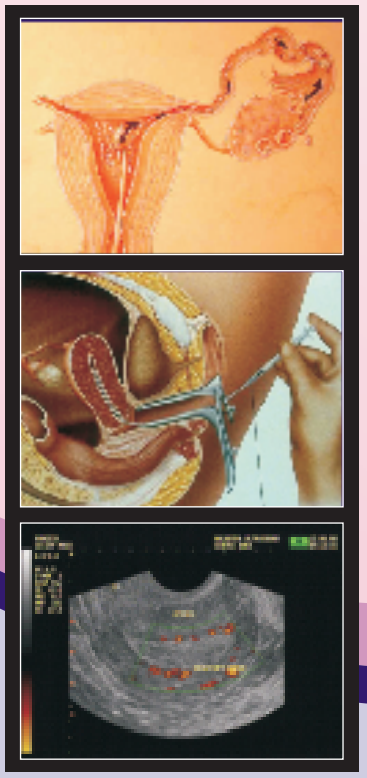
Intrauterine Insemination
(IUI)

Editor
Dr. Rishma Dhillon Pai



FOGSIFOCUS

Reaching the Unreached, FOGSI 2010 initiative"

President Message

Dr. Sanjay Gupte
President FOGSI

Dear Fogsians,

Greetings from the team of "Reaching the Unreached, FOGSI 2010 initiative".

It gives me great pride to bring forth the FOGSI focus on IUI (Intrauterine Insemination). There is a constant ongoing development in the field of gynecology, especially infertility & all of us strive for results to be able to serve a woman better. Constant updating of knowledge is fundamental. Many a practitioner due to busy schedule needs a focused capsule on a particular topic that he/she seeks & this focus offers just that.

I congratulate Dr. Rishma Pai, the senior Vice President, for the concept & the contents of this concise & precise focus. It has been a hard work & dedication that is seen in the completion of this issue. I also congratulate the contributors who have used this platform judiciously & thank them for sharing their knowledge with all of us.

There are a lot of projects planned this year which I urge you all to actively participate in. The agenda for the year is "standardization, data collection, clinical research & safe practice"

Remember FOGSI & all its endeavors are ours. FOGSI is us.

With warm regards

Editor's Desk

A new baby is like the beginning of all things wonder, hope, a dream of possibilities.
Eda J. Le Shan

Dr. Rishma Dhillon Pai
First Vice President

Dear Colleagues,
It gives me great pleasure to bring to you the first FOGSI Focus of the year 2010. The topic has been carefully selected considering that there are thousands of cycles of intrauterine insemination (IUI) being done in India annually. It has become a procedure of first choice due to its_simplicity, safety and good results in well selected cases of infertility. We have put together well written and researched articles by experts in the field, on every aspect of IUI.

Our President, Dr. Sanjay Gupte has really helped and encouraged us in the making of this FOGSI Focus. A very special thanks to Dr. Pratap Kumar, who reviewed the articles and gave us his very valuable feedback.

This FOGSI Focus would not have been possible without the support of Sun Pharmaceuticals, and we are really grateful to them for their wholehearted support. I thank our contributors who despite their busy schedules have taken the time to write these articles.

I hope this FOGSI Focus on IUI will bring to you all the updated knowledge and information that you seek and go a long way in your helping more woman achieve the dream of motherhood.

Yours in FOGSI

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IUI- Indications & Selection Criteria

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Intra-uterine insemination has become rather popular in recent years for a variety of indications. Some of the indications are backed by Level-I evidence while others are not supported by significant evidence but being offered because of simplicity & cost effectiveness of IUI procedure before moving onto more advanced procedures like IVF/ICSI. The concept of super ovulation & improvement in the methods of selection of motile sperms has helped to improve the results of intra-uterine insemination up to a certain extent. Proper Patients selection, individualization of stimulation protocols & exact timing of insemination are the key factors for a successful outcome in a IUI program.[1]

Thorough clinical evaluation of both male & female partner is mandatory before starting an IUI cycle. A detailed clinical history, physical examination, detailed semen profile, a base line TVS, a hysterosalpingogram or a video hystero-laparoscopy for tubal status, exclusion of major endocrinological disorders like hypothyroidism or hyperprolactinaemia & tests for sexually transmitted disease are basic investigation before starting the IUI cycle.[2] Basic evaluation helps in individualization of protocol, counseling & prognostication. Finally a informed consent must be obtained in all cases.

Indications:

Conflicting results have been reported in the recent literature making it difficult to identify groups of patients who will benefit from IUI. Some of the indications are largely empirical but offered IUI very frequently. Various clinical indications where IUI can be helpful in improving chances of conception are the following.[3]

1. Unexplained Infertility

2. Cervical Factor

3. Mild to Moderate Male Subfertility.
   - Oligospermia
   - Teratospermia
   - Highly Viscous Semen
   - Hypospermia
   - Asthenospermia
   - Oligo-asthenote ratozoospermia
   - Pyospermia
   - Delayed Lique faction

4. Endometrosis Mild & Moderate with Normal Tubo Ovarian relations.

5. Immunological factors: Presence of Antisperm Antibodies

6. Chronic Anovulation

7. Corrected Tubo-peritoneal factor

8. Anatomical Defects like Hypospadias/Retrograde ejaculation.

9. Psychosexual Dysfunction: like Erectile Dysfunction & Vaginismus

10. Neurological Disorders : Spinal cord Injury & Diabetic Neuropathy

11. Some special Indications : IUI with frozen husband's semen is indicated in the following circumstances.

- Absent ee Husband
- Husband on Anti neoplastic drugs
- Chr onic Medical Disorders

12. HIV Discor dant couples - HIV^{+ve} female with HIV^{-ve} male
HIV^{-ve} female & HIV^{+ve} male [4,5,6,7,8]

Indications for Donor Insemination :

- Azoospermia
- Failed Microsurgical Vaso-Epididymal anastomosis
- Persistent failure at IVF/ICSI
- Genetic disorder/Hereditary disorders
- Severe Seminal Defects
- Un-affordability of advanced procedures live IVF/ICSI
- Rh Iso -immunization
- HIV+ve Husband
- Same sex couples [9]
- Single women [9]

Unexplained infertility:

It is the second most common indication of IUI with evidence level I. A multicentre study revealed that super-ovulation with IUI achieved PR of 27%/ cycle as against 15.2% with super-ovulation alone & spontaneous conception of 11.1%.[10]

In another recent review of six trials, Authors concluded that Superovulation--IUI

significantly increased pregnancy rates when compared with timed intercourse (OR 1.68). [11]

Male infertility:

IUI is the most frequent used fertility treatment for male sub-fertility though its efficacy has not been proven specially in Moderate male sub fertility. In a recent meta analysis of RCT's, authors concluded that there was insufficient evidence to recommend or advice against IUI in male infertility & there is need for high quality randomized trials. Hence firm conclusion on its benefits cannot be drawn as yet.[12]

Cervical Factor:

Cervical hostility is one of the important indications for IUI as it is logical to bypass the hostile cervix, facilitating the ascent of motile sperms to increase the chances of conception . Numerous studies have confirmed the usefulness of IUI in cervical factor infertility.[13]

Endometriosis:

Patients with minimal & mild endometriosis can be benefitted from Superovulation-IUI. IVF seems to be more beneficial in severe endometriosis. [14]

Immunological infertility:

Several methods have been reported, however sperm washing is supposed to be one of the important methods using IUI, IVF or ICSI [15,16]. IUI is primary method & success can be enhanced by newer sperm preparation techniques. However ICSI is the method of choice in severe immunological infertility.[17]

Selection criteria:

Proper & careful selection is very important for a successful outcome.

Age:

Advanced age is a relative contraindication for IUI as the fecundity is quite low as age advances.[2]

Seminal Parameters:

Fecundity with moderate male factor is low & with severe OAT, very few conceptions are reported. So it is advisable to move on for ICSI in cases of severe OAT & 3 failed cycles of IUI in Moderate OAT.

A cut off limit of Absolute Motile sperm count of > 8 million sperm after wash has been suggested for satisfactory outcome. Is is also suggested that a absolute sperm count of < 5 million in post wash sample is a indication for ICSI as fecunidity is very low with IUI in such cases.[3] A poor sperm survival test has been suggested as relative contraindication for IUI.[3]

Tubal Pathology:

Bilateral Tubal disease is an absolute contraindication for IUI. As least one patent Tube with normal Tubo-ovarian relation is mandatory for IUI to be considered. Patients with corrected tubal pathology may be considered for three cycle of IUI before moving on to IVF. A visible pelvic mass/ or intrauterine irregularity is a relative contra indication for IUI. Ideally these patients on TVS should be scheduled for a video Hysteroscopy/ Laparoscopy before deciding the line of treatment.

Hormonal Profile:

Correction of major endocrinological disorders like Hypothyroidism / Hyperprolactinemia should be done before starting the IUI cycle.

Miscellaneous considerations:

Patients with multiple etiological factors have low fecundity & should be counseled thoroughly for advanced procedures like IVF/ICSI rather than IUI. However, a limited trial of IUI can be given in this group of patients.

A basic TVS on Day 2 or D 3 of cycle to rule out persistent follicular cyst or thick unfavorable endometrium is advisable before starting the stimulation.

Psychosexual Dysfunction:

Patients with Psychosexual Dysfunction usually have problem of producing semen on demand, specially on day of IUI. It is advisable to have frozen semen as back up before starting the actual cycle.[2]

Identification of poor prognostic factors is important & the patients should be counseled in detail for limitation of IUI & option for other ART procedures should be given.

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Physiological aspects of Intra Uterine Insemination (IUI)

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Introduction:

Intra Uterine Insemination (IUI) is the most widely used medically assisted conception (MAC) technique in patients with moderate male factor, cervical mucus hostility, and unexplained infertility as a first step before undergoing more complex programs such as in vitro fertilization. Separation of seminal fluid and other contaminants such as dead sperm, cell debris, prostaglandins, and microorganisms from inseminate is the initial important step to enhance the functional capabilities of the spermatozoa. Several methods such as swim-up and swim-down procedure, density gradient centrifugation, have been used for the separation of motile spermatozoa. The purpose of sperm wash technique is to separate spermatozoa from the seminal plasma and to improve the general sperm quality prior to assisted conception.

Sperm motility in female reproductive tract:

This aspect needs to be understood to get to know the principle of physiological aspects of IUI. Testicular spermatozoa of humans have faint or no motility which is apparently due to the immaturity of the plasmalemma. During passage through the epididymis, spermatozoa undergo substantial maturational changes that result in their acquisition of motility. After ejaculation when the spermatozoa are mixed with the secretion of the accessory glands spermatozoa undergo motility activation.Passage of sperm through the female reproductive tract is regulated to maximize the chance of fertilization and ensure that sperm with normal morphology and vigorous motility will be the ones to succeed. Because spermatozoa are terminally differentiated cells, deprived of an active transcription and translation apparatus, they must survive in the female reproductive tract. Spermatozoa are subjected to physical stresses during ejaculation and contractions of the female tract, and also subjected to oxidative damage. They may encounter the immune system of female body since they are allogenic to female [1]. Out of millions of sperm inseminated at coitus, only a few thousand reach the Fallopian tubes and, ordinarily, only a single sperm fertilizes an oocyte.

Transport in the fallopian tube:

Sperm transport within the tube appears to be a discontinuous process. As sperm pass through the uterotubal junction and enter the tubal isthmus, they may be trapped and held in a reservoir. Entrapment and storage of sperm in the initial segment of the tube may serve to prevent polyspermic fertilization by allowing only a few sperm at a time to reach the oocyte in the ampulla. In addition to providing the right kind of support, the storage reservoir maintains the fertility of sperm until ovulation. Sperm undergo two changes in preparation for fertilization: capacitation and hyperactivation. Capacitation involves changes in the plasma membrane, including shedding of proteins and cholesterol [2] which could reduce their affinity for the endosalpingeal epithelium. Hyperactivation, on the other hand, is a change in flagellar beating that typically involves an increase in the amplitude. This can provide the force necessary for overcoming the attraction between sperm and epithelium[3].

Hyperactivation of sperm:

Sperm becomes hyperactivated in the female tract, most likely in the Fallopian tubes. In addition to its possible role in detachment from endosalpingeal epithelium, hyperactivation is required by sperm to progress towards the oocyte in viscoelastic tubal lumen and penetrate. Hyperactivation also endows sperm with greater flexibility for turning around in pockets of mucosa[4]. Movement characteristics and acrosomal status of rabbit spermatozoa recovered at the site and time of fertilization[5]. In addition to assisting sperm in reaching the oocyte, hyperactivation also aids sperm in penetrating the zona pellucida.

Taxis of sperm towards oocyte:

Sperm taxis is defined as an oriented movement in response to a chemical or physical gradient, resulting in approaching or repelling from the attractant or repellant respectively. It is a key event in reproduction as the spermatozoa must be guided towards the eggs over long distance. There is evidence for the existence of two complementary guidance mechanisms i.e thermotaxis (temperature dependent) operating within the fallopian tube[6].

Gamete interaction and fertilization:

Capacitation:

The physiological changes in sperm cells that are required for acrosome reaction and oocyte binding are collectively termed capacitation. Capacitation may enable the female tract to control

the speed with which sperm gain the fertilizing capacity and thereby enable the delivery of freshly capacitated sperm to ovulated eggs. Under *in vivo* situation the capacitation occurs in female genital tract. During capacitation, the decapacitation factors from seminal plasma which are adsorbed on the surface of the spermatozoa are removed from the sperm surface especially during their migration through cervical mucus [7].The reactive oxygen species (ROS) at very low level acts as positive modulator of capacitation in female reproductive tract as well as under *in vitro* situation. Although there is no definitive marker to identify the capacitation, hyperactivated motility and exocytosis of acrosomal contents may represent the end points of capacitation.

Acrosome reaction:

Upon completion of capacitation, the spermatozoa are ready to undergo acrosome reaction, which is an exocytotic event. The release and dissolution of acrosomal contents are absolutely required for the successful penetration of spermatozoa through the cumulus oophorous and zona pellucida. The major inducers of acrosome reaction are progesterone and zona protein. Follicular fluid mixed with oviductal fluid at the time of ovulation is a rich source of progesterone. Binding of spermatozoa with progesterone induces a massive calcium influx. The enzymes hyaluronidase and acrosin are released in vesicles. Hyaluronidase may help in digesting the cumulus cells and help in penetration of sperm to reach the zona. The acrosin has trypsin like activity which softens the zona pellucida glycoproteins. Vigorous motility of acrosome reacted sperm and digestive activity of acrosin together may help the spermatozoa in piercing the zona to enter the perivitelline space.

Sperm physiology and enhancement of functional capabilities *in vitro*

The recovery of sperm after swim up varies according to the various modifications of the techniques used. Harris *et al.*[8] reported a recovery of 58% for normozoospermic men. Purvis and Egdetveit [9] reported a reduction in sperm recovery in normozoospermic men. Adiga and Pratap Kumar[10] observed poor sperm recovery in the samples of men having normal semen parameters. This may be due to reduced possibility of sperm movement from the lower portion of the centrifuged pellet, when the sperm are more densely packed. It may also reflect damage to the sperm by free oxygen radicals associated with centrifugation[11]. In normozoospermic men, the proportion of motile sperm entering the overlaying medium remained relatively constant at about 40% with increasing sperm concentration in the seminal plasma[9]. In such situations where sperm density is very high in the semen, swim up may be performed in more than one tube depending on the sperm count in the semen or the size of the pellet after first centrifugation. Methods utilized in sperm preparation should be capable of selecting and concentrating the

subpopulation of normal motile spermatozoa in the ejaculate. Such methods aim to remove seminal plasma, dead, damaged and abnormal spermatozoa, immature germ cells, bacteria, other cells such as white blood cells, and cellular debris. In so doing, they closely mimic the action of the cervix and cervical mucus on ejaculated semen. Removal of seminal plasma is important because it contains prostaglandins which if inseminated directly into the uterine cavity may stimulate very strong and painful uterine contractions. Furthermore, some other constituents of seminal plasma stabilize the sperm membrane and prevent capacitation and hyperactivation which normally precede the acrosomal reaction that is necessary for successful sperm invasion of oocyte investments and subsequent fertilization. The particular culture medium used for sperm preparation depends on factors such as individual preference, easy availability and cost considerations.

Use of density gradient separation:

Reactive oxygen species cause lipid peroxidation of the sperm membrane, thereby leading to a loss of motility and decreased fertilizing ability [12]. While normal spermatozoa do produce small amounts of these superoxide radicals, production is many times more in dead or defective spermatozoa and white blood cells found in the ejaculate [13]. In view of this, it is important to separate normal motile spermatozoa from these noxious constituents of the ejaculate at the outset of the preparation process. Incorporation of centrifugation of the semen sample through buoyant density media as the first step in the sperm preparation technique fulfils this requirement and avoids or significantly decreases the collection of these unwanted cells and debris in the pellet that forms at the bottom of the centrifuge tube.

The ideal sperm separation technique should (i) be quick, easy and cost-effective, (ii) isolate as much motile spermatozoa as possible, (iii) not cause sperm damage or non-physiological alterations of the separated sperm cells, (iv) eliminate dead spermatozoa and other cells, including leukocytes and bacteria, (v) eliminate toxic or bioactive substances like decapacitation factors or reactive oxygen species (ROS), and (vi) allow processing of larger volumes of ejaculates. Since none of the methods available meets all these requirements, a variety of sperm separation techniques is mandatory in clinical practice to obtain an optimal yield of functionally competent spermatozoa for insemination purposes. Depending on the ejaculate quality, these methods have different efficiency and areas of use. In the conventional swim-up technique, functional spermatozoa can come into close cell-to-cell contact with defective sperm or leukocytes by centrifugation, thus causing massive oxidative damages of the sperm plasma membrane by ROS and consequently of sperm functions . Therefore, the quality of the ejaculates has direct consequences on the choice of a sperm separation method. Major disadvantage of this technique is the fact that for its use spermatozoa are pelleted, thus coming into close cell-to-cell contact with each other, cell debris and leukocytes, which are known to produce very high levels

of reactive oxygen species (ROS). Due to the extraordinary high amount of poly-unsaturated fatty acids in the sperm's plasma membranes, these ROS cause lipid peroxidation and therefore a dramatic decrease in sperm functions, including motility. Overall, although many men's spermatozoa may not be impaired to the extent of inhibiting fertilization, some couples' chances of successful IVF will certainly be compromised. It is therefore not reasonable to continue and to use a technique, such as swim-up from pelleted semen with the inherent potential to cause irrevocable damage to spermatozoa prejudicial to a desired functional endpoint. Eventually, this knowledge led to the development of other more gentle sperm separation methods that also allow a higher recovery of motile and functional spermatozoa.

Conclusions:

A proper knowledge of the physiological process of the sperm production, transport,maturation, capacitation and fertilization is essential to understand the physiological aspects of IUI for better outcome.

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Setting up an IUI laboratory

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The idea of introducing seminal fraction containing motile sperm into female reproductive tract by means other than copulation to impregnate a woman was successfully applied nearly 200 years ago by Dr John Hunter in 1770's. Since then, many modifications of the technique were tried with varying success[1,2,3,4]. Intra uterine insemination (IUI) or artificial insemination can be considered as the first line of treatment for infertility as it is simple, noninvasive and less expensive as compared to more complicated and expensive procedures like varicocele surgery, microsurgery, In Vitro Fertilization (IVF), and Gamete Intra Fallopian Transfer (GIFT). The success rate varies from 6% to 26% depending upon age, indication, and extent of male factor [5]. The laboratory setup and skill involved in semen preparation technique has a major role in deciding its outcome.

A good IUI laboratory can give consistent high pregnancy rates. Therefore it should be set according to the stringent international standards.

Layout-

An IUI laboratory is divided into following areas-

1- **Patient's waiting room**.

A pleasant atmosphere always makes a difference. A spacious, well ventilated waiting room with a smiling, ready to help secretary and basic facilities like drinking water, reading material, TV and WC makes patient's approach more positive towards treatment.

Fig.1 Waiting room

2- **Semen collection room**.

In many laboratories, this is the most neglected section. One should realize that it can be really difficult task to produce semen sample on demand without natural sexual arousal. Particularly, when person knows that outside the room, there are few more patients waiting to give sample and the lab staff is in a hurry to finish the work and go home. Semen

collection room should be isolated, its entrance away from main waiting room. It should be neat and clean, should not be congested. Some times the female partner is required to assist the male, therefore sufficient area with a bed, chair and a disinfected platform to keep the container be provided. A urinal, washbasin with soap and sterile gauze to clean hands and penis also should be provided. Sometimes, patient is tensed and is unable to get erection. Under these circumstances, providing erotic materials like books, magazines, videos and vibrators certainly help. If still patient is uncomfortable collecting sample, he should be allowed to bring it from home if he can get it within 30-40 minutes. Giving proper instructions particularly while collecting by split ejaculation- is an integral part of good laboratory practice; otherwise if part of the sample gets spilled on the floor, patient may simply picks it and puts it back into the bottle without informing staff.

3- Semen preparation area.

Fig.2 IUI workstation

This area is the key factor in achieving good pregnancy rate. Depending upon the location, the air quality of the laboratory should be evaluated for presence of Volatile Organic Compounds (VOCs), dust, suspended particles and any other impurities. These can be effectively removed using HEPA filters, activated carbon filters and positive air pressure that flows unidirectional towards outer side of the lab. 7-10 times air change per hour is another effective method to get clean air in the room. Depending upon the workload, this area should be sufficiently large enough for at least 2 people to work comfortably without interference. Fig. 2

4- Insemination room.

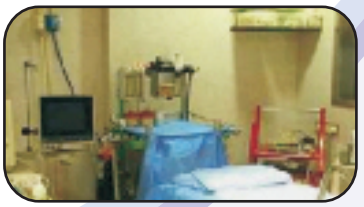

Fig.3 Insemination room

This room is equivalent to an operation theatre that needs IUI tables which can be adjusted for height and can be inclined as per requirement. This should be preferably sterile or at least a semi sterile room. Fig.3

5- Recovery room.

Patient can be shifted to this room post IUI for up to 30 minutes to take rest. In case of complications during treatment like excessive bleeding, there has to be a trained nurse to handle the situations.

6- Storage room.

There should be a clean place to store disposables like test tubes, pipettes, glassware, catheters etc. This place can also be used to store frozen semen samples.

7- Record room.

As required by ICMR guidelines, there should be a provision to maintain records of all patients for 10 years, particularly when donor's sample is used.

Equipments -

Intra uterine insemination needs very few equipments. However, those required should be of standard, good quality. The required equipments are -

1. **Laminar air flow hood**.

   This can be a small 2 x 2 feet or 4 x 2 feet, depending upon space available and workload. Generally a vertical type hood is sufficient to provide enough sterile workspace. Fig.4

Fig.4 Laminar air flow hood

2. **Centrifuge**.

   A good quality centrifuge is an important part of an IUI laboratory. However, its RPM should be counterchecked frequently to avoid fluctuations. Fig.5

Fig.5 Centrifuge

3. **Compound microscope.**

   A binocular microscope with good optics and clear visibility gives reproducible results and reduces eye stress of people working in the lab. Fig.6

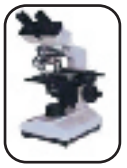

Fig.6
Compound microscope

4. **CO2 incubator.**

   This is the key factor in getting good results. When a physiological media with bicarbonate buffer system is used, supplying proper CO2 concentration in the incubator maintains pH of the media in the range of 7.2 to 7.4, thus optimizing sperm recovery. Fig.7

Fig.7 CO2 Incubators

5. CO_2 cylinders.

The CO_2 which is filled in the cylinders is generally not categorized as 'medical grade', but as 'food grade'. As it is manufactured as a byproduct of other chemicals, one should insist on its purification certificate to evaluate extent of impurities present.

