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EXcellence in PCOS & Expertise in Reproductive Technology

MODULE 1:
OVULATION INDUCTION IN POLYCYSTIC OVARY SYNDROME

Brought to you by

THE PCOS SOCIETY

An Initiative of PCOS Society (India)

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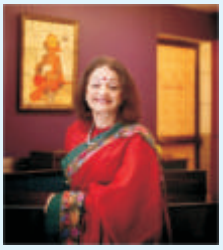
Foreword



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

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Ovulation Induction

Going Back in History

Infertility and its associated emotional burden, have been known since ancient times. Before recent advances in this field, there were not many treatment options available to help overcome this affliction. Ovulation induction, a pillar of infertility treatment, was just an abstract thought in the 1960s, and medical treatment was limited to psychological support and proper technique, timing, and frequency of intercourse. However, since the early 1960s, we have witnessed many amazing breakthroughs in infertility treatment.[1]

The first successful induction of ovulation with human gonadotropins was evidenced in 1958, materializing in pregnancy evidenced in 1960. In 1961, the first successful application of clomiphene citrate (MRL-41) was documented by Greenblatt *et al*. A major step forward was the application of human pituitary gonadotropins in hypophysectomized patients to achieve pregnancy and application of urine from menopausal women to enable ovulation induction in amenorrheic women.[1]

During the 1970s, the application of gonadotropin-releasing hormone in pulses, which were precisely spaced in time, proved to be successful in ovulation induction among women with hypogonadotropic amenorrhea of hypothalamic origin. The period between 1974 and 1977 was significant for further research in this field. During this period, many publications presented and discussed new evidence that demonstrated the efficacy of bromocriptine in reducing the circulating levels of prolactin and restoring the luteinizing hormone (LH) balance pulsatility, thereby helping reestablish normal menstruation and ovulation.[1]

Aim of Ovulation Induction

Ovulation induction is often the first-line approach for treating infertility, including cases of unexplained infertility, irregular menstrual cycles, and anovulation. The aims of ovulation induction are as follows:[2–4]

- To induce mono-follicular development and ovulation in anovulatory infertile women
- To overcome natural follicular selection process to increase the number of oocytes available for fertilization
- To restore normal fertility by generating normo-ovulatory cycles
- To mimic physiology and induce single dominant follicle selection and ovulation
- To augment ovulation in unexplained infertility
- For controlled ovarian hyperstimulation (COH) in intrauterine insemination (IUI) and assisted reproductive treatment (ART)

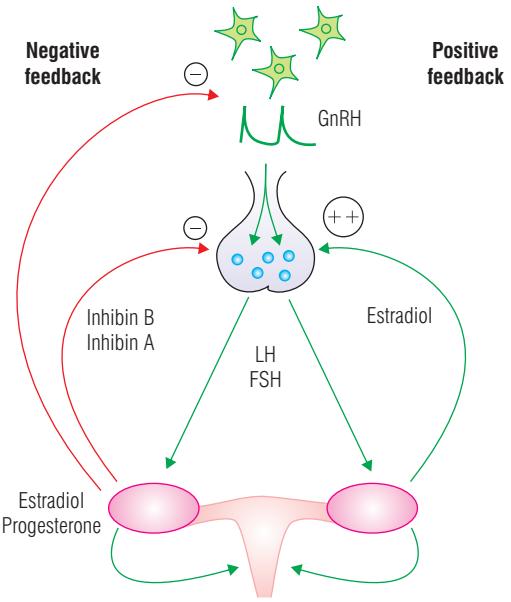
Physiology of Ovulation Induction

Ovulation is the result of a well-ordered series of events primarily controlled by the hypothalamic-pituitary-ovary axis. The menstrual cycle is directed by complex functional interactions between the ovaries and the hypothalamus-pituitary system which control each other by means of positive or negative feedback mechanisms.

The reproductive system functions in a classic endocrine mode initiated by pulsatile secretion of gonadotropin releasing hormone (GnRH) from the hypothalamus into the pituitary portal venous system. GnRH regulates the synthesis and subsequent release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary into the circulation. FSH and LH stimulate ovarian follicular development, ovulation, and corpus luteum formation and the coordinated secretion of estradiol, progesterone, inhibin A, and inhibin B (Figure 1).[5,6]

Ovarian steroids and inhibin have a modulatory effect on gonadotropin secretion, acting either directly at the pituitary level or through alterations in the amplitude or frequency of GnRH secretion. Negative feedback of ovarian steroids on FSH secretion is critical to the development of the single mature oocyte.

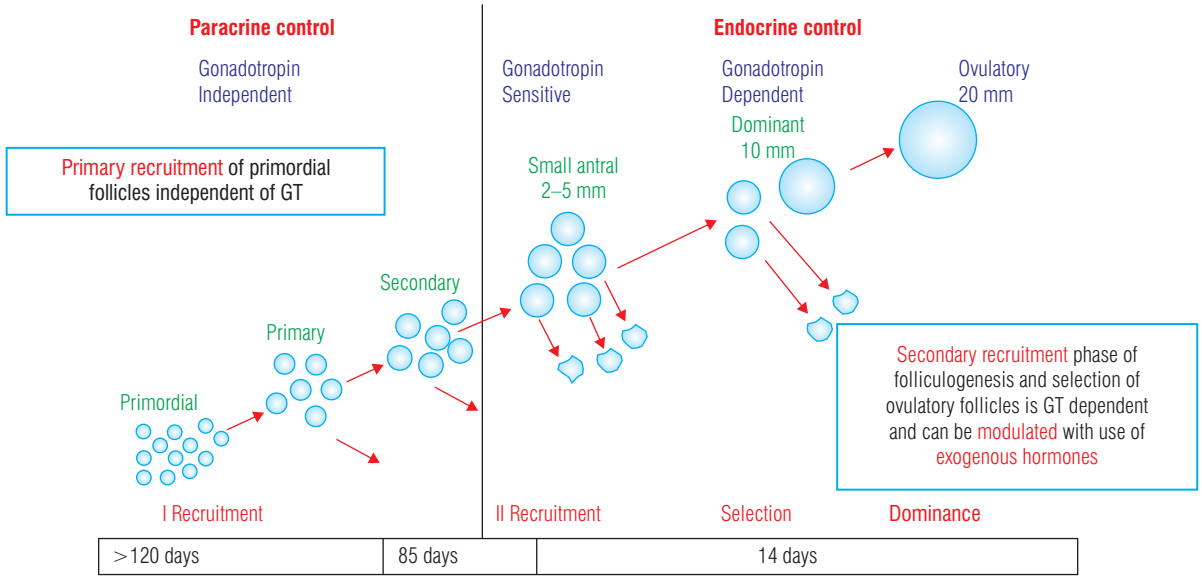
Figure 1: Neuroendocrine control of reproduction.



Folliculogenesis

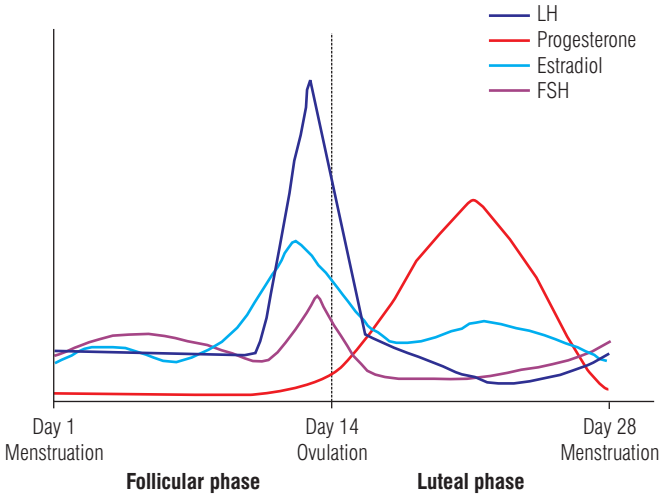
The follicular phase encompasses the period of recruitment of multiple follicles and emergence and growth of the dominant follicle. The number of follicles that starts growing in each cycle appears to be dependent upon the

Figure 2: Physiology of ovulation induction.



size of the residual pool of inactive primordial follicles. The total duration of time to achieve preovulatory status is approximately 85 days and the follicle destined to grow in a particular cycle are thus recruited 85 days before the actual ovulation. This primary recruitment is independent of the pituitary gonadotropins (Figure 2).

Figure 3: Hormonal levels during an ovulatory cycle.



An increase in FSH level above the FSH threshold of the cohort of follicles that has started developing at the beginning of the cycle is critical for rescuing follicles from undergoing apoptosis. FSH is responsible for the growth of the small antral follicles and also for aromatization of androgen in the granulosa cell resulting in production of estrogen. Together, FSH and estrogen increase the FSH receptor content of the follicle. Selection of the dominant follicle is established during days 5–7, when the follicle becomes 10 mm. Estradiol levels, derived from the dominant follicle, increase steadily and, through negative feedback effects, exert a progressively greater suppressive influence on FSH release. As the follicle achieves dominance LH receptors start appearing on the granulosa cells as a result of FSH action, so that despite the falling levels of FSH the follicle keeps growing as it can now also utilize LH. While directing a decline in FSH levels, the midfollicular rise in estradiol exerts a positive feedback influence on LH secretion. As the follicle reaches its pre-ovulatory stage once the follicular size is >16–18 mm, the rising estradiol levels result in a positive feedback on the LH secretion. Rise in LH during the mid-follicular phase results in reinitiating of the meiosis and ovulation. Luteinizing hormone surge also results in luteinization of the granulosa, and synthesis of progesterone and prostaglandins within the follicle.

This progesterone enhances the activity of proteolytic enzymes and together with prostaglandins results in digestion and rupture of the follicular wall. The progesterone-influenced midcycle rise in FSH serves to free the oocyte from follicular attachments, to convert plasminogen to the proteolytic enzyme, plasmin, and to ensure that sufficient LH receptors are present to allow an adequate normal luteal phase. Acting through its receptors, LH initiates luteinization and progesterone production in the granulosa layer.

The preovulatory rise in progesterone facilitates the positive feedback action of estrogen and may be required to induce the midcycle FSH peak. A midcycle increase in local and peripheral androgens occurs, derived from the theca tissue of lesser, unsuccessful follicles.

