eXpert

EXcellence in PCOS & Expertise in Reproductive Technology

MODULE 6:
ASSISTED REPRODUCTIVE TECHNOLOGY

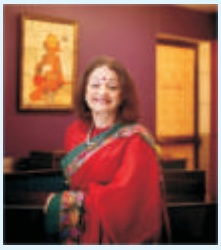
Brought to you by

THE PCOS SOCIETY

An Initiative of PCOS Society (India)

eXpert
EXcellence in PCOS & Expertise in Reproductive Technology

Foreword



Duru Shah
Founder President,
The PCOS Society of India,
Course Director, **EXPERT**

Madhuri Patil
Scientific Co-ordinator,
The PCOS Society of India,
Course Director, **EXPERT**

PCOS is quite often associated with infertility, especially in women with irregular periods and hyperandrogenemia. It is one of the most treatable forms of infertility, if ovulation induction is optimal. Unfortunately, many PCOS women, both lean and obese, behave erratically during ovulation induction sometimes leading to ovarian hyperstimulation syndrome which can become a serious iatrogenic complication. Keeping in mind that many PCOS women approach us for management for their infertility issues, it is important for us to understand the principles of management. Which when applied correctly, they can give us great success in making PCOS women pregnant. Of course experience counts, and as we continue treating women, we learn how to tweak our stimulation protocols to avoid complications and give us the best results!

After initiating the Basic Course on Infertility in 2018, we are delighted to introduce to you the Advanced Course called **"EXPERT"- (Excellence in PCOS and Expertise in Reproductive Technology")** a Certificate Course brought to you by the PCOS Society of India, through an unrestricted educational grant by Sun Pharma, Inca Life Sciences.

"EXPERT" will be presented to you in a set of 6 Modules which will update you on various aspects of the management. Infertility in PCOS, from minimal intervention to Assisted Reproduction.

Once you complete the 6 Modules, you could participate in an Online Exam, Assessment and on clearing it, you will be eligible to receive a beautiful certificate from the PCOS Society of India, which you will be extremely proud to display! To own this Certificate, you need to be a member of the PCOS Society, India!

To become a member, please log on to http://www.pcosindia.org/ to download your form and become a Life Member or Patron Member of the PCOS Society of India

If you have any queries, please write to us at thepcossociety@gmail.com

Both Madhuri and myself have worked hard on creating this program and we thank the team at Sun Pharma for their support in making this program a reality !

Enjoy reading.

With warm regards,

Duru Shah
Founder President, The PCOS Society of India.
Course Director, EXPERT

Madhuri Patil
Scientific Co-ordinator, The PCOS Society of India
Course Director, EXPERT

eXpert
EXcellence in PCOS & Expertise in Reproductive Technology

Contents

ASSISTED REPRODUCTIVE TECHNOLOGY

eXpert
EXcellence in PCOS & Expertise in Reproductive Technology

ASSISTED REPRODUCTIVE TECHNOLOGY

Assisted Reproductive Technology: An Overview

Incidence of Infertility: Global and Indian Data

Infertility can be described as the inability of any individual to conceive despite 2 years of cohabitation and exposure to pregnancy. The International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) define infertility as the "failure to achieve a pregnancy after 12 months or more of regular unprotected sexual intercourse."[1,2]

According to a study conducted by the WHO in 2002, approximately 187 million ever-married women of reproductive age and more than 48.5 million couples (~8%–12%) suffer from infertility worldwide. However, the prevalence rates of infertility vary widely, depending upon the age range of the population, regional variations, units of measurement, and relationship status.[1,2]

Estimates suggest that in developing countries, the overall burden of infertility is over three times higher than that in developed countries. In India, the burden of primary infertility among couples ranges between 4% and 17%. As per estimates from Indian census data for 2001, 1991, and 1981, approximately13% of ever-married women of reproductive age were childless in 1981, which increased to nearly 16% in 2001.[1,2]

Etiology of Infertility

Causes of Male Infertility

- **Abnormal sperm production or function** due to undescended testicles, genetic defects, health issues (such as diabetes), or infections (such as chlamydia, gonorrhea, mumps, or human immunodeficiency virus [HIV]). Enlarged veins in the testes (varicocele) can also affect the quality of sperms.
- **Problems with the delivery of sperms** due to sexual problems, such as premature ejaculation; certain genetic diseases, such as cystic fibrosis; structural problems, such as a blockage in the testicle; or damage or injury to the reproductive organs.
- **Overexposure to certain environmental factors**, such as pesticides and other chemicals, besides radiation. Cigarette smoking, alcohol, marijuana, or taking certain medications, such as select antibiotics, antihypertensives, anabolic steroids, or others, can also affect fertility. Frequent exposure to heat, such as in saunas or hot tubs, can raise the core body temperature and may affect sperm production.
- **Damage related to cancer and its treatment**, including radiation or chemotherapy. Treatment for cancer can impair sperm production, sometimes severely.

Causes of Female Infertility

- **Ovulation disorders:** These include hormonal disorders, such as polycystic ovary syndrome, hyperprolactinemia, and thyroid disorders (hyperthyroidism or hypothyroidism). Other causes may include excessive exercise, eating disorders, injury, or tumors. Table 1 presents the causes for anovulatory infertility.

The causes of hypothalamus–pituitary failure are provided in Table 2.

Table 1: Causes of anovulation

Group I: Hypothalamic/ pituitary failure	Weight loss, systemic illness, Kallmann's syndrome hypogonadotropic hypogonadism	5%
	Hyperprolactinemia, Hypopituitarism	
Group II: h/p dysfunction	PCOS	90%
Group III: Ovarian failure	Premature ovarian failure (POF) Resistant ovary syndrome (ROS)	5%

Table 2: Causes for hypothalamus–pituitary failure

Hypothalamic causes (hypogonadotropic hypogonadism)	• Weight loss • Exercise • Chronic illness • Psychological distress • Idiopathic
Causes of hypothalamic/pituitary damage	• Tumors (e.g., craniopharyngiomas) • Cranial irradiation • Head injuries • Sarcoidosis • Tuberculosis
Systemic causes	• Chronic debilitating illness • Weight loss
Endocrine disorders	Thyroid, Cushing's syndrome?

- **Uterine or cervical abnormalities:** These include congenital anomalies of the uterus, intrauterine pathologies (polyps, submucus fibroids, intrauterine adhesions, and endometritis), and uterine anomalies (intramural fibroids >5 cm, adenomyosis)
- **Fallopian tube damage or blockage:** It is caused by pelvic inflammatory diseases (which are usually caused by tuberculosis and sexually transmitted infection), endometriosis, or adhesions
- **Endometriosis:** Endometriosis can affect the function of the ovaries, uterus, and fallopian tubes
- **Primary ovarian insufficiency:** The cause for primary ovarian insufficiency is often unknown. Certain factors are associated with premature ovarian insufficiency, including immune system diseases; genetic conditions (such as Turner syndrome or carriers of Fragile X syndrome); radiation or chemotherapy treatment; and smoking.
- **Pelvic adhesions:** These are caused due to pelvic infection; appendicitis; or abdominal or pelvic surgery
- **Cancer and its treatment:** Both radiation and chemotherapy may affect ovarian and uterine function, resulting in infertility.

eXpert
EXcellence in PCOS & Expertise in Reproductive Technology

- **Other conditions:** Medical conditions associated with delayed puberty or amenorrhea, such as celiac disease, poorly controlled diabetes, and some autoimmune diseases, such as lupus, can affect a woman's fertility.
- **Genetic abnormalities**

Risk Factors of Infertility

- **Age:** A woman's fertility gradually declines with age, especially in her mid-30s, and it drops rapidly after the age of 37. Infertility in older women may be due to the number and quality of eggs, or due to health-related issues. Men more than 40 years of age may be less fertile compared with younger men and may contribute to higher rates of certain medical conditions, such as psychiatric disorders or certain cancers, in the offspring.
- **Tobacco use:** Smoking tobacco or marijuana by either partner reduces the likelihood of pregnancy. Smoking also reduces the possible benefit of fertility treatment. Miscarriages are more frequent in women who smoke. Smoking can increase the risk of erectile dysfunction and may result in a low sperm count in men.
- **Alcohol use:** For women, there is no safe level of alcohol use during conception or pregnancy. Alcohol consumption by women may contribute to infertility and may increase the risk of birth defects in the infants. For men, excessive alcohol consumption can decrease sperm count and sperm motility.
- **Being overweight:** Inactive lifestyle and being overweight may increase the risk of infertility. A man's sperm count may also be affected due to being overweight.
- **Being underweight:** Eating disorders, such as anorexia or bulimia, and a very low-calorie- or restrictive diet increase the risk of fertility problems in women.
- **Exercise issues:** Insufficient exercise contributes to obesity, which increases the risk of infertility. Although rare, ovulation problems may be associated with frequent strenuous, intense exercise in women who are not overweight.

Factors Determining Selection of ART Treatment Modality

There has been an increased demand for effective assisted reproductive technology (ART) treatment, with the increase in the prevalence of infertility over the past decade. Although ART was initially developed for patients with tubal infertility, it is currently employed for all types of infertility problems, owing to its high success rates. The choice of treatment, however, is influenced by the following factors (Table 3).[3,4]

Absolute Indications for ART

- Bilateral tubal factor infertility
- Advanced-stage endometriosis resulting in tubal disease or dysfunction
- Severe male factor infertility (azoospermia, aspermia, or significant oligoasthenospermia)

Table 3: Factors influencing the choice of ART[3,4]

1. Age of the women
2. Duration of infertility
3. Tuboperitoneal pathology
4. Semen quality and sperm parameters
5. Cervical or immunological factors
6. Etiology of infertility male and female factors
7. Presence of PCOS
8. Presence of endometriosis

- Menopausal states requiring egg donation
- Under circumstances that require pre-implantation genetic screening (PGS) or pre-implantation genetic diagnosis (PGD), to prevent aneuploidy and genetically inherited diseases

Absolute Indications for ICSI

- Surgically retrieved sperm
- Severe oligoasthenoteratozoospermias (OATs)
- Previous complete fertilization failure with *in vitro* fertilization (IVF), where clinical and laboratory problems are ruled out
- Poor post-thaw parameters after sperm-freezing
- Increased sperm DNA fragmentation index (DFI)
- Immotile sperm/non-HA

Conditions for Which IVF is not Required

- **Anovulatory infertility:** Medical management using ovulation-inducing agents.
- **Uterine factor infertility:** Surgical management, such as hysteroscopic polypectomy, metroplasty, myomectomy, and adhesiolysis, shows a significant post-surgical reversal of infertility without the need of further intervention.
- **Coital dysfunction (such as inability to have intercourse):** Placing sperm in the reproductive tract at the time of ovulation should result in normal fecundability.

