschi, G., Haahr, T. & Humaidan, P. 2018. Intracytoplasmic sperm injection for male infertility and consequences for offspring. Nat Rev Urol, 15, 535-62.
11. Khalafalla, K., Majzoub, A., Elbardisi, H., Bhathella, A., Chaudhari, A., Agarwal, A., et al. 2021. The effect of sperm DNA fragmentation on intracytoplasmic sperm injection outcome. J Androl, 53, 141-5.
12. Oseguera-López, I., Ruiz-Díaz, S., Ramos-Ibeas, P. & Pérez-Cerezales, S. 2019. Novel techniques of sperm selection for improving IVF and ICSI outcomes. Front Cell Dev Biol, 7, 298-305.
13. Kwan, I., Bhattacharya, S. & Woolner, A. 2021. Monitoring of stimulated cycles in assisted reproduction (IVF and ICSI). Cochrane Database Syst Rev, 1-13.
14. Balli, M., Cecchele, A., Pisaturo, V., Makieva, S., Carullo, G., Somigliana, E., et al. 2022. Opportunities and limits of conventional IVF versus ICSI: it is time to come off the fence. J Clin Med, 11, 5722-30.
15. Geng, T., Cheng, L., Ge, C. & Zhang, Y. 2020. The effect of ICSI in infertility couples with non-male factor: a systematic review and meta-analysis. J Assist Reprod Genet, 37, 2929-45.
16. Kasak, L. & Laan, M. 2021. Monogenic causes of non-obstructive azoospermia: challenges, established knowledge, limitations and perspectives. Hum Genet, 140, 135-54.
17. Tharakan, T., Luo, R., Jayasena, C. N. & Minhas, S. 2021. Non-obstructive azoospermia: current and future perspectives. Fac Rev, 10, 1-12.
18. Chen, H., Luo, T., Chen, T. & Wang, G. 2018. Seminal bacterial composition in patients with obstructive and non-obstructive azoospermia. Exp Ther Med, 15, 2884-90.

19. Mishra, S., Jain, B. & Shankar, S. 2021. Comparison of physiological-ICSI (PICSI) with ICSI in cases of moderate to severe oligoasthenoteratozoospermia (OAT)− a randomized control study. Hum Fertil, 8, 44-50.
20. Li, W., Zhu, L., Jia, M., Yin, S., Lu, G. & Liu, G. 2020. Missense mutation in DNAJB13 gene correlated with male fertility in asthenozoospermia. J Androl, 8, 299-306.
21. Máté, G., Balló, A., Márk, L., Czétány, P., Szántó, Á. & Török, A. 2022. Magnetic-Activated Cell Sorting as a Method to Improve Necrozoospermia-Related Asthenozoospermic Samples. J Clin Med, 11, 2914-22.
22. Crafa, A., Condorelli, R. A., La Vignera, S., Calogero, A. E. & Cannarella, R. 2023. Globozoospermia: a case report and systematic review of literature. World J Men's Health, 41, 49-7.
23. Fesahat, F., Henkel, R. & Agarwal, A. 2020. Globozoospermia syndrome: an update. J Androl, 52, 56-6.
24. Tavalaee, M., Nomikos, M., Lai, F. A. & Nasr-Esfahani, M. H. 2018. Expression of sperm PLCζ and clinical outcomes of ICSI-AOA in men affected by globozoospermia due to DPY19L2 deletion. Reprod Biomed Online, 36, 348-55.
25. Silva, A. F., Ramalho-Santos, J. & Amaral, S. 2021. The impact of antisperm antibodies on human male reproductive function: an update. Reproduction, 162, 55-71.
26. Abdallah, K. S., Hunt, S., Abdullah, S. A., Mol, B. W. & Youssef, M. A. 2020. How and why to define unexplained infertility? Rev Aquac, 38, 55-60.
27. Greco, E., Litwicka, K., Minasi, M. G., Cursio, E., Greco, P. F. & Barillari, P. 2020. Preimplantation genetic testing: where we are today. Int J Mol Sci, 21, 4381-90.
28. Sung, S. & Abramovitz, A. 2019. Tubal ligation. J Androl, 10-7.
29. Ku, F. Y., Wu, C. C., Hsiao, Y. W. & Kang, Y. N. 2018. Association of sperm source with miscarriage and take-home baby after ICSI in cryptozoospermia: a meta-analysis of testicular and ejaculated sperm. J Androl, 6, 882-89.
30. Gnessi, L., Scarselli, F., Minasi, M. G., Mariani, S., Lubrano, C., Basciani, S., et al. 2018. Testicular histopathology, semen analysis and FSH, predictive value of sperm retrieval:

supportive counseling in case of reoperation after testicular sperm extraction (TESE). BMC Urol, 18, 1-8.

31. Suzuki, T. & Perry, A. C. 2018. Intracytoplasmic sperm injection (ICSI): Applications and insights. J Androl, 169-81.
32. Baldini, D., Ferri, D., Baldini, G. M., Lot, D., Catino, A., Vizziello, D., et al. 2021. Sperm selection for icsi: do we have a winner? Cells, 10, 3566.
33. O'neill, C., Chow, S., Rosenwaks, Z. & Palermo, G. 2018. Development of ICSI. Reproduction, 156, F51-F8.