Normal luteal function requires optimal preovulatory follicular development (especially adequate FSH stimulation) and continued tonic LH support. Regression of the corpus luteum may involve the luteolytic action of its own estrogen production, mediated by an alteration in local prostaglandin and involving nitric oxide, endothelin, and other factors.

In case pregnancy occurs the hCG produced by early pregnancy rescues the corpus luteum, maintaining luteal function until placental steroidogenesis is well established.

Causes of Anovulation

The causes of anovulation have been described in Table 1.

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 (RIS)	5%

After an initial workup to determine the cause of anovulation and going through the required lifestyle changes, the timing of ovulation induction plays a critical role in determining outcomes. Commonly, treatment is initiated in the luteal phase to minimize the consequences of flare effects, which is usually seen in the first few days of treatment. Optimal timing is determined from the diameter of preovulatory follicles, their ultrasonographic appearance, and circulating estradiol-17β levels during spontaneous or induced cycles. Premature or delayed induction is detrimental to the follicle and can lead to unfortunate outcomes and probable complications.[7,8]

Diagnostic Hormonal Levels in Anovulatory Infertility

Diagnostic hormonal levels in anovulatory infertility have been described in Table 2.

Table 2: Diagnostic hormonal levels in anovulatory infertility

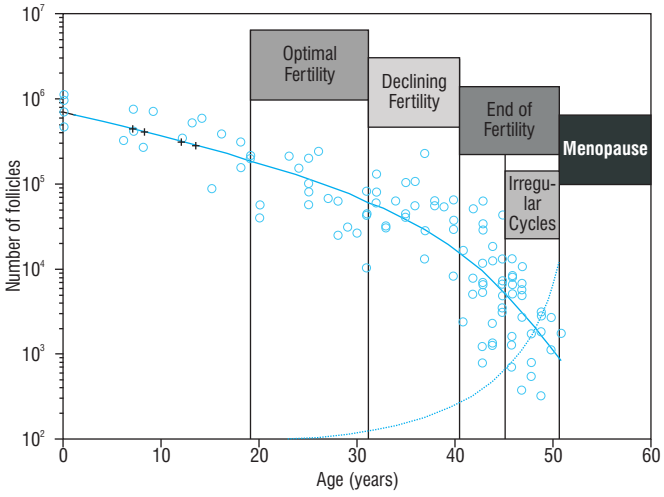
Hypothalamic: Underweight	n/↓FSH, ↓LH, ↓E2
Hyperprolactinemia	↓FSH, ↓LH, ↓E2
Ovarian failure/menopause	↑↑FSH, ↑LH, ↓E2
Mid-cycle	↑FSH, ↑↑LH, ↑E2
PCOS	↓/n FSH, ↑/n LH, ↑/n E2

What is Ovarian Reserve?

The loss of oocytes is a continuous process that begins just after the establishment of the oocyte pool during fetal life. At approximately 20 weeks gestation, the ovaries in the female fetus contain 6–7 million oocytes, but this number decreases rapidly. In total, 1–2 million oocytes remain at birth. At menarche, a female has about 300,000 ovarian follicles, which reduce to about a 1000 during menopause. With advancing maternal age, the number of egg cells that can be successfully employed for a possible pregnancy declines; thus, there is an inverse correlation between age and female fertility. Studies have suggested that the ovaries begin to go through an accelerated decline in fertility as early as 13 years prior to menopause (Figure 2).[9–11]

Ovarian reserve is a term used to establish the competence of the ovary to provide oocytes that are capable of fertilization, resulting in a healthy and successful pregnancy. It includes the secondary, preantral, and antral ovarian follicle pool.[10]

Figure 4: Quantitative (solid line) and qualitative (dotted line) decline of the ovarian follicle pool.[11]

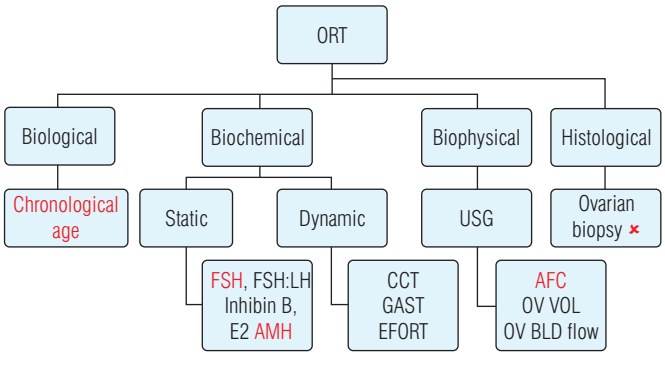


So ovarian reserve is the number of oocytes within the ovaries, growing follicles, small antral follicles, follicles that can be stimulated by FSH and oocytes that can be recovered after FSH. The main goal of individualized treatment in *in vitro* fertilization (IVF) cycles is to facilitate a superior probability of success with a lower risk from ovarian stimulation. This principally includes stimulation of the ovary and suppression of the pituitary. The treatment protocols in IVF are based on the most accurate calculation of ovarian response in an attempt to prevent poor and hyperresponse.[10,12]

Predictors of Ovarian Response/Reserve

Ovarian reserve tests (ORTs) (Figure 5) help to predict ovarian response, based on which the treatment plan is formulated. Hence, they should be easy to execute, reproducible, and should help distinguish between a normal, hyper and poor ovarian response. However, recent advances indicate that they are effective only in predicting the ovarian response to stimulation but are not accurate predictors of pregnancy or its outcome. Biological (age), biochemical, biophysical, and histological tests fall under the category of ORTs. Ovarian reserve test includes concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, inhibin, anti-Mullerian hormone (AMH); ovarian volume; ovarian antral follicle count (AFC); and ovarian biopsy. Most frequently done test include AMH and AFC with a good sensitivity and specific apart from age.[13,14]

Figure 5: Ovarian reserve tests.



Inhibin B and E2 are produced by early antral follicles in response to FSH, having the classical feedback loop of pituitary gonadal axis. With the decline of the follicle pool, serum levels of Inhibin B and E2 decrease leading to a rise in serum FSH level. Because these factors are part of feedback system, their serum levels are not independent of each other, and hence, they have to be measured collectively. Separately, their levels are poor predictors of ovarian reserve because their levels vary widely by assay, laboratory, population, and reproductive aging.[15]

Comparison of AMH and AFC for Assessment of Ovarian Reserve

Anti-Mullerian hormone produced by the pre-antral follicles witnesses its maximum expression in granulosa cells of secondary, pre-antral, and small antral follicles up to 6 mm in diameter. Thus, the level of AMH may correspond to the population of these follicles. Moreover, AFC is thought to correspond to the quantitative facet of ovarian aging. To compare significance, role, and reliability of AMH versus AFC in assessment of ovarian reserve, a study was conducted with 75 patients with PCO (polycystic ovaries) undergoing IVF and 75 non-PCO patients. Both (AMH and AFC) were found to have similar significance in oocyte retrieved on OPU (ovum pick up) in PCO patients. Difference in correlation for AFC and AMH was minimally significant in non PCO and not significant in PCO patients. Practically, AFC was found to be sufficient in terms of ova pickup.[12]

With the ovarian test one can only predict response and not the number of oocytes retrieved. So far, AFC (follicles of 2–10 mm size), which quantifies the number of antral follicles in the ovary by ultrasonography on day 3 of menstrual cycle, best predicts the quantitative aspect of ovarian reserve. However, it might be sometimes difficult for the patient to get ultrasound done on a specific day; additionally, it requires measurement of AFC by additional transvaginal ultrasound examination during early follicular phase. Therefore, in search of a better, time-independent parameter, serum anti-Mullerian hormone (AMH) emerged as a promising test to assess the ovarian reserve.[15]

Limitations of AMH and AFC

- AMH predicts extremes of response as compared to FSH[12,13]
- Significant negative interaction between age and AMH[12,13]
- AMH and age are independent determinants of oocyte yield[12,13]

Efficacy of Combined Markers

The efficacy of combined markers has been given in Table 4.

Table 3: Efficacy of combined markers

Markers vs. eggs collected/amp FSH	Regression co-efficient	Significance
Day 3 FSH+E2	0.37	p<0.01
Day 3 FSH+E2+AFC	0.7	p<0.001
Day 3 FSH+E2+AFC+ Inhibin B	0.8	p<0.001
Day 3 FSH+E2+AFC+AMH	0.73	p<0.001
AFC+Inhibin B	0.78	p<0.001
AFC+Day 3 AMH	0.7	p<0.001
AFC+Inhibin B+Day 3 AMH	0.78	p<0.001

Summary

- Age alone does not reflect the reproductive potential of ovarian reserve, of a woman
- Circulating AMH may accurately reflect the total developing follicular cohort - which may or may not represent the total ovarian reserve
- Available tests reflect, directly or indirectly, the size of the preantral and small antral (2–5 mm) follicle pool
- AFC and AMH are equally accurate predictors of high ovarian response to COH and allow us to identify the patients who are at increased risk of OHSS
- Relationship between AFC and AMH concentrations and prediction of response is more reliable than that observed with FSH, inhibin B and oestradiol on cycle day 3
- AMH best single marker and Day 3 FSH + E2 + AFC + Inhibin B are best combined markers to predict poor response to GT stimulation
- CCCT superior with regard to identification of potential low responders, & EFORT superior to identify hyper-responders but with a very high rate of false positive results
- AMH predicts extremes of response as compared to FSH
- Significant negative interaction between age and AMH
- AMH and age are independent determinants of oocyte yield
- Ovarian Stimulation is maximal with 150-200 IU of FSH
- Ovarian Response is mainly determined by the size of the FSH sensitive follicle cohort
- Poor response can be caused by ovarian ageing OR under dosing
- Prediction of Low Response possible but has only limited value
- A first cycle Low Response combined with an abnormal ORT is Poor Prognosis
- Prediction of Excessive Response allows dose reduction
- In ART population, first cycle IVF still remains the most informative test in terms of how woman will respond to ovarian stimulation

Drugs Used for Ovulation Induction

Clomiphene Citrate

Clomiphene citrate (CC) is the first treatment of choice in the management of infertility in normally estrogenized, anovulatory women, primarily in PCOS patients. It helps displace endogenous estrogen from estrogen receptor sites in the hypothalamus and pituitary leading to a favorable modification in pulsatile gonadotropin-releasing hormone (GnRH) secretion by blocking the negative feedback mechanism. Studies have shown >50% increase in endogenous FSH and a significant increase in LH concentration with clomiphene citrate.[14] It is administered orally from day 2, 3, 4, or 5 of spontaneous or induced MC for 5 days. The starting dose is 50 mg and most women (52%) show an ovulatory response to this dose.[16] The maximum dose of CC that can be administered is 150 mg. The dose correlates with body weight, age, indication for use (anovulation, PCOS,

COH), past response and cannot be accurately predicted. It requires empiric incremental titration to establish lowest effective dose. Treatment with CC should be discontinued if 2 consecutive cycles are anovulatory or when 6 ovulatory cycles fail to yield a pregnancy and if endometrial thickness <7 mm at the time of ovulation.