First-Line vs. Last Resort Uses of IVF

- Unexplained infertility
- Mild male factor infertility
- Endometriosis without tubal disease
- Unilateral tubal blockage
- Diminished ovarian reserve
- Age >40 years with good ovarian reserve

Factors That Will Guide the Choice of Treatment Between Timed Intercourse, IUI, IVF, and ICSI

- Semen parameters (Table 4)
- Other factors that affect the chance to pregnancy
- Special situations

Table 4: Selection of treatment modality depending on semen parameters

Parameter	TI/IUI	IVF	ICSI
Concentration	>10 million/mL	1–10 million/mL	<1 million/mL
Total motile count	>5 million/mL	1–5 million/mL	<1 million/mL
24 hours sperm survival	>70%	<70%	<70%
TZI	<1.6	1.6–1.84	>1.84
HOS	>60%	50–60%	<50%
DFI	<15%	15–30%	>30% >60% TESA/ICSI

Other Factors that Determine the Selection Process

- Presence or absence of cervical factors
- Endometriosis
- Tubal pathology
- Uterine pathology: Congenital anomalies, polyp, submucus myoma, and IUA
- Antisperm antibody in male and female partners
- Pelvic factors: History of previous pelvic or abdominal surgeries
- Age of women: Women above 40 years of age should receive gonadotrophins (GT) for ovulation induction (OI) and should consider IVF early in the treatment protocol.

Special Situations that Need Consideration: Whether IVF Should be the First-line Therapy or the Last Resort?

1. Unexplained infertility
2. Mild factor male infertility
3. Presence of mild-to-moderate endometriosis without tubal involvement
4. Unilateral tubal block
5. Tubal ligation reversal
6. Advanced age and decreased ovarian reserve

Common ART Procedures

ART treatments are offered when the chance of conceiving by other means is unacceptably low or the woman's age leaves insufficient time for other treatments. Table 5 summarizes the most common ART procedures employed worldwide.[4]

Table 5: The most common ART procedures employed worldwide[4]

Type of ART	Procedure
In vitro fertilization (IVF)	A four-stage procedure that includes controlled ovarian hyperstimulation, oocyte retrieval under transvaginal ultrasound guidance, fertilization with sperm *in vitro*, and embryo replacement into the uterus.
Gamete intrafallopian transfer	The oocytes and sperms are injected into the fallopian tube under laparoscopic guidance, and fertilization takes places within the body.
Intracytoplasmic sperm injection	A single sperm is injected directly into the oocyte to facilitate fertilization; at least a few viable sperms must be present in the ejaculate, epididymis, or testis.
Microsurgical epididymal sperm aspiration	For treatment of obstructive azoospermia when no sperms are found in the ejaculate; sperms are directly recovered from the epididymis through needle aspiration.
Testicular sperm extraction	For treatment of obstructive or non-obstructive azoospermia when no sperms are found in the ejaculate or the epididymis; sperms are directly recovered from the testis through open biopsy.
Frozen embryos	Excess embryos can be frozen and stored in liquid nitrogen for future use.
Oocyte donation	The donor is stimulated, and the eggs are extracted and fertilized with the sperm of the recipient's partner; the resultant embryos are then transferred into the recipient's uterus.
Embryo donation	An embryo is formed from donated oocytes and sperms, which is then transferred into the uterus of the recipient.
Sperm donation	Donated sperms are used for conception either for intra-uterine insemination (IUI) or for IOVF or ICSI
Surrogacy	Embryos formed from the use of sperm and oocytes of the commissioning couple are transferred into the uterine cavity of the surrogate who will carry pregnancy to term and, after delivery, will hand over the baby to the commissioning couple.

Summary

- Choosing the best modality of treatment: Despite the extensive literature on the subject, controversy remains about the order of treatment and the effectiveness of stimulated IUI cycles in relation to IVF and ICSI.
- Expectant management remains a respectable option in couples with favorable prognosis, including women with previous unilateral tubal disease.
- Choice between IUI, IVF, ICSI, and other modalities of treatment, such as sperm/oocyte/embryo donation and surrogacy, will be governed by the age of the patient, etiology of infertility, duration of infertility, and the previuos treatment taken.

IUI

IUI using the partner's sperm can be used as a potentially effective treatment for subfertility due to all causes in women under the age of 40 years, except for cases with

- Tubal block
- Severe tubal damage
- Very poor egg quality
- Ovarian failure (menopause)
- Severe male factor infertility

IUI is carried out after sperm-processing, and the aim is to obtain enriched fraction of motile and morphologically normal spermatozoa with better fertilizing ability.

Rationale for Intrauterine Insemination

Intrauterine insemination uses concentration of most motile and morphologically normal mature sperm cells. It increases the density of both eggs and sperm near the site of fertilization, which increases the likelihood of pregnancy.

Indications

Unexplained infertility ???	**Erectile/ejaculatory dysfunction**
Male factor infertility???	**Anatomic defects of penis (hypospadiasis)**
Endometriosis???	**HIV-discordant couple**
Ovulatory dysfunction???	**Cryopreservation of sperm in cases of cancer treatment**
Immunological causes???	
Cervical hostility???	

IUI Outcome is Linked to the Following Factors

High motile sperm count before preparation ($>20^{10/6}$/mL)
High inseminating motile sperm count (IMSC) ≥5 million/mL CPR per couple — TMS count 28.5% — <5 million 44.3% — >5 million
24-hour survival of processed sperm ≥70%
Better sperm morphology (>4%) in unprocessed and processed samples CPR per couple decreased from 40.7% to 21.4% if teratospermia was high
TZI <1.85
HOS>60%
DFI <15%
CPR: Couple protection ratio; HOS: Hypo-osmotic swelling; TMS: Total motile sperm; TZI: Teratozoospermia index.

Sperm Processing

How Does Sperm Preparation Help?

Removal of	Decreases
• Non-motile spermatozoa • Leukocytes and immature forms • Seminal plasma: PG and antigenic proteins responsible for contraction and cramps • Seminal plasma microorganisms might induce an inflammatory process	• Release of lymphokines and/or cytokines • Formation of free oxygen radicals, leading to functional demise of spermatozoa and sperm oocyte interaction

Collection of Sperms for IUI

- By masturbation
- By electroejaculation
- By collection form the culture medium (performed in patients with very low semen volume or those with increased viscosity and delayed liquefaction)

Methods of Sperm-Processing

Selecting the Sperm Preparation Technique According to the Individual Semen Sample is Very Important

1. **Swim-up technique:** Optimal results; normal/near-normal semen
2. **Discontinuous density gradient:** Asthenospermia; one day or less abstinence can improve motility

Selection of Procedure is Individualized Depending on the

- Count
- Motility
- Morphology
- Source of sample
- Debris and other cellular contaminations

Choice of Method

- Swim-up method is performed if the sperm concentration is >20 million/mL with good forward progressive motility.
- Gradient separation method is performed if the samples have large amount of debris, extreme oligozoospermia, and severely compromised motility.
- Samples collected using electroejaculation and gradient separation is used for low motile sperm concentrations.

Methodology

Semen samples were allowed to liquefy for 30 minutes at 37°C. The samples were then examined by placing a drop of undiluted semen on Makler's chamber to assess the count and motility.

eXpert
EXcellence in PCOS & Expertise in Reproductive Technology

Swim-up Technique:

Swim-up can be performed using four different methods:

1. Seminal plasma overlaid directly with culture medium
2. Seminal plasma under-layered below culture

   Both are then followed by:
   - The tube is inclined at a 45-degree angle and incubated for 1 hour at 37° C
   - It is then gently returned to the upright position and the uppermost 1 mL of seminal plasma containing motile sperms is removed.
   - This supernatant is centrifuged twice with sperm washing media.
   - The final pellet is then re-suspended in approximately 0.5 mL of media.
3. Assessment of sperm concentration:
   - The semen sample is diluted and centrifuged for 10 minutes; supernatant removed; pellet resuspended with culture media and centrifuged again for 10 minutes; and supernatant removed.
   - Pellet is loosened and overlaid with the culture medium.
   - The tube is inclined at a 45o angle and incubated for 1 hour at 37° C
   - The uppermost 1 ml containing motile sperms is removed.
4. Assessment of sperm concentration and motility:
   - Semen sample centrifuged without prior dilution, and pellet loosened and overlaid with the medium.
   - The tube is inclined at a 45° angle and incubated for 1 hour at 37° C.
   - It is then gently returned to the upright position and the uppermost 1 mL containing motile sperms is removed.
   - Assessment of sperm concentration and motility.

Disadvantages of the Migration Technique

- Reactive oxygen species produced by sperm pellet has marked deleterious effect
- Damage during centrifugation can impair the fertilizing ability
- Sperms may not reach the interface of the culture medium, thus rendering low yield.
- Sub-optimal sperms from original ejaculate may be included.
- It is not useful for individuals with severe OATs.

