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Donor Insemination

Introduction

Donor Insemination i.e. instilling donor semen in a woman with an idea to impregnate her has been used as a reproductive technique by thousands of couples with male factor infertility. The history of therapeutic insemination dates back to Biblical times and the first donor insemination was first performed in Philadelphia (USA) in 1884 [1]. Donor insemination is a simple and cost effective technique for treating infertility due to azoospermia in male partner. Until 1990 fresh semen was used for insemination but due to the concern regarding transmission of infectious diseases now frozen semen is used exclusively for donor intrauterine inseminations (IUIs) and it has proved to be highly effective in achieving pregnancy. With a frozen thawed sample, IUI or intra cervical insemination (ICI) can be performed to achieve pregnancy. Pregnancy rates in donor insemination is 4% higher compared to partner insemination, which is close 12% [2]. With the advent of Intra Cytoplasmic Sperm Injection (ICSI) many couples with oligoasthenospermia prefer homologous conception. However, some may still choose donor insemination as IVF and ICSI is costly, has its own complications and there is a possible risk involved to the offspring with ICSI.

Indications for donor insemination

- azoospermic male partner
- non-correctable ejaculatory dysfunction
- to avoid transmission of genetic and infectious diseases
- same sex female couples and single women desirous of pregnancy

Despite being a widely practiced and well-established treatment, donor IUI remains psychologically stressful and emotionally draining for couples especially when the time interval to achieving a successful pregnancy increases.

Transmission of infections

Sperm donors need to undergo rigorous medical evaluation or screening tests to ensure that no sexual or genetic diseases are passed on to the potential offspring. The screening process includes taking a medical history and laboratory tests on the semen sample. According to FIGO recommendation donors of genetic material should be healthy persons of normal reproductive age free from sexually transmitted diseases and hereditary disorders. A mandatory quarantine is recommended prior to use to prevent transmission of sexually transmissible infections. However, post thaw sperm survival, motility and pregnancy rates are adversely affected by the cryopreservation technique and the cryoprotectant medium used, reducing fecundity from 18.9% to 5% with fresh and frozen-thawed sperm respectively [3]. Therefore more treatment cycles are required to achieve comparable success rates using fresh sperm [4].

Timing of Insemination

Insemination procedure must coincide with ovulation. To overcome this number of inseminations should be increased to consecutive days using a combination of IUI-IUI or ICI-IUI. Data for the optimal number of inseminations to be performed in a cycle is available from fresh insemination cycles. A recent meta-analysis of six randomized studies in infertile couples with unexplained infertility concluded that in a cycle double inseminations did not increase the odds of clinical pregnancy as compared to single insemination using fresh sperm [5]. In a previous systematic review and meta-analysis that included couples with all causes of infertility, the pregnancy rates were significantly higher in double insemination of fresh sperm compared to single insemination [6]. The outcome regarding double versus single IUIs with fresh sperm cannot be applied to donor cycles where frozen thawed sperm is used and remains a topic of research. Current evidence supports the use of single IUI rather than single ICI in women undergoing donor insemination [7]. In most of the published studies insemination is performed 32-36 hours post hCG administration.

Mode of Insemination

After thawing the frozen sperm, the suspension is deposited in the cervix or the uterus with intrauterine insemination being preferred. It is performed by introducing a 0.2–0.5 ml sperm suspension into the uterus with a small catheter, usually without ultrasound guidance. For frozen semen, IUI is better than intracervical insemination (ICI). In a recent Cochrane systematic review, Besselink et al reported a significantly higher pregnancy rate (OR= 3.37 95% CI 1.9-5.96) and live birth (OR=1.98 95% CI 1.02-3.86) after 6 cycles of frozen thawed donor IUI compared to ICI [8].