Use of clomiphene citrate requires careful monitoring to achieve favorable results. Clomiphene citrate has shown successful ovulation in 60%–90% patients and pregnancy rates in about 22% per cycle and a cumulative pregnancy rate between 60 and 70% in six cycles. Failure to ovulate may be due to high free androgen index, BMI, LH or insulin levels. Ovulation but no conception is due to its anti-estrogen effects on the cervical mucus or endometrium and high LH. Anti-estrogen effect on endometrium results in endometrial thinning in about 15–50%. It results in ER downregulation and depletion and suppresses pinopode formation. No pregnancies when endometrial thickness at midcycle <7 mm and is not dose related and recurs in repeat cycles.

The estimated rate of associated multiple pregnancies is about 2–13%. Sometimes, mild ovarian hyperstimulation syndrome (OHSS) is observed in 1–6 % of cases treated with clomiphene. Other minor side effects include visual disorders, flushes, nausea, and breast discomfort. Cases of ovarian cancer have also been reported; however, the association remains uncertain. CC treatment should be limited to six ovulatory cycles and additional cycles of ovulation induction with CC (maximum of twelve cycles) may be individually evaluated based on the cost-effectiveness and age of women and after discussion with the couple. [14,17,18]

The advantages of CC use are low cost, oral administration, few side effects and induction of monofollicular development in most cases.

Tamoxifen

Tamoxifen is a selective estrogen receptor modulator (SERM) that acts as an agonist on the estrogen receptors of the vaginal mucosa and endometrium. It is given in the dose of 20 mg from day 2 or 3 of the menstrual cycle for 5 days. If no response dose increased to maximum of 40 mg and discontinued if patient remains anovulatory despite 40 mg in two consecutive cycles.

It is at par with clomiphene citrate in efficiency. Limited evidence shows 50%–90% ovulation rates and 30%–50% pregnancy rates with Tamoxifen. TMX not the first-line treatment for OI in patients with adequate endometrium but is promising alternative to CC for ovarian stimulation in the subgroup of patients who failed to develop an adequate endometrial thickness in a previous CC induced OI cycle. This is primarily attributed to the higher score of cervical mucus and better functioning of the corpus luteum with tamoxifen. The drug has shown to increase the risk of endometrial cancer; however, it is improbable with short-term use. Despite exhibiting its usefulness, further studies with tamoxifen are warranted. [19,20]

Aromatase Inhibitors

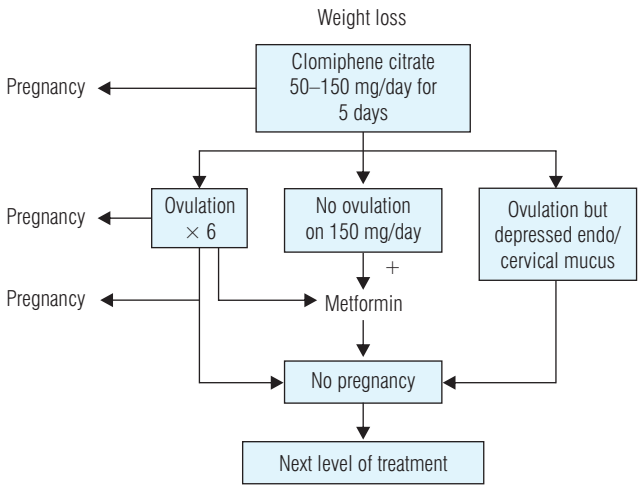
Aromatase inhibitors, a common regimen in the management of post-menopausal breast cancer, are a new group of drugs to join the arsenal of infertility treatment. Third-generation aromatase inhibitors, primarily letrozole and anastrozole, are used for ovulatory disorders and superovulation.[21] Several publications and even WHO are recommending letrazole as the first-line of drug for ovulation induction in women with PCOS. Their mechanism of action is based on reducing the peripheral conversion of androgens to estrogens in ovarian granulosa cells by blocking aromatase. Consequently, a decrease in estrogen serum levels and in its negative feedback in the hypothalamus and pituitary gland is noted, resulting in increased endogenous gonadotropin release. Therefore, aromatase inhibitors may be best suited for restoration of monofollicular growth.[21]

Letrozole has a short half-life, and no adverse impact is anticipated on the endometrium and in the luteal phase if it is administered in the early follicular phase. Letrozole displays an ovulation rate, live-birth rate, and miscarriage rate comparable to those of clomiphene citrate with a significantly thicker endometrium.[21]

A recent meta-analysis publishes on OI in women with WHO group II anovulation, showed both letrozole and the combination of clomiphene and metformin are superior to clomiphene alone in terms of ovulation and pregnancy. Letrozole is the only treatment showing a significantly higher rate of live birth when compared with clomiphene alone.[22]

Figure 6 shows the treatment strategy to induce ovulation with oral ovulogens.

Figure 6: Treatment scheme for oral ovulogens.



Failure to conceive within 6 CC/Letrazole/Tamoxifen-induced ovulatory cycles is indication to expand diagnostic evaluation to exclude other infertility factors or to change treatment strategy if evaluation already complete.

Gonadotropins

Gonadotropins, are the second-line pharmacological treatment and include follicle-stimulating hormone (FSH) or human menopausal gonadotropin (hMG). Urinary or recombinant gonadotropins are commonly used for ovulation induction, usually after other less complicated and costly methods have failed. However, premature ovarian failure does not respond to gonadotropins. It primarily causes an FSH upsurge, which fuels the proliferation of granulosa cells and results in

follicular growth. Due to the higher cost of this therapeutic modality, an evaluation of the tubal patency is recommended prior to initiating the ovarian stimulation with gonadotropins if this procedure was not performed prior to initiating CC treatment. Although the primary objective is to promote the development of a single mature follicle, it is common to expect multiple pregnancies. Other adverse outcomes include premature rupture of follicles (if GnRH analogs not used) and OHSS. [23,24]

Indications for gonadotropin therapy are

- Clomiphene / Letrazole/ Tamoxifen resistance
- Clomiphene / Letrazole/Tamoxifen failure
- Persistent hypersecretion of LH
- Negative post-coital test
- IUI/ART cycles

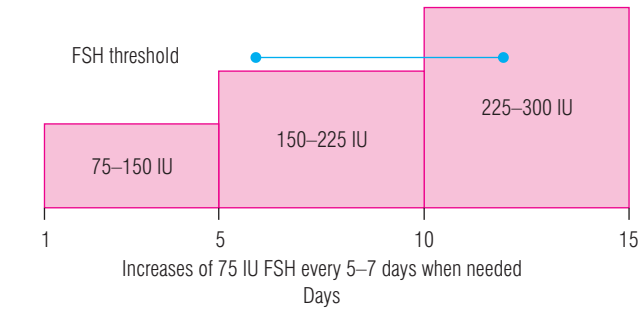
All gonadotropin cycles require specific protocols & stringent monitoring by TVS for follicular growth and endometrial thickness and serial serum E2 if hypo or hyper response.

Regimens of Gonadotropin Therapy

Conventional Step-up Protocol

The protocol aims at maintaining the FSH level above the threshold value. Starting dose is 50–75 IU for an IUI cycle and 150–225 for an ART cycle on day 2 or 3 of the menstrual cycle. The dose is increased by 37.5–75 IU for another 5–7 days, if the follicular and estradiol response is inadequate. Once response is seen the same dose is continued till the day of hCG. (Figure 7). The disadvantage of this protocol is that the supraphysiological doses of FSH provoke initial development of a large cohort, stimulate additional follicles, and even rescue those follicles destined to undergo atresia and can result in multifolliculogenesis with the risk of OHSS and multiple pregnancies. [25,26]

Figure 7: Conventional step-up protocol.[2,25]

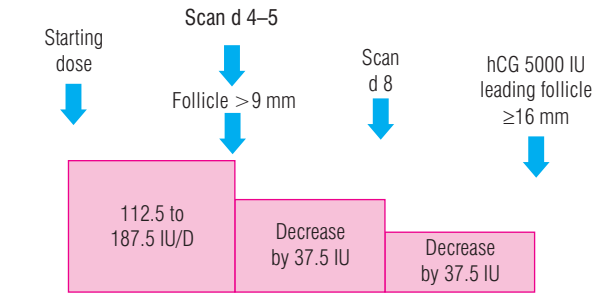


FSH: Follicle-stimulating hormone.

Step-down Protocol

The step-down protocol tries to replicate the normal physiological hormonal profile of FSH in the natural menstrual cycle. The starting dose is 100–150 IU from day 2 or 3 of menstrual cycle, and then decreased by 37.5 or 50 IU every 3–5 days. hCG is administered to trigger ovulation once the follicular diameter is 18 mm (Figure 8). The advantage of this protocol is that it results in monofollicular development as it is physiological and thus reduces the incidence of OHSS and multiple pregnancies. [2,25]

Figure 8: Step-down protocol.[2,24]

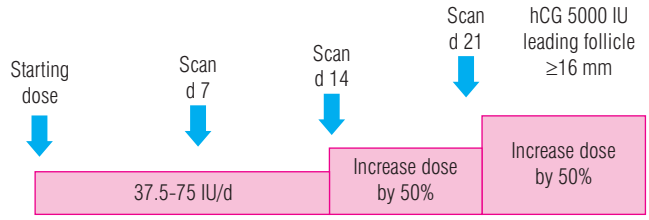


hCG: Human chorionic gonadotropin.

Chronic Low-Dose Protocol

Most suitable protocol in women with PCOS. The starting dose is 35.5–75 IU. The first increment in the dose is done on 14th day either by 50% or 37.5 IU. The next increment is only after 7 days. Dose increment can be done to a maximum of 225 IU/day. Once dominant follicle emerges, dose of FSH maintained same until the follicle reaches 18 mm when hCG trigger is given (Figure 9).