6. Semen freezing machine.

This is optional equipment as same results can be obtained by freezing semen samples by vapor freezing technique by directly suspending semen vials over LN2 for 30 minutes and then plunging into LN2. Fig.8

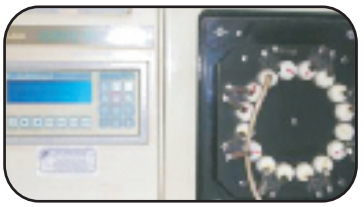

Fig.8 Semen Freezing machine

7. Liquid nitrogen cylinders.

There should be 2 types of cylinders. One for storing semen samples, other for storing LN2. It is important to maintain LN2 level in the storage tank to retain viability of frozen sperms.

8. Refrigerator.

The temperature of medium in which semen samples are processed should be maintained at 4°C till equilibration, as some ingredients are susceptible to degeneration at higher temperatures. It is necessary to disinfect refrigerator on a weekly basis to avoid contamination.

Materials -

IUI laboratory needs

1. Sterile, wide mouth semen collection jars. These should be made of non-toxic plastic material. Good grade polypropylene plastic containers should be used instead of ordinary plastic. Contact of rubber with the sample should be avoided.

2. Tissue culture grade conical centrifuge tubes. They should have provision for tightly fitting lid at the time of centrifugation and also loose fitting lids while equilibration.

3. Fyrite kit/ digital CO_2 analyzer. This is necessary to countercheck the display readings on the incubator panel.

4. Makler chamber/ Neubar counting chamber. Makler is preferred for its simplicity in taking count and a quick evaluation of sperm parameters

5. Sterile Pasteur pipettes. To handle semen samples efficiently and safely.

6. Culture media. These are specially designed for improving acrosome reaction and capacitation. Care has to be taken of proper protein supplementation and proper concentration of calcium, sodium and potassium. Alkaline pH between 7.1 and 7.4 of the medium should be maintained.

7. Sterile catheter or canula for IUI. The catheter should be non toxic, with smooth inner and outer surfaces.

Semen preparation techniques-

The aim of sperm preparation techniques is to recover maximum number of motile sperms and separate them from dead cells, debris and seminal plasma. Washing procedure also removes prostaglandins, infectious agents and antigenic proteins. This may enhance sperm quality by decreasing the release of lymphokines and/or cytokines and by reducing formation of reactive oxygen species [6]. Sample collection in split ejaculation is preferred over single ejaculation in oligozoospermic patients.

Swim-up Technique

Most commonly used techniques are swim-up and density gradient method. Swim-up is a simple technique based on the normal tendency of live sperms to migrate from denser pellet to above layered medium for free movement. The liquefied semen sample is mixed with sperm wash medium and centrifuged at 200 g for 5 minutes. The supernatant is removed and the pellet is layered with about 0.5 ml sperm wash medium. The tube is kept in CO2 incubator at 37^0C for 30 minutes. The supernatant is removed and checked for motile sperm count. [7,8]

Density Gradient technique

Gradient technique is based on separation of different cells according to their differential densities. This medium consists of sillane coated silica particles. It forms self generated gradients which trap cells of different sedimentation coefficient Fig.9. Live sperm settle at the bottom & leucocytes, pus cells, debris, dead sperms & seminal plasma remain in between the gradient layers. The pellet formed at the bottom of the tube is taken into another test tube & one step swim-up is performed. [9,10]

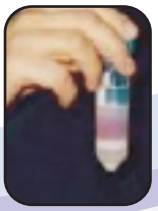

Fig.9 Density Gradient technique

Though IUI is relatively simple technique, it plays very important role in making infertility treatment a cost effective and less taxing to patient. A sophisticated, well maintained laboratory is the key factor in getting good pregnancy rate.

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Ovulation induction in IUI

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IUI is one of the safe, least invasive and cost effective forms of assisted reproduction. Ovulation induction is an important step in this process, as it aims at stimulating maturation of more than one oocyte at a time, which improves the chances of fertilization and pregnancy.

Rationale for Inducing Ovulation while carrying out IUI

Ovarian stimulation has been shown to significantly improve the outcome in IUI cycles. Ovarian stimulation may improve the results of IUI by following two mechanisms :

- By increasing the number of eggs available for fertilization.
- By overcoming a subtle defect in ovulatory function and luteal phase.

Numerous studies have highlighted the benefits of ovarian stimulation with IUI. From a retrospective analysis of 45 studies, Guzick et al concluded that the combined pregnancy rates of ovulation induction with IUI were better (8.3-17.1%) than isolated ovulation induction without IUI or COH[1].

Ovarian Stimulation or Induction

Patients requiring ovarian stimulation or induction can be categorized in two groups.

Ovulatory Patients (Ovarian Stimulation):

In these patients there is an established ovulatory pattern. Multiple studies have shown improved pregnancy rates with ovarian stimulation in these patients as compared to nonstimulated natural cycles.

Anovulatory Patients (Ovarian Induction):

These comprise about 20 to 30 percent of all female factor infertility.
Anovulatory patients are further divided by WHO into 3 categories:

Group I: Hypogonadotrophic hypogonadism
Group II: PCOS
Group III: Ovarian failure

The aim of ovarian stimulation in ovulatory patients is to bring about multiple follicular development in order to increase the number of eggs produced & hence the number of embryos potentially available for implantation. When anovulation is the cause of infertility, ovarian stimulation is aimed at achieving monofollicular development.

Tests before Ovarian Stimulation

A number of tests help in predicting the ovarian response to external stimulation and thus helps determining the dosage. Of these basal FSH and ultrasound estimation of antral follicle count are the ones most commonly used. A basal FSH of >10mIU/ml, indicates a low ovarian reserve and a higher stimulation dose will be required. A basal antral follicle count helps to establish the number of primordial follicles and is a good predictor of ovarian reserve. Other tests which can be done include Ovarian volume, serum AMH, and serum Inhibin[8]. We at Lilavati hospital IVF centre also prefer to check the patency by hysterosalpingogram or laparoscopy before we go ahead with the ovulation induction and IUI.

Various regimens commonly used for Ovulation Induction are:

1. Clomiphene citrate(CC)
2. Letrozole
3. Gonadotropins
4. Clomiphene with Gonadotrophins
5. Letrozole with gonadotrophins
6. Gonadotrophins with GnRH analogues
7. Gonadotrophins with GnRH antagonists
8. CC/ Letrozole with FSH with GnRH antagonist(the soft protocol)

1. Clomiphene Citrate (CC)

Clomiphene citrate is a selective estrogen receptor modulator with a structure that allows it to bind to hypothalamic estrogen receptors which interferes with estrogen receptor replenishment in the hypothalamus, resulting in increased pituitary release of FSH. Increased FSH release drives folliculogenesis at the level of pituitary[2]. It is simple to use, cost effective and associated with fewer complications. It is the drug of choice for inducing ovulation in women with oligoovulatory and anovulatory cycles. In such patients, it has been reported to induce ovulation in 70-80% women with cumulative pregnancy rates varying from 30-50%[3]. In combination with IUI, it has been also successfully used in ovulating patients.

Clomiphene citrate is administered in dosage varying from 50 to 250 mg per day for 5 days starting from the early follicular phase that is second to fifth day of cycle. The dose of CC is increased by 50 mg per cycle till ovulation is achieved. However it is seen that cumulative conception rate does not increase beyond 150 mg dosage, as the anti estrogenic properties of CC manifest more with greater dosage which interferes with implantation and pregnancy. We recommend a dose of 100mg per day for five days.

Outcome after Clomiphene Citrate:

Pregnancy:	30%
Failure (no pregnancy despite ovulation):	40%
Resistance: no ovulation:	25%
Antiestrogenic effect:	5%

2. Letrozole

Letrozole is an aromatase inhibitor which is now commonly used for ovulation induction. Aromatase is a member of cytochrome P450 enzyme complex which catalyses the final rate limiting step in production of estrogen. It is required for hydroxylation of androstenedione to estrone and testosterone to estrogen. Letrozole acting as an aromatase inhibitor decreases the estrogen production. The reduced estrogen levels cause release of estrogen negative feedback at the hypothalamus and pituitary resulting in increased gonadotropin production and stimulation of ovarian follicles[4]. Unlike Clomiphene citrate, letrozole does not have any effect on endometrium or cervical mucus because of absence of estrogen receptor depletion and its short half life.

Letrozole and Ovulation Induction

The use of aromatase inhibitors for ovarian stimulation is indeed promising, especially in patients who have failed to respond to clomiphene, either because of clomiphene resistance or thin endometrium. These patients have responded well to aromatase inhibitors, and it is associated with good ovulation rates, thicker endometrium, and considerable increase in pregnancy rates[5,6]. Letrozole is assiociated with thicker endometrium and increased stromal blood flow, thereby providing a better uterine environment more favorable for implantation. Compared to clomiphene, letrozole has been shown to have higher pregnancy rates.

A number of randomised controlled trials have shown that letrozole is comparable to gonadotropin with regard to pregnancy rates in IUI cycles, with significantly lower cost & better patient compliance.

Addition of letrozole to gonadotropin stimulation protocols decreases the gonadotropin requirement and increases the number of preovulatory follicles[7,8]. Especially in patients who are poor responders, aromatase inhibitors decrease the negative feedback on FSH secretion and increase the ovarian sensitivity to FSH.

Doses

It is administered in dose of 2.5-5mg/ day for 5 days starting from day 3 of the cycle. Few clinicians have used a single dose of 20 mg on day 3. However the information regarding single dose is still limited. The single dose may be beneficial by causing maximal estrogen suppression early in the cycle and of its early clearance from circulation.

Extended Letrozole Therapy

In a recent study conducted by Badawy et al, **extended letrozole therapy** (2.5mg daily from day-1 of menses for 10 days) was used for CC resisitant PCOS women. They observed that the long letrozole protocol (10 days) can produce more mature follicles and subsequently more pregnancies than the short letrozole therapy (5 days)[9].
Ten day letrozole protocol extends FSH window

- Higher number of patients ovulated
- No of dominant follicles were more
- Pregnancy rates were significantly greater
- No extra cost

Letrozole Step up Protocol

- Recently a new protocol known as letrozole step up protocol has been reported by Mitwally et al. In this protocol letrozole was administered in the step up doses consisting of one, two, three, and four tablets of letrozole (2.5mg) daily on menstrual cycle days 2, 3, 4 and 5 respectively. When compared with standard clomiphene citrate protocol (CC 100mg for 5 days starting on day-3), the step up letrozole protocol was associated with multifollicular development and a higher clinical pregnancy rates/ treatment cycle (27.3% vs 11.8%)[10]. This protocol extends FSH window by prolonging the suppression of estrogen levels.
- Prevents rising estrogen from suppressing endogenous FSH.
- Controls break through estrogen production due to proliferation of granulosa cells.
- Increases the duration of elevated FSH, resulting in multifollicular development.

3. Gonadotropins

Gonadotrophins are usually indicated for patients in whom both clomiphene citrate and letrozole have failed to induce ovulation. Gonadotrophins are the drugs of choice in WHO Group 1 patients (Hypogonadotrophic hypogonadism). They yield high pregnancy rate (17%) but their use is plagued by a high incidence of multiple gestation (30-40%).A recent Cochrane review (2007) on the available results of gonadotrophins suggested that gonadotropins might be the most effective drugs when IUI is combined with ovarian hyperstimulation[11].

Choice of Gonadotrophins

The choice of gonadotrophins to be used depends upon the day 2 plasma LH / FSH /E2 levels. If serum LH is elevated, FSH containing gonadotrophins are indicated, whereas if serum FSH is elevated (>10 mIU/ml), a combination of LH and FSH is used for ovarian stimulation. For ovarian stimulation in patients with hypogonadotrophic hypogonadism, a combination of LH and FSH is used.

Factors influencing the dose of Gonadotrophins:

1. BMI: Dosage is directly proportional to patient's BMI.
2. Ovarian reserve: FSH level above 10 IU/L indicates a need for higher dose.
3. Age of the patient: Patients above 35 years of age need higher dose.
4. Cause of Infertility: Pts with PCOS need lower dosage whereas patients of unexplained infertility and hypogonadotrophic hypogonadism need higher doses.
5. Dose needed for stimulation in previous cycle.

Regimens

Three different regimes of gonadotrophins are used for ovulation induction. They include:

A) Conventional Regimen:

This regime has been used with success in clomiphene resistant and clomiphene failure cases. In this protocol a daily dose of 75-150 IU of gonadotropins is started from day-2 or day-3. Serial USG for follicular monitoring and Serum Estradiol (E2) is performed from day 8 onwards. This protocol has yielded acceptable pregnancy rates of upto 30%.

B) Low dose step-up Regime

This regimen has especially been useful in women with PCOS. The principle behind this regimen is to find the "threshold "level of FSH which will lead to the development of a single preovulatory follicle.The key feature of this regimen is the low starting dose (37.5- 75 units/day) of drug, and a stepwise increase in subsequent doses, if necessary with an aim of achieving the development of a single dominant follicle rather than the development of many large follicles, so as to avoid the complications of OHSS and multiple pregnancy[12]. Serum E2 level is measured and USG is performed on day 7. If on day 8 the Serum E2 is > 200 pg/ml or follicle size is above10 mm, the same dose is continued. If, however, serum E2 level or the follicle size is inadequate, the dose is increased by 37.5 units/day every week till serum E2 level rises adequately.

Low dose step up regimen is a popular regimen for ovulation induction, as it is associated with acceptable success rate and decreased incidence of complications. However this therapeutic approach is very unphysiological. Low dose regimen results in elevated levels of FSH during late follicular phase contrary to the natural cycles.

C) Step down Regime

The step down regime mimics the hormonal pattern in normally ovulatory women and induces development of one follicle at a time in anovulatory women. In a natural cycle FSH promotes growth because of two events, the FSH threshold and FSH window. FSH threshold is the level of FSH below which no follicular growth can be initiated .The FSH window is the number of days that FSH levels are above the threshold, which accounts for the total number of follicles that are activated. Since sensitivity of follicle increases with development, the required FSH for a follicle will decrease. Balance between the decreasing levels of FSH and increasing FSH sensitivity is responsible for the growth of the dominant follicle and atresia of remaining follicles.

In a step down protocol HMG/FSH therapy at a daily dose of 150 units is started on Day 2 & continued till a dominant follicle of > 10 mm is observed on TVS. After this the dose is decreased to 112.5 units IM per day for 3 days, followed by 75 units IM per day for next 3 days. This dose is then continued till the day of HCG injection. Rest of the regimen is same as in the step up regime.

4. Clomiphene Citrate with Gonadotrophins:

Sequential use of CC and Gonadotrophin (HMG or FSH) therapy has become an increasingly utilized method for COH for patients who fail CC therapy. In this protocol CC 100 mg is administered from day 2 to day 6 and Inj.FSH/HMG 75/150 units is given on day 6 & day 8. Transvaginal sonography is done from day-8 onwards and in case the follicle growth or number is inadequate, additional FSH/HMG injections are administered.

A combination of clomiphene citrate with gonadotropins has following advantages:

1. Higher pregnancy rate than with CC alone[13,14].
2. More cost effective, as the dosage of gonadotrophins is reduced[14,15].
3. Lesser multiple pregnancy rate than with gonadotrophins alone .
4. Lower incidence of OHSS, as compared to the conventional regime.

Disadvantage

The disadvantage of adding CC has been its antiestrogenic effect which has an adverse pregnancy outcome.

5. Letrozole with Gonadotropins

Aromatase inhibitors are also being used in combination with gonadotrophins, in order to reduce the requirement of gonadotrophins and the side effects of high dose gonadotropin therapy. It appears to be a good alternative to CC in patients with unexplained infertility undergoing gonadotrophin stimulated COH cycles combined with IUI therapy.

In a prospective nonrandomized study by Mitwally and Casper it was shown that aromatase inhibition with letrozole reduced the dosage of FSH required for COH without any undesirable antiestrogenic effects, which are commonly observed with use of CC in combination with gonadotrophins. The pregnancy rate achieved was also significantly lower in the CC + FSH group (10.5%) compared with the letrozole + FSH group (19.1%) and FSH only group (18.7%).[16]

6. GnRH analogue in combination with Gonadotrophins

In almost 15-20 % of cycles, which have been stimulated with gonadotrophins or CC, the exaggerated oestradiol level due to the multifollicular development often provokes higher LH levels during the follicular phase or an untimely LH hormone surge, which leads to cycle cancellation.Therefore, in order to avoid interference from endogenous gonadotrophin secretion, a combination of gonadotrophins and GnRH analogues has being used for ovulation induction. Although GnRH analogues are routinely used in IVF cycles, their routine use in IUI cycles is not recommended. Recent Cochrane review has concluded that GnRH analogues do not significantly improve pregnancy rates in IUI.

7. GnRH antagonists

GnRH antagonists act by competitive inhibition of GnRH receptors, which results in rapid decline in FSH /LH levels thus preventing premature LH surge. The drug can be given in a single dose or daily dose regimen.

The 2 protocols for administering are:

1. Lubeck Protocol:

Gonadotrophins are started as usual and antagonist is started when the follicle reaches a size of 14 mm, or from from 6th day of stimulation onwards in a dose of 0.25mg / day till the day of HCG injection[17].

2. French Protocol:

Gonadotrophins are started as usual and a single dose (3 mg) of antagonist is given when serum E2 level is about 150-200 pg/ml and follicular size is 14 mm[18].

Both these protocols are equally effective in preventing premature LH surge. Their efficacy in preventing LH surge and pregnancy outcome has been compared to GnRH agonist and is found to be equally effective. It has also been shown that administration of antagonists to patients having COH cycles with multifollicular development, significantly improves the pregnancy rates.

Advantages of Antagonist Protocol.

1. Use of antagonist allows the manipulation of follicular development so that IUI can be avoided at weekends without any detrimental effect on PR.

2. When compared to agonist it is relatively simple and inexpensive. There is no suppression of oestrogen and the effects are easily reversible.

3. Antagonists are associated with lower rates of OHSS. The preserved pituitary response with antagonist has opened new paths in the treatment of patients at high risk of developing OHSS, as ovulation induction is possible by giving GnRH agonist and so the deleterious effects of HCG are avoided.

Follicular Monitoring

The follicular growth can be monitored with the aid of **serial transvaginal ultrasonography , serial serum estradiol levels & urinary LH assays**.

A. Serial Transvaginal Ultrasound

Serial Ultrasound f4rom day 8 provides a direct assessment of follicular development. Both the number as well as the size can be studied. The follicles normally grow at the rate of 2 -3mm a day once the leading follicle reaches 10-12 mm size. Serial ultrasound helps us to determine the exact time for triggering ovulation, especially in stimulated cycles. By counting the number of follicles one can also predict the probability of developing OHSS. The endometrium is also assessed for thickness & reflectivity (appearance). A triple line endometrium with a thickness of more than 9 to 10 mm is best conducive to pregnancy. Colour Doppler ultrasonography of uterine & ovarian blood flow, IUI under ultrasound control & endometrial peristalsis after IUI can also be done for improving & predicting success after IUI.

b. Serial Serum Estradiol Levels:

Plasma estradiol correlates closely with the stage of development of the dominant follicle in a natural cycle. This is not true in stimulated cycles as the estradiol reflects the total output of all developing follicle irrespective of size. In most cycles ultrasound has replaced estradiol monitoring. Another problem of serum estradiol estimation is the inconvenience of blood testing faced by the patient, both in terms of disruption of day to day routine as well as cost.

Practically serum estradiol level is done in pure gonadotrophin stimulated cycles with or without GnRh analogue on Day 8 of stimulation, to assess follicular response. A value of more than 200 pg/ml indicates adequate dose of gonadotrophins.

It is also indicated when there are more than four follicle of > 16 mm or more than 8 follicle of more than 12 mm. An estradiol level of more than 1500 to 2000 pg / ml would indicate withholding of ovulation trigger and cancellation of cycle. In case the estradiol level is < 1500 pg / ml one can use a GnRh analogue to trigger ovulation, provided that the ovarian stimulation is not in down regulated cycle.

c. Urinary LH assay

In patients who are undergoing ovarian stimulation, & who are not using GnRha for downregulation, there is possibility of having a premature endogenous LH surge prior to the administration of HCG to bring about ovulation. In case of premature LH surge, ovulation will occur in relation to the surge, rather than occurring in relation with the HCG injection. In such conditions IUI will have to be planned prematurely to time with the LH based ovulation.

Premature LH surge is known to occur in 20 to 24% of patients undergoing ovarian stimulation after the leading follicle reaches 16 mm. The LH surge can be detected either by doing a daily blood or urinary LH assay, once the leading follicle exceeds 16 mm diameter .If the LH surge is detected , Injection HCG 10000 units is given immediately, and an insemination is carried out on the same day. A repeat insemination is carried out the next day. The HCG injection is necessary as the LH secreted by the body may not be adequate enough, to induce the necessary maturational changes in all oocytes, if there are many follicles in the ovary. Numerous urinary LH kits are available to detect LH surge. They are easy to use and are cost effective.

Ovulation Trigger

The ovulation is triggered by administration of HCG injection when the following conditions are met:

1. Leading follicle is 18 -20 mm.
2. The number of follicles more than 16 mm is not more than 4 or follicles more than 12 mm not more than 8 in number.
3. Serum E2 is not more than 1500 to 2000 pg/ml.(serum Estradiol is done only if the total number of follicles exceed 12 & there is a potential chance of OHSS).

Summary

All the protocols have been used in COH with IUI with varying success rates. The drug most commonly used for ovulation induction is clomiphene citrate. It alone successfully induces ovulation in upto 70% of patients. Gonadotrophins may be good alternative in women who fail to ovulate or get pregnant with clomiphene therapy.

<u>Pts less than 40 yrs</u>

- C C- 10% PR
- Gonadotrophins only 20-30% PR

<u>Pts older than 40 yrs.</u>

- L etrozole + gonadotrophins- 10 -15 % PR

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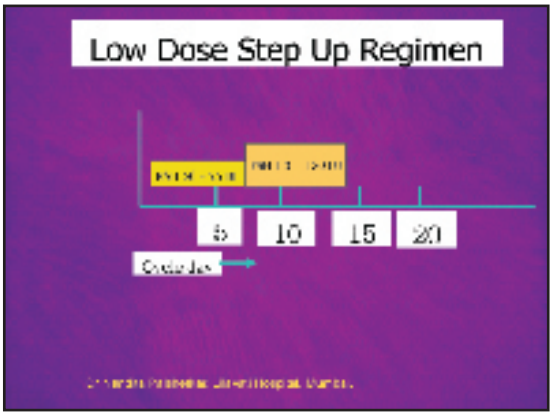
Low Dose Step Up Regimen
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15
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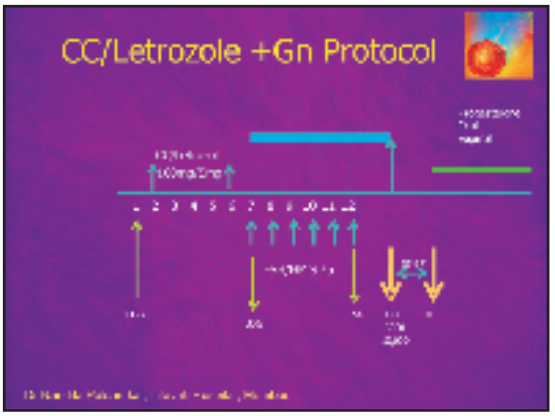
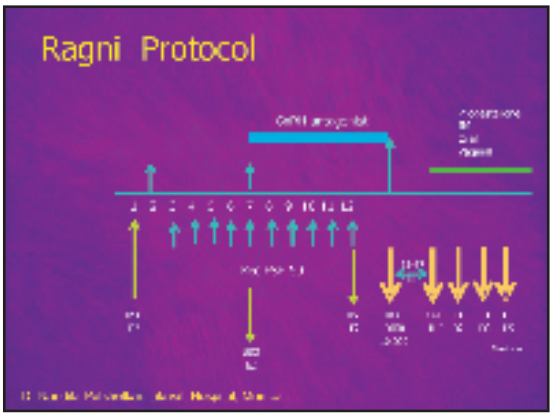
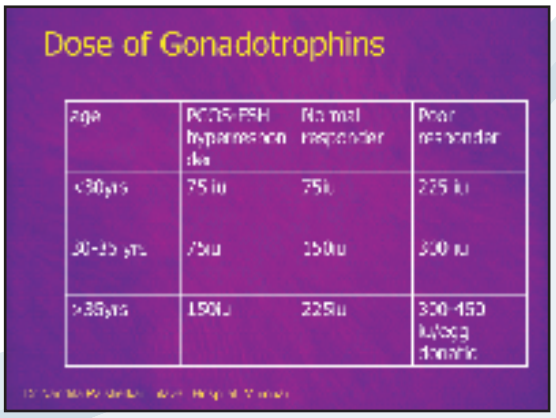

CC/Letrozole +Gn Protocol


Ragni Protocol


Dose of Gonadotrophins
Age
Normal responder
Poor responder
<30yrs
75iu
75iu
225 iu
150iu
300 iu
>35yrs
150iu
225iu
300-450 iu/day

Monitoring of IUI Cycles

Dr. Jaideep Malhotra
2nd Vice President FOGSI

Dr. Narendra Malhotra
Director Ian Donald School of Ultrasound

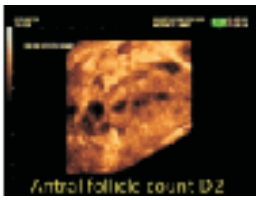
Monitoring of IUI cycles is as important as that of IVF cases. We know that results with IUI are fairly low, and this may further fall with poor selection of patients, improper cycle monitoring and imperfect semen preparation and insemination techniques.