Density Gradient Separation

- Pipette 1–3 mL of 90% gradient media into 15-mL sterile conical-bottomed tube.
- Gently overlay with 1–3 mL of 45% gradient media.
- Gently layer 1–2 mL semen over the gradient.
- Balance the tubes and centrifuge at 500 g for 20 minutes.
- Remove the supernatant.
- Re-suspend the pellet at the bottom of the 90% fraction in 4–5 mL of medium and centrifuge at 500 g for 10 minutes.
- Remove the supernatant and re-suspend the pellet in 0.5–0.8 mL medium.
- Estimate the sperm concentration and motility or assess the sperm function.
- Incubated the sample at 37° C in 5% or 6% of CO_2.

Advantages

- Rapid, simple, and provides highly uniform specimen with improved sperm quality
- Significantly eliminates abnormal and immotile sperms; debris; and other seminal constituents
- Reduces the production of reactive oxygen species
- Effective for severe OAT samples

Disadvantages

- Percoll (silica-coating) decreases motility and acrosomal reaction of human sperms. Hence, silane-coated silica of clinical grade is used.
- Selected sperms may have different surface properties, which may interfere with fertilization.
- Gradient material may damage sperms.

Damage to Sperms can occur During the Following Procedures, Which Must be Minimized

- Dilution (minimized by performing dilution slowly)
- Temperature change (minimized by changing the temperature gradually)
- Centrifugation (centrifugal forces should be low)
- Oxidative damage caused by free oxygen radicals released from leukocytes or abnormal sperms (Aitken RJ, Gordon E, Harkiss D; Mortimer D)
- Exposure to potentially toxic materials

An Increasing Success Rate With IUI

Success Rates With IUI are Contingent Upon the Procedure being Performed

1. For correct indication
2. Avoiding performance of IUI when CI exists
3. Whether women are ovulating normally on her own or will require ovulation induction

Factors Affecting the Success of IUI

Couple:

- **Age:** Success of IUI decreases after the age of 35 years. With age, there could be fewer oocytes per cycle, low E2 on the day of hCG, lower implantation rates, increased risk of miscarriage, and increased chance of chromosomal abnormalities.

- **Duration of infertility:** There is a steep decline in the success rate of IUI when the duration of infertility is more than 4 years.
- **Cause of infertility:** Best results of IUI are seen in cases of unexplained infertility, ovulatory dysfunction, and mild male factor infertility. Least success is noted among women with endometriosis.

Therapies:

- **Semen-processing technique:** Choosing the method of sperm wash on the basis of semen parameters will increase the success rate of IUI. A total sperm motile fraction of more than 5 million/mL post wash has a good success rate.
- **Protocol of controlled ovarian hyperstimulation (COH):** Obtaining three dominant follicles on the day of human chorionic gonadotropin (hCG) release increases the success rate with IUI. Using controlled ovarian stimulation (COS) increases the success rate by
  - Increasing the number of oocytes available
  - Increasing the chance of fertilization
  - Increasing the steroid production
  - Increasing the chance of implantation
  - Correct subtle ovulatory disorders, such as luteinized unruptured follicle syndrome
  - Determining an exact time to ovulation and insemination
- Luteal-phase support in IUI is absolutely necessary when COS is used for ovulation induction with or without GnRH analogs. It is used empirically in most other cycles (IUI in natural cycle or cycles in which oral ovulogens are used).

Moreover, stringent monitoring of IUI cycles by transvaginal sonography is essential to detect the time of ovulation.

- Timing of insemination: Viable sperms should be present at the time of ovulation. This is essential as IUI bypasses the cervix, which acts as the reservoir for sperms at mid-cycle to be released for 48–72 hours.

  Ovulation can be detected by:

  - Serum or urinary LH: IUI 24 hours later
  - TVS: Leading follicle >18–20 mm, hCG 5000 IU/Rec hCG: 250 mcg SC/GnRha 1 mg IUI 36–42 hours later

The insemination volume can vary from 0.3 to 0.8 mL. Moreover, it has to be performed immediately after loading the syringe. Wait for 15–20 minutes between loading the syringe and placing the processed sperms into the uterus results in significant loss of sperm motility, thus compromising the results with IUI.

- Catheter type: Open-ended/rounded tip teflon catheters are least traumatic and most efficient. An easy and traumatic transfer is essential for successful IUI.
- Bed rest: A 10-minute bed rest after IUI has a positive effect on pregnancy rates.
- Total number of cycles of IUI: A dramatic fall in PR after four cycles was noted; therefore, continued IUI after four cycles is not recommended.

PR per Cycle and NNT per Cycle

Treatment	PR per cycle	NNT
IUI	5	32
COS+IUI	12	11

Maintenance of the Equipment's Working Environment in the Lab is Very Important for Optimal IUI Results

- Daily, weekly, and yearly lab cleaning should be performed and schedule formulated for proper working should be maintained.
- The lab should be ready for any equipment failures.
- UPS for CO_2 incubator and back-up generator for other equipments should be used.
- Regular microbiological screening of incubator water, media, and lab surfaces should be done to ensure continued absence of pathogenic microorganisms.

Summary

- IUI is relatively simple, non-invasive, cheap, and easily repeatable.
- Careful selection of patients is important.
- IUI and endometriosis IUI with COS increase the live birth rate but at the cost of risk of OHSS and multiple pregnancies.
- Stimulated IUI is ineffective for male infertility.
- OI with GT and IUI treatments yield better PRs than CC but have a higher incidence of OHSS and multiple pregnancies, averaging to 13%.
- Total motile sperm concentration is the most important parameter predicting pregnancy.
- With an increase in abnormal forms, the success rate of IUI falls significantly.
- Although IUI may take relatively more treatment cycles to achieve pregnancy, there are considerable advantages in terms of risk/benefit ratio and financial cost as compared with ART.
- End point of IUI is still a dilemma.
- Failure of four to six trials of GT stimulated IUI in unexplained or mild male infertility, and mild-to-moderate endometriosis is an indication for IVF.

ART—Oocyte Retrieval and Embryo Transfer

Oocyte Retrieval

Laparoscopic oocyte retrieval is an invasive procedure that was used in earlier days. Today, what we perform is transvaginal follicle puncture under USG control, which has become the method of choice worldwide, as it is less invasive and is associated with less complications.

Transvaginal Oocyte Retrieval

- The vagina was cleaned with an isotonic saline solution and covered with a sterile surgical sheet.
- The gynecologist wore sterile surgical gloves.
- The ultrasound transducer was covered with a sterile plastic sheet.
- The needle guide is attached to the transducer over the sterile cover to guide the insertion of the aspirating needle.
- Oocyte aspiration is performed with a 17–20-G single-lumen needle or a double-lumen needle (Figure 1).

Figure 1A: Single-lumen needle.

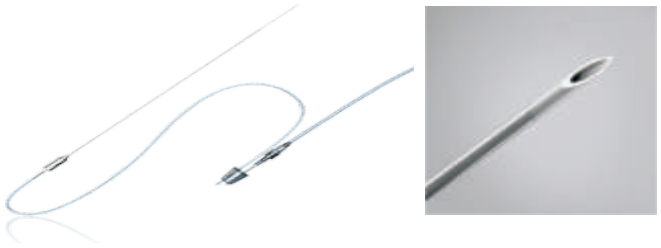
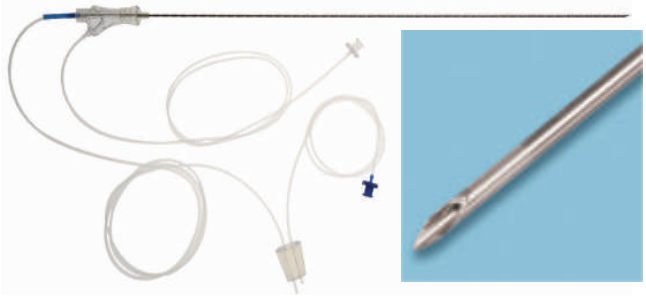


Figure 1B: Double-lumen needle.



When a double-lumen needle is used, flushing media is introduced through the flushing channel during aspiration, which increases the turbulence within the follicle that assists in dislodging the cumulus oocyte complex from the follicular wall, thus increasing the chance of oocyte recovery. This procedure can help women with poor ovarian reserve or response. However, evidence has not shown any significant difference in the number of oocytes retrieved, fertilization rate, and pregnancy rate when follicles were flushed. On the contrary, there was a significant shortening of operating and aspiration time in the aspiration-only group where a single-lumen needle was used (Gary Levy *et al*. *Human Reproduction*. 2012).

The tip of the needle should be sharp and have a reflective surface that would make it visible in ultrasound once it enters the ovary. Fingertip handle on the distal end of the needle allows puncture of the follicle with good clinical touch.

The tubing for the single- or double-lumen needle (17–20 G/35–40 mm) is connected via a Teflon tubing with silicone rubber cork to the collecting tube (kept in the test tube heater), to which the suction tubing from suction pump (to create negative pressure) is also attached (Figures 2 and 3). The suction used is 90–120 mmHg for mature follicles and 40–60 mmHg

Figure 2A: Integrated oocyte retrieval procedure. 2B Silicone rubber cork connected to the collecting tube.