Figure 9: Chronic low-dose protocol.[2]



hCG: Human chorionic gonadotropin.

The disadvantage of this protocol is that the treatment cycles are as long as 28–35 days, which requires proper counselling of the patient and patience in both doctor and patient. The advantage is that there is reduced multiple folliculogenesis and risk of OHSS. [2,27]

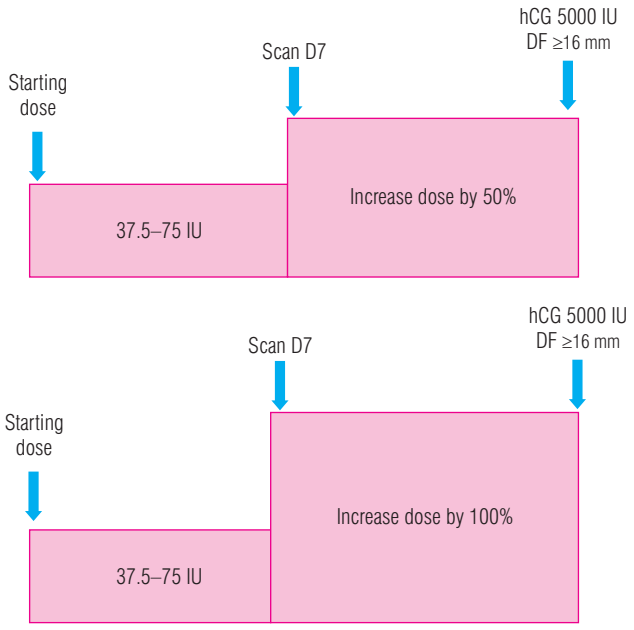
Low-Dose Protocol

To overcome the disadvantage of long treatment cycle one can use the low dose protocol. Here the starting dose is 37.5–75 IU per day and the dose is increased every 7 days either by 50% or 100% till dominant follicle emerges and then the same dose is maintained till hCG administration (Figure 10).

Sequential Protocol

The starting dose is 37.5–75 IU. The first increment in the dose is done on 14th day either by 50% and next increment is only after 7 days. The FSH

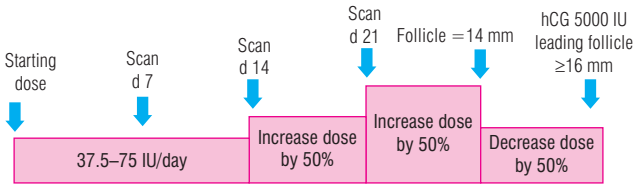
Figure 10: Low-dose protocol.[2]



hCG: Human chorionic gonadotropin.

threshold dose decreased by 50% when leading follicle is 14 mm and continued in the same dose until the follicle reaches 18 mm when hCG trigger is given (Figure 11).[2]

Figure 11: Sequential protocol.[2]



HCG: Human chorionic gonadotropin.

Newer Gonadotropins Used for Ovulation Induction

Corifollitropin Alfa

Corifollitropin alfa is the first long-acting recombinant FSH that has been approved by the European Commission for COS. It is a recombinant fusion molecule of FSH and the carboxy terminal peptide (CTP) of hCGb subunit with sustained follicle stimulating activity. It is a gonadotropin with different pharmacokinetic properties but similar pharmacologic features and interacts only with the FSH receptor and lacks LH activity. It has a half-life of 65 hours and t_{max} -of 25–45 hours and can sustains multifollicular development for one week. The dose is either 100 μg (<60 kg) or 150 μg (> 60 kg). After 7 days, if criteria for hCG administration is not reached Rec FSH 150–200 IU is added daily till hCG criteria is reached. Effective in stimulation of multi-follicular growth for IVF but less suitable for induction of monofollicular growth and therefore contraindicated in IUI cycles. Ovarian response induced may decrease with the patient's age and ovarian reserve.

Corifollitropin alfa in both doses was safe and well-tolerated with incidence of OHSS requiring hospital admission in 2 to 3%. Less suitable in cases with known risk of hyperresponse in women with PCOS, previous history of OHSS.[28,29]

Combination of Follitropin Alfa and Lutropin Alfa in the ratio of 2:1 ratio (Pergoveris) (r-hFSH 150 IU and r-hLH 75 IU).

Can be used in women with suboptimal response in previous cycles, young poor responders and older women (>35 y) and those women who have low basal LH.[30]

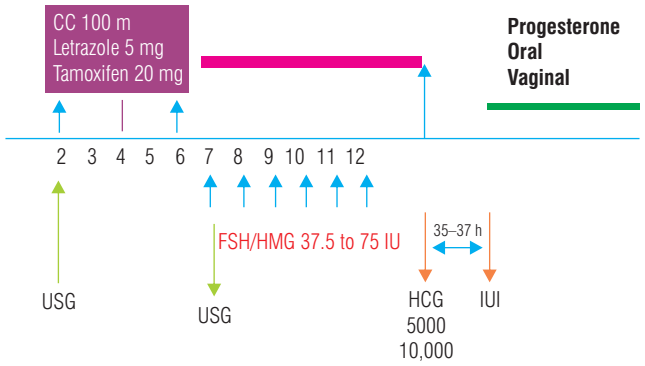
Recombinant LH

The physicochemical, immunological, and biological activities of rLH are comparable with those of pituitary LH. Recombinant LH (rLH) may help in situations where there is an inadequate response to FSH or those women with LH deficiency like WHO type I anovulation and in women where LH decreases after administration of GnRH agonist or antagonist.[31]

Combination Therapies Oral Ovulogens and Gonadotropins

FSH and hMG can be used alone or in combination with CC/Letrazole/ Tamoxifen (Figure 12). Oral ovulogens stimulates recruitment of number of small follicles and GTs sustains the growth of recruited follicles.[32]

Figure 12: Combination of oral ovulogens and gonadotropin protocol.



Gonadotropin-releasing Hormone – Agonist and Antagonist

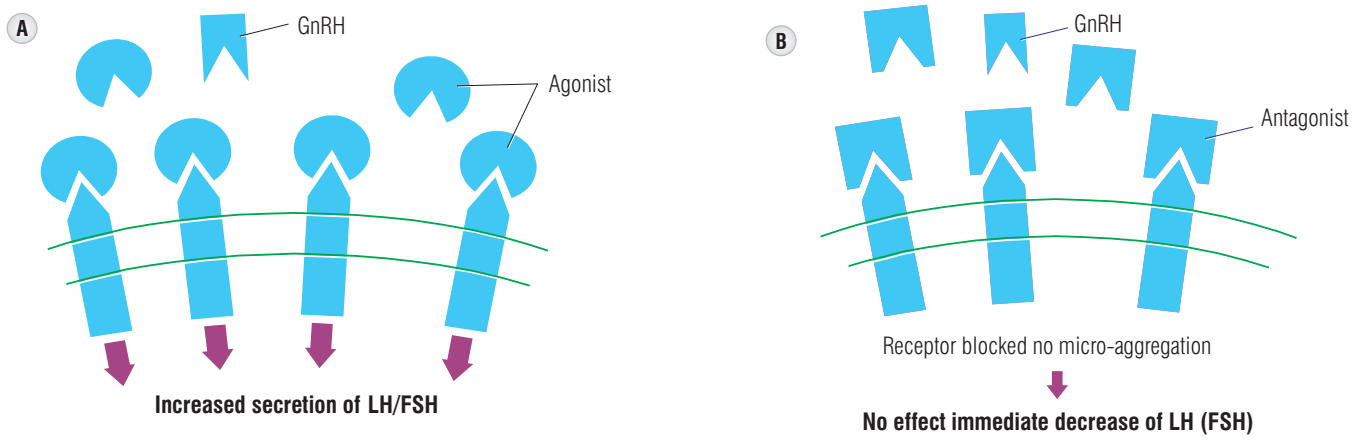
For years GnRH agonist have been used in the long and short protocol to prevent premature LH surge. With the introduction of GnRH antagonist early in the 21st century to prevent premature LH surge has several advantages like avoidance of an acute stimulation of endogenous gonadotropins (GT), a dramatic reduction in the length of analog treatment because of their ability to inhibit directly the premature LH surge and a reduction in the gonadotropin requirement used for ovarian stimulation.

With both GnRH agonist and antagonist present in our armamentarium for COS, we also need to know whether the probability of live birth per started cycle is dependent on the type of analog used? The initial use showed that the probability of clinical pregnancy was significantly lower with GnRH antagonist compared with GnRH agonists. But today the GnRH antagonist is the first-choice analog instead of GnRH agonist after the meta-analysis published by Cochrane in 2002[33], which showed no significant difference in the probability of clinical pregnancy and live births. Moreover, use of GnRH antagonist is associated with lower incidence of ovarian hyper stimulation syndrome (OHSS), where there is an option of using GnRH agonist for trigger. Efficacy and safety of GnRH antagonist has made it the analog of choice. The hypothalamic decapeptide gonadotropin-releasing hormone (GnRH) binds to specific receptors on pituitary gonadotrophs. GnRH agonist result in initial flare effect and with prolonged activation of GnRH receptors by GnRH leads to desensitization and consequently to suppress gonadotropin secretion. By contrast, GnRH antagonists compete with GnRH for receptors on inhibit GnRH-induced signal transduction and consequently gonadotropin secretion. These compounds are free of agonistic actions, which might be beneficial in certain clinical applications (Figure 13).[34]

Advantages and Regimes

The advantages and regimes of GnRH have been listed in Table 4.

Figure 13: Mechanism of action of GnRH agonist (A) and antagonist (B).[33]



GnRH: Gonadotropin-releasing hormone; FSH: Follicular stimulating hormone; LH: Luteinizing hormone.