Luteal phase support after donor insemination

In ovarian stimulation cycles there is development of multiple follicles. The luteal phase of these cycles is characterized by high levels of estradiol or progesterone or both hormones and together with Inhibin A they suppress the levels of LH and FSH to very low levels [9]. The low levels of LH may result in lack of luteotrophic support manifested by low levels of progesterone and/or short luteal phase. If IUI is used in spontaneous or in mildly stimulated (1–2 follicles) cycle treatment with hCG or progesterone in the luteal phase may not improve the pregnancy rate [10]. Nevertheless the addition of progesterone, hCG and/or other substances is an established clinical practice even in the absence of any robust evidence of effectiveness.

Offspring created as a result of donor insemination

One of the many issues is whether or not to disclose to the resulting child about their donor conception. By not disclosing information about conception to a resulting child may lead to adverse social, emotional, and identity development and may cause high levels of concern and stress to parents as well. The effects of secrecy have been shown to spill over into marital relationship as well. It may be predicted that donor insemination children whose parents are not open about the donor conception may be more at risk for psychological problems than those whose parents decide to disclose.

Ethical issues regarding sperm donation

The importance of limiting the number of donor offspring from a single sperm donor relates to preventing accidental consanguinity between donor offspring. Different countries have their own guidelines for limiting the number of donor offspring depending upon the size of the country's population, density of population and mobility of population. In a country like China, each sperm donor can only impregnate five women through donor insemination or *in vitro* fertilization (IVF), whereas the American Society for Reproductive Medicine (ASRM) recommends a limit of 25 children per population of 800,000 for a single donor. In the United States, there is no federal or state law limiting sperm donation. ASRM has recommended that all institutions, clinics and sperm banks should maintain sufficient records, which allows a limit to be set for the number of



pregnancies for which a single donor is responsible. The Human Fertilization and Embryology Authority (HFEA) is United Kingdom's independent regulating authority overseeing the use of gametes and embryos in fertility treatment and research. HFEA categorically states that gametes (or embryos created using gametes) from an individual donor should not be used to produce children for more than 10 families, as a result of licensed assisted conception services. As regards the age of the donor, HFEA states that gametes should not be taken from anyone under the age of 18 for the treatment of others while ASRM guidelines state that the donor should be of legal age and, ideally, less than 40 years of age, because increased male age is associated with a progressive increase in the prevalence of aneuploid sperm. In India regulation regarding donor insemination is still awaited.

Sperm Banking

For successful sperm cryopreservation the requirements are maintaining post-thaw structural and functional integrity. The first successful human pregnancy from frozen sperm was reported in 1953 by Bunge and Sherman using dry ice for freezing. As cryopreservation of human semen results in a significant loss of spermatozoa motility and viability, only semen derived from a highly selected population of males is suitable for the purposes of insemination. With gradual improvement over time, new techniques have evolved for freezing and storing semen by immersing it in liquid nitrogen at -196.5°C . This has improved the fertilizing capacity of frozen semen and has led to the emergence of a number of commercial human sperm banks.

Uses of Sperm Banking

- Couples with male factor infertility - nearly 30% of the infertile population
- Post vasectomy
- Freezing semen for use at a later date, to ensure future fertility
- Before cancer treatment as gonadal damage may occur

Procedure

An informed consent is necessary as the process of collecting, storing and using semen is for therapeutic purposes. Following the infection of four recipients with human immunodeficiency virus (HIV) after insemination with semen from a seropositive donor, the use of quarantined cryopreserved semen became mandatory in donor insemination programs [11]. Another safe approach to cryostorage involves using small secondary tanks for 6 months before placing the samples into a main storage system [12].

Recipients should be informed that there is no guarantee that pregnancy will result from the use of donor semen that has been frozen and stored over a protracted period of time. The concerned infertile couples are more than willing to consent to any procedure which promises them the slightest hope of conception. They remain vulnerable to exploitation and it is unethical to take advantage of their desperation by creating false expectations of a future pregnancy.

Duration of Sperm Banking

Recent guidelines from ASRM for sperm donation do not include any recommendations or instructions concerning the duration of donor sperm storage. From a biological perspective, sperm can be banked indefinitely or for a very long period of time. Functional tests have revealed that the sperm acrosome reaction and zona pellucida binding were retained even after 28 years in liquid nitrogen [13].