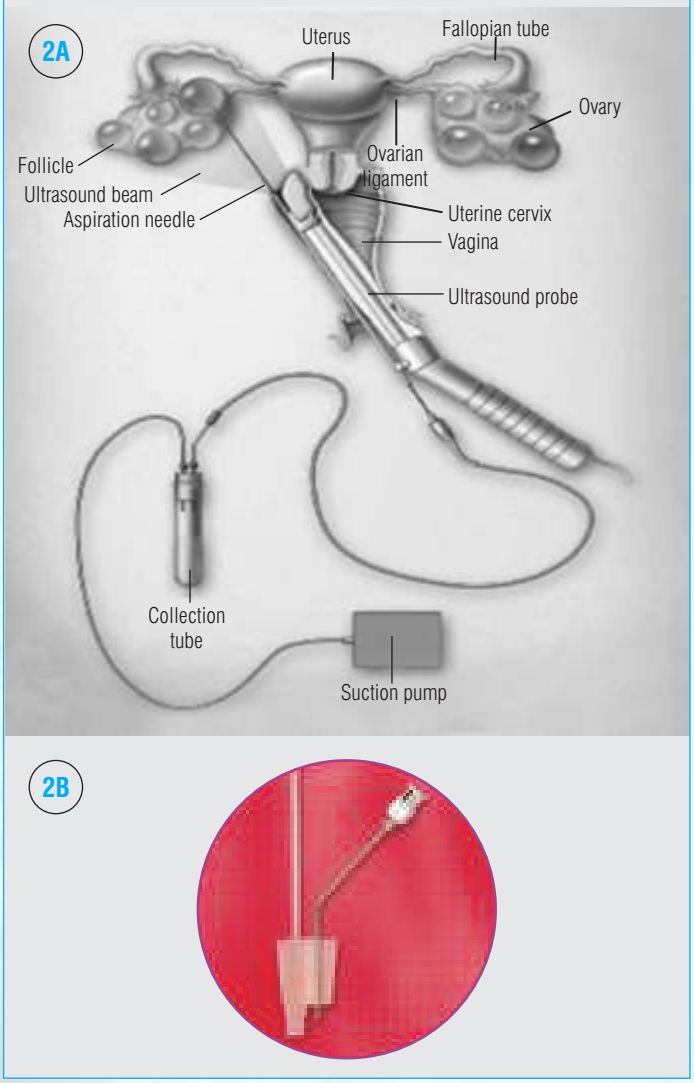
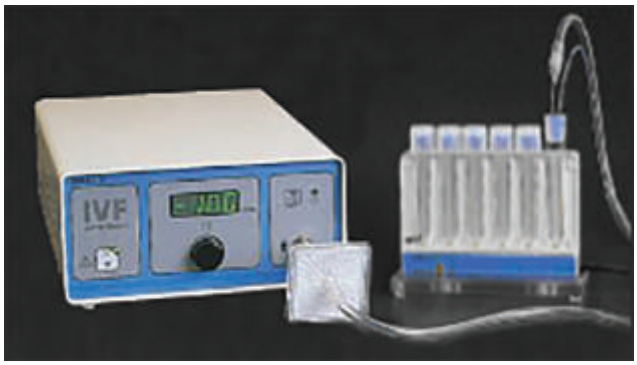


Figure 3: Connection from the suction pump to the collecting tube.



for smaller follicles, which are aspirated in IVM. As long as the inner diameter of the needle is 0.8–1 mm, the oocyte cumulus complex remains unaffected, provided that the aspiration pressure is <120 mmHg.

- The procedure is performed either under general anesthesia or with intravenous analgesia. Analgesia can be combined with a paracervical block pain relief.
- The aspirating needle is introduced through the guide attached to the transvaginal probe. The ovary should be lined up in the most accessible position by manipulating the transvaginal probe. The follicles are then lined up against the biopsy line. The needle should be inserted carefully into the follicle by looking for the reflective wall of the needle, which helps to identify its path. The needle tip can be observed and maneuvered within the ovary to aspirate all the follicles. The negative pressure is applied only when the needle tip is within the follicle to aspirate the oocyte–cumulus complex with the follicular fluid. Complete aspiration of the follicle is confirmed by complete collapse of the follicular wall. It is important that one should always visualize the tip of the needle on the ultrasound screen. During aspiration of follicles at the periphery of the ovary, one can rotate the TV probe to differentiate between a vessel and a follicle. The vessel will become elongated with change in the direction of the probe, whereas the follicle will remain round or ovoid. Avoid multiple puncture of the ovary to prevent hemorrhage from the surface of the ovary.
- At the end of the procedure, the vagina was thoroughly examined with a speculum and, if necessary, local compression was applied to allow hemostasis.
- One dose of intravenous antibiotic is given.

Complications of Oocyte Retrieval

The various complications associated with oocyte retrieval include:[33]

- Complications of anesthesia (very rare)
- Vaginal bleeding (3/100), which requires compression for 5–8 minutes. If no response occurs a suture can be taken.
- Severe intra-abdominal bleeding (<1/1000), which requires laparoscopy or laparotomy to stop the bleeding. Blood transfusion may be required if detected late.
- Injury to organs (1/1000), which requires laparoscopy or laparotomy to stop the bleeding.
- Pelvic infection (1-5/1000): Pelvic abscess, ovarian abscess, or infected endometriotic cyst
- Adnexal torsion (<1/1000)
- Severe pain (3/100): The pain level increased with the number of oocytes retrieved.
- Surgery for complications of oocyte retrieval (rare)[33]

  Rare complications include:(1) Ruptured endometriotic or dermoid cysts, which present with acute abdominal symptoms requiring laparotomy; (2) Acute appendicitis with puncture holes in the appendix; (3) Injury to the ureter: Ureterovaginal fistula; (4) injury to the ureter: Acute ureteral obstruction; (5) Rectus sheath hematoma: TAOR; (6) vaginal perforation in older patients with a history of repeated OR, particularly when the ovaries are difficult to visualize; (7) Vertebral osteomyelitis: Severe lower-back pain.

Unsuccessful Oocyte Retrieval

Empty follicle syndrome is defined as no oocytes being retrieved from apparently normal ovarian follicles with normal steroidogenesis after ovarian stimulation and meticulous follicular aspiration. Incidence of empty follicle syndrome is 1%–7% of cycles. There are two types of empty follicle syndrome.

Types 1: *Genuine*: 33% failure to retrieve oocytes despite optimal hCG levels on the day of oocyte retrieval. It may be caused due to genetic disorders or due to early oocyte atresia.

Type 2: *False*: 67% failure to retrieve oocytes in the presence of low hCG (<40 IU/L) due to an error in the administration or the bioavailability of hCG.

Causes of False Empty Follicle Syndrome

1. hCG-related faults are the main cause. (hCG injection scheduled later than 11 h before retrieval), failure of the hCG injection, confirmed by the undetectable hCG serum concentrations.
2. Rapid metabolic clearance
3. Manufacturing defects in hCG production
4. Low bioavailability of hCG after bariatric surgery may induce empty follicle syndrome (EFS)

Risk Factors for EFS

1) Advanced age (37.7±6.0 years vs. 34.2±6.0 years, $p<0.001$)
2) Longer infertility duration (8.8±10.6 years vs. 6.3±8.4 years, $p<0.05$)
3) Higher baseline follicle stimulating hormone (FSH) levels (8.7±4.7 IU/l vs. 6.7±2.9 IU/l, $p<0.001$)
4) Lower E2 levels before the hCG injection (499.9±480.9 pg/mL vs. 1516.3±887.5 pg/mL, $p<0.001$)
5) Low ovarian reserve

Therapeutic Approach for Prevention of Empty Follicle Syndrome

1. Assessment of serum hCG the day after the trigger
2. Re-administration and re-aspiration of hCG, if the hCG value is low after the retrieval of oocytes from one ovary.
3. Using recombinant hCG (Ovitrelle) to trigger ovulation
4. Increasing the dose of hCG
5. Prolonging the interval between ovulation trigger and OPU
6. Inducing ovulation using GnRHa

For maintaining the practice standards, the physicians should perform 20 follicular aspirations under their direct supervision prior to independent practice (ASRM, 2008). Proficiency scores (PS) should be obtained for all performing oocyte retrieval, which is obtained by dividing the number of

oocytes retrieved by the number of oocytes predicted based on the total number of follicles (≥12 mm) measured by ultrasound on the day of hCG trigger.

Summary

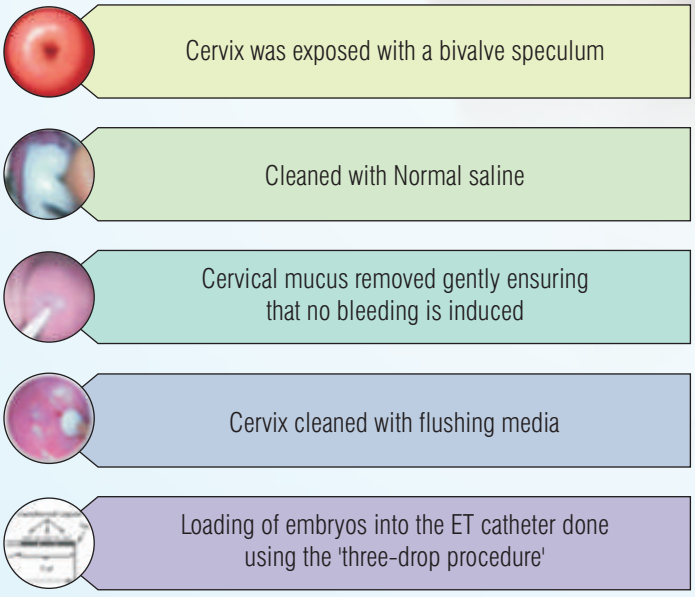
- Prophylactic use of antibiotics and antimycotics
- Proper vaginal sterilization with normal saline
- Minimal number of vaginal punctures
- Ultrasound visualization of peripheral follicles in a cross-section before puncture decreases the complication of internal bleeding
- Gentle manipulation of the needle all through the procedure
- Proper visualization of the tip of the needle all through the procedure
- Progesterone to be started after oocyte retrieval
- Administration of progesterone before oocyte retrieval is associated with a lower pregnancy rate than the administration of progesterone after oocyte retrieval.

Embryo Transfer

Why Should One Optimize Embryo Transfer?

Apart from embryo quality and ER, ET procedure is a basic and important factor that determines the final outcome of an IVF cycle. It is probably the least successful step in IVF, with ±30% of failure in ART due to its poor performance.

Transfer Technique (Steps are Shown in the Figure Below)



- Loaded catheter is passed through the external os to reach the internal os.
- Inner catheter is then advanced into the uterine cavity to expel the embryos about 1.5–2 cm below the fundus, without touching the fundus.
- The catheter is kept in place for 10–15 seconds after the embryos are expelled.
- The catheter is then drawn back into the introducing cannula and the two are removed together.
- The cannula is then flushed with medium under a stereomicroscope to ensure that the embryos were released into the uterine cavity.