Table 4: Advantages and regimes of GnRH agonists and antagonists[34–36]

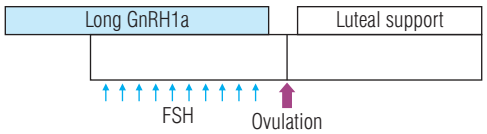
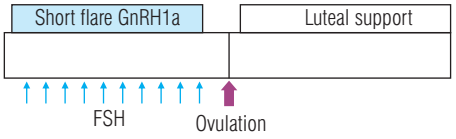
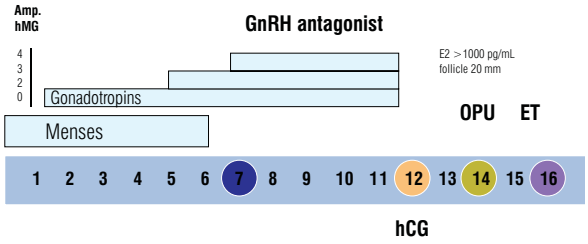
Advantages	GnRH agonist	GnRH antagonist
	• Fewer cycles cancelled • Eliminates LH surge • Controls basal LH secretion • Oocyte recovery programed • Enhances intrafollicular growth and recovery of better quality oocytes • Widens the window of uterine implantation thus increasing pregnancy rates	• For prevention of premature LH surge • Immediately suppress GTs by blocking GnRh receptor, restricting treatment only to those days when LH surge likely to occur • Mechanism of action dependent on equilibrium between endogenous GnRh and dose of applied antagonist • Lower GT consumption • Lower risk of OHSS • Opportunity to give GnRH agonist for trigger • Reduces the burden of treatment
Regimes	• **Long or desensitization protocol**  • **Short protocol** 	• **Multiple-dose protocol (Lubeck protocol)** 

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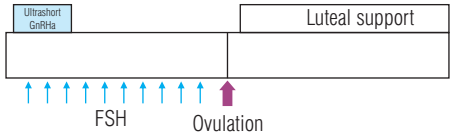
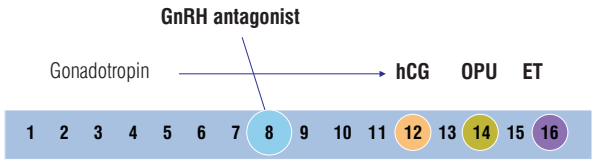
Table 4: Advantages and regimes of GnRH agonists and antagonists[33–35] *Continued from previous page*

Advantages	GnRH agonist	GnRH antagonist
	• **Microdose flare protocol** ◆ Similar to the short protocol with reduced agonist dose • **Stopping gonadotropin-releasing hormone agonist protocol** ◆ Started in the luteal phase 1 week before the expected start of menses, and stopped at the initiation of gonadotropin therapy. However, it is not popular due to the erratic response. • **Ultrashort protocol** 	• **Single-dose regimen (French protocol)** 

Induction of Follicular Maturation

1. Urinary hCG: 5000–10,000 IU IM
2. Rec hCG: 250–500 mcg SC
3. GnRha-1 mg SC: Requires a modified luteal phase support

Function

Cellular and nuclear maturation for final meiotic resumption after sperm entry

Follicular changes for follicular rupture and ovulation

Table 5 summarizes the treatment modalities in different anovulatory states

Table 5: Treatment modalities in different anovulatory conditions

Condition	Treatment
Hypothalamo-pituitary failure	• Gonadotropins • GnRH pulses Dose - 15 ug or 20–25 ug either IV or SC, respectively at 90 minutes interval till hCG administration
Hyperprolactenemia	Dopamine agonists • Bromocriptine 2.5–20 mg daily • Cabergoline 0.25–1 mg twice weekly • Quinagolide 25–150 daily
Hypothalamo – Pituitary dysfunction PCOS	• Weight loss • Clomiphene citrate/Letrazole/Tamoxifen • Gonadotropins • Ovarian surgery - LOD • Insulin sensitizers???
Hypergonadotropic hypogonadism	• Success rate poor • GnRHa in ultra-short protocol with hMG • Oocyte donation/Adoption • HRT to prevent osteoporosis and CVS disease

Adjuncts to Ovulation Induction

At times, adjuvant drugs are required along with ovulation induction to optimize ovulation. Table 6 gives the different adjuvants used in different conditions.

Table 6: Adjuvants in ovulation induction

Adjuvants in treatment of PCOS	Adjuvants in treatment of poor responders	Other adjuvants
• Glucocorticoids-prednisone, methyl prednisolone and dexamethasone • Metformin/Myoinositol • N Acetyl cysteine • Melatonin • Vitamin D • Chromium polynicotinate • L Methyl folate	• Growth Hormone/GH-releasing factor (GHRF) • Pyridostigmine • DHEA/Testosterone • L-arginine • Aromatase inhibitors • Estrogen pre-treatment • Antioxidants • GCSF	• Antioxidants • Micronutrients • Dopamine agonist • Aspirin • Sildinafil

The following 3 Tables describe the evidence for the adjuvants to be used in PCOS, poor responders and general adjuvants to increase implantation and pregnancy rates.

Table 7: Adjuvants in poor responders

Adjuvants in poor responders	Application
Growth Hormone	• GH enhances GT effects on granulosa cells • Stimulation of the insulin-like growth factor-I (IGF-1), which stimulates follicular development, E production, and oocyte maturation • Initial studies showed beneficial effect on the probability of clinical pregnancy and live birth but recent studies showed no statistically significant benifit
Testosterone and DHEA	• Pre-treatment with DHEA or testosterone may be associated with improved live birth rates • The overall quality of the evidence is moderate • There is insufficient evidence to draw any conclusions about the safety of either androgen • Definitive conclusions regarding the clinical role of either androgen awaits evidence from further well-designed studies
LH/ hCG or aromatase inhibitor	• Androgen-modulating agents • No beneficial effect on PR but decrease duration & dose of GT
L-arginine	• No beneficial effect on PR
Low-dose aspirin	• Beneficial effect of low-dose aspirin is not currently supported
Colony stimulating factor 1 (CSF-1)	• Controlled trials required to assess the efficacy of CSF-1 as adjuvant therapy for poor responders

Table 8: Adjuvants in PCOS

Adjuvant in PCOS	Application
Dexamethazone	• Beneficial in elevated DHEAS androgen levels • Highly effective adjunct to clomiphene citrate in PCOS women • Should be avoided in women with diabetes
Metformin	• Decreased the risk of OHSS when gonadotropins used for OI • Increase the LBR among women undergoing OI with GTs
Myo-inositol	• Insulin sensitizer which has beneficial effects on ovarian function and response to ART in women with PCOS • No data on its effects on PR and LBRs
Vitamin D	• Influence ovarian endocrine function and likely hood of pregnancy • Inverse associations 25-hydroxyvitamin D levels and insulin resistance, features of hyperandrogenism and circulating androgens in women with PCOS
N-acetylcysteine	• Improves insulin sensitivity & decreases androgen level • can improve the ovulation and pregnancy rates • May also have some beneficial impacts on endometrial thickness
Melatonin	• Regulates a variety of important central and peripheral actions related to circadian rhythms and reproduction • Powerful free radical scavenger and has a broad-spectrum antioxidant property • Melatonin deficiency seems to be involved in pathophysiology of PCOS
Chromium polynicotinate	• Active component of glucose tolerance factor which is responsible for binding insulin to cell membrane receptor sites • Improves insulin sensitivity • Stimulates the metabolism of sugar, fat, & cholesterol

Monitoring Ovulation Induction Cycles

Monitoring helps the physician to choose the most suitable protocol, to obtain best possible outcome avoiding complication. Base line USG provides invaluable information on ovarian morphology and allows to choose appropriate stimulation regimen to prevent OHSS & multiple pregnancies and prediction of patients response to ovarian stimulation.

Induction of ovulation and IVF protocols can be monitored successfully by measuring endometrial thickness and size of ovarian follicles using trans vaginal ultrasound. USG also allows diagnosis of disorders and complications of ovulation induction.[36,37] Monitoring ovulation induction cycle improves chance of safe and effective treatment with OI. The main complications of ovulation induction include OHSS, multiple pregnancies, and spontaneous abortions.[38]

Table 9: Other adjuvants

Other adjuvants	Application
Antioxidants	• Supplementing diets with mitochondrial nutrients such as CoQ10 and r-alpha lipoic acid may potentially be beneficial • Increased oxidative stress results in ◆ altered glucose metabolism ◆ antiglycation defenses ◆ mitochondrial dysfunction ◆ progressive metabolic impairment
Micronutrients	• Iron: Reduces risk of anovulatory infertility • Green tea: Has positive effect on glucose metabolism • Zinc: Plays an important role in ovulation • Vitamin B12, folic acid pyridoxine: Reduces homocysteine levels, which if raised can lead to defective ovulation • Chaste berry: Used to treat hormonal imbalances in women because it has an immediate effect on pituitary gland • L Arginine: Helps to –optimize oocyte quality & maturation • Alpha lipoic acid: Modulates insulin sensitivity
L-methyl folate	• Role in cell growth & differentiation • Reduces homocysteine levels and prevent cardiovascular risk factors associated with PCOS
Coenzyme Q10	• Functions as an intercellular antioxidant. Intermediate of the electron transport system in the mitochondria. • Necessary for ATP production. • Important for aging ovary, PCOS.
Vit D_3	• Sufficient levels of Vit D_3 improves menstrual dysfunction and insulin resistance in PCOS. • Sufficient levels of Vit D_3 associated with more clinical pregnancy rates following IVF.
Dopamine Agonists	• Treatment of hyperprolactinemic disorders, such as pituitary adenomas and idiopathic hyper-prolactinemia • Reduces the production of VEGF by follicles after hCG administration and thus occurrence or intensity of OHSS
Aspirin	• More research is needed on potential effects of low-dose aspirin on fecundity and implantation
Sildenafil	• Increase uterine artery blood flow and endometrial thickness by augmenting the vasodilatory effects of nitric oxide by preventing the degradation of cGMP • May have beneficial impact on the endometrial thickness • Data are limited regarding live- birth rate

Monitoring luteal phase helps confirm ovulation and pregnancy by beta hCG assay and USG documentation of pregnancy (20 days post-ovulation when beta hCG is 1000 mIU/mL), an end point desired of tracking ovulation.

Table 10 gives us the reason why monitoring of OI cycles is necessary.

Table 10: Why monitor ovulation induction cycles?

