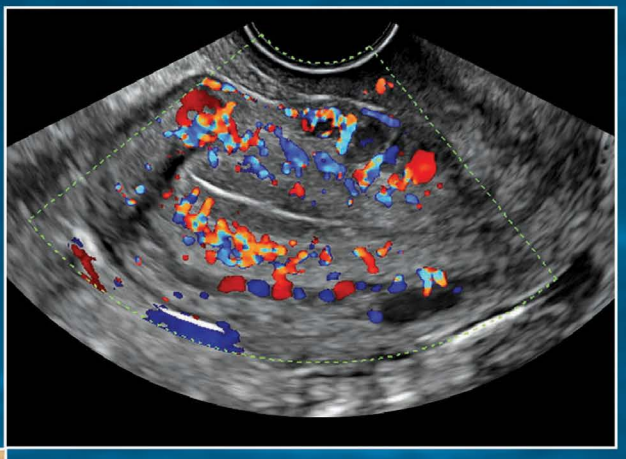
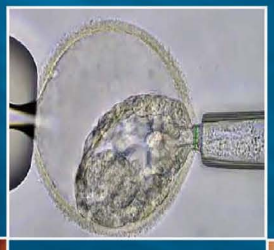
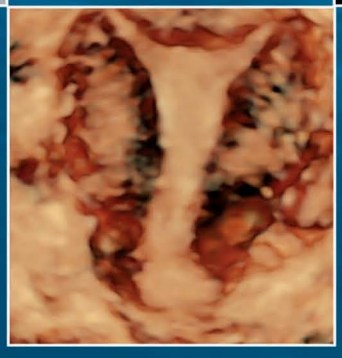
Inter-University School of Medical Ultrasound
Ian Donald

Donald School Textbook of

Human Reproduction and Gynecological Endocrinology







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CHAPTER

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Ovulation Induction

Chaitanya Nagori

INTRODUCTION

Ovulation induction is the most important aspect of any assisted reproductive technology (ART). The success of the ART is dependent on the selection of ovulation induction protocols.

The drugs that are available for the purpose can be used individually or in combination. These chiefly are of two types. These are pharmacological agents, which mimic endogenous gonadotropins to stimulate ovarian function or are agents that manipulate the endogenous gonadotropin secretions through effect on estrogen receptors or estrogen production to stimulate ovaries for producing follicles. The first group are usually injectable gonadotropins and the second group are oral ovulogens. Although researches are being done to produce oral gonadotropin preparations. Amongst the oral ovulogens, those which act on estrogen receptors are called selective estrogen receptor modulators (SERMS) like clomiphene citrate (CC) and those which affect estrogen production are aromatase inhibitors, of which the most commonly used and found to be most effective is letrozole. Gonadotropins commonly used are [follicle-stimulating hormone (FSH)], luteinizing hormone (LH), and human menopausal gonadotropins (HMG). These were earlier produced from menopausal urine but now are produced as recombinant forms.

For the judicious use of these chemicals, it is essential to understand the pharmacology and action of each.

CLOMIPHENE CITRATE

Introduction

Ovarian induction with CC was first published by Green-Blatt et al. in 1961.[1] CC now available commercially is a mixture of two isomers, enclomiphene (trans) 62% and zuclomiphene (cis) 38%. Enclomiphene is more antiestrogenic, and, therefore primarily responsible for ovulation stimulation and has a half-life of few days.[2] Zuclomiphene is more potent, more estrogenic, and responsible for ovulation inducing actions. It is in clinical use since 1967 for dysovulatory infertility. The efficacy of the drug varies in clinical practice, if the proportion of enclomiphene and zuclomiphene are not maintained.[3]

Mechanism of Action

It acts as antiestrogenic agent. It binds with estrogen receptors at hypothalamus signaling lack of estrogen to hypothalamus. This in turn releases gonadotropin-releasing hormone (GnRH) that stimulates pituitary to secret more FSH and LH, which in turn stimulates the ovarian follicular development. As the duration of action is longer, (half-life of 5–21 days, depending on the isomer),[2] it allows more and more FSH and LH and leads to multifollicular development. Clomiphene has direct effect on ovary to stimulate follicular estradiol synthesis also. It increases the GnRH pulse frequency in ovulatory patients and pulse amplitude in anovulatory patients like polycystic ovarian syndrome (PCOS). Clomiphene does not display progestational, corticotropic, androgenic, or antiandrogenic properties.