Ultrasound-Guided Embryo Transfer

- Confirms the position of the tip of ET catheter within the uterine cavity and the site of embryo deposition
  - Avoiding contact with the fundus
  - Avoiding endometrial indentation
- Distended bladder alters uterocervical angle and allows easy transfers, requiring less frequent use of tenaculum or obturator.

Factors That may Affect the Success of Embryo Transfer (ET) are Highlighted in Table 9

Table 6: Factors affecting the success of embryo transfer

• Ease of procedure	• Presence or absence of the blood on the catheter tip
• Embryo transfer catheter type soft vs. hard ET catheter	• Retention of embryos in the catheter
• Removal of cervical mucus	• Excessive uterine contractions after ET
• Use of ultrasound guidance	• Microbiological factors in the cervix and bacterial contamination of the catheter
• Position of embryo deposition in the uterus	• Rest after ET
• Position of the air-medium content in the catheter and amount of media transferred	• Experience of the physician
• Duration of embryo transfer	

Precautions to Avoid Initiation of Uterine Contractions

- Use soft catheters to avoid trauma to the endocervix or endometrium. They are malleable enough to find their way into the uterine cavity.
- Avoid contact with the uterine fundus, as it is associated with the initiation of uterine contractions.
- Gentle manipulation should be done to prevent the release of oxytocin. Therefore,
  - Holding the cervix by vulsellum should be avoided.
  - Introduction of speculum should be gentle to avoid unnecessary pushing of the cervix.
- Administer single oral dose of 10 mg of piroxicam for 1–2 hours before embryo transfer.

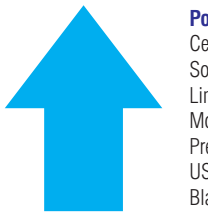
eXpert
EXcellence in PCOS & Expertise in Reproductive Technology

Solutions for Difficult Embryo Transfers

- Mock transfer
- Use of stiffer and more rigid catheter systems when there is resistance at the internal ostium (Mansour *et al*., 1990; Sharif *et al*., 1995).
- Maneuvering the vaginal speculum gently.
- Carrying out the transfer when the bladder is partially full.
- Moderate cervical traction to straighten the uterus.
- Use of malleable obturator and then the inner catheter.
- Use of a co-axial or echo tip catheter system (Patton and Stoelk, 1993).
- Use of USG guidance to facilitate ET.
- Trans-myometrial (vaginal or abdominal) surgical ET can be used in rare cases (Kato *et al*., 1993; Groutz *et al*., 1997).
- Use of trans-tubal ET.

Degree of Impact of Variables Affecting the Success of Embryo Transfer

Figure 5: Impact of different variables on the success rate of embryo transfer.



Positive	Degree of impact
Cervical lavage	+
Soft catheter	+
Limiting volume and air when loading	++
Mock transfer	+++
Pre-cycle cervical dilatation if necessary	++
USG guidance for ET	++
Blastocyst transfer	+

Negative	Degree of impact
Blood on catheter tip	+++
Mucus on catheter tip	+
Retained embryos	+
Bacterial contamination of catheter	+++
Excessive uterine contraction	++
Difficult transfer	+

Key Elements for Successful ET are Given Below
(Schoolcraft. Embryo transfer technique. *Fertil Steril.* 2016)

Goal	Protocol
Easy, atraumatic transfer without blood or mucus	Trial transfer, ultrasound, soft catheter
Proper placement	Inject embryos slowly 1.5 cm from fundus, as confirmed by ultrasound
Minimize embryo stress	Minimize transfer time, control temperature/pH
Negotiate a difficult/stenotic cervix	Pre-cycle dilatation, malleable stylet, ultrasound
Optimize implantation, minimize contractions	Oay-5 ET, FET cycles, avoid trauma to cervix or fundus, Piroxicam 10 mg 2 hrs before ET

Summary

Use of USG-guided soft catheter embryo transfer technique shows an improved result and lowers the variability of the results by making it less traumatic, standardized, and technically precise.

ART in Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is an endocrine and reproductive disorder reported in women of reproductive age. Polycystic ovary syndrome is the primary cause of hyperandrogenism and oligo-anovulation and is often associated with infertility and clinical and metabolic disorders.[9]

The prevalence of infertility in women with PCOS varies between 70% and 80%. The principal infertility treatment for PCOS includes the use of drugs to induce mono-or bi-follicular ovulation. At later stages, therapeutic modalities, such as exogenous gonadotropins or laparoscopic ovarian drilling, which are considered to be second-line treatments—or ART (such as IVF), which is a third-line treatment—may be employed.[9]

Polycystic ovarian syndrome is the most common endocrine malady and a frequent cause of infertility in women. Proper diagnosis and management of PCOS is essential at the right time for proper management. Various treatment modalities are available for PCOS. Lifestyle intervention, along with insulin sensitizers, leads to improvement in regulation of menstrual cycle and fertility. Clomiphene citrate and letrazole are both considered as the first-line of management, followed by gonadotropins or laparoscopic ovarian drilling. Excellent results have been achieved by *in vitro* fertilization and optimal luteal support.

Oocyte quality is dictated by events that occur during both growth and maturation stages of development.

It is influenced by:

Intrinsic factors such as

- Age
- Genetics

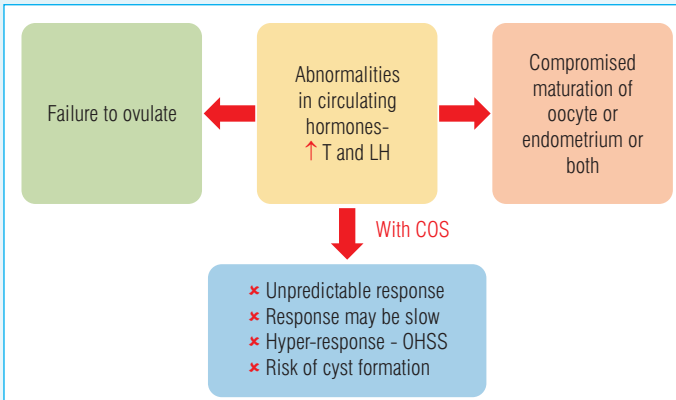
Extrinsic factors such as

- Stimulation protocols
- Culture conditions
- Nutrition

Controlled Ovarian Stimulation (COS) in PCOS

- Rescue of oocytes may be intrinsically abnormal.
- Oocytes intended to be atretic are forced to be recruited and ovulated.
- Disruption of the intrafollicular physiology overrides the endogenous pattern of control of the oocytes, which optimizes the normal selection process.

Reproductive Impairment in Women with PCOS



Difficulties Encountered During COS in PCOS

With the multiple "explosive" follicular development, a greater sensitivity to GT stimulation has been noted. This is due to a sixfold increase in the density of pre-antral follicles compared to normal ovary (Webber *et al*., 2003) and this large cohort of small follicles arrested in development are capable of responding to exogenous FSH.

Problems Caused by COS in PCOS

- Abnormal folliculogenesis and uncertain response with under-stimulation or hyper-response, resulting in OHSS
- Hyper-response to GT leads to high E2, which is detrimental to implantation and pregnancy
- Relative GT resistance and increased GT consumption due to obesity and insulin resistance (Fedorcsak *et al*., 2001)
- Multiple pregnancies
- Early pregnancy losses

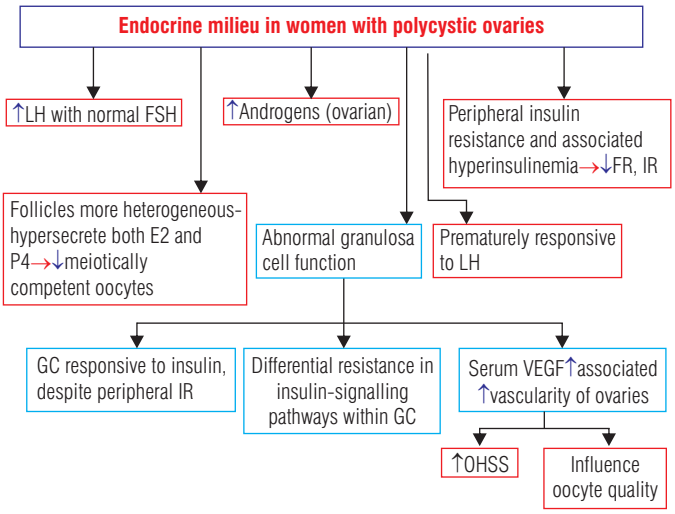
Table 7 provides the reason for abnormal folliculogenesis (Chavez-Ross *et al*., 1997).

Table 7: Causes for abnormal folliculogenesis

1. **Primary cause of follicular dysfunction**
   At the level of ovary and not pituitary
   ↓
   Characterized by variable responsiveness of follicles to GT
2. **Abnormal pre-antral follicle development**
   Due to precocious acquisition of LH receptors in granulosa layer
   ↓
   Results in arrest of follicular growth
3. **Abnormal response to GT**
   Characterized by enhanced relative sensitivity to FSH and LH in a subpopulation of follicles
4. **Follicles hyper-responsive to GT**
   Prematurely reach level of maturity
   ↓
   Produce sufficiently high concentration of circulating EZ
   ↓
   Suppress FSH to a level that is too low to encourage further development of healthy follicles in the cohort

The endocrine milieu is responsible for an abnormal response to gonadotropins in PCOS women (Figure 6). (Willis *et al*., 1998; Franks *et al*., 2000.; Book and Dunaif, 1999; Yen, 1980; Franks, 1995; Argrawal *et al*., 1998).

Figure 6: Endocrine milieu in PCOS.

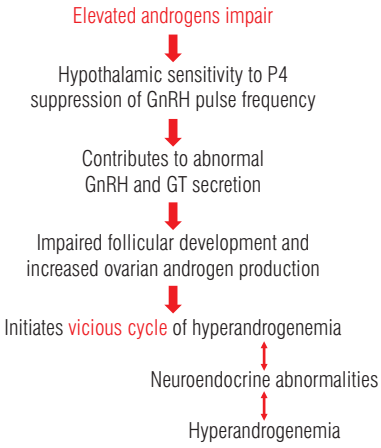


Gonadotropin secretion in women with PCOS is different from that in normo-gonadotropic women.