Patient's initial parameters	Ovarian response to ovulation induction	Completion of therapy
• Baseline Scan–TRO Ovarian or uterine pathology, AFC • Baseline hormonal profile–Ovarian reserve, FSH: LH ratio, androgen excess, thyroid profile, and hyperprolactinemia • Choose appropriate stimulation regimen to prevent OHSS, multiple pregnancy, and predict response to ovarian stimulation	• Confirmation of downregulation after GnRH agonist • Determine response to drug • Determine the dose and length of GT TX • Determine optimal time for hCG administration • Detect ovulation • Time OR • Identify-poor responders and women at risk of OHSS	• Diagnose complications of OI 1. Premature luteinization 2. LUF 3. Endogenous LH surge 4. Retention/Functional cyst • Confirm pregnancy • TRO multiple pregnancy • TRO late onset OHSS

- **Serial transvaginal (TVS) ultrasound:**

  Ultrasound provides information on:

  - Follicular growth
  - Timing of ovulation
  - Presence of cyst
  - Ovarian morphology/reserve/blood flow
  - Endometrial thickness/morphology/texture/blood flow
  - Feasibility of oocyte retrieval

  Baseline scan on day 2[36] is essential to

  - Identify morphology of ovary and adenexal abnormalities – ovarian cyst, hydrosalpinx
  - Assess the ovarian reserve (AFC)
  - Identify uterine abnormalities – myoma, adenomyosis, polyps, intrauterine adhesions, endometrial abnormalities, congenital anomalies
  - Decide the stimulation protocol for adequate response

OI initiated on day 2/3 only if

- Follicular size is <10 mm
- Absence of ovarian cyst
- Endometrial thickness <6 mm
- E2 levels <50 pg/mL and P4 <1.5 ng/mL

Adjustment of GT dose is done with serial USG and values of log increment in estradiol levels. In an oral ovulogen cycle after day 2 subsequent scan is done 8 days later to determines timing of next scan. If dominant follicle >14 mm next scan done within 24 hours as rate of follicular growth is about 2–4 mm/day. If the follicular size is less than 14 mm the subsequent scan is after 48 hours. hCG is administrate when the dominant follicle is 20 mm. In a gonadotropin stimulated cycle after day 2 scan the subsequent scan is done 4 days later to determine the response. The next scan is done 2 days later to determine the timing of subsequent scan and GT dose. Administration of hCG is done when dominant follicle 18–20 mm.

Sonographic Indicators of Ovulation

- Sudden collapse of growing follicle
- Central echoes within the follicle
- Crenation of the follicular wall
- Decreased follicular size
- Appearance of follicular fluid in cul-de-sac
- Formation of CL – internal follicular area becomes isoechogenic with respect to surrounding ovary

Monitoring endometrial changes when tracking follicular growth

Endometrial thickness - a reliable bioassay of the patient's estrogenic status

Endometrial changes correlate with plasma E2 & P4 levels (Figure 14)

- Early proliferative phase – translucent and thin on either side of midline echo
- Late proliferative phase – increase in thickness with hyporeflective area in the center
- Following ovulation – shrinks in thickness, becomes dense echogenic on either side of midline echo
- Serial serum estradiol E2 levels: They correlate closely with the stage of development of the dominant follicle. A value of more than 200–250 pg/mL in an natural cycle indicates impending ovulation. Estradiol levels in an gonadotropin cycle need to be monitored more frequently in an hypo- or hyperresponder more stringently.[36] Values correlate with the endometrial thickness and morphology as well as the number and size of follicles.

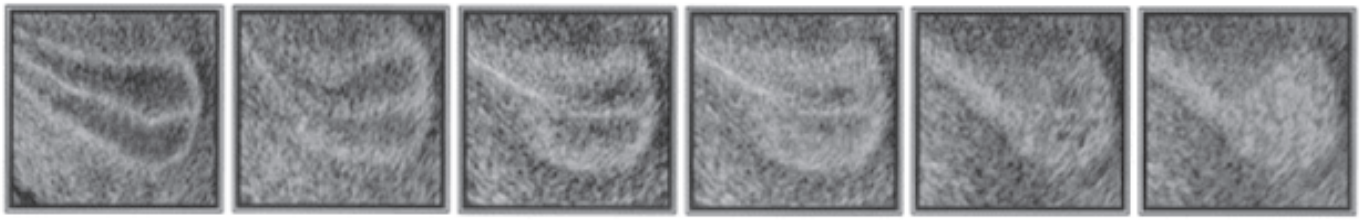
Cancellation of ovarian stimulation cycles

- Definite Indication
  - Poor follicular growth
  - E2 levels <100 pg/mL on day 5–6
- Possible Indication
  - Adenexal cyst secondary to GnRh agonist
  - Endogenous LH surge
  - Steady decline in E2 levels
  - E2 <250 pg/mL per mature follicle on day of hCG

Endocrine consequences of GT-hCG include:

- Abnormal FSH/LH ratio
- Blockade of LH surge
- Hyperprolactenimia
- Premature lutenization
- Short luteal phase
- Follicular atresia/dyssynchrony
- Hetrogenecity of estradiol response

Figure 14: Different stages of endometrial development.



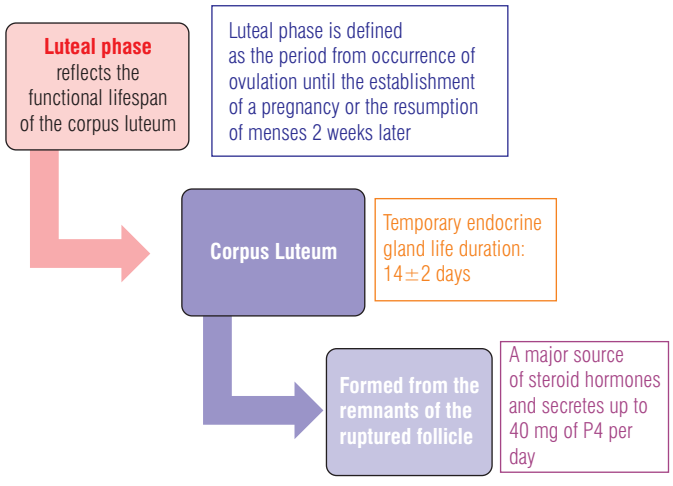
Abnormal response to controlled ovarian stimulation

1. Premature lutenization
   - Follicle <15 mm with echoes
   - Correlates with high P4 levels in follicular phase
   - Premature & suboptimal LH surge
   - Progesterone production but no ovulation
   - Oocyte maturation without follicular rupture
   - Poor quality oocytes and embryos
   - Endometrium out of phase
   - Reduces implantation rate
2. LUF
   - Diagnosed when dominant follicle is still apparent 48 hours after administration of hCG or LH surge
   - Size 34–36 mm with internal echoes
   - Perifollicular RI 0.51–0.59
3. Endogenous surge
   - Seen on USG as premature rupture of follicles at diameter <16–17 mm
   - Compromised oocytes and embryo quality-result of exposure to inappropriate LH levels
   - Requires extensive endocrine monitoring
   - Prevented with use of GnRH agonist or antagonist
4. Functional cyst
   - Presence of cyst at pre-stimulation base line scan following GnRh agonist stimulation for downregulation
   - Characterized by sharp edges and anechogenic contents
   - Initial FSH surge when GnRh agonist is commenced triggers functional cyst formation
   - Requires USG guided aspiration before commencing OI
5. Persistent/Retention cyst
   - Presence of cyst at baseline scan – follicle from previous cycle or persistent CL

Corpus Luteum Support

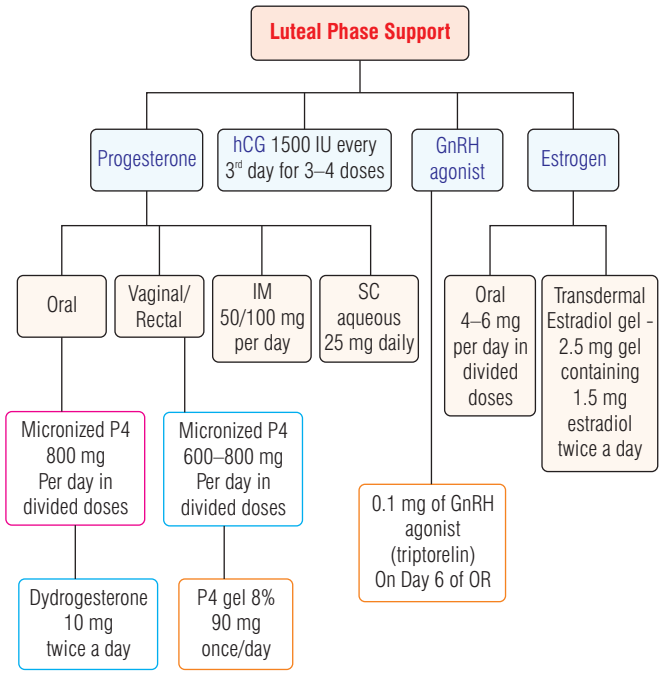
Figure 15 highlights the information about the function of corpus luteum.

Figure 15: Function of corpus luteum.



One could use progesterone, estrogen, hCG and GnRH agonist for luteal phase support. Figure 16 gives an overview of different agents that can be used for luteal phase support.

Figure 16: Drugs used to support corpus luteum function.



Important points regarding LPS

- P4 levels >15 ng/mL in luteal phase of stimulated cycle appear to reduce early pregnancy loss and increase implantation rate
- LPS improves PRs of IUI only in GT cycles and not in natural or CC cycles
- Significant effect in favor of P4 for LPS
- Addition of E2 or hCG to P4 did not improve outcome
- hCG or hCG plus P4 associated with higher risk of OHSS hence, should be avoided
- Addition of GnRHa to P4 appears to improve outcomes
- No evidence favoring a specific route or duration of administration of P4
- Administration of P4 before ovulation is associated with a lower PR due to premature secretory changes in the endometrium and subsequent decreased implantation rate.
- GnRHa used for triggering ovulation as surrogate for mid-cycle LH surge results in shorter duration of LH surge and the luteal phase needs to be modified for optimal results by giving estrogen along with progesterone or give a bolus dose of 1500 IU hCG on day of oocyte retrieval.
- Usually given till 8 weeks, when the placenta takes over the function of producing hormones
- Selection of protocol

Summary

- New treatment modalities have revolutionized infertility treatment with good rates of successful pregnancies.
- Clomiphene citrate is the first treatment of choice in the management of infertility in estrogenized, anovulatory women; it is used primarily in PCO patients and has shown successful ovulation in 60%–90% patients and pregnancy rates in 10%–40% women.
- Tamoxifen is an antiestrogenic compound that acts as an agonist on the estrogen receptors of the vaginal mucosa and endometrium, its efficiency being at par with clomiphene citrate.
- Gonadotropins are hormones synthesized by the anterior pituitary gland; they mainly include FSH and LH. They play a crucial role in the process of ovulation and are usually used after other less complicated and costly methods have failed.
- Regimens of gonadotropin therapy used commonly include conventional step-up protocol, step-down protocol, chronic low-dose protocol, and sequential protocol.
- GnRH agonist regimens include long or desensitization protocol, short protocol, microdose flare protocol, stopping gonadotropin-releasing hormone agonist protocol, and ultrashort protocol.
- GnRH antagonist regimens include multiple-dose protocol (Lubeck protocol) and single-dose regimen (French protocol).
- Off-label prescription of adjuvants for the treatment of female infertility is widespread.
- Many supplements are inexpensive and generally well tolerated, such as low-dose aspirin and DHEA, but evidence is insufficient to demonstrate that they increase live-birth rate.
- GH, data support their effect in increasing LBR, but they are expensive, and their use is associated with significant precautions, so appropriate counseling should be provided.
- Significant precautions, so appropriate counseling should be provided.
- In the field of reproductive medicine, it is commonplace for an innovative practice to become widely used before demonstration of its efficacy.
- Patients with infertility are particularly vulnerable to trying new treatments in hopes of conceiving.
- Good medical practice dictates that the physician keep the best interest of their patients in mind and counsel patients appropriately about the best evidence available and potential adverse effects of the treatments prescribed.