The disadvantage of CC is that when it binds with the estrogen receptors, it remains there for days or weeks, unlike natural estrogen, which stays there only for hours. This leads to gradual depletion of estrogen receptors.[1]

When to Use Clomiphene Citrate?

It will be effective as ovulation induction in patients in whom reasonable levels of endogenous estrogen are present and hypothalamo-pituitary–ovarian (HPO) axis is intact.

I. WHO group II patients: It is extremely effective in anovulatory patients in whom HPO axis is normal and have normal basal levels of endogenous estradiol (oligo-anovulation, PCOS, etc.). Normal thyroid function tests and prolactin should be confirmed before starting the treatment.[4] It is not effective in hypogonadotropic hypogonadism, i.e. WHO group I as there is no negative feedback mechanism of estrogen.[5]

II. Luteal phase defect (LPD): Short luteal phase is associated with low levels of FSH in follicular phase causing LPD. CC will increase FSH and LH in follicular phase, develops the good follicle, and corrects the abnormality in luteal phase. So CC is the drug of choice in LPD due to short luteal phase.[6]

III. Unexplained infertility: The rationale of using CC in unexplained infertility is that it corrects subtle defects due to deficiency of FSH and LH. It increases the number of follicles available for fertilization and corrects LPD.[7] Cochrane database supports the use of CC in unexplained subfertility.[8] Former belief of cervical dysmucorrhea and decreasing endometrial receptivity in unexplained infertility is not accepted and instead of contraindication, it is the indication for CC in modern era for unexplained infertility.

Clomiphene citrate should not be used in patients with ovarian cyst and abnormal uterine bleeding. It should also not be used in individuals having liver dysfunction, thyroid or adrenal dysfunction, or an organic intracranial pathology. It is a category X drug and is not recommended in pregnancy.

Treatment Regimes

Clomiphene citrate is given as 50 mg dose daily between day 2 and day 5 (both inclusive) for 5 days after spontaneous or induced bleeding. Ovulation, conception, and pregnancy rates are same independent of the day when CC is started. But day 5 is the most accepted day for starting the treatment.

Most women (up to 74%) respond to clomiphene with maximum 100 mg daily dose. Higher doses have very poor pregnancy rate (12% with 150 mg, 7% with 200 mg, and 5% with 250 mg).[9] Prof. Bruno Lunenfeild and Prof. Roy Homburg have recommended only one dose to start with and maximum 100 mg daily. The advantage of using this dose is that it can diagnose clomiphene resistant cases earlier and will cut down superfluous cycles of treatment till ovulation occurs. Highest pregnancy rates with CC has been documented in first three treatment cycles and the chances significantly decrease after six cycles; therefore, it is not recommended to continue CC beyond six treatment cycles.[10]

Monitoring of Clomiphene Citrate Cycles

Serum progesterone in midluteal phase if is more than 3 ng/mL, it is an evidence of ovulation. LH detection kit can help to know the LH surge. Normally LH surge occurs after 7 days of the last dose of CC. Ultrasound is a useful tool to monitor ovulation induction but serial sonography just for documenting ovulation, when no intervention is intended, is not justified.

It is justified to assess S. LH on day 8–9 of the cycle and if it is more than 10 IU/mL, success rate with CC is very low because of poor quality ova and embryo.

Efficacy of the Drug

Clomiphene citrate will induce ovulation in approximately 75–80% of cases. Up to 45–75% of cumulative pregnancy rate can be achieved in 3–9 months.[11] So, if the patient has not conceived within 3–6 months with CC, re-evaluation of the patient and switching over to further management may be required.