- Rapid GnRH pulse frequency signifies a failure of the systems that are necessary to suppress GnRH pulsatility, as a result of primary hypothalamic defects and abnormal hormonal milieu or a combination of the two.
- Increased luteinizing hormone (LH) pulse amplitude (Waldstreicher *et al*.)
- Exaggerated LH responses to exogenous gonadotropin-releasing hormone (GnRH)
- Low FSH basal concentrations(Cahill *et al*., 1994; Popovic-Todorovic *et al*., 2003b)

Elevated androgen levels also have an effect on the gonadotropin secretion (Figure 7).

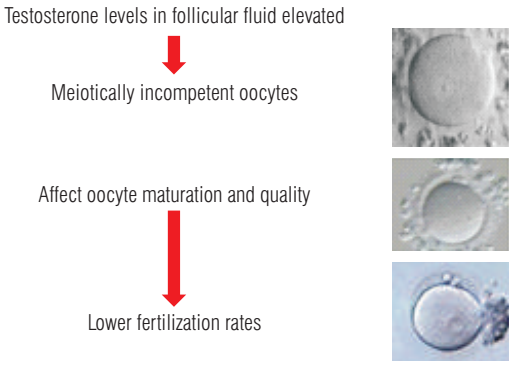
Figure 7: Effects of elevated androgen levels.



eXpert EXcellence in PCOS & Expertise in Reproductive Technology

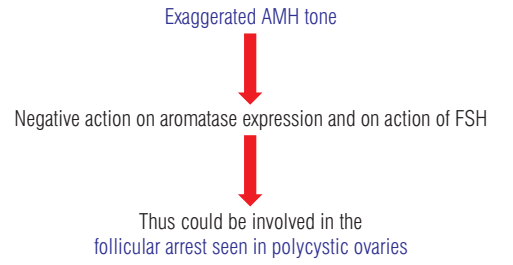
Androgens also affect the follicular microenvironment (Figure 8).

Figure 8: Effect of androgens on follicular microenvironment and oocytes.



Anti-Müllerian hormone (AMH) also plays a very important role in determining the response to COS in PCOS women. Very high AMH values (>7 ng/mL) are associated with poor response to COS in PCOS women. This is because of exaggerated AMH tone, which prevents recruitment of primordial follicles (Figure 9).

Figure 9: Effect of high AMH levels in PCOS.



Abnormal Response of polycystic ovary to COS is observed.

- Extremely difficult to find out the GT threshold
- Recruitable pool of follicles is increased
- Stromal hyperplasia contributes to more than normal amounts of androgens to the follicular microenvironment.
- Granulosa cell aromatase activity is normally decreased; however, it is readily stimulated by exogenous FSH.
- Follicular response is initially slow, but becomes explosive at later stages of stimulation.
- Obesity is also responsible for abnormal response.

  Increase in the number of fat cells

  ↓

  Increased leptin and insulin levels and a preferential increase in LH, but not in FSH

  ↓

  Stimulated partial development of follicles that secrete supernormal levels of T, which rarely ovulate; hence the low P4.

Indications for ART in PCOS

IVF is not the first-line of treatment for PCOS. It is the third-line treatment, the first-line being ovulation induction with oral ovulogens; if this fails one could try gonadotropins. Both these methods can be combined with IUI. Following are the indications for IUI:

1. Other factors of infertility (50%), such as tubal factor and male factor (Tannys, 2010)
2. Failure to conceive despite at least six ovulatory cycles (Adam, 2007)
3. Failure to conceive on gonadotropin therapy alone/IUI (Araki, 2011)
4. Failure of weight reduction, anti-estrogen therapy, or LOD (Eijkemans *et al*., 2005)
5. High response to FSH (four or more follicles) despite a low gonadotropin dose
6. To eliminate the chances of MP particularly for some older women in whom single ET is performed (Papanikolaou *et al*., 2006;Heijnen *et al*., 2007)

Is the Fertile Window Extended in Women With PCOS?

Effect of Aging on the Ovarian Reserve

- With age, women with PCOS gain regularity in their menstrual cycles (Elting *et al*., 2000, 2003).
- Age-related decrease in AFC and AMH is slower in women with PCOS compared to those without PCOS (Shalom-Paz *et al*., 2012).
- The reproductive lifespan of PCOS women extends, on average, to 2 years beyond that of normo-ovulatory women (Tehrani *et al*., 2010).
- However, older PCOS patients continue to have increased testosterone levels in comparison with their age-matched controls who have 40%–60% lower levels (Winters *et al*., 2000).

Table 8 shows age-specific AMH and AFC values in women with and without PCOS.

Table 8: Age-specific AMH and AFC values in women with and without PCOS

Age	20–31	32–34	35–37	38–40	41–43	>44
AMH (ng/mL; 95% CI)						
Control	3.15±2.90 (2.98–3.32)	2.28±1.98 (2.06–2.48)	1.99±2.01 (1.74–2.24)	1.44±1.31 (1.21–1.67)	1.43±1.37 (0.98–1.88)	0.92±0.89 (0.43–1.41)
PCOS	5.65±4.29 (5.10–6.20)	4.85±3.53 (3.77–5.92)	4.40±2.92 (3.14–5.67)	—	—	—
AFC (N)						
Control	15.45±6.09	13.13±5.73	12.46±5.44	10.25±4.29	9.76±4.61	7.13±4.32
PCOS	28.65±7.08	26.11±7.71	29.61±10.80	—	—	—

AMH and AFC decreased with age in non-PCOS women ($p<0.001$), which was not obvious in PCOS patients ($p>0.05$) (Yuqian Cui *et al*. Fertility and Sterility® Vol. 102, No. 1, July 2014).

eXpert
EXcellence in PCOS & Expertise in Reproductive Technology

Smaller follicular count with regular menstrual cycles in PCOS is associated with:

- Older age
- Higher FSH concentration
- Lower FSH-induced inhibin B increment

  Mariet W. Elting *et al*. Fertility And Sterility VOL. 79, NO. 5, MAY 2003

As the AFC and AMH values could be normal even at the age of >37 years, is the fertile window extended in women with PCOS? The answer is no, as the following observations were made (Anuja Dokras *et al*. Fertility and Sterility Vol. 100, No. 1, July 2013):

- Despite a higher oocyte yield in all age groups, women with PCOS over the age of 40 years had similar clinical pregnancy and live birth rates when compared to women with tubal factor infertility.
- Thus, the reproductive window may not be extended in women with PCOS and patients with infertility should be treated in a timely manner, despite indicators of high ovarian reserve.

Increasing the Efficiency and Reducing Complications of the ART Treatment in PCOS

Choosing the:

- Right GT and dose
- Right GnRH analog

Is Very Important in an ART Protocol to:

1. Increase the safety and effectiveness of ART
2. Optimize the treatment:
   - To increase the live birth rates
   - To decrease birth defects, imprinting and epigenetic disorders
3. To minimize the risk of OHSS and multiple pregnancies

This can be performed by choosing an individualized protocol (based on age, AMH, AFC, and BMI), substituting oocyte and embryo number, implantation and pregnancy rates per cycle by healthy-term live birth. This automatically reduces the risk, complications, patient discomfort, and cost of treatment.

The different ovulation-induction protocols have already been discussed in Module 1. The main challenges of ovulation induction in PCOS are to see whether the treatment

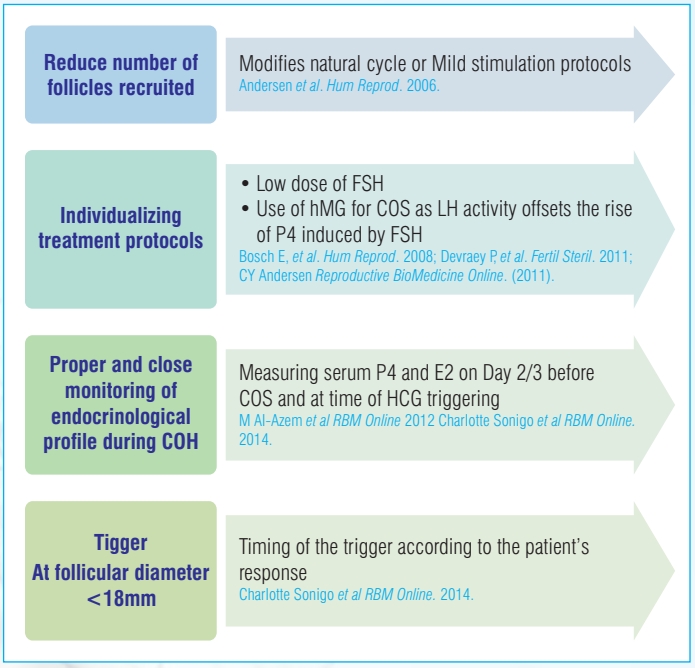
- Results in pregnancy
- Offers a low risk of OHSS
- Offers a low risk of multiple pregnancies
- Offers a better neonatal outcome

We can increase the efficiency by prevention of follicular phase P4 rise and by freezing all embryos to increase PRs. Decreasing the incidence of OHSS, multiple pregnancies, imprinting disorders, and epigenetic abnormalities will reduce the risk of complications associated with ART in PCOS.

Why are Serum Progesterone Levels in the Follicular Phase Important?