The potential use of semen banking for social purposes creates biological and social consequences for a broad spectrum of society and poses serious ethical issues. It would be best to utilize the services of a sperm bank for human needs without a commercial angle.

Donor Insemination and Sperm Banking

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In Vitro Fertilization is a fertility procedure which first succeeded in the year 1978 by Dr. Edwards (an embryologist) and Dr. Steptoe (a gynaecologist) in England with the birth of Louise Brown from a natural cycle IVF. Since then the technology has developed and many women are having babies with the help of this technique.

Indications Of Ivf

IVF today is used for many indications but when first developed it was to overcome infertility from irreparable tubal damage. It is a legitimate treatment in infertility due to severe tubal disease, severe male factor infertility or couples with multifactorial causes. IVF is appropriate for women with age related or unexplained infertility where other treatments have failed. In women with premature ovarian failure or reproductive aging IVF using oocytes from a young donor is possible and for women with normal ovaries but no functional uterus IVF with embryo transfer to a gestational surrogate can be done. IVF is used for treatment of infertility with one or more causes for which no other effective treatment has worked.

INDICATIONS FOR IVF¹

Tubal factor

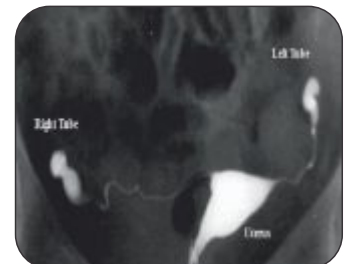
- Tubal block due to infection and its associated complications (especially in women with severe distal tubal disease, recurrent distal tubal obstruction) (Figure 2)
- Double block
- Patent but non-functional tubes
- Short tubes
- Hydrosalpinx (pre IVF, salpingectomy or cornual blockage is recommended) (Figure 1)
- Previously sterilized and in those whom recanalisation is not feasible
- Congenital absence of tubes
- Infertile 1 year post tubal surgery
- Altered tubo- ovarian relation
- Women with associated infertility factors (such as male factor infertility)

Unexplained infertility

- Following 3 or more failures with intrauterine insemination
- Elderly women with Unexplained Infertility

Ovulatory Dysfunction

- Women with ovulatory disorders (hypogonadotrophic hypogonadism, PCOS, Thyroid Disorder,



2. HSG film showing B/L fimbrial block with terminal Hydrosalpinx



1. Laparoscopic view of right Hydrosalpinx

Hyperprolactenemia), if women does not conceive after 3-6 cycles of treatment or there is an improper response to conventional ovulation inducing agents, IVF is an obvious alternative.

Endometriosis

- In cases of severe endometriosis
- In cases of mild to moderate where conventional treatment regimens have failed (as in unexplained)

(Figure 3-5)



3.Chocolate cyst



4.Fulguration of endometriotic spots



5.Flimsy adhesions in adnexa

Ovarian Failure or Diminished Ovarian Reserve

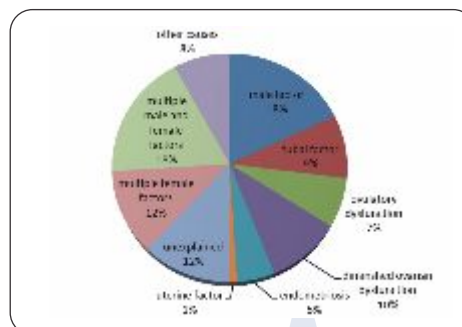
- IVF using oocytes from a young anonymous donor(esp. for women age >38 years, poor ovarian reserves test results, and in women with previous poor yield of embryos from IVF)