Before starting the IUI cycle on the selected couple,

The Male partner must undergo,
- Semen Analysis and, if possible, culture and sensitivity if the leukocyte count in semen is high.
- Blood group
- Hepatitis B
- Hepatitis C
- HIV test

In the female partner
- Tubal patency documentation (RCOG Guidelines for Infertility)
- TSH , Prolactin (FSH + LH not really mandatory)
- Transvaginal sonography (TVS) to rule out any pathology in the cervix, uterus ,ovaries and adnexa
- Uterus- mid cycle endometrial thickness
- Ovarian antral follicle count

Day 2 or 3 of the menstrual cycle the woman must undergo
- Antral follicle count
- TVS to rule out corpus luteum or follicular Cyst or pelvic pathology
- Endometrial shedding
- Start the desired stimulation protocol



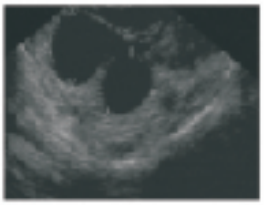
Day 6 or 7:

Follicular response & endometrial thickness synchrony(increase or decrease the dose according to response)
TVS - number of follicles > 10 mm in size.
If > 10 mm size follicles number is more than 4, no addition of FSH / HMG

If > 10 mm size multiple follicles are present -abandon the cycle (risk of multiple pregnancy and Hyperstimulation).

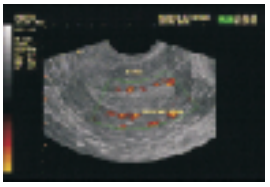
Day 9 or 10:

Follicular size and endometrial thickness
Follicle & endometrial synchrony
Timing of HCG to be determined.


Follicle measurements

If in clomiphene cycles, follicle and endometrial asynchrony is seen on ultrasound monitoring, then, a note can be made to switch over to aromatase inhibitors or gonadotropins in the next cycle.

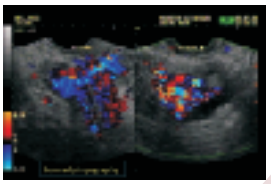
LH kits:


Secretory endometrium

Use of LH kits for documentation of LH surge for timing of insemination have not proved to be clinically important in improving results in IUI.

Color doppler in IUI cycles .

Three-dimensional power doppler ultrasound maybe useful for evaluating endometrial and subendometrial neovascularization in IUI cycles. Endometrial blood flow parameters may be useful predictors for pregnancy.

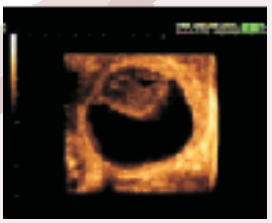


Peri Follicular vascularity does not predict the chance of pregnancy in women undergoing mild Controlled Ovarian Stimulation and IUI cycles.[1]

48 hr Post HCG injection:

Documentation of ovulation ; it is recommended that documentation of ovulation in IUI cycles does improve the pregnancy rates and waiting for the ovulation to occur before performing the IUI, does improve results.

Serum Estradiol / progesterone levels:

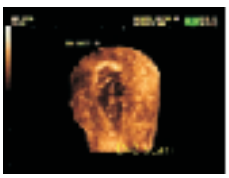
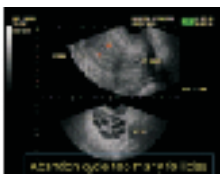
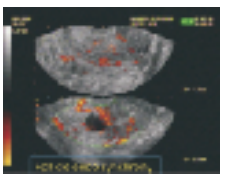

Corpus luteum

These are not recommended unless there is a specific reason such as multiple follicles, short luteal phase etc. Estradiol levels of >1000pg/ml indicate increased chances of hyperstimulation and multiple pregnancy rates in IUI cycles

The aim of monitoring IUI Cycles is to optimize the rate of success with minimum side effects, however there are many aspects that need evaluation and monitoring;

- Not more than 6 follicles altogether in both ovaries
- Follicle endometrial synchrony
- Add antagonist in case premature LH surge is expected (maybe present in 24% of stimulated Cycles)
- Timing of HCG
- Timing of IUI (38 hrs-40hrs yields same results) [2]
- Documentation of ovulation
- Prevention of OHSS (Ovarian hyperstimulation syndrome)

Minimum discomfort and low cost of cycle monitoring with a well performed TVS scan can give excellent insight into the IUI cycle. If required then color doppler of uterus and endometrial blood flow can be done , though it is not recommended routinely.

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Semen Collection for IUI

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Introduction

IUI is indicated for a variety of male and female problems hampering fertility. Ideally, a sample for IUI should be collected without spillage, in the clinic itself (to facilitate rapid processing), by masturbation. The sooner the sample is processed and inseminated the better will be the results[1].

In this overview we will discuss the ideal method for semen collection for IUI and strategies for managing men who are unable to collect semen.

Semen collection

If a man has normal semen parameters then the standard collection procedure is fine. He collects a sample by masturbation into a sterile jar, keeping 4 days of abstinence. If he is uncircumcised he should be instructed to ensure that the prepuce has been retracted and the glans cleaned prior to collection of the sample.

If the man has oligoasthenozoospermia then it would be worth experimenting with different periods of abstinence, ranging from 1 to 7 days, assessing semen quality, harvest and sperm functions. Often a short abstinence gives the best semen parameters[2,3] and pregnancy rates[4] but some men are unable to give an adequate sample without adequate abstinence. Thus, for a given individual the optimal abstinence period for obtaining the best sample can vary.

It is also sometimes useful to ask for 2 samples and pool the harvest; this may result in better count and motility.

Difficulty in collection

In many cases the man is unable to collect a sample in the clinic because of a variety of reasons[5]. These are listed below:

A. **Situational Anejaculation**: Many men are able to ejaculate during intercourse at home

but fail to do so by masturbation in the clinic due to anxiety or lack of arousal. There are various types of situational ejaculatory problems:

1. Clinic specific: can masturbate at home but cannot collect in the clinic

2. Masturbation specific: can collect by intercourse but not by masturbation

3. Peri-ovulatory: can ejaculate and collect semen routinely, but fails to do so when under pressure during an IUI procedure

B. Total Anejaculation: Total anejaculation refers to the total inability of the man to ejaculate by masturbation or intercourse while in the waking state. This can further classified as:

1. Anorgasmic Total Anejaculation: The man fails to ejaculate because he does not reach an orgasm /climax during masturbation or intercourse. This is due to psychological inhibitions or a high physiological threshold for reaching orgasm. There is no physical defect. Typically, nocturnal emissions will be present, and the presence of this is an important pointer in history that rules out any physical problem.

2. Orgasmic Total Anejaculation: In this case the man reaches a climax and experiences an orgasm but still does not ejaculate any semen due to a physical problem resulting from anatomical obstruction, neurogenic failure or an incompetent bladder neck. Common causes include spinal cord injury, lumbar sympathetic damage due to surgery or autonomic neuropathy, fibrosis of the ejaculatory ducts following genitor-urinary Kochs, and retrograde ejaculation. Since the problem is due to a physical cause these men will also have absence of any nocturnal ejaculation.

Managing Semen Collection problems

A good history will help identify and anticipate a semen collection problem before the IUI procedure starts. Never take it for granted that the man will be able to give a sample when and where desired. Always ask specific questions about whether he will be able to collect a sample, by masturbation, in the clinic, on demand. Also, enquire whether he has had difficulty collecting semen in the past. For such men, as well as those who are very tense, it is always better to obtain and freeze a couple of samples in advance.

If a man gives a history of clinic-specific anejaculation then assess whether he can bring a sample from home in an acceptable period of time. If he cannot do so then encourage the couple to rent a room close by or provide one in the clinic. If he is still unable to give a sample then he can be treated with a vibrator.

Men who have difficulty masturbating can be instructed to try collecting semen by coitus interruptus. Alternatively, the couple can use a special non-spermicidal condom to collect semen by intercourse. Finally, vibrator therapy can be offered.

If a man has stress-induced (peri-ovulatory) ejaculatory problems then prior cryopreservation is very useful in ensuring a sample when required and also in reducing the stress on the man. Prior training on the vibrator will ensure that he can use it successfully to provide semen on ovulation day, if a fresh sample is required.

Anorgasmic anejaculation can be successfully treated with vibrator stimulation in 70% of cases. The remaining men will need electro-ejaculation[6].

The management of Orgasmic Anejaculation is more complex since the etiology is so varied. In event of an anatomical obstruction due to fibrosis the only option is PESA-ICSI. Failure of emission due to neurogenic factors can be treated by vibrator therapy or electro-ejaculation[7, 8]. Men with retrograde ejaculation may occasionally respond to sympathomimetics. Otherwise sperm can be retrieved from the urine after giving medication to render the urine alkaline. These sperm can be processed and used for IUI.

When obtaining a sample for IUI in men with delayed liquefaction one should ask the patient to collect semen directly in to a beaker containing 5 ml of medium. This immediate dilution of the semen before it can coagulate will allow the sperm harvest to be done quickly without having to wait for the semen to liquefy,

Technique of Vibrator Therapy

The vibrator works by providing a high intensity stimulus to the penis. This stimulus is strong enough to overcome any psychological or situational inhibition[9].

The procedure is carried out in a room with complete privacy. Preparatory counseling is important: the procedure is explained and it is emphasized that ejaculation will occur automatically as a result of the vibratory stimulation - *the patient should not try and force ejaculation.*

The patient passes urine, takes off his clothes and sits on a bed with his legs apart. The vibrator is placed between the legs. The penis is placed upon the vibrating head such that the undersurface of the glans and distal shaft are stimulated. Once the patient is comfortable with the vibratory sensation, the glans is pressed down upon the vibrator such that the penis receives the maximum amount of stimulation. Keeping the vibrator in place he then closes his eyes and fantasizes sexually. Stimulation is continued till ejaculation occurs. This usually occurs in 10 to 30

minutes but some patients with anorgasmic anejaculation, who have never experienced orgasm, may take up to an hour of stimulation before they reach orgasm the first time! This period shortens during subsequent sessions. Some patients require a second or third session before they succeed.

Technique of Electro-ejaculation

Electro-ejaculation involves the direct electrical stimulation of the efferent nerves innervating the seminal vesicles and terminal vas. The most commonly used device is the Seager electro-ejaculator which delivers a sine wave alternating current. The procedure is carried out under general anaesthesia (except in paraplegic men with no sensations). The electrodes are mounted on a cylindrical rod which is lubricated and introduced per rectum with the electrodes facing the prostate gland. The voltage is turned up to 5 volts, held for 2 seconds and then turned back to 0 volts. For the next stimulus the voltage is increased to 10 volts. The stimulus is progressively increased till ejaculation occurs. Stimulation is then continued at that level till there is no more ejaculation. It is then increased further till ejaculation occurs once more. Usually, the first ejaculation occurs between 5 and 20 volts with a current of 50 to 200 mamps, but some men require a much higher current. If the antegrade ejaculate is scanty, the bladder is catheterized to check for retrograde ejaculation.

Electro-ejaculation will work in most cases where the vibrator has failed. However, it is an expensive device and hence most centres performing IUI may not have access to it. In our experience, electro-ejaculated sperm tend to loose motility earlier and hence we time the procedure once ovulation has been confirmed and process and inseminate the sample as early as possible.

Conclusion

Most men will give a semen sample for IUI without any problem. However, ejaculatory dysfunction is not uncommon in male partners of couples undergoing IUI. An alert infertility specialist can identify the problem before the induction and take proper remedial measures.

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Antagonists - Their Role in Controlled Ovarian Stimulation Today

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Chairman, Infertility Committee, FOGSI

GnRH antagonist in controlled Ovarian Stimulation Intrauterine Insemination Cycles

GnRH antagonists are peptide molecules that are made up of multiple, often synthetically produced amino acids. GnRH antagonists compete with natural GnRH for binding to GnRH receptors, thus decreasing or blocking GnRH action in the body.

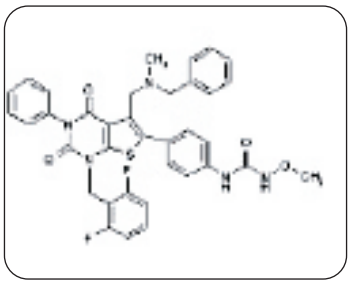
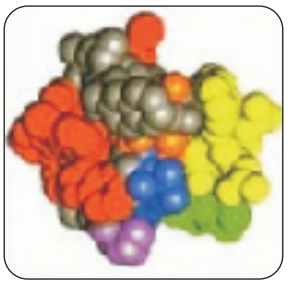


Figure1

Figure 2



Mechanism of action:

GnRH antagonists competitively and reversibly bind to GnRH receptors in the pituitary gland, blocking the release of luteinising hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary. This leads to suppression of oestrogen release from the ovaries. Unlike the GnRH agonists, which cause an initial stimulation of the hypothalamic-pituitary-gonadal axis (HPGA), leading to a surge in oestrogen levels, GnRH antagonists have an immediate onset of action, rapidly reducing sex hormone levels without an initial surge.

Figure 3: Effects of GnRH antagonist versus agonist treatment on levels of luteinizi ng hormone (LH) and follicle stimulating hormone (FSH)

Currently approved GnRH antagonists include the following:

- Cetrorelix
- Ganirelix
- Abarelix
- Degarelix

GnRH antagonists are administered by either intramuscular injection (abarelix) or subcutaneous injection (cetrorelix, degarelix and ganirelix).

The GnRH antagonists that are currently licensed for use in fertility treatment are cetrorelix and ganirelix.

Drug Preparations:

Cetrorelix: 0.25 mg and 3 mg. Only 0.25 mg preparation is currently available in India.
Ganirelix: 0.25 mg

Dosage Regimens:

GnRH antagonists are used in controlled ovarian stimulation. This could be with Clomiphene, Letrozole and / or Gonadotropins in IVF-ICSI or IUI cycles; the main aim being prevention of a natural LH surge, (as HPO axis is not down regulated in these cycles).

1. Multiple-dose regimen:

a) Fixed protocol : A dose of 0.25 mg of GnRH antagonist is given daily from stimulation day 5 irrespective of the follicular size and continued till the day of hCG administration.

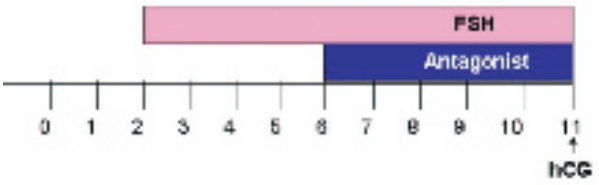


Figure 4 Multiple dose – fixed regimen

b) Flexible protocol : GnRH antagonist administration is started in a dose of 0.25 mg daily when the leading follicle is 14mm in diameter and continued till the day of hCG administration. Generally fewer doses of GnRH antagonist are used in this regimen as the antagonist is started only after the leading follicle is ≥ 14mm and the risk of an untimely LH surge is high.

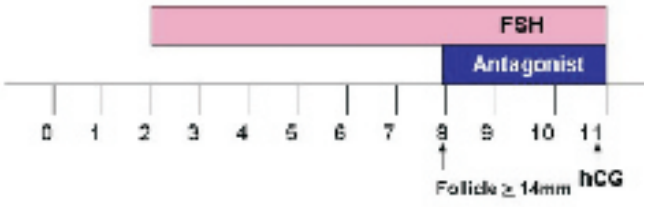


Figure 5 Multiple dose – flexible regimen

2. Single-dose regimen: A single dose of GnRH antagonist 3 mg is given when an adequate follicular response is seen (leading follicle of 14mm), usually stimulation on day 5 or 6 (range days 5-9). If hCG is not administered within the next 4 days, then 0.25 mg of GnRH antagonist is given daily until hCG is administered.

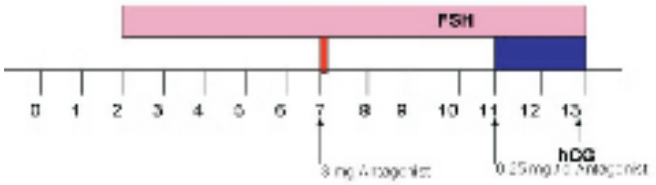


Figure 6 Single dose flexible regimen

Administration:

GnRH antagonist is administered by subcutaneous injection following proper aseptic procedures. Injections should be taken in the lower abdomen, preferably around the navel (but staying at least 1 inch from the navel). The injection site should be rotated daily. The needle should be inserted completely into the skin at a 45-degree angle.

Potential Advantages of Incorporating Antagonist in IUI cycles

1. Suppressing LH rise:
Antagonists, on either a fixed or a flexible protocol, have been proven successful in suppressing LH rise in superovulated cycles. Based on this fact, antagonists in IUI cycles have been recommended.

Increasing E_2 levels may induce an LH surge with disastrous effects for follicular progress and growth.[1] If a fertility facility and a clinician are available, IUI can be timed according to LH levels. Otherwise, premature LH rise leads to cycle cancellation. This is especially important if premature luteinization takes place on Friday and a weekend insemination is impossible. For that reason, some authors have administered a GnRH antagonist that rapidly inhibits LH rise.[2]

2. Follicle Endometrial dyssynchrony :

During some stimulated cycles for IUI, the endometrium lags behind and is poor [< 7mm] when the leading follicles have already reached 17 -18mm. In this situation, if the administration of hCG is delayed to allow the endometrium to grow further, the risk of LH surge is very high. In this situation, GnRH antagonists can be administered for a day or two to delay the IUI till the endometrium develops further.

GnRH antagonists are easy to incorporate in an IUI scheme by adding it in a flexible protocol. In addition, GnRH antagonists can be safely administered in IUI cycles without compromising the luteal phase.

3. High Risk of OHSS :

In patients undergoing COH and at risk of OHSS, GnRH antagonists have the advantage of combining GnRH agonist in place of hCG as ovulation triggering agent [4,] thereby reducing the risk of OHSS.

4. Easy Conversion to IVF:

In women showing excessive response to gonadotropins with multifollicular development, going ahead with IUI leads to a very high risk of multiple pregnancy(>20%). Conversion of such cycles to IVF is an alternative to cycle cancellation, with success rates comparable to conventional IVF cycles[5]. This can be easily achieved by adding an antagonist.

Conclusion:

GnRH antagonists can be used in IUI cycles to prevent cycle cancellation due to premature LH surge and to delay insemination if endometrium is lagging behind. Adding GnRH antagonist to controlled ovarian stimulation intrauterine insemination cycles significantly increases pregnancy rates in multifollicular, but not monofollicular, cycles[6].

However, the incremental cost of antagonist administration and the possibility of not improving pregnancy outcome must be considered. This might add to the reluctance to adopt this technique as a standard method of treatment in all IUI superovulated cycles. The small size of studies performed until now and the different schemes for antagonist administration might further reinforce this reluctance.

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Semen Analysis

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Any comprehensive infertility investigation undertaken for a couple unable to establish a pregnancy after one year of normal, unprotected intercourse begins with a complete semen analysis. An accurate semen analysis is a crucially important component of an assisted reproductive technologies laboratory. However, semen analysis is not an objective test for male fertility, since infertility is attributable to a complex combination of multiple factors inherent in both the male and female partners. Therefore a couple cannot be considered fertile based only on a normal semen analysis. Similarly, deficiencies revealed in 'infertile' patients may not be sufficient to prevent pregnancy from occurring.

It is well known that the intra-patient variability of semen specimens from fertile men can vary significantly over time. This decreases the diagnostic information that can be obtained from a single analysis, often necessitating additional analyses. The most accurate evaluation of semen is made by considering the results of two or more semen analysis's atleast a week apart. The commonly accepted standard for defining the normal semen analysis are the criteria defined by the world health organization (WHO). These parameters are listed in Table I.

Standard tests	**Normal values**
Volume	2.0 ml or more
pH	7.2-7.8
Sperm concentration	$20x10^6$ spermatozoa/ml or more
Total sperm count	$40x10^6$ spermatozoa or more
Motility	50% or more with forward progression or 25% or more with rapid progression within 60 min after Collection
Morphology	30% or more with normal morphology[b]
Vitality	75% or more live
White blood cells	Fewer than $1x10^6$/ml
Immunological tests	
Immunobead test	Fewer than 20% spermatozoa with adherent particles
MAR test	Fewer than 10% spermatozoa with adherent particles

Seminal plasma biochemical analysis

Epididymal markers	
α–Glucosidase (neutral)	20 mU or more per ejaculate
Carnitine	0.8-2.9 mmole per ejaculate
Prostate markers	
Zinc (total)	2.4 mmole or more per ejaculate
Citric acid (total)	52 mmole or more per ejaculate
Acid phosphatase (total)	200 U or more per ejaculate
Seminal vesicle marker	
Fructose (total)	13 mmole or more per ejaculate

[a]*WHO manual, 3rd edition, 1992.* [b]*empirical reference value.*

Abstinence from ejaculation for 2 to 4 days before producing the sample for analysis is necessary. A shorter period of time may adversely affect the semen volume and sperm concentration, although it may enhance sperm motility[1,2]. A longer period might reduce sperm motility. Masturbation is the preferred method of collection. The use of lubricants or saliva is discouraged since most are spermicidal. However some mineral oils and a few water-based lubricants are acceptable. The use of certain non-spermicidal condoms known as SCD's - seminal collection devices, during intercourse may be an acceptable second choice. Interrupted intercourse should not be considered, as the first few sperm rich drops of semen may be lost while also contaminating the container with bacteria[1,2]. Incase the sample is collected at a place other than the laboratory, it should be delivered within 60 minutes of collection, preferably kept at body temperature during transport to the lab.

When the semen sample arrives in the laboratory it is first checked for liquefaction and viscosity. Liquefaction is a natural change in the consistency of semen from a coagulum, which characterizes freshly ejaculated semen, to a liquid. This coagulum results from secretions from the seminal vesicles. The liquefaction of this coagulum over the next 15 - 30 minutes is a result of enzymatic secretions from the prostate. Viscosity refers to the liquefied specimens tendency to form drops from the tip of a pipette. Delayed or inadequate liquefaction, in the presence of a coagulum, or high viscosity may indicate prostate dysfunction[3].

A normal seminal volume is considered to be >2ml. 65% of the volume is from seminal vesicles, 30-35% is from the prostate and only 5% from the vasa[3]. Low volume is associated with absence or decrease of seminal vesicle component of the ejaculate either due to absence of seminal vesicles or complete or partial obstruction of the ejaculatory ducts or retrograde ejaculation. Normally the pH of semen is alkaline because of the seminal vesicle secretion. An alkaline pH protects the sperm from the acidity of the vaginal fluid. An acidic pH (pH<7.2) suggests problems with seminal vesicle function. It is usually found in association with a low volume of the ejaculate and the absence of fructose. Complete obstruction of the ejaculatory ducts may be associated with azoospermia coupled with low volume, acidic pH and absent fructose. pH more than 8.0 may be associated with prostate infection.