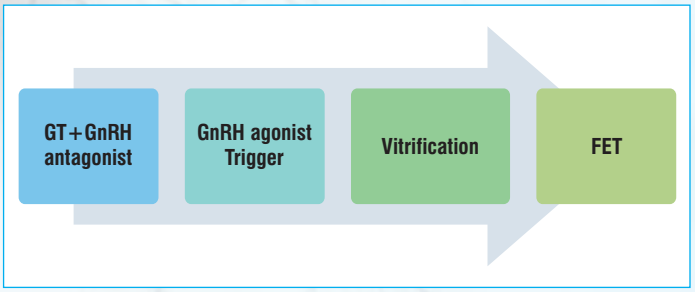
LBRs significantly decreased with progesterone elevation on the day of hCG due to a decreased endometrial receptivity; moreover, it correlated with a high ovarian response and is associated with a greater dose of FSH.

Prevention of Follicular Phase P4 Elevation



In cycles with elevated pre-ovulatory P4, the probabilities of IR, CPR, and LBR increased if all embryos are cryopreserved and subsequently thawed, followed by being transferred in a natural or hormone replacement therapy cycle (Richard Fleming; Reproductive BioMedicine Online 2010).

Freeze-All Protocol



The Table 9 depicts the advantages of the freeze-all policy.

Disadvantages of the Freeze-All Policy

Frozen embryo transfer cycles are associated with

- A higher incidence of large for gestational age (Wennerholm *et al*., 2013; Pinborg *et al*., 2014)
- A higher risk of placenta accreta (Ishihara *et al*., 2014; Kaser *et al*., 2015)

Table 9: Advantage of the freeze-all policy

Increase Effectiveness	Increase Safety
Improving Pregnancy Rates by improving embryo implantation by ↑ endometrial receptivity 32% ↑ in ongoing pregnancy rate (Roque *et al*., 2013)	Prevention of OHSS
Decreased ER related to Endometrial advancement as a result of COS (Ubaldi *et al*., 1997; Kolibianakis *et al*., 2002) Altered genes that are crucial for the endometrium-embryo interaction (Horcajadas *et al*., 2005; Labarta *et al*., 2011)	Decrease incidence of ectopic pregnancy which may be more as COS associated with increase in uterine contractility and embryoendometrium asynchrony (Huang *et al*., 2014; Landro et *al*., 2015)
Altered trophoblast expansion and invasion due to COS is, in part, responsible for obstetric and perinatal complications (Mainigi *et al*., 2014)	Lower risk of low birth weight (Pinborg *et al*., 2010; Li *et al*., 2014; Ishihara *et al*., 2014) Pre-term birth (Pelkonen *et al*., 2010; Pinborg *et al*., 2013; Wennerholm *et al*., 2013; Ishihara *et al*., 2014; Schwarze *et al*., 2015) Small for gestational age (Ishihara *et al*., 1014; Li *et al*., 2014; Pinborg *et al*., 2014)

Cost Effectiveness of the Freeze-All Policy

(Roque *et al*. *JBRA Assisted Reproduction*. 2015;19(3):125–130)

- Lack of studies evaluating the cost effectiveness of the freeze-all policy
- Roque *et al*. concluded that this strategy was cost-effective when compared to fresh embryo transfers.
- More studies are necessary to evaluate the incremented costs when performing elective cryopreservation of all embryos.

Prevention of OHSS by:

- Identifying high-risk patients and cycles
- Using low-risk treatment
- Using specific measures in individual cases

Risk Factors Include

- Prior AMH level at the cutoff of 3.36 ng/mL has a sensitivity of 90.5% and a specificity of 81.3%. Elevated AMH levels (>5 ng/mL and >10 ng/mL) had significantly higher rates (more than threefold) of OHSS.
- Rapidly increasing E2 levels of >75% from the previous day or E2 level of >3500 pg/mL on the day of hCG
- Optimum cutoff value for AFC is ≥14
  - Sensitivity 82%
  - Specificity 89%
  - Risk of OHSS from 2.2% (AFC <24) to 8.6% (AFC >24)
- Emergence of a large number of small- and intermediate-sized follicles (10–14 mm)on USG
- Presence of >19 large-/medium-sized follicles before hCG
- Retrieval of >20 oocytes
- Occurrence of pregnancy
- Blood group A associated with early-onset OHSS, via elevated Von Willebrand factor (VWF) and factor VIII

Use of Low-Risk Treatment

- iCOS and mild ovarian stimulation has grade A evidence (RBM online 2017).
- GnRH antagonist instead of GnRH agonist has grade A evidence (Papanikolau *et al*. (2006), Mathur *et al*. (2000). *Fertil Steril*).
- GnRH agonist trigger in antagonist cycle has Grade A Evidence (Humaidan *et al*. 2010;Castillo *et al*. 2010; Papanikolau *et al*. 2010; Engman *et al*. 2008).
- Administration of lower-dose or recombinant hCG has Grade C Evidence (Driscoll *et al*., 2000; Chang *et al*., 2001).
- Recombinant human LH for triggering has Grade B Evidence (Shoham Z, Schacter M, Loumaye E, *et al*.)

Thus, the Key to the Prevention of OHSS is:

- The OI therapy and recognition of risk factors for OHSS
- Highly individualized OI regimens, carefully monitored with USG and E2
- Use of minimum dose and duration of GT therapy necessary to achieve the therapeutic goal

GnRH Agonist for Triggering Ovulation

Most commonly used GnRHa-triggering doses are:

- Buserelin 0.5 mg s.c.
- Triptorelin 0.2 mg s.c.
- Leuprolide 1 mg s.c.

Strategies after Using GnRH Agonist Trigger

- Supplementing with one bolus of 1500-IU hCG: Grade B evidence (Humaidan *et al*., 2010; Radesic *et al*. 2011; *Hum Reprod*. vol.26:3437–3442; Andersen *et al*. *Reprod Biomed Online*. 2014;28:552–559).
- Three doses of 500 IU of hCG as LPS-safe and efficient alternative in high responders (Castillo *et al*., 2010).
- Luteal Rec LH: Rec LH for 10 days from the day of OPU (5000–30000 IU)
- 10,000 IU: Adequate, but the ideal dose needs to be evaluated (Papanikolau *et al*. 2010)
- Intense P4 and E2 luteal support
- Freeze all embryos
- Combination

Specific measures in individual cases, with the evidence of their use are shown in the Figure 10.

Figure 10: Specific measures in individual cases to reduce the incidence of OHSS.

The GnRH antagonist protocol coupled with the GnRH agonist triggering is the best, efficient, safe, and simple method to prevent OHSS.

Preventing Multiple Pregnancies

Preventing multiple pregnancies is of utmost importance, as with multiple pregnancies the ART results shift from success to complications. Multiple pregnancies, especially higher-order multiples, are associated with increased morbidity and mortality both in the mother and the neonate. Table 10 enumerates the complications of multiple pregnancies.

Table 10: Complications of multiple pregnancies in the mother and child

Maternal mortality	X 2 or 3	Transfer to NICU	X 15.5
Preeclampsia/HELLP syndrome		Severe prematurity	X 4
Acute fatty liver		SFGA	X4
Thromboembolism		Infant mortality	X 5
Postpartum hemorrhage		Cerebral palsy	X 5 to 10

The solution to multiple pregnancies is single embryo transfer (SET) by choosing the best embryo for transfer. This can be carried out by blastocyst transfer with good morphology, embryo assessment by embryoscope, and aneuploidy assessment by PGS. The remaining embryos to be used in the subsequent cycle should be frozen by vitrification. To implement SET, patient education to provide correct information in an appropriate way is important.

COS can result in imprinting disorders and epigenetic modification, and therefore should be prevented.

Imprinting disorders and epigenetic modification can result in:

- Fertilization failure and pre-implantation embryonic-development arrest
- Implantation and placentation defects, along with spontaneous abortions
- Increased risk of pre-eclampsia, pre-term births, LBW, various syndromes, and cancer

Imprinting disorders and epigenetic modifications can be prevented by:

- Mild stimulation protocols
- Improving *in vitro* culture systems
- Diet and supplementation of adequate folate

A tailored approach to COS could reduce the incidence of OHSS, multiple pregnancies, imprinting disorders, and epigenetic modifications in women predicted to show excessive response.

Summary

- Modifying conventional stimulation protocols according to patients' characteristics and ovarian reserve makes it patient-friendly and optimizes the chance of LBR.
- Choosing the protocol on the basis of patient characteristics and LH levels, and using the appropriate gonadotropin type and dose with an appropriate GnRH analog will definitely increase the LBR, efficacy, and safety, while reducing the complications.
- Increased BMI results in an increased dose and unpredictable response; therefore, weight loss should be the moto before we embark on ART if the woman is <35 years of age.
- iCOS, freeze-all, and eSET could help in increasing the success rate of ART and in reducing stress and anxiety, thus leading to lesser complications.

Clinical Advances in ART

Advances in Ovarian Stimulation

Many new technologies have been developed over the last 5 years, which have focused on the improvement of oocyte quality, especially in older women. New stimulation protocols have been developed that may improve the number of mature oocytes retrieved during an IVF cycle. The major advances in understanding of ovarian physiology have paved the path for a better understanding of the clinical condition to individualize therapy. New gonadotropins, including recombinant(r) FSH, rLH, rhCG, and GNRH analogs, have been identified. Also, there has been an increase in the availability of self-administered hormones.[36]

GnRH Agonist Triggers for Ovarian Hyperstimulation Syndrome (OHSS)

Krishna *et al*. (2016) conducted a prospective, randomized study to evaluate whether the gonadotropin-releasing hormone agonist (GnRHa) trigger is a better alternative to hCG for PCOS among the Indian population undergoing IVF cycles with GnRH antagonists for the prevention of OHSS. A total of 227 patients diagnosed with PCOS undergoing IVF in an antagonist protocol were recruited and randomly assigned to two groups: (i) Group A: GnRHa trigger 0.2 mg ($n=92$) and (ii) Group B: 250 μg of recombinant hCG as a trigger ($n=101$) 35 hours before oocyte retrieval.[37]

The incidence of moderate-to-severe OHSS was 37.6% in the hCG group and 0% in the GnRHa group ($p<0.001$). The GnRHa group had significantly more mature oocytes retrieved, more fertilized oocytes, and a higher number of top-quality cleavage embryos on day 3 compared with the hCG group.[37]