Selection of Protocol

Aim of ovulation induction is to overcome natural follicular selection process to increase the number and quality of oocytes available for fertilization. Patients respond differently to ovarian stimulation and so our objective should be to optimize response and outcome and at the same time minimize the risks .

Controlled ovarian stimulation (COS) results in

1. Rescue of oocytes that may be intrinsically abnormal
2. Oocytes intended to be atretic are forced to be recruited and ovulated
3. Disruption of the intra-follicular physiology which overrides their endogenous pattern of control which optimize normal selection process

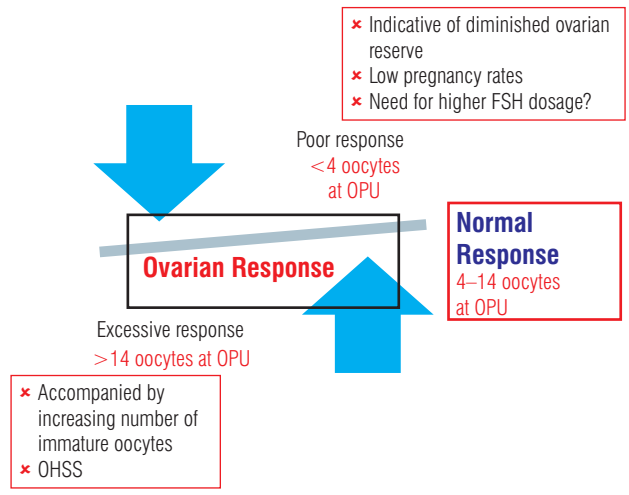
Before starting ovarian stimulation, it is important to analyse the ovarian reserve, define goal of ovarian stimulation and select the correct stimulation protocol based on age, AMH, and AFC amended further for the BMI.[23,29,38]

Prediction of Ovarian Response

Successful end-point of any ART treatment is live birth and this depends on various factors including adequate number of follicles being stimulated, adequate number of oocytes retrieved and quality of oocytes, which in turn depends on ovarian response. Markers of ovarian reserve, in particular, AMH and AFC, allow identification of women who are likely to show a high or low response to controlled ovarian stimulation (COS).

With COS we can expect three types of response as seen Figure 17.

Figure 17: Types of ovarian response to gonadotropin stimulation.



Choosing the protocol and dose of gonadotropin depends on whether we desire mono-follicular or multi-follicular development (Figure 18). It also depends on whether we are using COS for anovulatory infertility. There is no significant difference in the results when FSH and hMG are compared.

One could use the AMH and AFC tailored protocols as seen in the table below. Dosage is subject to change as per patient profile and increases with BMI and Age (≥36)[23,29,38]

AFC value of >16 is used to predict a high response with a sensitivity of 89% and a specificity of 92%. AMH at a cut off 3.36 ng/mL has a sensitivity of 90.5% (95%CI 69.6–98.5) and specificity of 81.3% (95% CI 75.8–86.0) for prediction of hyperresponse.

Figure 18: Choosing the starting dose depending on the indication for ovulation induction.

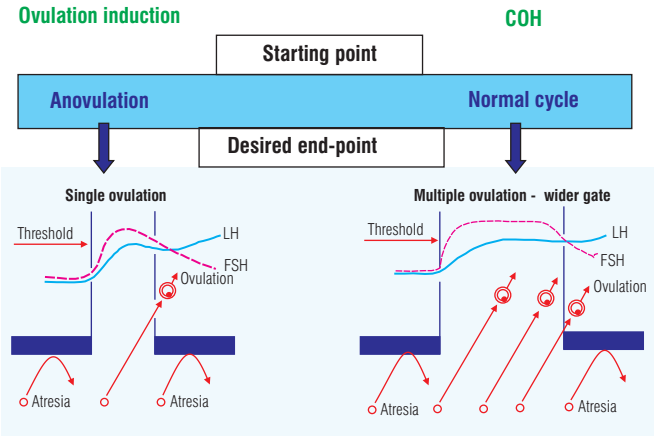


Table 11: AMH and AFC tailored protocols

AMH/AFC	Treatment
AMH <2.2 pmol/L: (0.3 ng/mL) AFC <2	Exclude, counsel Offer alternative ART Flare GnRH agonist protocol
AMH 2.2–15.6 pmol/L: (<2.2 ng/mL) AFC - 2–8	300 IU GT Flare agonist GnRH antagonist
AMH 15.7–28.6 pmol/L: (2–4 ng/mL) AFC - 8–15	200 IU rFSH or 225 IU HMG GnRH agonist/antagonist
AMH >28.6 pmol/L: (>4 ng/mL) AFC - 16 or >	150 IU GT + GnRH antagonist With GnRH agonist trigger

Whereas for poor ovarian response optimum cut off value for AMH is 0.99 ng/mL and post-test probability was highest at cut off levels of 0.59 ng/mL. For AFC the optimum cut off value was </= 10 but the post-test probability was highest at cut off levels of <8.[13]

We need to identify women at a risk of developing poor and hyperresponse to individualize protocols.

Women at risk for hyperresponse

- AFC >14
- AMH >3.5 ng/mL
- PCOS
- Excessive ovarian response in the past
- Younger women <30 years
- Low BMI
- High GT dose for OI
- Increased hCG exposure – LPS with hCG
- Previous OHSS
- Rapidly rising serum E2 levels
- E2 > 3500 pg/mL on day of hCG

Prediction of poor response

There are other group of patients which we need to keep in mind are those mentioned below

- Young, normo-gonadotrophic women, with normal ovarian reserve who show sub-optimal or unexpected poor response to exogenous FSH, which reflects hyposensitivity of granulosa cells to standard FSH dose
- FSH and LH receptor polymorphism

Table 12: Prediction of poor response

Predictive criteria	Previous response to COS
Biological criteria • Advanced reproductive age (>36) • Basal FSH (>10 or >12 mIU/mL?) • AFC ≤6 • Day 3 Inhibin - B <45 pg/mL • AMH - <0.35 ng/mL • Day 2/3 - E2 >75 pg/mL • P4 - Day 10 of COH >1.1 ng/mL **Clinical criteria** • Shortening of menstrual cycles • Repeated pelvic surgery • Previous chemo-radiotherapy • Familiar early menopause	• Cycle cancelled because <3 follicles • <4 oocytes reterieved • Estradiol <500 pg/mL • Correlation between FSH dosage and number of oocytes recovered (≤3-5 oocytes and >3000 IU of gonadotropin used)

FSH: Follicle stimulating hormone
AFC: Ovarian antral follicle count
AMH: Anti-Mullerian hormone
COH: Controlled ovarian hyperstimulation

Prevention of OHSS

Caution is indicated when any of the following indicators for increasing risk of OHSS are present during COS:

- The emergence of large number of small and intermediate sized follicles (10–14 mm) on USG
- Presence of >8 –10 dominant follicles
- Enlarged ovaries
- Presence of free fluid in POD
- Rapidly rising serum E2 levels
- E2 > 3500 pg/mL on day of hCG

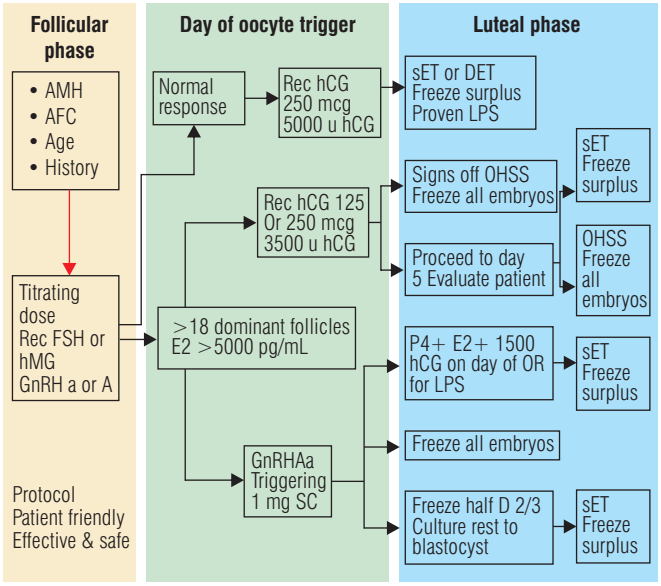
Key to prevention of OHSS

1. Experience with OI therapy and recognition of risk factors for OHSS
2. Highly individualized OI regimens carefully monitored with USG and E2
3. Use of minimum dose and duration of GT therapy necessary to achieve the therapeutic goal

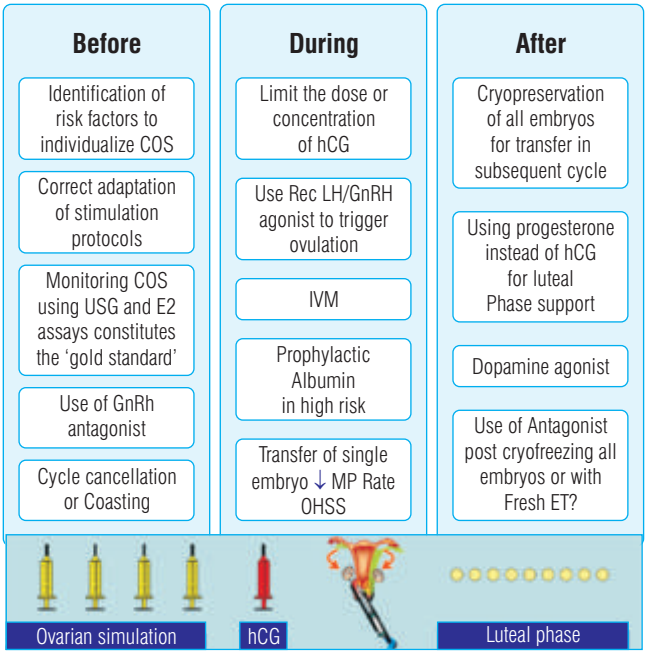
The tailored approach in the figure below will help in selecting the best protocol and thus optimize the results in both normal and hyperresponders.