Sperm concentration can be determined by a hemocytometer or the Makler counting chamber. Normal semen is defined to have > 20 million/ml sperm. Oligozoospermic samples have less than 20 million/ml sperm and azoospermic samples have no sperm.

The quality of sperm, characterized by its motility and morphology are often more significant than the count. Motility is one of the most important prerequisites for achieving fertilization and pregnancy. Motility can be expressed on a subjective scale based on the quality of sperm progression into a) active b) sluggish or c) vibratory & non-progressive. Normal sperm morphology is directly related to fertilizing potential. This may be due to inability of abnormal sperm to deliver normal genetic material to the cytoplasm of the egg. Sperm morphology can be assessed in different ways. The most common classification systems are the 3rd edition WHO standard and the 4th edition WHO standard that incorporates the Kruger strict criteria. WHO criteria for assessing normal forms include the following: "

- Head - oval and smooth heads are normal; round, pyriform, pin, double and amorphous heads are abnormal.
- Mid-piece - a normal mid piece is straight and slightly thicker than the tail.
- Tail - single, unbroken, straight tails, without kinks or coils are normal.

A normal semen analysis should contain at least 30% normal sperm using WHO 3rd edition criteria[3]. The Kruger criteria for assessing normal forms include the following:

- Head - smooth; oval configuration; length 5-6µm, diameter 2.5-3.5µm; acrosome must constitute 40 - 70% of the sperm head.
- Mid piece - slender, axially attached; <1µm in width and approximately 1.5X head length; no Cytoplasmic droplets >50% of the size of sperm head.
- Tail - single, unbroken, straight, without kinks or coils approximately 45µm in length.

According to Kruger's strict criterion[6], the presence of 15% normal sperm morphology should be interpreted as normal result. Normal morphology of 4 - 14% should be considered to be borderline, and normal morphology below 4% is abnormal[4,7].

Sperm vitality tests are useful to determine the presence of live sperm in samples with total astheno-zoospermia. Live sperm can be identified either with eosin staining, or the Hypo-osmotic swelling (HOS) test or treatment with pentoxiphylline. Amongst the three, treatment with pentoxiphylline has been found to be most accurate since it not only differentiates live sperm, but also initiates or enhances motility, causing sufficient twitching to choose sperm for ICSI.

All semen samples have white blood cells or leukocytes. If WBC's are present in concentrations of more than 1Million/ml, then it may be suggestive of infection. However first these leukocytes need to be differentiated from other round cells such as immature germ cells using immunohistochemical methods. Leukocytospermia (WBC in semen) in addition to being a sign of bacterial infection also has ability to stimulate the release of reactive oxygen species thereby inhibiting sperm motility and sperm function. The seminal plasma contains number of antioxidants that protect sperm from oxidative damage from exposure to ROS. Men who have higher concentrations of such antioxidants may be able to tolerate greater levels of seminal leukocytes.

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Semen Preparation Techniques for IUI

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Introduction

Ejaculated semen is a mixture of spermatozoa and seminal plasma. Seminal plasma is constituted by secretions of epididymis, seminal vesicle and prostate. The semen may also contain other cellular components like microorganisms and leukocytes. The main objective of different semen preparation techniques is to harvest the most functional sperms from this ejaculate with minimal damage.

Advantages of semen preparation:

Removal of seminal plasma during semen preparation has numerous benefits:

1. Insemination with raw semen can lead to pelvic infection as it may be contaminated with bacteria and other microorganisms.

2. Seminal plasma is a rich source of prostaglandins that can lead to uterine contractions if inseminated in high concentration.

3. Sperm washing significantly reduces the viral load. It is an ideal treatment option when the male partner is HIV positive and the female is HIV negative

4. Antibodies in seminal plasma are removed with semen preparation. It also removes antigenic proteins, which may stimulate an immune reaction in the females.

5. It helps in removing decapacitation factors and other detrimental factors like dead cells & debris.

6. During semen preparation certain active substances may be added which improve the motility of spermatozoa.

Semen Collection methods: Points to remember:

1. It is important to select patients properly. Husbands with normal semen counts (as per WHO criteria) or with mild male factor infertility yield the best pregnancy rates. Semen with prewash counts of more than 10 million per ml, with motility > 50% & morphology > 30% have the best chance of a positive outcome. Similarly a post wash sperm insemination load of forward progressive motile sperms of > 5 million /ml gives the best pregnancy rate.

2. If facilities are available besides doing routine semen analysis one can also do semen culture, sperm antibody testing and sperm morphology tests using Kruger strict criteria. Patients with positive semen culture can be treated with appropriate antibiotics.

3. Males with idiopathic oligoteratozoospermia can be empirically treated with broad spectrum antibiotics, antioxidants and other dietary supplements, vitamins and cofactors as well as some antiestrogenic drugs to boost their counts and motility prior to subjecting them to IUI . One can co treat these patients in association with an andrologist. If there is a presence of varicocele, it can be surgically treated (if indicated) prior to starting IUI.

4. The abstinence period prior to IUI should not be more than 3 days. Longer duration may lead to poor results.

5. The time taken from the ejaculation and preparation of the semen till insemination, should not be more than 90 minutes. Longer duration would result in poor results. The clinician should give the ovulating dose of HCG at the appropriate time, such that the insemination should occur 36 to 40 hours post HCG and not exceed the 90 minutes time from ejaculation to insemination.

6. Semen collection should be done in a special secluded collection room , having an attached toilet with a bed or couch . Audiovisual aids and magazines should be available to aid the semen collection. If needed the spouse should be able to assist her husband in semen collection.

7. The male should wash his hands and dry them prior to producing semen. The penile part should not be washed with soap &/or water. The male should be warned against using any lubricant or soap to aid him in masturbation, as this can be toxic to the sperm.

8. Collection should be done using sterile non toxic plastic containers. The containers should be labeled, both on the lid as well as on the side walls with alcohol based pen markers, thus avoiding any mix up.

9. At any given point in time, only one semen sample should be processed on the laboratory bench or laminar flow. Plastic tubes & pipettes used for processing sample should be labeled with the patients name and discarded as soon as their function is over. This will avoid mix up.

10. Prior to insemination, the lab technician /embryologist should confirm and tally that the patient to be inseminated with the prepared sample is the correct one. The embryologist, nurse and the doctor can loudly utter the name of the husband written on the tube , prior to insemination.

Types of Techniques

Over the years several techniques have been developed for semen preparation prior to Intra Uterine Insemination (IUI).The method used to prepare a semen sample depends on the following parameters:

1. Volume
2. Viscosity
3. Motile Sperm count
4. Presence of leucocytes, other cells and debris
5. Whether it is for IUI, IVF or ICSI

The most commonly used methods of semen preparation for IUI are

1. **The pellet & Swim Up technique**
2. **The Overlay technique or the direct swim up**
3. **The Density gradient centrifugation technique**

A normal ejaculate contains a large number of defective spermatozoa and granulocytes which on centrifugation can generate a high number of ROS (reactive oxygen species) and sperm DNA damage[1, 2, 3]. These ROS in turn attack fatty acids present in plasma membrane of the sperm, resulting in oxidative stress which can damage sperm function. Hence, preparation methods, which do not use centrifugation, are in general, advantageous

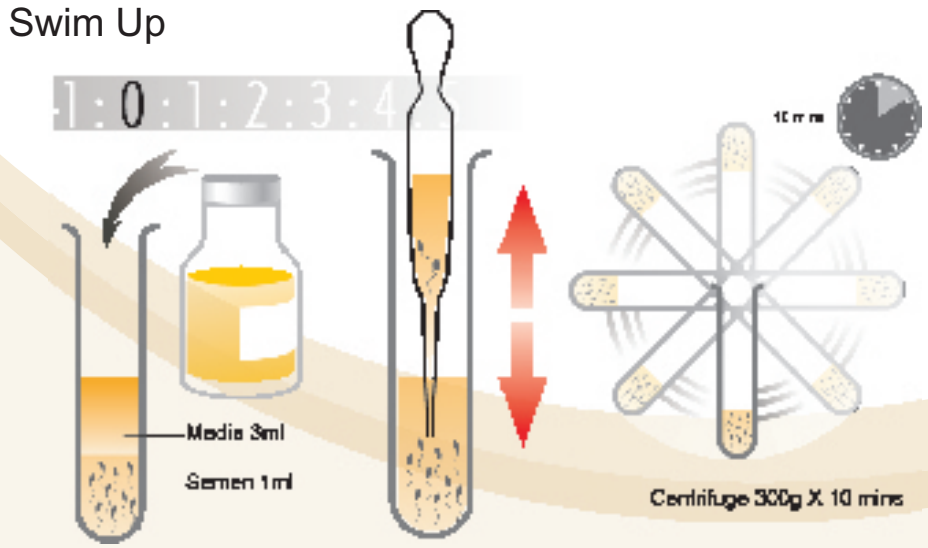
1. The Pellet & Swim Up Technique

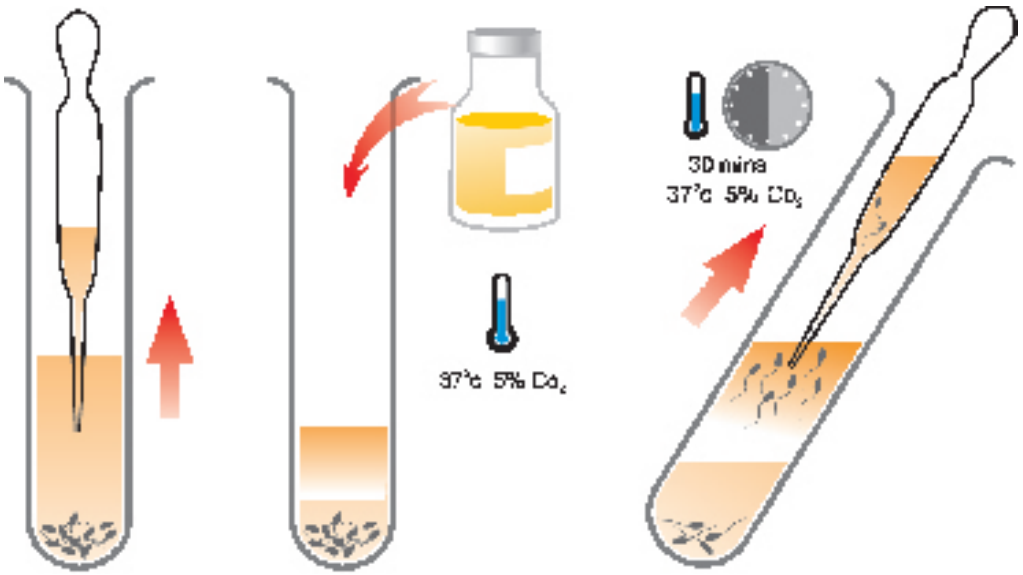
It is the earliest and most widely used technique of sperm preparation[4]. In this procedure, the innate capacity of motile sperms to migrate against gravity is used to select a motile sperm population, leaving behind seminal plasma, immotile sperms, extraneous cells and debris. It is a simple, easy to perform and a cost effective technique. Another advantage is that a good number of highly motile sperms are recovered. It is also effective for viscous samples which are collected

in culture medium. It is restricted for normal or marginally abnormal ejaculates only as it does not separate morphologically normal sperms from abnormal ones.

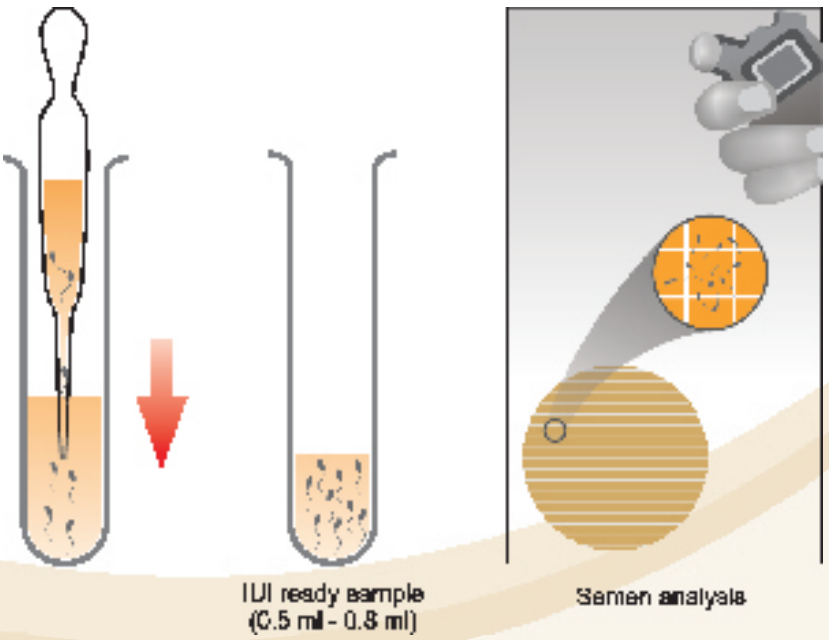
Steps

1. Perform semen analysis (count and motility) using a Makler chamber.
2. Take two round bottom 6 ml test tubes. Label them and place 1 ml. of sperm sample in each tube.
3. Add 2 ml. of flushing media to each tube. Mix the samples thoroughly.
4. Centrifuge for 10 minutes at 300g (1500)rpm.
5. Aspirate out the supernatant without disturbing the pellet. Discard the supernatant.
6. Gently layer the pellet with 1 ml. of flushing media/culture media & place the tubes in the heating block or container. Then place the block in a heater or incubator, for 40 to 60 minutes.
7. After 30-40 minutes remove 0.5 ml of the supernatant from each tube to make it 1 ml and put it in another clean tube.
8. Determine the count and motility of the supernatant on the Makler chamber.
9. The supernatant is used for intra-uterine insemination.




30 mins
37°c 5% CO2
37°c 5% Co2

Swim Up

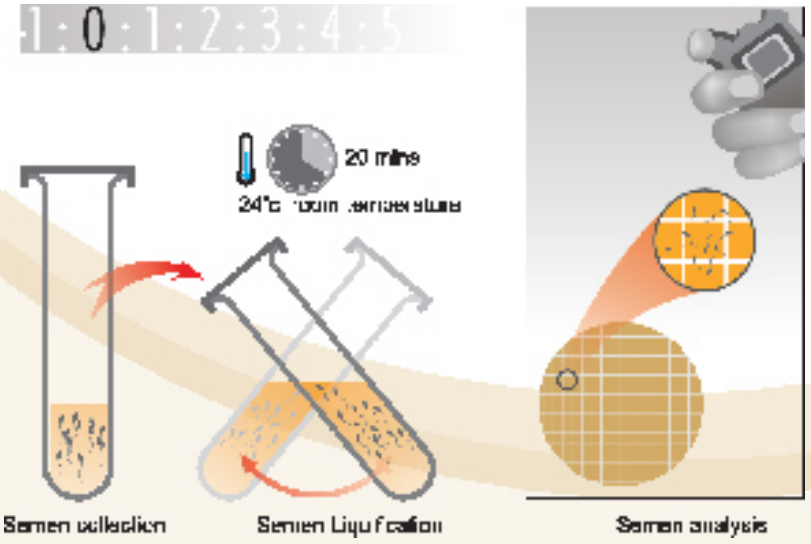

IUI ready sample
(0.5 ml - 0.8 ml)
Semen analysis

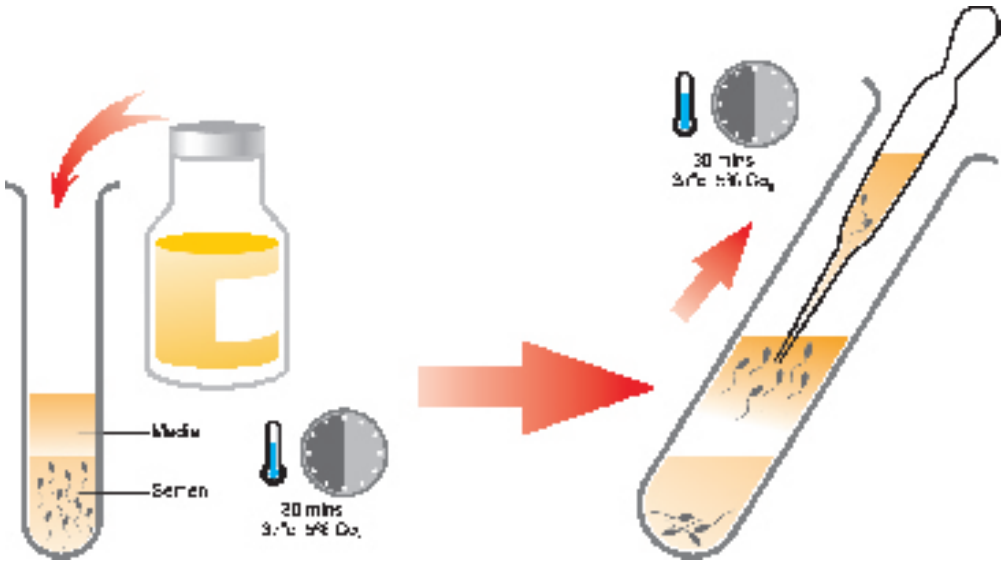
2. The Overlay Technique or the direct swim up.

Basically, this technique is performed in the same way as standard swim up but avoids centrifugation which might damage spermatozoa. It is useful only for ejaculates with normal semen parameters that contain high degree of progressive motile spermatozoa. It cannot be used for viscous samples.

1. Take a 10ml round bottom plastic test tube and label it with the patients ID.
2. Pipette 2 ml of culture medium.
3. Gently pipette 1.5 ml of neat semen underneath the medium. One has to be careful not to disturb the interface formed between the semen and medium.
4. Cap the test tube and put it in the incubator carefully for 30 mins to 1 hour.
5. Pipette the top layer and the cloudy middle layer of the supernatsnt and discard the rest.
6. Transfer the supernatant into a 6ml centrifuge tube and add 1 to 2 ml of culture media. Spin at 300G for 5 minutes. Discard the supernatsnt without disturbing the pellet at the bottom.
7. Resuspend pellet in 0.5 to 1.0 ml of media. Sperm count and motility is assesed.
8. Keep the sample in the Co2 incubator or on a bench till the IUI is performed.

Direct overlay





3. The Density Gradient Technique

The basis of this method is that it separates motile spermatozoa from other components of seminal plasma based on their size, motility and specific density. The most progressively motile sperms reach to the higher density portion at the bottom of the tube ,with the aid of the density gradient and centrifugation force. Therefore, a sample devoid of dead sperms and debris and containing highly motile spermatozoa, with optimal morphology is attained. However, it is more expensive and time consuming and the lower sperm recovery rate remains an issue.

Steps

1. Allow the sperm sample to liquefy for 20 minutes. If it does not, then pass it through a 23-gauge needle.

2. Take one drop and put it on the Makler chamber to take a count and determine the motility.

3. Take two conical bottom test tubes and label them with patient ID.

4. 1 to 1.5ml. of 90% sperm gradient is layered in each conical tube with a sterile pipette.

5. 1 to 1.5 ml. of 45% sperm gradient is then gently layered on top of it with another sterile pipette.

6. 1-2 ml. of sperm sample is then gently layered on top of the two layers. Care is taken not to add too much sample as it results in poor separation.

7. Without disturbing the layers, the tubes are centrifuged at 300 g (1500 rpm) for 20 minutes.

8. The supernatant is then pipetted out and discarded leaving the pellet with as little of the 90% solution as possible.

9. Take a new test tube & add 5-10 ml. of flushing medium to it, transfer the pellet to this tube.

10. It is then centrifuged at 300 g for 10 minutes. The supernatant is pipetted out and discarded. Add 5 ml. of media and repeat this step.

11. For samples with good motility a swim up procedure is now carried out in a 6 ml test tube and the pellet is layered with 1 ml of culture medium and incubated for 30 minutes in Co2 incubator. The upper layer is carefully pipetted out in another clean test tube.

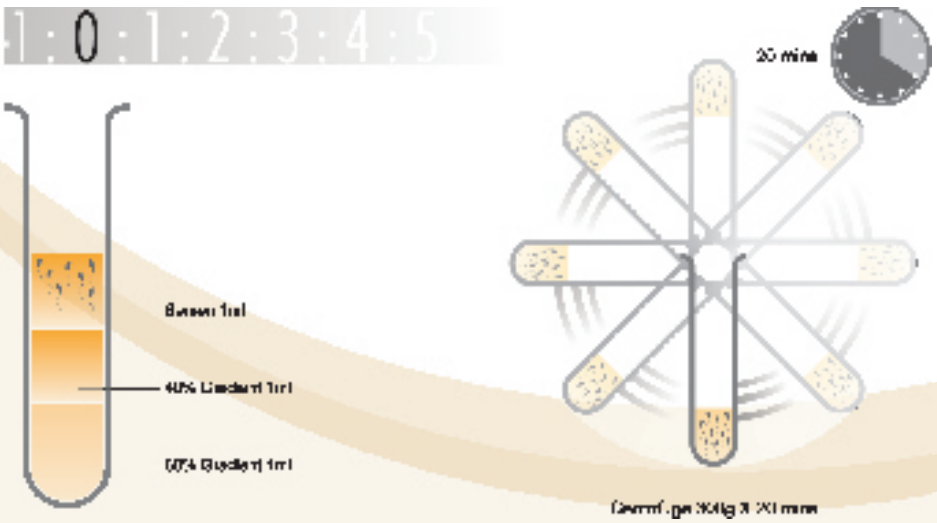
12. For samples with poor motility the pellet is gently layered with 0.8 ml culture medium and incubated for 60 minutes. The supernatant is carefully pipetted out in a clean test tube.

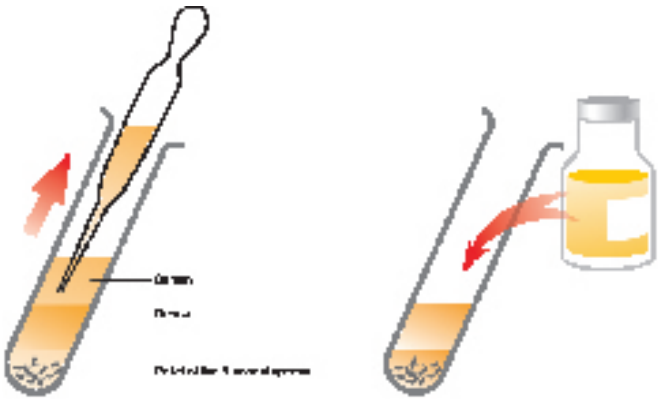
13. Alternately, the pellet is re-suspended in 0.5 ml. of culture media or flushing media.

14. It is then checked for motility and concentration.

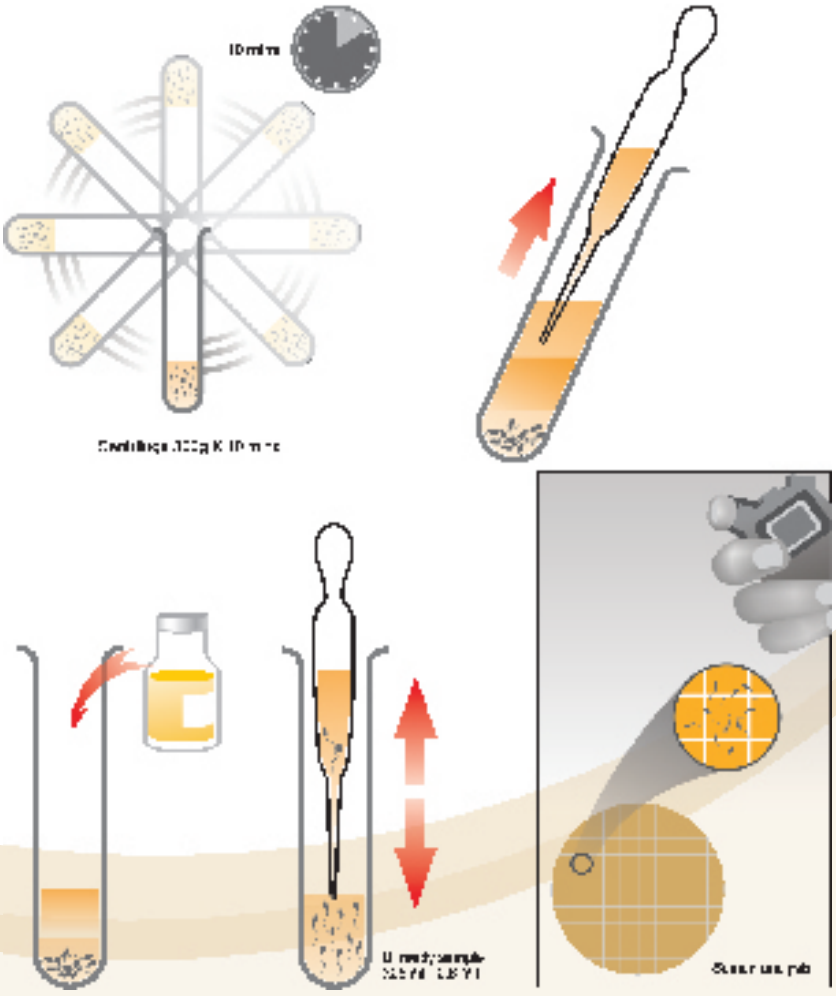
15. The sample is ready for either IUI, IVF or ICSI.