Male Partner

Mild to moderate oligoasthenoteratozoospermia

Other indications for IVF

- Fertility preservation in men/ women undergoing treatment for cancer or other illnesses requiring treatments (chemotherapy/ radiotherapy) that possess a serious threat to fertility may be candidates for urgent IVF and preservation of embryos before treatment begins and time and health allows.
- For women with normal ovaries but no functional uterus (mullerian agenesis, previous hysterectomy, severe intrauterine adhesions) and those women in whom pregnancy is to be avoided due to severe health issues IVF with embryo transfer to a gestational surrogate offers the possibility of a genetic offspring.
- For couples at risk of transmitting a specific genetic disease or abnormality to their offspring, IVF with pre-implantation genetic diagnosis (PGD) provides means to identify and exclude affected embryos and avoid the risk.
- HIV / AIDS, Hepatitis A, B, C affected male partner (as sperm washing reduces chances of transmission of the virus).



Diagnosis Among Couples Using ART¹

Intra Cytoplasmic Sperm Injection (ICSI) is a specialized technique which was devised for severe male factor infertility but has now found its place along with conventional IVF. Though initially used for men with severe count and motility problems and as an adjunct to sperm retrieval procedures like PESA (percutaneous epididymal sperm aspiration) and TESE (Testicular sperm extraction), it is now used in cases with previous failed fertilization, oocyte factors, age of the woman more than 35 years (usually at least 50% of the oocytes are injected) and unexplained infertility.

ICSI is performed using high power, specialized microscope, where the sperm is injected into the egg through a micro pipette, with the aid of fine coordinated movements (micromanipulation) (Figure 6). It achieves high fertilization rates, sometimes, but not always better than conventional IVF. When used in appropriate situations it gives a good success rate.



6. ICSI

Indications for intracytoplasmic sperm injection²

Obstructive azoospermia (following MESA/TESE) in patients with

- Congenital absence of the vas deferens (CAVD)
- Acquired vas obstruction
- Irreparable epididymal obstruction
- Postinfectious epididymal obstruction
- Conservatively untreatable ejaculatory disturbances
- Failed microsurgical reversal for vasectomy

Non-obstructive azoospermia (following TESE) in patients with

- Germ-cell aplasia, maturation arrest, and tubular sclerosis/atrophy, all with
- Focal spermatogenesis
- Sertoli-cell only syndrome
- Persistent azoospermia post chemotherapy
- A history of orchidopexy
- Spinal cord injury
- Seminiferous tubule dysgenesis (Klinefelter syndrome 47, XXY)
- Presence of acrosome less or immotile spermatozoa. E.g. inherited disorders of the sperm tail for which no other IVF technique is effective (ex: Kartagener's Syndrome).

High risk of fertilization failure due to

- Subnormal sperm samples - semen parameters below the threshold for standard IVF treatment e.g. oligoasthenoteratozoospermia (OAT)
- Severely oligozoospermic (< 5 million) and teratozoospermic men (strict normal sperm morphology 5%) with a very high (>70%) frequency of defective sperm-zona pellucida (ZP) interaction and hence a high risk of low or zero fertilization rate in IVF.
- Sperm autoimmunity (high titres of antisperm antibodies/sperm-bound antibodies- interference with gamete interaction)

Previous fertilization failures with conventional IVF

When preimplantation genetic diagnosis (PGD) is indicated in pregnancies that are at high risk of aneuploidy because of genetic factors associated with azoospermia, to avoid contamination by extraneous DNA in the case of Polymerase chain reaction (PCR)- based testing and to increase the number of embryos available for testing.

PROCEDURE OF IVF

Before starting IVF :

A successful IVF procedure requires a normal oocyte, normal sperm and a receptive endometrium. Before IVF, both the partners are counselled. The female partner is tested for ovarian reserve [if required _ advanced age, previous poor response] and the endometrium is examined by sonography and/ or hysteroscopy if required. A basic semen analysis is done and a sample is frozen for future use in case the male partner is unavailable at the time of IVF cycle. If the male partner is azoospermic then testicular biopsy is performed to look for sperms and the sample frozen. Routine haematological tests are carried out to confirm suitability for anaesthesia and pregnancy.