Strategies to prevent OHSS are highlighted in the table below. Highly individualized OI regimens carefully monitored with USG and E2 and use of minimum dose and duration of GT therapy necessary to achieve the

Figure 19: Individualization of Protocols to reduce OHSS.



Figures 20: Methods to prevent OHSS before during and after COS.



therapeutic goal. In the past apart from cancellation, none of the approaches were totally efficient, although they decrease the incidence in patients at high risk of OHSS. But today we have an option of GnRH agonist trigger in an GnRH antagonist cycle with Cryopreservation of all embryos to be transferred in subsequent cycles. HCG is primary stimulus for the syndrome and withholding hCG is the main preventive measure.

GnRH agonist trigger and cryopreservation of all embryos to be transferred in the subsequent cycle reduces the probability of occurrence of OHSS.[39,40] But if the embryo transfer is done in the same cycle one needs to modify the luteal phase support as it results in massive and irreversible luteolysis and also has a direct effect on endometrial receptivity thus decreasing the pregnancy rates. Modifying the luteal phase support by give hCG 1500 IU on day of oocyte retrieval or giving estrogen in addition to progesterone in the luteal phase can rescue the cycle.[39,40,41]

Figure 20 shows the different methods of OHSS prevention before, during and after initiating ovarian stimulation.

GnRH antagonist protocol coupled with GnRHa triggering is efficient, safe and simple technique to prevent OHSS.

Current Strategies in the Management of Poor Responders

The European Society of Human Reproduction and Embryology (ESHRE) consensus (Bologna criteria) defines "poor response" to ovarian stimulation as presence of at least two of the following three features:[42,43]

At least two of the following three features must be present:

I. Advanced maternal age (≥40 years) or any other risk factor for POR

II. A previous POR - ≤3 oocytes with a conventional stimulation protocol

III. An abnormal ovarian reserve test - AFC <5–7 follicles or AMH - 0.5–1.1 ng/mL

Bologna criteria had an supplemental criteria to diagnose poor responders which included presence of two episodes of POR after maximal stimulation in the absence of advanced maternal age or abnormal ORT.

As the Bologna criteria did not look at the heterogeneity of subgroups, include specific profiles of abnormal ovarian response (hypo- and sub-optimal) and age-related aneuploidies the POSEIDON Working Group (Patient-Oriented Strategies Encompassing Individualized Oocyte Number) came with a new classification as seen in the Figure 21.[44]

This classification is based on follicle output ratio, where we can have patients with good ovarian reserve but few follicles growing and the other way round where there are few follicles but we are able to recover all the follicles (Figure 22).[44]

The common causes or risk factors present in poor responders include advancing age, structural chromosomal aberrations or mutations or early menopause, primary ovarian insufficiency, pelvic infection, ovarian endometriomas, ovarian surgery, and chemotherapy. One stimulated cycle is deemed essential for the diagnosis of poor ovarian response (POR). However, patients over 40 years of age with an abnormal ORT may be classified as expected PORs.[43]

Managing Poor Responders

It is extremely difficult to establish criteria to define the population that should be treated and/or how to treat them. All types of empirical interventions are being tried, some with a hypothesis behind them, which might be biologically plausible, others with less plausibility. The Table 13 gives the different interventions to enhance IVF outcome in women with poor ovarian response.

Figure 21: Low prognosis group by POSEIDON classification.

Group 1
Young patients <35 years with adequate ovarian reserve parameters (AFC ≥5; AMH ≥1.2 ng/mL) and with an unexpected poor or suboptimal ovarian response

Subgroup 1a: <4 oocytes*
Subgroup 1b: 4–9 oocytes retrieved*

*after standard ovarian stimulation

Group 2
Older patients ≥35 years with adequate ovarian reserve parameters (AFC ≥5; AMH ≥1.2 ng/mL) and with an unexpected poor or suboptimal ovarian response

Subgroup 2a: <4 oocytes*
Subgroup 2b: 4–9 oocytes retrieved*

*after standard ovarian stimulation

Four groups of patient with lower prognosis

Group 3
Young patients (<35 years) with poor ovarian reserve prestimulation parameters (AFC <5; AMH <1.2 ng/mL)

Group 4
Older patients (≥35 years) with poor ovarian reserve prestimulation parameters (AFC <5; AMH <1.2 ng/mL)

Figure 22: Follicle output ratio.

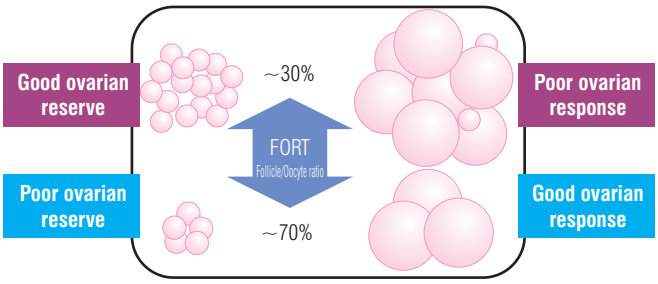


Table 13: Interventions in women with poor ovarian response

Adiuvant therapy	Change in OI protocols and ART
• Growth hormone (GH) or GH-releasing factor (GHRF) • Pyridostigmine • Androgens - Transdermal testosterone, DHEA • Aspirin • L-arginine • Aromatase inhibitors • Antioxidants	• Modifications of the long GnRH-a protocol • Use of GnRH agonist short protocol • Use of GnRH antagonist protocol • Natural cycle IVF • Modifications of ovarian stimulation • Use of LH in COS • Intracytoplasmic sperm injection • Day 2 versus day 3 ET

We have already discussed the adjuvants above and now let us see what evidence for change in treatment strategies in ART.

1. Change in GnRH analog and their protocol used: There is no evidence that one protocol is better than the other. GnRH antagonist and long agonist protocols have similar outcome except for the fact that the antagonist protocol is associated with less treatment burden as the gonadotropin dose required is much lower than the antagonist protocol. No significant difference in pregnancy rates was seen when short agonist protocol was compared to natural cycle IVF.
2. Change in stimulation protocols:
   - High fixed-dose GT regimen does not improve the probability of pregnancy
   - Initiation of FSH during the luteal phase has no beneficial effect on total number of oocytes retrieved
   - High dose step down protocol – do not increase PRs and result in more unnecessary interventions and also increase the cost of treatment
   - Only addition of LH/hCG has shown to be beneficial
3. Role of mild IVF and modified natural cycle IVF have been controversial and not shown to have increased pregnancy rates.
4. Double stimulation may have a role in obtaining more number of oocytes and correspondingly more embryos in one cycle to be transferred subsequently in an HRT cycle.
5. Early oocyte retrieval would improve outcome by avoiding exposure of oocytes to premature luteinization?
   - Retrieval at 14–16 mm follicle size instead of 19–21 mm
   - 30 hours instead of 36 hours after hCG This option though has shown good results it still requires to be explored further with large scale randomized controlled trails.
6. Shortening the duration of embryo culture has been associated with an improvement in PRs by increasing the number of embryos available for transfer. Cleavage stage better than blastocyst and day 2 better than day 3.
7. Selecting an euploid embryo by performing PGS could increase the chance of pregnancy but one must remember that the number of embryos in a poor responder are few and one can be in a situation where no euploid embryos are present. Moreover, if we take mosaicism into consideration when PGS has been performed, one may even discard a normal embryo.
8. Newer interventions like accumulation of oocytes and embryos and *In Vitro* Activation of ovaries can be considered.

Whether it is use of adjuvants or changing the ART treatment strategy, no 'positive' intervention is supported by more than one 'positive' RCT.

Management Strategies for Normal Responders

- Bologna criteria, while being a step forward, may still not be perfect
- Most studies are underpowered and single center based
- No evidence for any particular COS protocol to improve treatment outcome
- GnRH-antagonist protocols may reduce treatment burden
- LH seems to increase the number of oocytes retrieved (+0.75) and CPR (+30%)
- Insufficient evidence for most of the adjuvants to improve outcome
- Growth hormone/Testosterone gel priming may improve outcome?
- Role of PGS?
- IVA may be promising in selected patients
- Poor responders are not homogenous for pregnancy prospects
- Female age and number of oocytes retrieved will modulate the chances for pregnancy in current and subsequent cycles
- Management of POR still represents a therapeutic challenge for the clinician
- POR are a heterogeneous group with no uniform protocol
- We cannot recruit follicles that do not exist in a case of DOR!
- Egg quality fundamentally cannot be altered

Summary

- Choice of ovulation induction protocol is dictated by the planned treatment for the patient
- Anti-estrogens appear to be cost effective in IUI programs, although less effective as compared to gonadotropins. Both letrozole and CC have similar results, When gonadotropins are used they need to be given on daily basis. Low dose protocols do not differ significantly in success, when compared to high dose, which in turn increase the risk of multiples and OHSS. There is no role for GnRH-agonist in IUI programs as they increase costs and risk of MP and OHSS. Role of GnRH-antagonists in mild COS/IUI programs needs still to be determined. There is no difference in the probability of conception whether one uses urinary or Recombinant GT.
- Success of OI in an ART cycle depends on several factors like gonadotropin type and dose, GnRH analog used, trigger for final oocyte maturation, age and ovarian reserve, duration and cause of infertility, lab quality, embryo transfer policy, cryo program, physical and psychological stress, risk of complications and financial support.
- Modifying conventional stimulation protocols according to patients' characteristics and ovarian reserve makes it patient-friendly and optimizes the chance of LBR.
- Our aim should be using proper drugs after proper evaluation and investigations at its proper timing to obtain an optimal outcome

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