Density Gradient





Density Gradient



The Direct Swim Up is used for semen with normal parameters, that is, a count of more than 20 million/ml with more than 50% motility. It is not used for viscous samples.

The Pellet and Swim Up method is used for normal or marginally abnormal sperm and viscous samples or samples that are collected in medium.

The Gradient method is effective for abnormal sperm. The density gradient method not only removes the abnormal sperm but also microbes, debris and other cell contaminates from the sample.

The sperm preparation for IUI, IVF and ICSI is basically the same, the only difference being in the quantity of sperm required for each procedure.

Other Semen Preparation Techniques:

a. Simple wash technique

The semen sample is mixed with culture medium (3: 1) and centrifuged. Supernatant is discarded, the pellet is resuspended in 0.4 to 0.6 ml of culture media.

The simple wash method generally recovers the highest number of spermatozoa from a given ejaculate. It is useful in cases of severe oligospermia. However, the inseminate retains a mixture of motile, immotile, and immature sperm as well as nonspermatozoal cellular elements found in the semen.

b. Glass wool filtration technique

The semen is filtered through a glass wool column. It is not used routinely used.

c. Sperm select method technique

It's a modification of overlay method where instead of culture medium, hyaluronic acid is used. The advantage of hyaluronic acid is that it mimics cervical mucus in composition[5] .However this method requires the semen to contain spermatozoa of fairly good motility.

Comparison of Various Methods

In a RCT in 2005, Soliman et al.[6] compared wash and pellet method with Density Gradient Method (DG). Wash and pellet method was found to be more cost effective, took significantly less time and had same pregnancy rate as the gradient method. In another study of 166 couples, Nayar et al[7] compared various semen preparation techniques for IUI and found that swim up has higher pregnancy rates and is more cost effective than DG. Gradient method was found to be useful for

poor quality samples. Simple washing is effective in samples with extremely low sperm densities. In another study Pranav et al.[8] found density gradient with percoll produces a significantly greater number of sperms with normal morphology than swim up method. In a recent Cochrane review[9] various techniques were compared. They included 5 RCTs including 262 couples in total. There was no evidence of a difference between pregnancy rates for swim-up versus a gradient or wash and centrifugation technique (OR 1.57, 95% CI 0.74 to 3.32; OR 0.41, 95% CI 0.15 to 1.10, respectively); nor in the two studies comparing a gradient technique versus wash and centrifugation (OR 1.76, 95% CI 0.57 to 5.44).There was insufficient evidence to recommend one technique over the other.

Managing difficult samples

Viscous samples

A sample which fails to liquefy within 30 min requires liquefaction before processing. Passing the sample through a 23 gauge needle is usually effective. Alternately, the husband can ejaculate in a container filled with 5 to 10 ml of culture media.

Excessive pus cells

In general these samples should be discarded and a semen culture should be done to rule out any infection. If culture is sterile, repeated washing should be done to reduce the load of infection.

Low volume

Sometimes there is only a drop of semen. This drop should be immediately dissolved in medium to prevent any drying and then processed normally. For future, sample should be collected in medium.

Teratozoosperia

Density gradient technique is better in these cases.

Antisperm antibodies

Sample should be collected in medium to avoid tagging antibodies to spermatozoa. It significantly reduces the percentage of antibody bound sperm[10].

HIV infection

There is a growing number of serodiscordant couples where only one partner is infected. In couples with HIV positive husband and an HIV negative wife , semen wash followed by IUI or ICSI has been routinely used to achieve pregnancy without the risk of HIV transmission to the wife . Semen does contain virus particles and it's been seen that sperm washing can significantly reduce the viral load[11,12].

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Clinical Aspects of IUI

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The success of Intrauterine insemination (IUI) depends largely on good insemination techniques. There are several important aspects to be considered.

1. The time of insemination
2. The number of inseminations
3. Time interval between collection of semen sample and insemination.
4. The method of semen preparation
5. The type of insemination canulae
6. Quality and quantity of inseminate
7. Technique of insemination
8. Difficult situations in IUI
9. Post insemination instructions.

1. The time of insemination:

It is important to time insemination as close to the period of ovulation as possible. There are various methods to determine ovulation time.

Basal body temperature which is simple and inexpensive, but is also inaccurate. The rise of temperature occurs late in the ovulation window.

Cervical mucus assessment This is also unreliable. Templeton et al.[1] showed that in 35% of cycles, the optimum mucus score was observed the day before the LH surge, in 44%, it was optimum on the day of the LH surge and in 18% on the day after LH surge.

Ultrasonograpy: There is a wide variation in the size at which the follicle ruptures. If the scan is done once daily, the ovulation may have occurred 23 hours earlier. After ovulation, the follicle decreases in size and becomes irregular, there is an increase in density of internal echoes and the presence of free fluid in the pouch of Douglas.

LH Surge: This can be determined by urine dipstick method or daily blood tests near ovulation time.

Daily morning urine sample is checked or even twice a day checking can be done. The ovulation usually occurs within 14-26 hours of the detection of the urine LH surge and may happen upto 48 hours later. Testing can be started from the 9th day of the cycle or earlier in case of short cycles. The day after the first positive test is the best day for IUI.

HCG injection: If ovulation determination tests are not done, then IUI should be timed 36-40 hours after the HCG dose. Kucuk T et al. [2] in their study analysed that the clinical pregnancy rate was 23.5%, when IUI was done when follicle rupture was evident by TVS whereas it was only 8.8% when follicle rupture was not evident. However other studies such as by Wang YC et al.[3] notes that pregnancy rates were similar when IUI was performed at 24 or 36 hours after HCG injection.

GnRH Antagonist use: This is becoming the most accepted method to prevent LH surge from occurring and hence being able to time IUI at ease and without risk of cycle cancellation. GnRH antagonist can be started daily from when the follicle is 13 mm in size (flexible start day protocol) or by the fixed start day protocol or the single dose protocol.

Kosmas IP in 2008[4], conducted a meta analysis of prospective RCT's and concluded that allowing for follicle growth and avoiding premature LH surge with use of GnRH antagonists resulted in increased pregnancy rates.

2. The number of inseminations:

Whether one should do 1 or 2 inseminations in an IUI cycle has continued to be a subject of debate. Two inseminations can be done, one 12-14 hours after the HCG injection and the second one 36-40 hours later. A meta analysis of RCTs by Polyzos in 2009 [5]concluded that double IUI offers no clear benefit in overall pregnancy rates compared to a single well timed IUI in unexplained infertility.

Randal GW et al.[6] found that double IUI's at 18 and 36 hours following HCG or the day of and following the LH surge was superior to a single IUI at 36 hours following HCG or the day following LH surge, in cycles using gonadotrophins for ovarian stimulation or within the ovulatory dysfunction and male factor diagnostic categories.

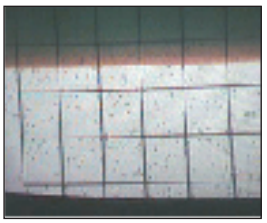
3. Time interval between semen collection and insemination:

Semen processed within 30 minutes after collection resulted in a higher pregnancy rate (48%) than that processed 30 60 minutes after collection (18%). IUI performed within 90 minutes of collection, gave a higher pregnancy rate. This is due to temperature and PH changes. The PH of sperm wash media changes if kept outside an incubator when media such as Hams F-10 is used. Hepes buffered media may not require incubator use.

4. Method of semen preparation:

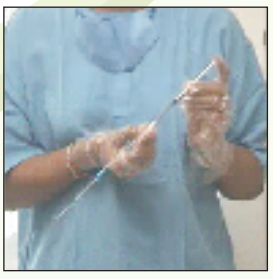
Semen preparation techniques were developed to separate the morphologically normal spermatozoa and remove the leucocytes, bacteria and dead sperms which produce oxygen radicals which negatively influence the ability to fertilize the egg. The commonly used methods are the density gradient method which gives good count of motile and normal sperms and is more efficient in removing debris, however the percentage recovery of sperms is low. The swim up method is simple and gives a good yield of motile sperms.

Cochrane database review in 2007 [7] concluded that there was no difference in pregnancy rates if swim up or gradient method was used.



5. Insemination Canula:

The ideal insemination canula should be easy to use, non toxic and disposable, soft or semi rigid with a small dead space and atraumatic to use.



Soft canulas eg Wallace, Cook, Gynetics Firm Cannulas eg - Tomcat, Makler

Abou Set ta.[8] reviewed literature and noted there was more difficulty with soft catheter and more patient discomfort with firm catheter. However there was no difference in pregnancy rates whichever catheter type was used.

6. Quality and Quantity of inseminate:

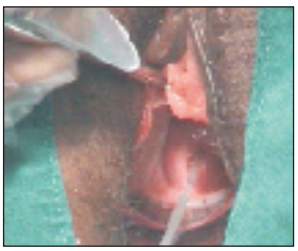
After sperm preparation, to get good results, the sperm count should be 5 10 million / ml;, motility 80% (progressive 60-70%), Velocity (straight line) 20-25 µm / sec.

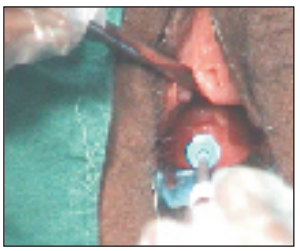
According to Badawy A et al[9] IUI used for treating male factor infertility has very less chance of success when the woman is older than 35 years, the number of motile spermatozoa inseminated is $< 5 X 10^6$ or normal sperm morphology is < 30 %

The quantity of inseminate should be about 0.25 ml. Volume of more than 0.4 ml reaches the fallopian tube. Large volumes causes distension of uterine cavity, discomfort and pain.

7. Techniques of insemination:

Patient is given lithomomy position. Vagina is cleaned with saline and cervical mucus is removed. The cervix is exposed using a bivalve speculum or sims speculum with anterior wall retractor. The cannula is loaded without keeping any dead space and introduced into the cervical canal so as to reach 1-2 cm from the uterine fundus. Sperm injection is done slowly over 1- 2 minutes. Avoid any trauma to cervix or endometrium as this can reduce pregnancy rates. The patient rests for a few minutes.





8. Difficult situations in IUI.

Difficulties arise in cases with an acutely anteverted uterus, where filling the bladder helps to correct the ante version. Also in acutely anteverted or retroverted uteri, the cervix may be held and gentle traction given or a metal canula is used or else the insemination is done under sonography control.

With a stenosed cervix, a soft, thin catheter may be tried or sonographic guidance can be used. Rarely, dilatation can be done gently. Ideally, dilation should be done on the 1st or 2nd day of the next menstrual cycle.

9. Post Insemination Instructions.

There is not much that a woman can do to improve her chances of pregnancy. She can get back to normal work as well as a regular sex life. Luteal support has been shown to be beneficial. Pregnancy rates per cycle were 21% with the use of luteal support and 12% without. Live birth rates were also higher with luteal support.[10] A pregnancy test is done 14 days after the IUI.

IUI helps many couples experience pregnancy, childbirth and the joy of raising children. However to achieve triumph in this low success zone, great attention is to be paid to all aspects of the procedure.

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Luteal Support in IUICycles

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Issues Addressed :

1. Importance of normal luteal phase
2. Is luteal phase compromised in ovulation induction cycles ?
3. Does evidence support LP supplementation in IUI cycles ?
4. Choice of therapeutic agents for Luteal support.
5. Route , Dosage & Duration of Luteal support.

Introduction

From the point of establishing a pregnancy, the Luteal phase of the menstrual cycle is all important. Embryonic implantations occurs during the "implantation window" of the Luteal phase during which perfect synchronization of the embryonic and endometrial signals are essential. The state of endometrial receptivity is dependant on ovarian steroids, progesterone being the most important.

Progesterone is the "**Pregnancy Hormone**", the dominant Luteal Phase Hormone.

Normal Luteal phase is characterized by a normal hormonal environment, normal progesterone secretion by the corpus luteum and adequate endometrial secretory transformation. Formation and continuing function of the corpus luteum is dependant on LH Stimulation, which in turn is dependant on GnRH secretion.[1]

In ART cycles, as a result of long term suppression of GnRH (Agonist cycles) luteal phase LH levels are suppressed which in turn leads to low progesterone levels. Non agonist (antagonist) cycles also interrupt luteal phase by short term suppressing GnRh secretion.[2,3]

Thus, Luteal support is definitely indicated in ART cycles. But, what about IUI cycles, where ovarian stimulation is carried out using clomiphene citrate , Letrozole, gonadotrophins or combinations of oral & injectables?

Thus - Is there any rationale in using luteal support for IUI cycles?

Rationale for Luteal support in IUI cycle

1. Evidence of luteal insufficiency in ovulation induction cycles :

☞ Number of studies have shown Short Luteal phase in ovulation induction cycles using only gonadotropins [4].

☞ Defective LH secretion [5], multi follicular growth and supra physiological steroid concentrations, leads to direct intibition of LH via negative feed back at Hypothalamo - pituitary axis and results in early luteolysis. [6,7]

☞ Exogenous HCG reduces LH concentration by a short loop feed back mechanism, further contributing to LPD. [8]

Thus abnormal LH secretion during luteal phase as a result of high steroidal environment due to multi follicular development leads to Luteal phase defect. [4,5,6]

No studies/data available about the status of luteal phase in ovulation induction cycles with monofollicular development.

2. Evidence of abnormal endometrial changes :

Cycles with multi follicular growth, lead to an advancement in endometrium in early luteal phase, asynchrony between stromal and glandular component in the late luteal phase [9]. Thus there is evidence to indicate that in ovulation induction cycles with multifollicular development, *endometrial receptivity* may be compromised.

Not many studies have addressed the issue of IUI and luteal support. There has been in fact, only one recent study addressing the issue of luteal supplementation after ovarian stimulation (Gonadotropins only) for IUI in a prospective randomized study. [10]

There have been conflicting reports with couple of other studies [11,12],however they are smaller studies , with 'n' numbers being less.

They have concluded that luteal support with vaginal Progesterone is clearly beneficial in OI & IUI cycles. The study indicates that luteal support is mandatory , in OI cycles with gonadotropins, where multi follicular response has been achieved.

More data are necessary to decide role of luteal support in cycles with monofollicular response (cc, letrozole).

Thus <u>does evidence support luteal phase supplementations in IUI cycles the answer is "Yes".</u>

Choice of therapeutic agent for luteal support.

Natural progesterone and HCG are the common therapeutic agents used for luteal support. Because HCG use is likely to increase the chances of OHSS , and theoretically hyper stimulation can occur only with clomiphene also , **progesterone** is the preferred drug for luteal support in infertility.

Natural progesterones are superior to synthetic progesterones. Synthetic progesterone derivatives have been linked to increased risk of *fetal congenital malformations* , adverse effects on High Density Lipoproteins (HDL) & psychological effects severe enough to limit usage [13,14,15]

Natural progesterones are more effective in inducing secretory features in the endometrium (Peller etal, 1989). Oral, vaginal and I.M routes of natural progesterone have been used.

Oral use is ineffective due to rapid metabolism and hepatic 1st pass effect.

Vaginal use achieves high serum concentrations, slower metabolism as it avoids hepatic first pass metabolism, higher local endometrial concentrations. [16,17].

I.M route has been shown to achieve high and persistent serum concentrations, and good histological co-relation[17]. Daily injections are however difficult & painful for the patient.

Both vaginal Vs I.M route have been considered adequate. Superiority of one over the other has not been established. The optimal route of progesterone administration is not yet known. (Cochrane database syst. Rev. 2008 Jul 16;(3) : (D004830.) ASRM Practice committee opinion 2008)

Dydrogesterone is a potent orally active progestogen that has been used in clinical practice for over 40 years. Chemically, it belongs to the class of retrosteroids. Dydrogesterone is closely related to endogenous progesterone. It differs from most other synthetic progestogens in that it has no estrogenic, androgenic, glucocorticoid, or anabolic effects. This progestogen also has been used orally for luteal support, & studies support its efficacy comparable to micronised natural progesterones. [18]

Dosage of progesterones for luteal support

Practice varies greatly between physicians and locality. Majority would agree on the following :

IM, 50 mg / daily once (In sesame oil, peanut oil or ethyl oleate)

Vaginal capsules 200 mg TID

Vaginal Gel 90 mg/daily once

Oral not recommended

When to start

One or two days **after** confirming ovulation or from the expected time of ovulation.

How long to continue

Available evidence says that support is justified until about the time of diagnosis of pregnancy. However it is common practice to continue luteal support upto the detection of a fetal heart beat or at gestational age of 10-12 wks. Part of the reason for continuing so long is the psychological " feel comfortable "factor, and in case the patients miscarry after they have stopped progesterone, they think stopping it is the cause.

However strong support by way of lutectomy studies have shown that exogenous progestogens or even progesterone from corpus luteum should not be necessary after 9 wks of gestation.

Key Learning Points

A. *Luteal phase is likely to be compromised in OI cycles.*
B. *Luteal support is neccessary for all OI cycles with a multifollicular development.*
C. *Natural micronised progesterone Vaginal or I.M is the best drug .*
D. *Progesterone supplement should start from 1-2 days from ovulation.*
E. *To be continued atleast till scan detection of fetal heartbeat.*

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Problems and Complications in IUI

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Infertility though not a physically disabling disorder has far reaching emotional and social consequences. Attempts to overcome sub fertility have been going on since the advent of medical science but it is only in recent years that one has seen a great improvement in treatment, due to advances in reproductive research. Intrauterine insemination is the first step in the ladder of fertility management. Since it is practiced so extensively a thorough understanding of the underlying principles, methodology and associated complications is mandatory to achieve good results.

The underlying principle for the use of intrauterine insemination (IUI) is to reduce the effect of factors such as vaginal acidity and cervical mucus hostility and to deposit a bolus of concentrated motile morphologically normal sperm as close as possible to the oocytes in the hope that this might help in achieving fertilization and thereby pregnancy. The development of sperm preparation techniques like 'swim up' and 'double density gradient' for IVF led to the use of washed motile capacitated sperm for IUI. Sperm preparation removes leucocytes and dead sperms, which generate free oxygen radicals that reduce the functional capacity of the intact sperm. The use of washed prepared sperm for IUI has resulted in a significant reduction in the side-effects associated with the earlier use of neat semen for IUI, such as painful uterine cramps, collapse and infection. Success achieved with the procedure and its inherent simplicity became instrumental in making it the technique of first choice amongst the assisted conception techniques. Tubal patency of course, is an essential pre-requisite to the use of this procedure.

Intrauterine insemination may be carried out in either a ***natural or stimulated cycle***. The rationale for the use of superovulation with IUI is to increase the number of oocytes available for insemination and thus the chance of implantation. The rise in steroid levels with ovarian stimulation is also thought to correct subtle ovulatory defects. Unfortunately multifollicular development increases the cost and chances of complications such as ovarian hyperstimulation syndrome, multiple pregnancy (Fig.1& 2)and an increased incidence of maternal and neonatal complications associated with multiple pregnancies[1].

Though the process of semen preparation and insemination are fairly simple it would be prudent to bear in mind some of the associated problems and complications which may compromise results.

1. Problems related to sperm preparation

a. Increased viscosity of semen this prevents adequate semen processing and hence poor sperm recovery.

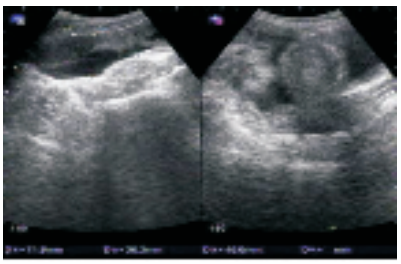
b. Poor semen volume and/or sperm count and motility on the day of IUI leading to cancellation of the cycle or a sub-optimal procedure.

2. Problems related to ovarian stimulation

a. Anovulatory cycle leading to cancellation of procedure

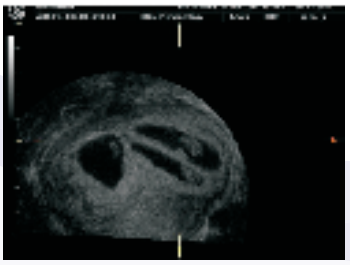
b. Multiple follicular development increases pregnancy rates but has many associated Problems.

i. Increases the risk of OHSS resulting in hospital admission, increased morbidity and occasional mortality. It is very important to keep the possibility of an ectopic pregnancy in mind when the patient shows signs of OHSS brought on by rising HCG levels in early pregnancy. Enlarged ovaries with ascitic fluid and symptoms of severe OHSS could mask the picture of a bleeding gestational sac[2]. Judicious use of ovarian stimulation drugs will go a long way towards prevention of OHSS.



Ohss

ii. Increases risk of multiple pregnancy and heterotropic pregnancy. Multiple pregnancy results in higher pregnancycomplications, preterm births and fetal reduction.



Multiple_preg

c .Pr ematurе LH surge. Seen often in women with PCOS when they undergo ovarian stimulation compromises pregnancy rates.

d .Aberrant/Blun ted LH surge leading to anovulation. Administration of an ovulatory trigger of HCG takes care of this and has become the norm in IUI cycles.

e. Leutinized unruptured follicle is generally encountered in women with endometriosis and pelvic adhesions. There is however an iatrogenic reason for LUF as well and that is administration of HCG before oocyte has attained maturity. This is seen when HCG is administered based on follicular measurement alone without an associated Estradiol check. In the event of an inability to perform blood E2 levels it is wise to wait till atleast cycle day 11/12 before giving the HCG trigger even if follicle size has reached 18mm before that. Large follicles on day 8/9 of the cycle are seen often with letrozole and clomiphene citrate.

f. Early rupture of the follicle generally seen in older women and those with poor ovarian reserve. Monitoring should be started early day7/8 of the cycle and followed closely as they tend to ovulate as early as day10 of the cycle.

3. Problems related to timing of IUI

A well timed insemination increases the possibility of pregnancy in an IUI cycle. It is currently believed that insemination at 32-38 hours after HCG administration provides the best results[3]. Semen preparation capacitates the sperm which then reduces sperm survival tim post preparation. The ovum in turn needs to get fertilized within 6-10hrs after ovulation. This calls for precise timing in IUI. In order to time the IUI accurately one can test for the onset of the LH surge either in blood or urine. In a prospective study of 1540 cycles of IUI with donor sperm at Cleveland Fertility Centre, to determine the best time interval between the onset of the LH surge and IUI, women were divided into five subgroups according to the positive urine LH test-IUI time interval and the pregnancy rate and live birth rate per cycle were calculated for each group. The first positive LH surge test was most frequently (44.5%) found at lunch-time (11:00-15:00). The live birth per cycle achieved was 5.6% when the insemination was performed 18-23 h from the first detection of the LH surge, and 11.7% when it was performed between 24 and 42 h. The live birth rate declined to 6.5% when IUI was performed later than that. Overall, no significant differences were discovered in live birth or pregnancy rate when insemination was performed at any of the time points between 18 and 53 h. Their conclusion was that lunch-time is the best time to check for the LH surge using urine dipsticks and insemination at any time between 18 and 53 h after the onset of the surge will produce optimal results[4]. The advent of GnRH Antagonists has made it easier to time an IUI more precisely and also avoid weekend procedures.