There are basically five steps in the IVF and embryo transfer process which include the following:

1. Controlled ovarian hyperstimulation and monitoring.
2. Oocyte retrieval.
3. Sperm collection and processing.
4. Insemination and Fertilisation check.
5. Embryo transfer.

Step 1: Controlled Ovarian Hyperstimulation and Monitoring :

Natural cycle IVF has lower pregnancy rates per cycle of treatment. Hence, Ovaries are stimulated by ovulation induction medicines to produce multiple follicles. Monitoring is carried out using transvaginal ultrasound to monitor the development of the follicles and endometrium.

Stimulation protocols

There are different types of ovarian stimulation protocols, but IVF usually uses three, the long, the short, and the antagonist ones. The duration and type of GnRH analogue administered is the key difference in the different protocols and the decision to offer which protocol is individualised.



1. Long protocol

The long protocol has two stages, ovarian suppression and stimulation. The first stage of suppression begins in the mid luteal phase [around day 21] in a woman having a regular 28-day menstrual cycle. GnRH agonist analogues are administered on a daily basis. A sonography and blood estradiol measurement is performed to check pituitary and ovarian suppression on the 2nd or 3rd day of next period. If suppression is adequate, ovarian stimulation is started.

Gonadotropins (recombinant or urinary products) are administered on a daily basis at a dose recommended by the doctor. Transvaginal sonography is carried out every 3 to 5 days to monitor ovarian response and the dose modified accordingly. The duration of stage two is approximately 10-14 days i.e. the long protocol will last approximately one month.

2. Antagonist protocol

In the GnRh antagonist protocol, stimulation with gonadotropins begins on the 2nd or 3rd day of the cycle. GnRH antagonist is administered when the leading follicle reaches the size of 14 mm to prevent premature ovulation. Initiation of antagonist administration may take place on the 6th day of gonadotropin stimulation [fixed protocol] or based on sonography [flexible protocol].

3. Short protocol

The short protocol (flare-up GnRH agonist protocol) is mostly chosen for women with a poor ovarian response or for women of an older age. It takes almost half the time it takes the long protocol (10-15 days) since suppression and stimulation phases take place almost concurrently. The GnRH agonist usually begins on the 1st-2nd day of the cycle and gonadotropins begin one day later. Monitoring is carried out as in the long protocol.

Ovulation Trigger :

When the follicles reach a size of 17 – 18mm and the endometrium is 8mm or more, final follicular maturation is triggered by administration of hCG.

Step 2: Oocyte retrieval

The egg collection is performed 34-36 hours after the hCG injection. It is carried out under short general anesthesia using transvaginal sonography. A needle is inserted through a guide attached to the vaginal probe to enter each follicle and the follicular fluid aspirated. The follicular aspirate is examined by the embryologist for identifying eggs. All eggs are identified, separated and placed in the incubator.

Step 3: Sperm collection and processing:

At the time of egg collection, a semen sample is collected and processed to separate the highly motile and sperms with normal morphology for insemination. The two most commonly utilised methods for sperm preparation are swim – up and density gradient method. A semen sample is generally cryopreserved before starting treatment. In case the husband is azoospermic, testicular biopsy is performed or donor sperms are used.

Step 4: Insemination and fertilization check :

In Conventional IVF, 1 to 3 oocytes are incubated with sperms 50,000 – 100,000 sperms in 4 well dish containing culture nutrient and placed in the incubator where they will remain for approximately 16-20 hours. The

Indications and Procedure of In Vitro Fertilisation

following morning, eggs are examined using a microscope and it is verified whether or not fertilisation has occurred. Approximately, 60 -70% fertilisation rate should be achieved. The embryologist records the number of eggs normally fertilised and monitors their normal development. In some cases where fertilization is suspected to be low, or severe male factor infertility, intracytoplasmic sperm injection (ICSI) is used. It is micromanipulation where, a single sperm is injected directly into the egg in an attempt to achieve fertilization. The eggs are monitored to confirm that fertilization and formation of embryos.