The study, therefore, demonstrated that the use of GnRHa trigger yields more mature oocytes and good-quality embryos compared with the hCG trigger.[37]

Ovarian Stimulation Protocols for Cancer Patients

Vitrification of oocytes and embryos has improved the chances of pregnancy for cancer patients in recent years, substantially increasing the uptake of fertility preservation before cancer treatment. Strategies for ovarian stimulation for such patients should optimize oocyte yield while avoiding the risk of ovarian hyperstimulation.[38]

Small-Molecule Agonists and Antagonists for LH and FSH Receptors

LH and FSH act on their respective receptors in the gonads to either promote follicular growth and differentiation in women or to stimulate the proper progression of spermatogenesis in men. The LH and FSH are currently used for the treatment of infertility. Small-molecule agonists of LHR and FSHR have the potential to become oral therapeutics for infertility treatment, whereas small-molecule antagonists of LHR and FSHR may find utility in oral contraception. Advances in molecular biology, high-throughput screening, and combinatorial chemistry have made significant contributions to the recent discovery of a variety of small-molecule LHR and FSHR agonists and antagonists, some of which have shown highly promising efficacy in animal models of fertility control.[39]

Summary

- New stimulation protocols have been evolved that may improve the number of mature oocytes retrieved during an IVF cycle. New gonadotropins, including recombinant(r) FSH, r LH, rhCG, and GNRH analogs, have been identified. Also, there has been an increase in the availability of self-administered hormones.
- Vitrification of oocytes and embryos has improved the chances of pregnancy among cancer patients in recent years, substantially increasing the uptake of fertility preservation before cancer treatment.

References

1. Broekmans Datta J, Palmer MJ, Tanton C, *et al*. Prevalence of infertility and help seeking among 15 000 women and men. *Hum Reprod (Oxford, England)*. 2016;31(9):2108–2118.
2. Sarkar S, Gupta P. Socio-demographic correlates of women's infertility and treatment seeking behavior in india. *J Reprod Infert*. 2016;17(2):123–132.
3. Peyromusavi F, Barouni M, Naderi T, *et al*. Factors affecting response to infertility treatment: Case of Iran. *Global J Heal Sci*. 2016;8(1):118–123.
4. Cheung LP. Patient selection for assisted reproductive technology treatments. *Hong Kong Med J*. 2000;6(2):177–183.
5. Kamath MS, Bhave P, Aleyamma T, *et al*. Predictive factors for pregnancy after intrauterine insemination: A prospective study of factors affecting outcome. *J Hum Reprod Sci*. 2010;3(3): 129–134.
6. Vilela M *et al*. ART, clinical: Prognostic factors. Abstracts of the 21st annual meeting of the ESHRE, 2005.
7. Good clinical treatment in assisted reproduction. ESHRE, 2008.
8. ESHRE Capri Workshop Group. Intracytoplasmic sperm injection (ICSI) in 2006: Evidence and evolution. *Hum Reprod Update*. 2007;13(6):515–526.
9. Melo AS, Ferriani RA, Navarro PA. Treatment of infertility in women with polycystic ovary syndrome: Approach to clinical practice. *Clinics*. 2015;70(11):765–769.
10. Practice Committee of the American Society for Reproductive Medicine. Obesity and reproduction: A committee opinion. *Fertil Steril*. 2015;104(5):1116–1126.
11. Kalra SK, Ratcliffe SJ, Dokras A. Is the fertile window extended in women with polycystic ovary syndrome? Utilizing the Society for Assisted Reproductive Technology registry to assess the impact of reproductive aging on live-birth rate. *Fertil Steril*. 2013;100(1):208–213.
12. Moolenaar LM, Nahuis MJ, Hompes PG, *et al*. Cost-effectiveness of treatment strategies in women with PCOS who do not conceive after six cycles of clomiphene citrate. *Reprod Biomed Online*. 2014;28(5):606–613.
13. Siristatidis C, Bhattacharya S. Unexplained infertility: Does it really exist? Does it matter? *Hum Reprod*. 2007;22(8):2084–2087.
14. Dovey S, Sneeringer RM, Penzias AS. Clomiphene citrate and intrauterine insemination: analysis of more than 4100 cycles. *Fertil Steril*. 2008;90(6):2281–2286.
15. Harris ID, Missmer SA, Hornstein MD. Poor success of gonadotropin-induced controlled ovarian hyperstimulation and intrauterine insemination for older women. *Fertil Steril*. 2010;94(1): 144–148.
16. Hansen KR, He ALW, Styer AK, *et al*. Predictors of pregnancy and live-birth in couples with unexplained infertility following ovarian stimulation-intrauterine insemination. *Fertil Steril*. 2016;105(6):1575–1583.
17. Reindollar RH, Regan MM, Neumann PJ, *et al*. A randomized clinical trial to evaluate optimal treatment for unexplained infertility: the fast track and standard treatment (FASTT) trial. *Fertil Steril*. 2010;94(3):888–899.
18. Ray A, Shah A, Gudi A, *et al*. Unexplained infertility: An update and review of practice. *Reprod Biomed Online*. 2012;24(6):591–602.
19. Armstrong S, Akande V. What is the best treatment option for infertile women aged 40 and over? *J Assist Reprod Genet*. 2013;30(5):667–671.
20. Goldman MB, Thornton KL, Ryley D, *et al*. A randomized clinical trial to determine optimal infertility treatment in older couples: The Forty and Over Treatment Trial (FORT-T). *Fertil Steril*. 2014;101(6): 1574–1581.
21. Macer ML, Taylor HS. Endometriosis and infertility: A review of the pathogenesis and treatment of endometriosis-associated infertility. *Obstet Gynecol Clin North America*. 2012;39(4):535–549.
22. Practice Committee of the American Society for Reproductive Medicine. Endometriosis and infertility: A committee opinion. *Fertil Steril*. 2012;98(3):591–598.
23. ESHRE. Endometriosis Guideline Development Group. September 2013.
24. Rose BI. Approaches to oocyte retrieval for advanced reproductive technology cycles planning to utilize in vitro maturation: A review of the many choices to be made. *J Assist Reprod Genet*. 2014;31(11):1409–1419.
25. Bjercke S, Tanbo T, Dale PO, *et al*. Comparison between two hCG-to-oocyte aspiration intervals on the outcome of *in vitro* fertilization. *J Assist Reprod Genet*. 2000;17(6):319–322.
26. Sohn SH, Penzias AS, Emmi AM, *et al*. Administration of progesterone before oocyte retrieval negatively affects the implantation rate. *Fertil Steril*. 1999;71(1):11–14.
27. Pinheiro OL, Cavagna M, Baruffi RLR, *et al*. Administration of β_2-adrenergic agonists during the peri-implantation period does not improve implantation or pregnancy rates in intracytoplasmic sperm injection (ICSI) cycles. *J Assist Reprod Genet*. 2003;20(12):513–516.
28. Kwan I, Bhattacharya S, Knox F, *et al*. Pain relief for women undergoing oocyte retrieval for assisted reproduction. *Cochrane Database Syst Rev*. 2013 31;(1):CD004829.
29. Cerne A, Bergh C, Borg K, Ek I, *et al*. Pre-ovarian block versus paracervical block for oocyte retrieval. *Hum Reprod*. 2006;21(11):2916–2921.
30. Ben-Shlomo I, Moskovich R, Golan J, *et al*. The effect of propofol anaesthesia on oocyte fertilization and early embryo quality. *Hum Reprod*. 2000;15(10):2197–2199.
31. Scott RT, Hofmann GE, Muasher SJ, *et al*. A prospective randomized comparison of single- and double-lumen needles for transvaginal follicular aspiration. *J In Vitro Fert Embryo Transf*. 1989;6(2):98–100.
32. Roque M, Sampaio M, Geber S. Follicular flushing during oocyte retrieval: A systematic review and meta-analysis. *J Assist Reprod Genet*. 2012;29(11):1249–1254.
33. Ludwig AK, Glawatz M, Griesinger G, *et al*. Perioperative and post-operative complications of transvaginal ultrasound-guided oocyte retrieval: Prospective study of >1000 oocyte retrievals. *Hum Reprod*. 2006;21(12):3235–3240.
34. Henkel RR, Schill W-B. Sperm preparation for ART. *Reprod Biol Eendocrinol*. 2003;1:108.
35. ESHRE. Capri Workshop Group. 2009.
36. Casper R, Haas J, Hsieh TB, *et al*. Recent advances in *in vitro* fertilization [version 1; referees: 2 approved]. *F1000Research* 2017. 6(F1000 Faculty Rev):1616.
37. Krishna D, Dhoble S, Praneesh G, *et al*. Gonadotropin-releasing hormone agonist trigger is a better alternative than human chorionic gonadotropin in PCOS undergoing IVF cycles for an OHSS Free Clinic: A randomized control trial. *J Hum Reprod Sci*. 2016;9(3):164–172.
38. Koch J, Ledger W. Ovarian stimulation protocols for onco-fertility patients. *J Assist Reprod Genet*. 2013;30(2):203–206.
39. Tao G. Small molecule agonists and antagonists for the LH and FSH receptors. *Expert Opi Therap Pat*. 2005;15(11):1555–1564.

Notes

You have reached the end of the module.
To view the module online and complete the assessment test, scan this **QR code**.
A certificate will be issued on completing all six modules.

Disclaimer: The matter published herein has been developed by clinicians and medical writers. It has also been validated by experts. Although great care has been taken in compiling and checking the information, the authors, BioQuest and its servants or agents, and sponsors shall not be responsible or in anyway, liable for any errors, omissions or inaccuracies in this publication whether arising from negligence or otherwise however, or for any consequences arising there from. The inclusion or exclusion of any product does not mean that the publisher advocates or rejects its use either generally or in any particular field or fields.

8344911 L21804E27

Developed & Designed by BioQuest Solutions **www.bioquestglobal.com**

For the use of Registered Medical Practitioner or Hospital or Laboratory only