4. Problems related to procedure.

a. **Trauma,** vaginal tear, bleeding from cervix and endometrium can result if the operator is not gentle with the use of instruments. Blood on the catheter after IUI signals a poor outcome in terms of pregnancy. Though vaginal and cervical tears are rare one should examine carefully before removing the speculum. In case a stitch is warranted please do not hesitate to put it, compression alone may not work and the patient maybe brought back to hospital in a Collapsed state later.

b. **Difficulty in negotiating cervical canal** because of fibrosis or a false passage is quite common. Even an acutely retroflexed or anteflexed uterus can create this problem. This can be overcome by doing an ultrasound guided IUI, and/or using a metal catheter(Fig.3). Using a metal catheter and holding the cervix with a tenaculum increases uterine contractions and may on occasion lead to severe cramping and anaphylaxis. Interestingly higher pregnancy rates have been reported in women undergoing IUI when uterine contractions occurred with the use of a tenaculum (Balci et al 2009)[5]. Bleeding from the cervical canal is a common feature with a difficult negotiation. It is also a good idea to negotiate the cannula before loading it with the sperm preparation. This way incase any difficulty is encountered you would have prevented contamination of the inseminate with blood.

c. **Retrograde flow**. If the internal cervical os is open or the catheter has got bent during insertion there may be a retrograde flow of the sperm preparation. Needless to say this is not a happy situation! Ensure that the catheter is well placed in the uterine cavity and deliver the inseminate slowly over a few minutes. Do not withdraw the IUI cannula immediately as a suction effect may be created. Give enough time to allow the sperm containing media to find its place into the tubes and in the endometrial folds.

d. **Anaphylaxis**- though rare has been reported after IUI. Such reactions are attributed to the bovine serum albumin in the media[6].

e. **Infection** -the prevalence of infectious complications is 1.83 per 1,000 women undergoing IUI. It could be a result of not maintaining proper asepsis during procedure. Infective organism can travel from the vagina or cervix into the uterus. Ensure that you have cleaned the cervix with a swab soaked in saline or distilled water before loading the IUI cannula. Do not touch the tip/ front portion of the cannula.

f. Infections like Hepatitis B,C and HIV can be transmitted to the woman in the event of infected semen.

Risk of Infection

Transmission of STD: pathogens from an infected donor to the recipient of a semen donation may result in acute pelvic infection. It also carries the risk of long-term reproductive complications or adverse outcomes of pregnancy, including infection of the offspring. Screening for Chlamydia trachomatis, Neisseria gonorrhoeae and other bacterial infections is strongly recommended. (Peeling & Embree)[7] .

Risk of HIV transmission: Discordant couples, in which the man is HIV positive and the woman is HIV negative, face limited options when they wish to produce healthy children whilst practicing safe sex. In order to conceive they must abandon unprotected sex, which carries high risk of HIV infection to the woman. A method for removing HIV infection from the semen has been developed and should be used when performing IUI in such couples. Motile spermatozoa are isolated from semen samples by the gradient and "swim-up" techniques. HIV-RNA is tested before and after the procedure in both the semen and the purified spermatozoa fraction. Insemination is performed only when the absence of any viral particles is verified by PCR Lowenstein et al. reported that this procedure is safe and enables biological parenthood for HIV-1 discordant couples without the risk of infecting the female partner[8]. This procedure has been used by other investigators with good results[9].

Hepatitis B infected males

It is believed that HBV infection can bring about mutagenic effects on sperm chromosomes. Integrations of viral DNA into sperm chromosomes which are multisites and nonspecific, can further increase the instability of sperm chromosomes. It is suggested that HBV infection can create extensive hereditary effects by alteration of genetic constituents and/or induction of chromosome aberrations, as well as the possibility of vertical transmission of HBV via the germ line to the next generation[10].

Conclusion

Though IUI is a simple procedure to perform there are certain problems related to the procedure. Taking care to avoid complications and pre-empting problems should help to improve patient experience and avoid lowering the already low pregnancy rates seen with IUI.

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Semen Cryopreservation

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History:

The history of human cryopreservation dates back to about two centuries when the first experiments involving cooling followed by re warming of spermatozoa were first published[1]. Despite the early success, it was not until the fortuitous discovery of glycerol as a cryoprotectant[2] and subsequent live birth of a calf in the early 1950s[3] that cryopreservation of human semen for assisted reproduction became a feasible option. Dr Jerome Sherman reported the first live human birth using cryopreservation and insemination[4] in 1953 and since then it has become an indispensable part of managing patients of Infertility.

Indications:

Some of the common indications of semen cryopreservation are:

1. Donor Insemination
   Artificial insemination with donor semen as a method of circumventing severe male infertility is the mainstay of treatment of infertility in such cases.

2. Social indications like infrequent cohabitation due to touring, jobs etc

3. All IVF-ET cycles:
   This prevents cycle cancellation in case of unavailability of male partner or if the male partner is unable to produce sample on demand.

4. Oligo-asthenozoospermia

5. Young male cancer patients:
   Advancements in oncology treatment with advanced post-therapy long term survival rates have resulted in an increase in demand of cryopreservation[5] in order to preserve the reproductive potential of young male cancer patients

6. Surgically retrieved spermatozoa (PESA/TESA/ TESE/Testicular biopsy) or sperms retrieved by procedures such as electro ejaculation:

ICSI is now routinely available in most reproductive medicine units and offered to an increasingly large group of men with severe sperm dysfunction. Lately several new methods have ensured successful freezing and storage of very small numbers of spermatozoa which maximizes the number of IVF attempts possible from a single surgical procedure.

7. Males unable to produce semen on demand

Screening of Patients and Donors:

There was little pressure to optimize semen cryopreservation protocols until the mid 1980s, but following infection of four recipients with Human Immunodeficiency Virus after insemination from a seropositive donor, the use of quarantined cryopreserved semen has now become mandatory. All donors should be screened for the presence of HIV 1 & 2 antibody, Hepatitis B surface antigen, Hepatitis C antibodies and syphilis by using appropriate serologic tests. All donors should be retested for these infections after six months and only on confirming non-infectious state should the samples be used. It is also noteworthy that according to the latest draft on ART bill and rules 2008, cryopreservation of a gamete cannot be for more than a period of ten years[6].

Effects of Cryopreservation:

It has been extensively studied and documented that cryopreservation of human semen results in a significant loss of spermatozoal motility and viability with considerable variation between ejaculate of different individuals. It is therefore recommended that semen from highly selected population of men is suitable for treatment purposes after cryopreservation. Reasons for such differences are yet to be completely elucidated and therefore the ability to predict post-thaw survival remains limited.

Techniques of Cryopreservation:

There are various techniques for semen cryopreservation and mentioned below is the one of the more commonly used protocols.

Semen samples are allowed to liquefy in an incubator at 37 degree centigrade for 30 minutes after collection. Washed spermatozoa can be prepared with a density gradient separation method and the final washed sperm preparation resuspended in washing media. Alternatively the liquefied semen sample can be cryopreserved directly.

Bring all reagents to room temperature before use.

Freezing Protocol:

1. Add one volume of freezing media drop wise over a 30 second period to one volume of liquefied semen or washed spermatozoa solution with continual mixing after each drop. This is to ensure adequate equilibrium of the sperm cells with the freezing media. Allow the mixture to equilibrate for three minutes after all the freezing media has been added.

2. Place the freezing medium/ sperm mixture into straws or vials and cool at 3 degrees /minute from 25degree C to -5 degree. Hold the cryocontainer at -5degreeC for three minutes. Seed the cryocontainers manually by touching them for about 1 second with forceps precooled in liquid nitrogen. Hold the cryocontainers at -5degreesC for another 7 minutes. Cool the cryocontainers at 10degreeC/minute from -5 to -80 degree. Plunge the cryocontainers in liquid nitrogen and then transfer them to storage canes.

3. Alternatively, suspend the vials/straws on aluminum canes and immerse in a container filled with approximately 600 ml of water at room temperature. Place the container of water holding the samples at 4 degrees C in the refrigerator for 30-60 minutes.

4. Transfer quickly to liquid nitrogen vapour at the top of a liquid nitrogen storage tank and leave for 30-45 minutes. Vials should be suspended about 10-20 cm above the surface of the liquid nitrogen. Straws should be laid horizontally at a similar height. Quickly transfer the vials/straws to final storage on labeled canes in liquid nitrogen.

5. The next day, or several hours later, thaw a test vial/straw and record all results on an appropriate report form

Thawing, Dilution, and washing

1. Thaw straws by placing them on the bench top (22 degree C). Cryovials need to be agitated in a water bath at 30-35 degree C.

2. Transfer the thawed sperm suspension to a culture tube of adequate volume and then slowly add drop wise 10 volumes of sperm washing medium to the thawed sperm suspension over a 30 second period with adequate mixing to ensure complete dilution of the medium. Motile spermatozoa are then recovered from the thawed, diluted suspension by density gradient centrifugation and washing.

Packaging and leakage

Cracking and leakage from cryopreservation bags has been reported and therefore it is recommended that all primary packaging should be robust and leak proof. In addition, to avoid

contamination of cryopreserved cells, all samples should be encased in a secondary container, "double bagged", to prevent external organisms infecting cells or tissue post thaw. It is also recommended that unscreened samples should not be stored with screened samples and samples from infected patients should be stored separately in dedicated containers[7].

Summary

It is now about 60 years since the birth of the first offspring following insemination with cryopreserved semen. Many couples continue to benefit from the combined use of cryopreservation of spermatozoa in conjunction with assisted fertilization technologies. Many males for whom infertility was almost impossible; as in cases of oncology treatment, infertility preservation has become a reality and fatherhood a prospect. Optimization of the technique of cryopreservation continues to be investigated and unraveled and this will in turn result in improved cryosurvival rates. The most important but often missed role is that of ART practitioners and regulatory authorities who play a major role in the introduction of peer reviewed guidelines and evidence based practice ensuring safety of the recipients and healthy offspring.

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Donor IUI

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Donor Insemination

Artificial insemination, sometimes known as "alternative insemination" or "donor insemination," is a procedure by which semen is injected into the uterus for the purpose of impregnation. Although it is used primarily to impregnate married women whose husbands suffer from infertility, it is also often used by lesbians and heterosexual single women who wish to conceive without sexual contact with males. It is also frequently the method of choice when gay men create families through surrogacy or through co-parenting.

Legal Issues

The idea of applying artificial insemination to human propagation was difficult enough for turn-of-the-century society to accept: to use the sperm of a man other than the woman's husband was scandalous.If any doctors were treating infertility through DI, they were doing it with the utmost discretion. DI remained virtually unknown to the public until 1954. That was the year the first comprehensive account of the process was published in The British Medical Journal.

As it had before, donor insemination provoked heated public debate. The Archbishop of Canterbury established the first in a long procession of commissions that, over the years, inquired into the development of the practice.

The first commission produced a report strongly critical of DI, and recommended that the practice be made a criminal offense. A Parliamentary Commission agreed. In Italy, the Pope declared DI a sin, and proposed that anyone using the procedure be sent to prison.

In that same year (1954), the Supreme Court of Cook County ruled that regardless of a husband's consent, DI was "contrary to public policy and good morals, and considered adultery on the mother's part." The ruling went on to say that, "A child so conceived, was born out of wedlock and therefore illegitimate. As such, it is the child of the mother, and the father has no rights or interest in said child."

This perspective was maintained as late as 1963, when a court in the United States held that a DI

child was illegitimate because the sperm donor was not married to the child's mother. Regardless of her husband's consent, the court stated, the woman's insemination constituted adultery.

But a year later, there were signs that attitudes were changing. In 1964 Georgia became the first state to pass a statute legitimizing children conceived by DI, on the condition that both the husband and wife consented in writing.

In 1973 the Commissioners on Uniform State Laws, and a year later, the American Bar Association, approved the Uniform Parentage Act. This act provides that if a wife is artificially inseminated with donor semen under a physician's supervision, and with her husband's consent, the law treats the husband as if he were the natural father of the DI child. The laws most states have enacted pertaining to DI have been based on this act. In every case, the statute makes it clear that the donor who provides the doctor or sperm bank with sperm is not the legal father of any child conceived by that sperm.

Since semen could be frozen and preserved, banks of semen by anonymous donors soon developed. Technically they were anonymous, but their medical and genetic records could be made available to the would-be mother.

Ethical Issues

A sperm donor may be classified as a donor or as a father. The former is generally anonymous and relinquishes all parental claims and responsibilities, while the latter is known to the mother and assumes parental responsibilities.

While most donors recruited by sperm banks are anonymous and legally relinquish parental rights and responsibilities, a few sperm banks allow children, with the consent of the donor, to initiate contact with their genetic father at a specified age.

When the sperm donor is known--as in cases where individuals such as a gay man and a lesbian (or a gay male couple and a lesbian couple)--decide to co-parent a child, it is very important that all parties be clear as to the legal obligations and consequences of the co-parenting arrangement. Since alternative families are not acknowledged in most jurisdictions, co-parenting may carry with it considerable legal risks.

Other issues involved in artificial insemination include health considerations, such as access to the donor's medical history and genetic heritage, and the emotional impact on the children of artificial insemination of not knowing their fathers or growing up in non-traditional families.

While many sperm banks and fertility specialists offer their services to lesbians, others do not, and several countries, including Germany, restrict access to sperm banks to married couples. However, because the procedure is relatively simple, donor insemination is frequently available to lesbians even in places where there are legal hurdles.

When used by lesbians and gay men, artificial insemination carries a number of legal and emotional considerations and risks. Perhaps the most important of these is the role of the parties in the rearing of a child.

Indian Perspective

In Indian mythology there are umpteen evidences of donor insemination. However, Delhi state was the first state to legalize the donor inseminations, vide Delhi artificial insemination act (human) 1995.Otherwise it has remained uncharted area with no proper control. In more than 80% of times the fresh semen sample is being used and only 20% of times frozen semen samples are used, that too in metropolitan cities where semen banks are available. All good centers follow the HFEA, ASRM and ICMR guidelines, which will become law in near future. However as on today there is no enforcement agency. ICMR guidelines are quite comprehensive and can be down loaded from the internet and followed.

Advantages Of Donor Insemination

a) The experience of pregnancy from the start to the birth, often seen as an important preparation for parenthood, is shared by the couple.

b) One parent has a biological and genetic link with the child.

c) By attending the inseminations the husband can share in the child's conception.

d) DI is a relatively simple & usually painless procedure requiring neither surgery nor a stay in hospital.

e) Public opinion is showing a far greater acceptance of DI as a means of having a family.

f) The treatment is confidential. Couples decide for themselves who knows that they are being treated.

g) The anonymity of the donor ensures against any legal, material or emotional claim by him on the couple or child and vice versa.

The Dilemmas Of Donor Insemination

a) Some religious groups are still opposed to donor insemination.

b) The secrecy that sometimes surrounds a DI conception can perpetuate the notion that it is naturally and ethically wrong. This can, in some cases, lead to feelings of guilt and fear in relation to the child's birth and nurture.

c) As with adoption, the husband has no hereditary or genetic relationship with the child, and his procreative desires cannot be fulfilled.

d) Both partners need to reflect on their attitudes and feelings towards a child conceived by donor semen and its impact on their relationship. A mutual acceptance is of the utmost importance but cannot always be reached.

e) The right of the child to know about the method of conception is a controversial question which some couples find hard to resolve.

f) Remarks about family likeness should be expected when the baby arrives. These are perfectly normal, but they can cause embarrassment if parents are not prepared for them.

g) Children conceived with anonymous sperm may have a difficult time emotionally because their biological father and his family could forever be a mystery.

Indications Of Donor Insemination

Historically, DI was primarily a treatment of male factor infertility. However, the indications for DI have expanded such that it has become an alternative approach to fertility for some women. The procedure can be considered in:

Gross male subfertility

Obstrucitve azoospermia, eg congenital bilateral absence of vasa deferentia

Non obstructive azoospermia, eg Klinefelter's syndrome

Severe oligoazoospermia

Severe asthenozoospermia

Severe tetratoazoospermia

Oligoasthenoteratozoospermia

Failed fertilization with ICSI

Infectious disease in the male partner such as HIV

Familial / genetic diseases eg. hemophilia, Huntington's disease

Severe rhesus incompatibility

Lesbian couples or single women

Couples who fail to achieve pregnancy with other assisted reproductive technology procedures

One must be psychologically ready to proceed with DI. It is recommended that any patients considering DI see a counselor who is skilled at clarifying feelings about infertility, and about trying DI. It is essential that both partners feel comfortable with the decision and that all fears and questions be openly discussed. For some, it may mean dealing with various moral and ethical questions; for others, exploring questions about donor selection and whether to be open about the decision to do DI and whether to tell a child conceived by DI how they were conceived.

Before starting DI, a careful medical and reproductive history should be taken on the woman and a rubella titer, blood type, and test for HIV, Australia antigen and syphilis should be done. It is important to document normal ovulation patterns and get a hysterosalpingogram done to document that the woman's fallopian tubes are open.

The DI procedure involves inseminating the woman as close to the time of ovulation as possible, which can be monitored by testing the urine for an LH surge which indicates that ovulation will soon take place. Inseminations are usually done about 24 hours after a surge of LH is noted on the urine test. One or two inseminations per cycle can be done per cycle. Follicular monitoring helps in timing the IUI.

Donor Selection

Couples or individuals usually have the right to decide which sperm bank and which donor to use. Information about a donor's physical characteristics, race, ethnic background, educational background, career history, and general health should be available. Many banks provide written profiles about the donors they have some sperm banks are open to providing non-identifiable information about the donor (even photographs) as well as providing a service for adult offspring to obtain information about the donor.

The American Society for Reproductive Medicine recommends that physicians use only frozen semen and that the specimen be frozen and stored for at least 180 days. The donor should have an initial HIV blood test and should then be retested and have a negative result on the HIV test before the frozen specimen is used.

All donors should have tests for certain infections such as HIV, syphilis , hepatitis B, cytomegalovirus (CMV), gonorrhea, chlamydia , streptococcal species and trichomonas. All these organisms can be transmitted via semen to woman. Some can have grave effects on the fetus; others principally affect the woman. The donor's semen should also be checked for the presence of white blood cells which can indicate an infection within the reproductive tract.

Donors are excluded from a donor program if he or his sexual partner have experienced any of the following: a blood transfusion within one year, a history of homosexual activity, multiple sexual partners, a history of IV drug use, or a history of genital herpes.

Frozen sperm are quarantined for a particular period and the donor will be tested before and after production of the sample to ensure that he does not carry a transmissible disease and then only used for intrauterine insemination.

Procedure

Some sperm banks process the sperm for intrauterine insemination before shipping. If not, the thawed specimen is processed to remove the seminal plasma and get maximum number of motile sperm cells After the sperm is processed, it is injected, using a syringe and thin catheter, into the uterus via the cervix at the time of ovulation. IUI is preferred over cervical insemination when using cryo-preserved donor sperms as it gives better pregnancy rate[1].

The woman is generally advised to lie still for a half hour or so after the insemination. Specially designed equipment/catheters are available for carrying out artificial inseminations. Semen is occasionally inserted twice within a 'treatment cycle'. If the procedure is successful, the woman will conceive and carry to term a baby. A pregnancy resulting from artificial insemination will be no different from a pregnancy achieved by sexual intercourse. However, there may be a slight increased likelihood of multiple births if drugs are used by the woman for a 'stimulated' cycle.

Success Rates

Success rates, or pregnancy rates for artificial insemination may be very misleading, since many factors including the age and health of the recipient have to be included to give a meaningful answer, e.g. definition of success & calculation of the total population[2]. For couples whose infertility is unexplained, unstimulated IUI is no more effective than natural means of conception[3]. Generally, it is 15-20% per cycle for IUI. In IUI, about 60 to 70% have achieved pregnancy after 6 cycles

The highest success rates for DI are reported in women who have no infertility problems, are under 35 years old and whose husbands have azoospermia. Lower success rates are reported where there is a female factor (ovulation problem, endometriosis,etc.) or the woman is over 35. Statistics show that for women under the age of 35 the success rate is about 14 per cent, falling to 8 to 9 per cent for women aged between 35 and 39, and to 4 to 5 per cent for women aged between 40 and 42. A promising cycle is one that offers two follicles measuring more than 16 mm, and estrogen of more than 500 pg/mL on the day of hCG administration[4].

Success rates vary from 60-80% but achieving pregnancy may take many cycles. In one study the overall cumulative pregnancy was 86% in the IUI patients and 49.5% in pericervical insemination group [5] Success rates for insemination may increase with two inseminations per cycle & correct timing.

If no pregnancy occurs after several cycles, then further evaluation of the woman involving a laparoscopy and hysteroscopy to ensure there are no adhesions or endometriosis, and an evaluation of the luteal (post-ovulatory) part of the cycle by endometrial biopsy and/or checking progesterone levels in the blood, is warranted. Other hormonal tests as well as ultrasound monitoring of follicular development may be indicated.

Ovulatory stimulating drugs such as clomiphene or injectable gonadotropins can be given to the woman. The pregnancy rate obtained with CC stimulation is approximately half that obtained with FSH. Each case should be considered on an individual basis and the treatment options discussed with patients. CC could be a reasonable approach for young women with good prognosis, whereas in the remaining cases FSH would be the preferable method[6].

Pregnancy rate also depends on the total sperm count or, more specifically, the total motile sperm count (TMSC), used in a cycle. It increases with increasing TMSC, but only up to a certain count, when other factors become limiting to success. The summed pregnancy rate of two cycles using a TMSC of 5 million in each cycle is substantially higher than one single cycle using a TMSC of 10 million[7]

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IUI in Viral Diseases

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Transmission of viral diseases are of major concern to reproductive specialists. The viral diseases that are causing most concern are human immunodeficiency virus (HIV) types 1 and 2, and hepatitis B (HBV) and C (HCV) viruses. These pathogens, which may cause incurable, often fatal, infections, have been transmitted through assisted reproductive technology (ART) and insemination procedures, and can be transmitted from infected mothers to the fetus or newborn. Other common sexually transmissible viruses include hepatitis A virus (HAV), human T-cell lymphotropic viruses (HTLV) I and II, human papilloma viruses (HPV), and several members of the herpes virus family: Epstein Barr virus (EBV), cytomegalovirus (CMV), herpes simplex virus 2 (HSV-2), and human herpes viruses (HHV)-types 6 and 8.

Sexually transmitted viruses can cause chronic lifelong infections. The past two decades of intensive virus research has not provided cures, they have produced a substantial body of information on mechanisms and risk factors underlying STI transmission, suggesting risk-reduction strategies. Sensitive and precise diagnostic tests allow the early detection and monitoring of viral infections, and new antiviral drugs make it possible to manage many chronic viral infections. Several couples seek fertility services to maximize reproductive potential and/or minimize the transmission risk to their partners and children. Fertility services cannot be withheld ethically from individuals with chronic viral infections, including HIV, if a center has the resources to provide care for the same. Those centers that do not have the resources or facilities to provide care should facilitate referral to a center with protocols in place to manage such patients.

When we talk about fertility in this group of patient following should be kept in mind:

1) Reducing viral load in infected partner(s)

2) Reducing exposure and susceptibility of a non infected partner

3) Frank and detailed discussion of available scientific evidence and risk-reduction strategies to provide a basis for informed consent.

Apart from the couples who are at risk of viral infections all semen donors should be screened for high-risk factors and clinical evidence of infectious diseases, and be tested serologically for

chronic viral infections, including HIV-1, HIV-2, HBV, HCV, HTLV-I and II, and CMV[1]. Use of semen from donors determined to be ineligible, based on their risk profile or screening test results, is not medically appropriate under any circumstances. Such screening can help to ensure that appropriate precautions are taken to minimize risk of viral transmission to partners and offspring. Couples in which one or both partners are positive for HIV, HBV, or HCV should be treated by fertility centers having the additional laboratory resources.