Step 5: Embryo transfer:

Embryo transfer is performed two [at 2 or 4 cell stage] or three [at 6 or 8 cell stage] days or on the fifth day [at the blastocyst stage] after oocyte collection. The embryologist selects the best embryos, loads them into a fine embryo transfer catheter which is passed trans cervically in to the uterus. The procedure is generally carried out under ultrasound guidance. The number of embryos transferred depends on many factors, and more the embryos transferred higher is the multiple pregnancy rate. Typically two to three embryos are transferred.

Luteal Phase Support:

Progesterone preparations in the form of vaginal suppositories, vaginal gel or intramuscular oil based injections or hCG injections are administered for support of the corpus luteum. This is initially given for 2 weeks and is continued if pregnant, till 12 weeks.

A blood test is carried out 14 day of after the embryo transfer to confirm pregnancy. If the test is positive, ultrasound is carried out 1 week later.

Cryopreservation:

Cryopreservation involves freezing of any tissue at -196 C in liquid nitrogen. Sperms, eggs and embryos can be preserved by either the traditional slow freeze method or by vitrification. Embryos preserved in such a way can be used for another cycle if the first cycle fails or for another pregnancy also.

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21 INTRA CYTOPLASMIC SPERM INJECTION (ICSI): INDICATIONS AND PROCEDURE



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After the introduction of intra cytoplasmic sperm injection (ICSI) (Palermo et al., 1992), this technique was rapidly integrated into the routine clinical use of fertility clinics offering assisted reproductive technology (ART) throughout the world. During recent years, ICSI has become the most frequently used method for fertilization in Europe, Australia and USA (Wright et al., 2007; de Mouzon *et al* 2010).

ICSI is a method of assisted reproductive technology that involves the selection of a single spermatozoon and then injection into the egg. In order to produce a healthy embryo by ICSI a spermatozoon containing a functional genome and centriole are required. A significantly higher fertilization rate can be achieved with ICSI than with conventional IVF in patients with borderline sperm characteristics.

Indications for ICSI

Most couples with severe male factor infertility can be treated with ICSI. In addition, ICSI can also be applied using sperm from the epididymis and testis in case of obstruction of the seminal excretory ducts. Testicular sperm extraction (TESE) followed by ICSI is useful in patients with aberrant spermatogenesis if sufficient number of sperm can be retrieved from the testicular tissue (reviewed by Devroey and van Steirteghem, 2004).

ICSI using ejaculated sperm helps when sperm characteristics are not suitable for conventional IVF as in case of oligoasthenoteratozoospermia. In addition, ICSI can be applied on patients who had total fertilization failure after conventional IVF, high concentration of antisperm antibodies, cryopreserved ejaculate of cancer patients where sample may be needed for multiple ART attempts, patients with ejaculatory disturbances, patients with retrograde ejaculation and in patients where semen was banked prior to vasectomy (Nagy et al., 1995; Chung et al., 1998; Benadiva et al., 1999; Nikolettos et al., 1999).

In patients with obstructive azoospermia, congenital bilateral absence of the vas deferens (CBAVD) and Young syndrome, sperm can be retrieved from the epididymis and then used for ICSI. Microsurgical epididymal sperm aspiration (MESA) is commonly used to collect epididymal spermatozoa. Since the sperm yield is usually higher in patients having obstructive azoospermia with normal spermatogenesis, excess sperm can be cryopreserved for subsequent ICSI attempts. Epididymal sperm can also be obtained by percutaneous sperm aspiration (PESA) (Tsirigotis et al., 1996). In case of HIV serodiscordant couples, ICSI can be effectively used as a risk reduction technique.

In all cases where fresh sperm can be used, it is also possible to use frozen–thawed sperm. This is particularly important in case of testicular spermatozoa where repeated biopsy and its eventual complications can be avoided by using frozen thawed sperm (Devroey et al., 1995; Habermann et al., 2000).