Contamination with HIV, HBV, and HCV has been documented in ART clinics [2,3,4] and blood banks [5]. It is highly recommended that samples from viral carriers be processed in a separate laboratory or designated space within the main laboratory, utilizing dedicated equipment, to minimize the risk of cross contamination. HIV, HCV, HBV, & possibly other viruses can survive in liquid nitrogen, making it possible for cross contamination of samples to occur in liquid nitrogen storage tanks. To protect cryopreserved specimens from potential cross contamination, separate storage tanks for HIV-, HBV-, & HCV-infected specimens are advised. The following measures have been proposed to further reduce the risks of cross contamination of samples in liquid nitrogen storage:

1) The use of specimen containers guaranteed by the manufacturer to withstand freezing temperatures and thawing cycles;

2) The use of "double bagging" or sealing techniques to prevent the direct contact of cryocontainers with liquid nitrogen;

3) The storage of samples in liquid nitrogen vapor instead of in liquid nitrogen itself;

4) The use of "sperm-washing" techniques to decrease the viral load before freezing semen samples[6]

5) A vertical laminar flow cabinet with 100% recirculation of filtered air offers a safe workplace to the laboratory workers

6) No mouth pipetting used

Requirements for treatment

Couples in which one or both partners are infected with a sexually transmissible pathogenic virus should receive pre-conceptional counseling on the risks of sexual & vertical transmission of their infections. Adoption &, in circumstances involving an infected man & uninfected woman, donor insemination should be presented as the safest options. Couples who decide to proceed with partner-IUI or other fertility treatment must agree to reasonable interventions aimed at reducing the transmission risk.

Counseling and education concerning safe sex practices should be provided and emphasized. In cases where the male, but not the female, partner is infected, the couple should understand the merits of using condoms throughout fertility treatment, pregnancy, and the postpartum period. Serial diagnostic testing of the uninfected partner is recommended throughout treatment and pregnancy, and for both mother and infant during the first year after birth. Informed consent should be explicit and as thorough as possible, emphasizing that risk of transmission cannot be completely eliminated even when specific risk reduction strategies are employed. Psychological, medical, and obstetrical care can be provided by a multidisciplinary medical team.

Sperm wash methods

Sperm-wash procedures involving density gradient centrifugation followed by a sperm swim-up step have been used to separate motile sperm from free HIV virus and HIV-infected somatic cells[7,8,9]. Quantitative assessment of HIV in semen before and after the sperm-wash procedure indicates that >99% of HIV is removed[8]. Virologic testing of the sperm fraction for the presence of residual detectable HIV prior to its use for insemination can provide an added measure of safety, as up to 5% to 10% of samples may contain residual virus after this procedure[9]. Similar sperm preparation techniques have been used to separate HCV from sperm[10] and may be useful for other viral infections where the majority of virus is found in free form or associated with semen somatic cells (i.e., white blood cells, epithelial cells). Some centers adopt routine testing of the washed semen product for HIV and/or HCV with a polymerase chain reaction technique to prevent infection in the inseminated woman.

Virus-specific risk reduction strategies

HIV

Because HIV is found primarily in white blood cells and as cell-free virions in semen , sperm-wash techniques that separate motile sperm from the round cell and seminal fluid fractions, including density gradient centrifugation and swim-up methods, can reduce HIV levels markedly prior to insemination). Where available, testing of the processed sample for HIV RNA prior to insemination may further reduce risk. Trauma to the cervix or uterus during the IUI procedure must be minimized.

Both partners should undergo a sexual health screen. Bacterial vaginosis and infections with HSV-2, *Trichomonas vaginalis*, *Chlamydia trachomatis*, *Neisseria gonorrhea* and *Treponema pallidum* can increase HIV-1 transmission[11] and should be treated. The use of condoms during sex should be reinforced. Practices and behaviors that lacerate mucosal surfaces are other risk factors for HIV transmission and should be avoided[12]. Both partners should undergo a fertility

assessment so that the number of exposures is minimized. HIV viremia should be minimized in the infected partner (peripheral blood viral load less than 10,000 copies/mL)[13] through use antiretroviral therapy, to reduce levels of HIV in semen.

Experimental approaches such as pre-exposure prophylaxis (PREP) with antiretroviral drugs[14,15,16] with a long half life (tenofovir) and locally applied vaginal estrogen gels[17] may further reduce the susceptibility of the uninfected female partner. The uninfected partner in a discordant couple should be tested for HIV serology and viral load at three-month intervals during treatment and pregnancy. If HIV infection is detected in the female partner during pregnancy, she should be referred to an obstetrical service experienced in managing HIV-infected women. Use of antiretroviral drugs during pregnancy and/or labor, use of cesarean section, and avoidance of breastfeeding can reduce the risk of vertical transmission of HIV to less than 2%.

Hepatitis B

In couples who are discordant for HBV infection, the partner who is seronegative should be vaccinated against HBV. Fertility treatments may be initiated once the vaccinated partner's anti-hepatitis B surface antibody titer (HBsAB) is positive. Modified sperm washing to reduce viral load is not required after the female partner is immunized against HBV. If the female is the infected partner and is HBsAg-positive, her newborn should receive immunoprophylaxis within 12 hours after birth. Immunoprophylaxis consists of both HBV vaccine and immunoglobulin, and is repeated at six months of life. Breastfeeding is not contraindicated in women chronically infected with HBV[18].

Hepatitis C

There is a small, but measurable, risk of HCV transmission via semen. All patients with viral hepatitis should be counseled about the risks of transmission to their partner, children, and their healthcare team. When the male partner is HCV-infected, sperm washing can reduce the viral load in semen and is recommended to reduce the risk of transmission to his partner[10].

If either partner is chronically infected with HCV (HCV-RNA positive), treatment with peginterferon-alpha and ribavirin should be considered prior to fertility treatment in order to reduce the infected partner's viral load[19]. The goal of therapy is to achieve a sustained virologic response, and the recommended duration of the initial course of therapy is 48 weeks. In addition, pregnancy should be deferred for an additional six months after conclusion of therapy, regardless of which partner is undergoing treatment. Because the drug is believed to cause new mutations, it is recommended to avoid pregnancy during treatment and for the first six months after discontinuation of therapy in either partner.

Hepatitis A Virus

The virus also can be found in semen, and epidemiologic studies indicate that it is sexually transmitted in high-risk groups such as sex workers and homosexual men. Hepatitis A vaccines are available for persons at increased risk of HAV infection, and immune globulin is used to protect against illness associated with HAV infection.

Human T-cell Lymphotropic Viruses I and II

Potential for transmission through ART procedures. Because HTLV-I and -II have several properties in common with HIV, risk reduction protocols devised for HIV-discordant couples that separate infected white blood cells and free virus from sperm (i.e., sperm washing before insemination) could be applied in cases where semen from directed donors infected with HTLV-I and -II will be used to inseminate an uninfected partner.

Human Papilloma Viruses

Genital HPV infections are transmitted primarily through sexual contact, and 50% of sexually active adults have been infected with one or more HPV type. HPV is detected frequently in semen and urethral swabs from normal men. Because these viruses are so prevalent, a coherent strategy for donor screening and risk reduction has not been developed. Since HPV appears in semen as cell-free virus and in infected epithelial cells, sperm-wash protocols may reduce the infectiousness of semen from HPV-infected men. A quadrivalent vaccine for HPV is effective when administered to young teenage women[20].

Herpes Viruses

Herpes viruses have been detected in semen, sexual contact is a significant mode of transmission for HSV-2, CMV, and HHV-8.

HSV-2 has been transmitted via IUI and can cause serious complications if the fetus becomes infected during maternal viremia[21]. To reduce the risk of HSV-2 transmission, semen collection should be avoided when a lesion is present, and infected male partners may be treated with a nucleoside analog against HSV-2 (i.e., acyclovir or valacyclovir) to reduce HSV-2 shedding[22]. Sperm-wash protocols also may be effective because HSV-2 normally appears in semen as free viral particles. Women infected with HSV-2 are treated with acyclovir during pregnancy to reduce the risk of vertical virus transmission. Acyclovir is a category B drug during pregnancy.

Semen donors are screened for CMV because the virus has been transmitted via IUI and primary infection during early pregnancy may have serious complications in the fetus and neonate. Because CMV is so common, insemination with semen from a CMV-infected man is permissible when the female partner is also CMV seropositive. Although the practice is not entirely without risk, because there are many strains of CMV and super infection is possible, the associated risk of newborn CMV infection is approximately 1% and such infants appear to have no significant illness or other abnormality.

Summary

The past two decades of research have produced sophisticated screening tools, new antiviral drugs and vaccines, and an intellectual framework for development of strategies aimed at reducing the risk for transmission of several pathogenic human viruses through artificial insemination and ART procedures. The field is evolving rapidly. It must be emphasized that although the risk-reduction strategies outlined are based on sound scientific and clinical principles, their efficacy has not been tested conclusively. Extensive discussion with patients on scientific evidence and options for risk reduction provides a strong basis for decision making and informed consent.

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Endometriosis-Infertility and IUI

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Introduction

Endometriosis, disease of the millennium, is a sticky, perplexing & unconquerable pathology. Amongst infertile women the prevalence is estimated to range between 30% and 50%. The exact nature of the relationship between endometriosis and infertility remains uncertain.

The controversy regarding whether endometriosis is a cause of infertility or an incidental finding is ongoing. The monthly fecundity rate (MFR) for normal couples of reproductive ages typically range around 30% for the first three cycles. However the MFR in couples diagnosed with both endometriosis & infertility is 2~10% [1]. Even minimal endometriosis can be associated with marked infertility. In an observational IVF study using natural cycles, the follicular phase was significantly longer and the fertilization rate lower in patients with minimal to mild endometriosis compared with women with tubal factor and unexplained infertility. Women with endometriosis were noted to have a slower follicular growth rate and reduced dominant follicle size, poor oocyte quality [2] & altered immunologic functions compared with women with unexplained infertility. It was found that pregnancy associated plasma protein (PAPP-A) is elevated in patients with endometriosis; aromatase activity takes place excessively in endometriotic tissue hence worsening the condition.

In addition sperm motility and sperm penetration is affected.

Fertilization rates are significantly reduced i.e. 33% as compared to 63% in unexplained infertility. Few embryos reach 4-cell stage at 48 hours, a reduced number of blastomeres at 72 hours, lower cleavage rates & less blastocsyt formation hamper the ART process in these patients.

Endometriosis associated impairment of implantation results from a compromise to the potential of the oocyte or early embryo and not the endometrium itself [3,4].

Surgical treatment of moderate and severe endometriosis improves the monthly fecundity rate. Surgical ablation of minimal and mild endometriosis seems superior to expectant management. Infertile patients with minimal and mild endometriosis can benefit from using clomiphene citrate and intrauterine insemination (IUI) or gonadotropins and IUI.

Role of IUI

Intra-uterine insemination (IUI) is a treatment that involves artificially injecting the partner's or a donor's sperm into the woman's uterus. Endometriosis~infertility & IUI involve controlled ovarian hyperstimulation, by clomiphene citrate, Letrozole or Gonadotropins & adjuvants. Apart from Endometriosis with different severity, if there is also a male factor then IUI, IVF or more likely ICSI is important as there are fertilization failures more in Endometriosis.

Success rates

The pregnancy rates achieved by women with minimal & mild endometriosis who undergo intra-uterine insemination with their partner's or a donor's sperm are lower than those of women without fertility problems. One study found that the success rate of insemination in women with endometriosis is about half of that of other women.
However, women with minimal mild endometriosis who undergo intra-uterine insemination and controlled ovarian hyperstimulation are more likely to conceive than those who try conceiving without such help. Furthermore, stimulating the ovaries with artificial follicle stimulating hormone results in higher pregnancy rates than stimulating them with clomiphene citrate

How many cycles of COH-IUI?

Research indicates that if the patient has not conceived after 3-4 intra-uterine insemination cycles, she is not likely to conceive. IVF~ ICSI is suggested, specially in late marriage & advanced age >37 years. A single trial that compares FSH plus IUI versus timed intercourse alone for unexplained subfertility, demonstrated a five-fold increase in cycle fecundity from 2 to 10% (Zikopoulos et al., 1993).

A related trial has made the same comparison in unexplained infertility associated with mild endometriosis.(Fig.1~4) Again, cycle fecundity was increased five-fold, from 2 to 11% (odds ratio 5.6, 95% CI 1.8, 17.4) [5].

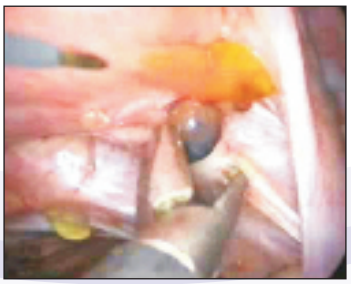

Fig. 1 Classical Pigmented Lesion

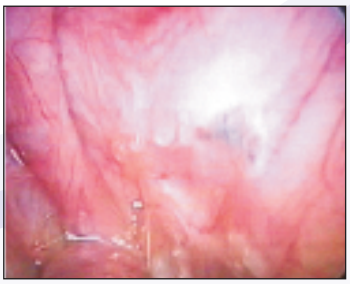

Fig. 2 Older Lesion brown

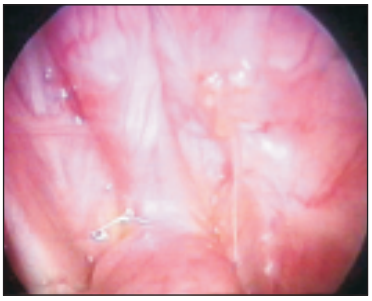

Fig 3 Yellowish Lesion

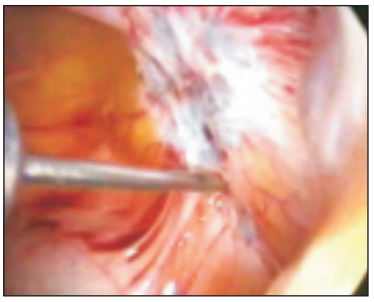

Fig 4 Deep fibrotic Lesion

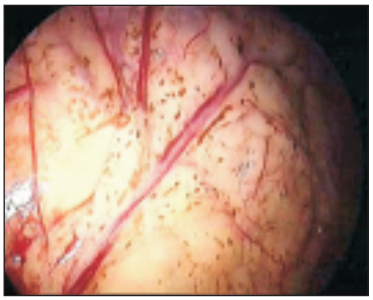

Fig 5 Scattered on omentum

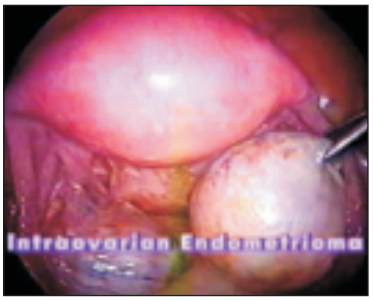

Fig 6 Intraovarian Endometrioma

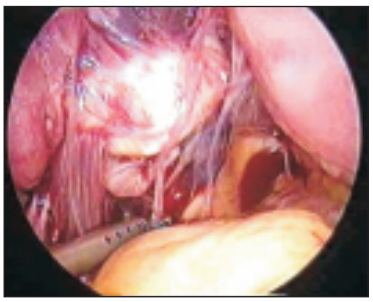

Fig. 7 Peritubal & ovarian adhesions in a case of severe Endometriosis

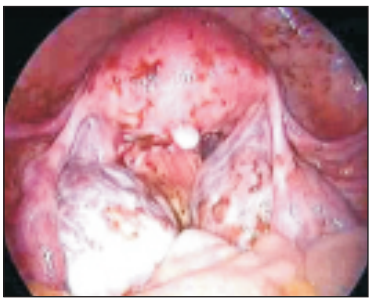

Fig. 8 Extensive bilateral Endometrioma

Superovulation with IUI seems effective in the treatment of unexplained infertility. However, evidence supporting this approach in patients with minimal or mild endometriosis is still limited. Omland et al. [6,7] conducted a prospective cohort study comparing 119 couples with unexplained infertility and 49 whose only anomaly was untreated stage I/II endometriosis at diagnostic laparoscopy, undergoing ovarian stimulation with AIH. No difference was observed as to stimulation regimen, dose of gonadotropin, estradiol concentration, days of stimulation and number of follicles prior to insemination, and semen characteristics. The pregnancy rate was significantly higher for the unexplained infertility group, being 33.3% compared with 16.3% for the endometriosis group.

Furthermore, an increased number of multiple gestations were obtained in the former group, resulting in a significantly higher implantation rate per cycle. The authors speculate that the observed difference reflects a difference in the etiology and pathogenesis of unexplained and endometriosis-associated infertility, and suggest that ovarian stimulation with IUI is indicated in the former situation, whereas in the latter the couples could be better off undergoing IVF-ET without undue delay.

Double IUI offers no clear benefit in the overall clinical pregnancy rate in couples with unexplained infertility [8] and endometriosis.

RCOG guidelines

Grade A recommendation

In cases of mild endometriosis, ovarian stimulation with IUI is more effective than no treatment or IUI alone in Endometriosis-associated infertility.

Hormonal therapy for ovulation suppression cannot be recommended as a standard therapy for endometriosis-associated infertility.

Grade B recommendation

Surgical treatment may improve fertility but controlled studies and comparisons with Assisted reproduction techniques are required (B).

ESHRE guidelines

For women presenting with symptoms suggestive of endometriosis, a definitive diagnosis of most forms of endometriosis requires visual inspection of the pelvis at laparoscopy as the 'gold standard' investigation.

In minimal-mild endometriosis, suppression of ovarian function to improve fertility is not effective, but ablation of endometriotic lesions plus adhesiolysis is effective compared to diagnostic laparoscopy alone. There is insufficient evidence available to determine whether surgical excision of moderate-severe endometriosis (Fig.6~8) enhances pregnancy rates. IVF is appropriate treatment especially if there are coexisting causes of infertility and/or other treatments have failed, but IVF pregnancy rates are lower in women with endometriosis than in those with tubal infertility.

ASRM guidelines

If the endometriosis is minimal or mild (based on the ASRM classification), there is no significant ovulatory abnormality, there is no male factor, the duration of the infertility is less than a year or two, and the woman is under 30 years of age, waiting 3-6 months to see if a spontaneous conception will occur is an option. However, more aggressive therapy using Super-Ovulation combined with IUI will significantly increase the woman's chances of becoming pregnant[9,10]

If the endometriosis is minimal or mild and the duration of the infertility is more than two years, the treatment of choice is Superovulation with Intra-Uterine Insemination. Gonadotropin therapy is the best treatment. If the endometriosis is low in the moderate range, a surgical laparoscopy followed by the institution of Superovulation therapy with IUI, is the treatment of choice.

If the endometriosis is high in the moderate range (according to the ASRM classification), a surgical laparoscopy followed by 6 months of GnRH followed by Superovulation with IUI is the best approach.

If the endometriosis is severe, a laser laparoscopy will be performed. Following this several months of GnRH depo suppression is instituted followed by a second look laparoscopy. After the second look laparoscopy, the GnRH suppression is maintained until the woman has been on the drug for a total of six months. Following this, Superovulation with IUI is instituted

If the Endometriosis is very severe (ASRM score > 70), the disease is frequently too extensive to safely and (more importantly) appropriately treat by laparoscopy. In such instances, after the initial laparoscopy, which assesses the severity of the disease, the woman should go on GnRH suppression for 2-3 months, followed by surgery, and followed by a second look laparoscopy. The GnRH is maintained for a total of 6 months

Women with early Endometriosis therefore fall into the same category as couples with "unexplained infertility" or "Minimal Abnormality Infertility". In such couples, COH / IUI has been definitely shown to significantly improve pregnancy rates.

In women with more advanced Endometriosis, there is no sense wasting time to see if she could conceive on her own. She will be most "fertile" immediately after she has completed her surgical therapy. Instituting COH / IUI/ IVF immediately will maximize that woman's chances of conceiving as quickly as possible.

Once a woman has completed whatever surgical therapies are appropriate followed by 6-9 good cycles of COH / IUI and has not conceived, then she has to go for In-Vitro Fertilization (IVF). This approach thus allows the maximum gain in the shortest period of time without wasting a lot of effort and money on diagnostic work-ups or treatment programs of dubious value and efficacy.

Discussion

In our opinion, in minimal to mild endometriosis the treatment is debateable. But if it is seen at laparoscopy, then fulguration of endometriosis is usually done at our centre. Later, COH-IUI is a better option than no treatment followed by dydrogesterone[12] for luteal support for 10 days from 15 to 25. Pregnancy rates at our centre are17-18% with this treatment.

In moderate to severe endometriosis, operative laparoscopy should be followed by dydogesterone 10mg twice a day from day 5 to day 25 & COH- IUI should be done if the tubes are patent as soon after surgery as it is the best period to conceive immunologically. After this, our pregnancy rates with IUI in<35yrs age are 20-22% and above 35 they are 12-14%.Same group IVF results are 29% in women <35 yrs and 23% above 35yrs.

In extensive endometriosis, operative laparoscopy and suppression with GnRH agonists should be followed by active fertility treatment & early IVF-ICSI.

In our experience, dydrogesterone after laparoscopic surgery increased the pregnancy rate to 58% and progestational support in HMG stimulated IUI / IVF cycles improved the pregnancy rate to 33%.

To summarise, treatment of endometriosis related infertility should be based on RCOG, ASRM and ESHRE guidelines. Also, surgeon's skills of operative laparoscopy, facilities for IUI and IVF-ICSI should be taken into consideration.

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PCOS and IUI

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Polycystic Ovarian Syndrome has evolved from a gynaecological curiosity to a multisystem endocrinopathy. It is probably the most common endocrine disorder in women, accounting for the majority of cases of hirsuitism, menstrual disturbances and anovulatory infertility. It is also one of the most poorly defined endocrinological conditions with a complex pathophysiology that has produced considerable scientific debate.

The journey of PCO women from adolescence to menopause is full of dilemma and emotional turmoil. Of these the most difficult period is that when this young, plump lady is trying desperately for conception. She may be suffering from oligo/ammenorhoea and thus unable to plan her relations for conception. She may be overweight or obese and unable to lose weight despite efforts due to the disturbed endocrinological environment. What may take further toll is the hyperandrogenic environment, which may not only give her difficulty in conception, but also cosmetic problems of hirsuitism and acne. The situation may be worse if this young lady has other infertility factors like male factor, etc further compounding her infertility problem.

The prevalence of PCO in Indian subcontinent Asian Women aged 18-40 years is 52%. Ethnic background of women with PCOS may affect the clinical, hormonal and metabolic characteristics of this condition.

Although the exact definition of PCO/PCOS has had different parameters, following a Consensus Conference held in Rotterdam in 2003, an internationally accepted definition has been adopted by the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine,[1]. Two of the following 3 criteria were sufficient to diagnose the syndrome : oligo or anovulation, clinical and / or biochemical signs of hyperandrogenism , and polycystic ovaries. The ultrasound diagnostic criteria rest on the observation of more than 12 discrete follicles of <10 mm, usually peripherally arranged around an enlarged hyperechogenic central stroma, in atleast one ovary, or increased ovarian volume (>10 cc), at either transabdominal or transvaginal ultrasound.[2]

There is substantial heterogeneity of symptoms and signs among women with PCOS. Ultrasound assessment of ovarian morphology is considered to be essential and the gold standard for defining polycystic ovaries.[3-4] Increased ovarian stromal blood flow in PCOS patients, especially in normal weight PCOS women, may lead to a greater delivery of gonadotropins to the granulosa

cells of the developing follicle. Therefore, we should incorporate the assessment of the ovarian stromal blood flow in the management of PCOS women undergoing ovulation induction in order to reduce the associated risk of OHSS.[5]

Alteration of the environmental components of this condition is fundamental to the management of the condition and pharmaceutical treatment should only be used after adequate counseling and action relating to lifestyle alteration. Attention to weight loss, altered diet, and exercise are the most important aspects to discuss with the patient as well as stopping smoking and improving psychological attitudes.

After weight loss, ovulatory dysfunction is treated initially by ovulation induction and timed sexual intercourse. However, success rate of ovulation induction and timed intercourse is quite low (5-8%), IUI with controlled ovarian stimulation gives a higher success rate up to 15-20%.

Clomiphene citrate was usually used as the first line drug to induce ovulation in women with PCOS. Successful ovulation is achieved in approximately 70-85% of women and 40-50% will conceive (The ESHRE Capri Workshop 1997)[6].Aromatase inhibitors such as letrozole are now replacing CC for ovulation induction with their many advantages like monfollicular growth, lack antiestrogenic action on endometrial lining and cervical mucus and proven safety .However, a subgroup of patients may be resistant to these oral ovulogens. Takahashi et al 1994 revealed that enlarged ovarian volume (>6.2 ml) were the most prominent transvaginal ultrasound findings associated with non-responsiveness to CC.[7]

Insulin resistance and compensatory hyperinsulinism are prominent features of PCOS. Increased insulin concentration also leads to hyperandrogenism by increasing ovarian androgen production and decreasing SHBG concentration, Metformin, an insulin sensitizing agent has been extensively used to induce ovulation in PCOS women either as a first line agent or along with oral ovulogens or in CC resistant cases. A metanlaysis[8] showed that metformin is effective in achieving ovulation in women with PCOS, with odds ratio of 3.88(95%) confidence interval for metformin compared with placebo and 4.41 for metformin and CC compared with CC alone.

Many herbal medicines like D-chiroinositol which reduce the insulin resistance and green tea extracts (epigallocatechin gallate) which exert beneficial effects in thermogenesis, glucose and lipid metabolism as well as the hormonal system, which are all very relevant in management of women with PCOS.

Some novel therapies like extended letrozole (2.5 mg from day 1-10) therapy for ovulation induction in CC resistant women with PCO has shown to produce more mature follicles and subsequently more pregnancies than the short letrozole group[9] .

Gonadotropin treatment can be offered when these anovulatory women fail to respond to oral ovulogens.While using gonadotropins, one has to keep in mind the risk of OHSS and multiple pregnancy. Hence, different protocols of administering gonadotropins such as fixed dose, low dose step up, and step down regimens have been developed.Andoh et al (1998) [10] concluded that the low dose step up regimen of gonadotropin for patient with PCOS may be the safest protocol for IUI as our aim is to develop one or two excellent follicles and not to blast the ovary with many follicles. Since PCOS patients already have higher levels of LH in circulation, recombinant or highly purified FSH is preferable to human menopausal gonadotrpins, though the use of menopausal gonadotropins may be more economical for IUI cycles.

The combination of GnRh antagonist with low dose gonadotropin is useful in women with high serum concentration of LH, who have repeated premature leutinisation, stubbornly do not conceive with gonadotropin alone, or who have conceived and had early miscarriage on more than one occasion. We recommend the use of antagonist 0.25 mg daily after the follicle reaches the size of 14-15mm.

We recommend the use of antagonist whenever there are more than 2 follicles to avoid premature LH surge. Antagonist is started when the follicle size is >13 mm and given daily till the day of HCG.

If the lady fails to conceive despite 4-6 cycles of IUI (ie their infertility remains unexplained), these women may be referred for IVF.

Conclusions:

1 IUI in women with PCOS helps enhance success rate after ovulation induction or may be indicated for any coexisting infertility factor such as mild to moderate male factor infertility.

2 Ovulation induction in women with PCOS is a delicate balance between poor response and hyper-response.

3 A multidisciplinary treatment approach including counseling, weight reduction, lowering LH and hyperandrogenism is beneficial in these cases.

4 A sound scientific knowledge and patient involvement on part of the physician and patience and compliance on part of the patient is needed to conquer the battle of infertility in a PCOS lady.

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IUI in Male Subfertility

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Introduction

Male factor infertility is responsible for nearly 40% of all cases of infertility. Intrauterine insemination (IUI) remains a widely used treatment option for many couples with unexplained infertility, cervical factor subfertility, physiological or psychological sexual dysfunction, mild endometriosis,and mild-to-moderate male subfertility.

Despite the extensive literature on IUI, controversy remains about the effectiveness of this very popular treatment procedure, particularly in relation to in vitro fertilization (IVF) and intracytoplasmic sperm injection(ICSI) [1].

To investigate the real value of IUI in male subfertility cases, this treatment option has to be weighed against

1. Expectant management,
2. Medical and surgical treatment,
3. Timed coitus,
4. IVF,
5. Intracytoplasmic Sperm Injection ICSI.

This comparison should involve 1.success rates, 2.cost-benefit analysis, 3.an analysis of the complication rates of the different treatment options, 4.the invasiveness of the techniques, and 5.couple compliance.

Success Rate

Contradictory results with IUI are observed because most studies are retrospective and pregnancy rates of between 10 and 18% per cycle are reported [2,3,4].

Keck C etal [5] reported a 6% pregnancy rate per cycle and 20% per couple with IUI done due to male factor infertility or unexplained infertility. Lowest values at which pregnancies were achieved were 0.8 mill sperm / ml and 11% motility after sperm processing and 8% normal morphology before semen preparation.

Results from 11 studies showed that the overall pregnancy rate with IUI in male factor subfertility was 4.8% compared to 11.6% in the unexplained infertility group. [6]

There are studies which show that for couples with mild male factor subfertility, primary offer of IVF is less expensive than IUI with its low success rate, followed by IVF. [7]

Minimal Semen requirements for IUI

A minimum of 5 milion motile sperms should be inseminated when the morphology of sperms after preparation is < 30%. When the number of motile spermatozoa inseminated (NMSI) is less than 1 million, the pregnancy rate is significantly lower (3.1%) than if the (NMSI) is > 2 million.

Pregnancy rates did not differ according to NMSI if the percentage of normal sperm after preparation was > 30% or according to percentage of normal sperm when the NMSI was > 5 million. [8]

Ovarian Hyperstimulation & IUI

The controlled ovarian stimulation use or nonuse is another important confounding factor, significantly influencing the success rate of IUI.

The meta-analyses analyzed by Cohlen et al. show the benefit of IUI with mild ovarian hyperstimulation (MOH) versus timed intercourse with MOH in couples with male subfertility and unexplained subfertility.

On the other hand, no difference in success rates could be observed in IUI with or without MOH, if a moderate or severe male factor was involved.

Published data comparing the cost of IVF versus IUI are scarce, but recent studies from The Netherlands, the United Kingdom, and the United States indicate that initiating treatment with IUI appeared to be more cost-effective than IVF in most cases of unexplained and moderate male subfertility [9,10,11]

Despite evidence-based arguments, most infertility centers abroad are still neglecting the possibility of using IUI as a first-line treatment.

Data from Australia and New Zealand clearly show that almost 80% of the centers are convinced of the cost-effectiveness of IUI, but nearly a third of the centers still promote IVF as a first-line treatment, even with patent tubes and normal semen. In case of male subfertility, IUI is rarely considered a first-line option [12]

Although IUI is a simple, noninvasive, and cheap first line treatment in most subfertility cases, the future of IUI will depend on our ability to maintain the multiple pregnancy rate at an acceptable level with avoidance of OHSS, & this will undoubtedly be the most important challenge in the near future.

Conclusion

It is clear that if the rate of multiple pregnancies & OHSS after IUI is comparable or lower than that after IVF, IUI with or without ovarian hyperstimulation should be used for most subfertile couples with mild male subfertility.

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Improving Success in IUI

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Introduction

Intrauterine insemination (IUI) is frequently used in the treatment of infertile couples with various causes of infertility including cervical factors, ovulatory dysfunction, endometriosis, immunological causes, male factors and unexplained infertility. It is also the mode of treatment for various ejaculatory and coital problems.

IUI is generally considered to be an intermediate step of low to moderate complexity before the application of more sophisticated assisted reproductive technologies (ART) such as IVF with or without ICSI [1] Although IUI is widely used; its effectiveness remains a matter of debate. IUI is less invasive and less expensive than IVF or GIFT; it should only be applied if the probability of conception is improved significantly as compared to natural chances of conceiving.

The overall success of IUI remains controversial and depends on several factors with published rates ranging from as low as 5% to as high as 70% per patient; however a 10-20% clinical pregnancy per cycle is an acceptable range for all etiologies [2] .IUI may be performed in natural cycles as well as in conjunction with controlled ovarian hyperstimulation (COH). When combined with COH in unexplained infertility, cumulative pregnancy rates may approach those of ART [3] .

Review of IUI:

In this review, daily dilemmas that the physician confronts in the clinical setting are addressed. The objectives were to (i) compare the success rate of IUI with that of timed intercourse (TI) and intracervical insemination (ICI); (ii) compare the success rate of IUI according to the protocols; (iii) review methods used to time the IUI with ovulation; (iv)identify factors to determine IUI outcome;(v) review risk for multiple pregnancies as a major risk of IUI.

In a postal survey conducted to determine the attitudes of ART consultants to the factors influencing IUI practice, it was found that the factor with the highest rating was the number of follicles on the day of hCG injection followed by the size of the follicle and the total sperm count. [4] .

IUI versus TI/ICI:

It has been well documented that IUI is superior to TI in couples with male sub fertility.[5] In Natural cycles six randomized trials indicated that IUI significantly improved probability of conception compared to TI with an OR of 2.5(95%CI of 1.6-3.9).[6] In COH cycles seven randomized trials showed improvement in pregnancy in cycles with IUI with an OR of 2.2 (95% CI 1.4-3.6) .[7]

Natural cycle versus ovarian stimulation in conjunction with IUI:

In general clomiphene citrate (CC) and /or gonadotrophins are used for COH in conjunction with IUI. For male sub fertility, COH obtained by CC does not seem to increase the efficiency of IUI.[8] When gonadotrophins were used for COH combined with IUI, the pregnancy was increased with an OR 2.0(95% CI 1.1-3.8) as compared to IUI only.[5]

Timing /Induction of ovulation, frequency of insemination:

Timing of ovulation appears to be one of the crucial factors to determine the success of IUI therapy. It is the major goal of treatment to provide sperms that are capable of fertilizing the oocyte at the site of fertilization during a narrow window, the so called peri ovulatory period. Various strategies have been developed to achieve this goal. Urinary LH peak monitoring, hCG injection to stimulate ovulation and scheduling IUI with different frequencies at different time points are some of the strategies. hCG is a well documented and accurate means of triggering ovulation by the time of optimal follicle maturation. However it does not have the superiority against spontaneous ovulation detected by urinary LH detection kits. Its main advantage is to give the physician a better control in the management of the cycle.

The most prospective randomized study of 449 COH/IUI cycles with CC and gonadotrophins indicated an increased cycle fecundity for double insemination performed 12 and 34 hrs after hCG administration as compared with both single at 34 hrs and double at 34 and 60 hrs after hCG.[9] Various other studies have shown similar results with probability of pregnancy OR 2.3 (95% CI 1.4-3.9). However further randomized controlled trials with better design are needed to confirm this findings.

Prediction of Pregnancy:

Factors related to the couple:

Of the several parameters related to the couple, the most important is the duration of infertility. Logistic regression analysis has shown 10% conception rate per cycle if the duration of fertility exceeded 6 years. For a shorter duration the conception rate was > 20%.

Female parameters:

It is a difficult task to isolate the influence of female factors on IUI outcome. However absence of history of any pelvic corrective surgery was one of the factors directly associated with a successful outcome.

A recent retrospective study identified unexplained infertility and anovulation as favorable factors to predict the likelihood of pregnancy as compared with other aetiological factors. A similar study revealed negative impact of the diagnoses of endometriosis or tubal factors on IUI outcomes. [10]

Other significant female factors that are associated with positive IUI outcomes are age, number of pre-ovulatory follicles, endometrial thickness by the time of ovulation, as well as indicators of vascular compliance in ovarian, uterine and spiral arteries. The age of the woman is a well known indirect indicator of the oocyte quality, a consensus that was reached as a result of several reports of ART.

Male parameters:

Since there is lack of standardization of semen analysis it is very difficult to determine IUI outcomes related to male parameters. The presence of severe male factor infertility is an indication to proceed to ART rather than IUI. Although other factors have been proposed, a combination of post semen preparation sperm concentration and motility seems to be a major predictive factor for success. Values of < 2 million post wash insemination resulted in the poorest outcome. [11] As reported by a logistic regression analysis of 2473 cycles, pregnancy rates of 5.3% and 12.8% with post wash insemination of < 5 million and > 5 million respectively were observed.

TABLE A

Post wash insemination /ml	Pregnancy Rate /cycle
< 5 million	5.55%
> 5 million	24.28%

Age of the female partner	post wash insemination / ml	Pregnancy rate/cycle
< 25 years	> 5 million	28.2%
> 35 years	< 5 million	no pregnancy
> 35 years	> 5 million	0.84%

Evidently morphology of sperm assessed by strict criteria of Kruger is one of the best predictors of IVF, its role in IUI is debatable. In a recent publication [12] Badawy et al have commented that IUI used for treating male factor infertility has little chance of success when the woman is older than 35 years, the number of motile spermatozoa inseminated is < 5 million or normal sperm morphology is <30%. (Table A) More robust studies are needed to establish role of sperm morphology in predicting IUI outcome.

Several other parameters related to sperm are under investigations. So far motion characteristics evaluated by computer assisted sperm analysis (CASA) have not indicated a consistent prognostic value. With the advent of different evaluation methods several parameters related to energy metabolism, membrane characteristics, nuclear maturity will need to be determined. More data is needed to examine predictive value of available sperm functional assays ie sperm zona binding tests and induced acrosome reaction testing on IUI outcome. [13]

Sperm processing Methods:

There is no consensus on the use of sperm processing methods for IUI. Till recently most centers perform swim up or density gradient centrifugation (DGC). Combined data from two studies have shown a borderline benefit in favor of DGC. However there are no good robust studies to prove the same. Hence it has been suggested that the sperm processing technique be tailored to the individual case.

Unfortunately the use of substances invitro to stimulate sperm function& /or metabolic activities has not yielded expected results. These stimulants are xanthine derivatives like caffeine, pentoxifylline , adenosine derivatives and analogues, kinin enhancing drugs, follicular fluid, & prostaglandins etc.[14] Although use of some of these substances clearly improves sperm functions the success rate in IUI is not improved.

Multiple Preganacy:

Multiple pregnancies impose a less favorable obstetric and perinatal outcome. A rate of 14 -39% has been reported. Major factors identified to predict multiple pregnancy outcomes include peak estradiol levels & number of pre-ovulatory follicles on the day of hCG. Aspiration of supernumerary follicles before IUI has been suggested to decrease the multiple rates without decreasing the overall pregnancy rate; it has not been well accepted as a routine practice. Multi follicular growth is associated with increased pregnancy rates in IUI with COH. Since in cycles with three or four follicles, the multiple pregnancy rate increased without substantial gain in overall pregnancy rate. Hence IUI with COH should not aim for more than 2 follicles. One stimulated follicle should be the goal if safety is the primary concern, whereas two follicles may be acceptable after careful couple counseling. [15] (Table B)

TABLE B

Pregnancy rates Odds ratio (OR)		Multiple pregnancy rates (OR)	
Two follicles v/s monofollicle	1.6	Two follicles v/s monofollicle	1.7
Three follicles v/s monofollicle	2.0	Three follicles v/s monofollicle	2.8
Four follicles v/s monofollicle	2.0	Four follicles v/s monofollicle	2.3

Conclusion:

The treatment of infertility with IUI is a very frequently used approach. In most programs twice as many IUI cycles are performed than ART procedures on a yearly basis. IUI is a very useful and cost effective treatment for some infertility etiologies. Cumulative pregnancy rates by the 4th to 6th cycle are considered as optimal.

Several factors have been proposed to influence the likelihood of pregnancy. Of these duration of infertility, age of the female partner, history of pelvic disease including endometriosis and severe male factor infertility have a negative impact on outcome. .Cervical factor , anovulatory causes and unexplained infertility have a better outcome. The addition of COH to IUI increases the success at the cost of increased expense and risk of multiple pregnancies which is a major drawback. The use of GnRh agonists as adjuvant in gonadotrophin treated cases or GnRH antagonist in CC/gonadotrphin or gonadotrophin alone may be indicated in selected cases to optimize ovarian response. The optimal timing for insemination and adequacy of luteal phase support needs further investigations. Percentage or actual number of motile sperms appears to have an important impact on outcome.

Purification of selected sperm population by new methodology and use of stimulants of defined sperm function will hopefully be added to the clinical armamentarium in the near future. In addition efforts need to be directed towards identification of local molecular regulatory factors within the uterine cavity and the fallopian tubes determine optimum environment for fertilization followed by successful implantation, is likely to play a significant role in determining the success in IUI.

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IUI-what the ICMR Guidelines Say?

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The field of human reproductive technologies illustrates the challenges posed by developments in medical science to social policy, ethics and the law. Arrays of controversial issues include medical, ethical and legal issues.

ICMR GUIDELINES were drafted by a team of 19 experts, selected by the ICMR, New Delhi, in collaboration with National Academy of Medical Sciences, New Delhi. The Panel includes -seven gynaecologists, one lawyer, two members from the Ministry of Health and Family welfare, embryologists and biologists of ICMR.

Code of Practice deals with all aspects of the treatment provided and the research done at registered clinics. Those aspects of the code of practice, related to intrauterine insemination, have been summarized below.

In this Act, **a) "artificial insemination"** has been defined as the procedure of artificially transferring semen into the reproductive system of a woman and includes insemination with the husband's semen or with donor semen;

b)"assisted reproductive technology", with its grammatical variations and cognate expressions, means all techniques that attempt to obtain a pregnancy by handling or manipulating the sperm or the oocyte outside the human body, and transferring the gamete or the embryo into the uterus;

c) "assisted reproductive technology clinic", means any premises used for procedures related to assisted reproductive technology;

Art Clinic-

Clinics offering any treatment, involving the use of gametes which have been donated or collected or processed *in vitro, except for* AIH, and for IUI by level 1A clinics who will not process the gametes themselves should be regulated, registered , supervised by the State Accreditation Authority /State Appropriate Authorities .

Primary (Level 1A) Infertility Clinics

These clinics will not require registration under the Act. They would be clinics where preliminary investigations are carried out and type and cause of infertility diagnosed. Depending on the severity of infertility, the couple could be treated at the Level 1A clinic or referred to a speciality (Level 1B,Level 2 or Level 3) clinic.

Primary (Level 1B) Infertility

Clinics engaging in artificial insemination using husband's semen (AIH), artificial insemination using donor semen (AID) or intrauterine insemination (IUI) using husband's or donor semen. These clinics will require registration under the Act.

The insemination in such clinics must be done under the supervision of a gynaecologist with a post-graduate degree.

Facilities

A) Assessment of follicular growth and ovulation by serial ultrasonography.

B) Hysteroscopy, laparoscopy and ultrasonography.

C) Facilities for semen preparation for IUI, including an appropriate clean room for IUI.

(The facilities for investigation and for semen preparation mentioned above could be shared with another accredited infertility clinic or semen bank or an accredited clinical laboratory.)

Semen Bank

Either an ART clinic or a law firm or suitable independent organization may set up a semen bank. The bank will ensure the requirements of the sperm donor are fulfilled, recorded & maintained for 10 years. A bank may advertise suitably for semen donors who may be appropriately compensated financially.On request for semen by an ART clinic, the bank will provide the clinic with a list of donors giving all relevant details. The semen bank shall not supply semen of one donor for more than ten successful pregnancies. It will be the responsibility of the ART clinic or the patient, to inform the bank about a successful pregnancy. The bank shall keep a record of all semen received, stored and supplied, and details of the use of the semen of each donor, and is liable to be reviewed by the accreditation authority .The bank must be run professionally and must have facilities for cryopreservation of semen, following internationally accepted protocols. Each bank will prepare its own Standard Of Protocol for cryopreservation. Semen must be

cryopreserved for at least six months before first use, at which time the semen donor must be again tested for HIV, hepatitis B & C.

INFORMATION AND COUNSELLING-Patient should be provided information regarding limitations of treatment, the results, side-effects and cost. Pamphlets (one-page on each technique in all local languages and English)which give clear, precise and honest information about the procedure recommended to be used will help the couple make an informed choice. Counselling regarding rights of the child, especially, in case of donor insemination must be done.

CONSENT-Written Informed consent of the couple with regard to sperm freezing and artificial donor insemination.

Desirable practices /prohibited scenarios

Storage and handling of semen -highest possible standards, security, recording & identification should be maintained.

Age of the donor should be between 21 45 years .

The individual must be free of HIV and hepatitis B and C infections, hypertension, diabetes, sexually transmitted diseases, & identifiable and common genetic disorders such as thalassemia .

A semen analysis must be carried out on the semen of the individual, preferably using a semen analyzer, and the semen must be found to be normal according to WHO method

No woman should be inseminated with sperms derived from more than one man in one treatment cycle. Semen from two individuals must never be mixed before use, under any circumstance .

A third party donor must be informed that the offspring will not know his/her identity. ART clinic is responsible to obtain sperm from appropriate banks. Neither the clinic nor the couple shall know the donor identity and address.It will be the responsibility of the semen bank and the clinic to ensure that the couple does not come to know the identity of the donor.

Blood group and Rh status ,height, weight, age, educational qualifications, profession, colour of the skin & the eyes, record of major diseases including any psychiatric disorder, & the family background in respect of hist. of any familial disorder, freedom from any known diseases or carrier status(eg. hepatitis B or AIDS), ethnic origin, & the DNA fingerprint (if possible) of the donor, must be recorded by the semen bank. The ART clinic & the couple, before accepting the donor semen, shall have the right to have the fullest possible information from the semen bank on the donor

The ART clinic will be authorized to appropriately charge the couple for the semen provided and the tests done on the donor semen.

Use of sperm donated by a relative or a known friend of either the wife or the husband shall not be permitted.

Unmarried woman-There would be no bar to the use of artificial insemination by a single women . No ART clinic may refuse to offer its services. The child thus born will have all the legal rights on the woman .The ART clinic must not be a party to any commercial element in donor programmes.

Confidentiality of donor-A semen bank may store a semen preparation for exclusive use on the donor's wife or on any other woman designated by the donor

Legitimacy of the child born through AID - A child born through AID is presumed to be the legitimate child of the couple, born within wedlock, with consent of both the spouses, and with all the attendant rights of parentage, support and inheritance. Sperm donor shall have no parental right or duties in relation to the child, and their anonymity shall be protected .

Adultery in the case of AID-ART used for married woman with the consent of the husband does not amount to adultery on part of the wife or the donor. AID without the husband's consent can, however, be a ground for divorce or judicial separation. AIH does not necessarily amount to consummation of marriage and a decree of nullity may still be granted in favor of the wife on the ground of impotency of the husband or his willful refusal to consummate the marriage

Posthumous AIH through a sperm bank-A child born to a woman artificially inseminated with the stored sperms of her deceased husband must be considered to be a legitimate child.

Conclusion

The principle is maximizing good, minimizing harm and justice. The advances in ART in the past 25 years have been remarkable. The exponential development of technology in assisted reproduction has brought new ethical challenges to the forefront. There are numerous ethical issues raised by egg donation, sperm donation, embryo donation and surrogacy. There is no total consensus regarding these issues. The ICMR guidelines exist as of now but the law is in the process of being formulated. However, careful screening, counseling and medical care can allow these treatments to be done with focus on the welfare of the child and the couple.

Achieve higher pregnancy rates and lower miscarriage rates

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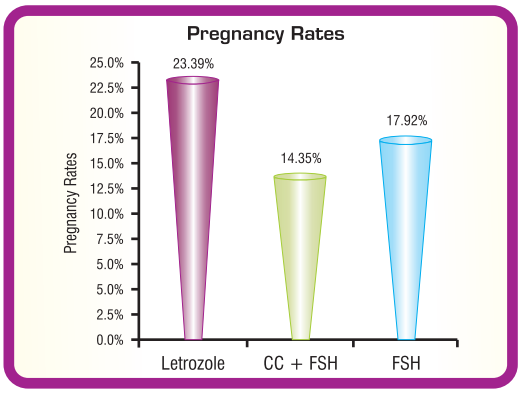
- Ensures successful ovulation as well as implantation
- Better pregnancy rates compared to clomiphene citrate
- Offers higher pregnancy rates[1]



- Lower miscarriage rates[1]

Ref: 1. J Assist Reprod Genet. 2009 Jan; 26(1):19 - 24

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