Instrumentation:

An already existing successful IVF program is a prerequisite for the success of any ICSI program, as all the equipment, environmental requirements and skills in gamete handling that are needed for oocyte and embryo culture *in vitro* are essential to the establishment of good success by ICSI. In addition, a good micromanipulator is an essential requirement for efficient, smooth and confident translation of hand movement to the clearly visualized specimen.

There are two basic types of manipulation system available today, motorized and mechanical. The micromanipulator set-ups have a motorized coarse movement with joystick and hydraulic fine movement translated through a separate joystick. Quality tested consumables are manufactured and supplied by many companies worldwide.

ICSI is usually conducted using an inverted microscope between 200-400X magnification and therefore equipped with 10, 20 and 40X objectives. Also, the microscope should have a long working distance condenser. Hoffman or similar optical systems offers special image enhancement that is of great value in these procedures. A steady heat stage is required to prevent temperature shock to the gametes. Vibration can cause significant disruption to ICSI procedure and to minimize the vibration, commercially available anti-vibration table can be used.

Procedure:

Setting up of ICSI workstation

It is important to set up the manipulators to ensure they provide the maximum range of movement around the centre of the microscope field of view. This can be achieved by executing the following steps (Fleming & King, 2003).

- Ensure the controls of the micromanipulators and microscope stage are adjusted so they are in the centre of their movement range.
- Ensure that oil column in the injector and silicon tube is continuous.
- Fit the pipettes into the pipette holders of the microinjectors, and fit the pipette holders into the universal joints of the micromanipulators.
- Using the freedom of movements of the ball joints, the micromanipulator-coarse positioned joint, and the coarse positioned-mounting adapter adjustment, position the pipette tips in the centre of the field of view of the microscope, in focus.

Sperm immobilization and aspiration

- Align and equilibrate injection pipette in PVP droplet using 4X or 10X objective and then by 20X objective.
- Look for healthy, morphologically normal sperm & refocus the sperm tail, edge of the PVP streak & pipette tip.
- Immobilize the sperm by applying gentle pressure in the tail.
- Apply very slight negative pressure to the injection pipette by turning the injector screw marginally anticlockwise, aspirate sperm tail first. Care need to be taken to control the movement of sperm inside the pipette.

ICSI

- Align the first polar body at right angles to the line of the micropipettes (6 or 12 o'clock position)
- Lower the injection pipette, and keep it almost adjacent to the zona pellucida at the midline of the oocyte and raise or lower it until its very tip comes into sharp focus. At this stage, both oolemma and injection pipette tip should be in focus concurrently. This ensures that the injection pipette will initially pierce the oocyte and its midline.
- Turn the injector screw clockwise to move sperm towards tip of the injection pipettes.



Intra Cytoplasmic Sperm Injection (ICSI): Indications And Procedure

- The injection pipette may then be advanced carefully in a straight line towards the oocyte to penetrate the zona pellucida. Confirm that the injection pipette will enter the oocyte at its midline by observing the pattern of indentation that it creates.
- Once the injection pipette has penetrated the zona pellucida, the pipette may be withdrawn slightly, but it is important to ensure that ooplasm remains around its opening.
- Aspirate ooplasm into injection pipette and then carefully deposit ooplasm that has been aspirated along with the spermatozoa, into the oocyte.
- Once the sperm has excited the injection pipette, withdraw the injection pipette slowly.

Ovarian stimulation and oocyte collection methods are similar as for conventional IVF. In the majority of cases the combination of GnRH agonist/antagonist and urinary or recombinant gonadotrophins followed by hCG trigger 36 h prior to oocyte collection is being practiced in most of the ART centers.

ICSI is the most successful technique used to overcome fertilization failure. In addition, it has helped to understand better some of the key steps of the fertilization process. Complete fertilization failure after ICSI is very unusual, and in most cases is probably due to either failed oocyte activation or incomplete de-condensation of the sperm. The potential effects of ICSI on child development should not be underestimated and continuous and vigilant screening should be practiced.

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