Side Effects

Though serious side effects are extremely rare, transient hot flushes (13.6%), mood swings (64–78%), breast tenderness, nausea (2.2%), and pelvic pain (5.5%) may occur.[12] Some may also complain of headache or visual disturbances.[13]

Antiestrogenic effect of CC is one of the major side effects of CC.

Antiestrogenic effect on cervix, endometrium, ovary, ovum, and embryo have been described, but there is no objective evidence that these effects occur or have clinical consequences. Endometrial thickness is within normal range in majority of cases. Very rarely endometrium suppression is observed, which is less than 6 mm in spite of a good mature follicle. It also leads to reduction in glandular density and increase in the number of vacuolated cells.[14] In these cases, patients may be shifted over to other therapy. Decreased uterine blood flow during the early luteal phase has also been found in patients on CC.[15]

Cervical dysmucorrhea can be overcome by intrauterine insemination (IUI). Exogenous estrogen supplementation is of no benefit as the receptors are blocked.[16]

Starting CC early in the cycle or using other SERMs have also been not proved of any benefit.[17,18]

Risks of Clomiphene Citrate

Multiple pregnancy (5–8%) is one of the important unwanted effect of CC due to its long half-life and continued production of FSH and LH.[19] It is because of this effect

that the risk of ovarian hyperstimulation is also associated with CC therapy. Though a dreaded complication, it may be seen only in PCOS patients when CC is used as ovulation inducing agent. CC treated patient when conceived had a 23.7% risk of pregnancy loss as compared to 20.4% in spontaneous pregnancies.[20] There is no substantial evidence that CC increases the risk of congenital fetal anomalies or causes any particular birth defects.[21,22] Previous studies suggested that if CC is used for more than 12 months, it can increase the risk of ovarian malignancy. But subsequent studies have proved that fertility drugs are not associated with any invasive cancers. CC does not increase the risk of breast cancer.[23-25] Women on clomiphene therapy must be counseled that no causal relationship has been established between ovulation inducing drugs and breast or ovarian cancer. However, prolonged treatment with CC is futile and should be avoided because it has little success.

Alternative Treatment Regimes with Clomiphene Citrate

Extended Clomiphene Treatment

In patients who did not respond to standard regimes, CC is given for 7–10 days in a dose of 150–250 mg per day or 50 mg/day may be given for 5 days and increment of 50 mg/day is done every 5 days to reach 250 mg/day.[26] No factor has been identified that can predict which patient will respond to extended regime. Such extended regimes have extremely poor pregnancy rates and are not much helpful in clinical practice.

Combination of Clomiphene Citrate with Other Drugs

Clomiphene citrate with glucocorticoids: This combination is very useful in chronic anovulatory patients. It leads to androgen suppression and, therefore, it can be given to those patients who have high dehydroepiandrosterone sulfate (DHEAS). It can be given to all the patients who show increase in adrenal androgen production. It has given good results even with normal DHEAS and unselected population and even PCOS having clomiphene resistance.[27] Those patients who are resistant to 100–150 mg of CC, start ovulating when glucocorticoids are added. Glucocorticoids are given in the form of dexamethasone 0.5 mg daily or prednisolone 5 mg daily continuously for 30 days at bed time.[28] It can be given continuously for 3–6 months. No serious side effects are seen, though water logging and weight gain have been documented. The dose can be reduced to 0.25 mg/day or can be stopped in second phase of the cycle.

Clomiphene citrate with estrogen: Estrogen was tried to improve cervical mucus and endometrial thickness but it has already been discussed earlier that adding estrogen does not help to correct the side effects of CC on cervical mucus or endometrium.[16]

Clomiphene citrate with bromocriptine: Bromocriptine is given to the patients of hyperprolactinemia. But there are two definite indications even with normal prolactin level.

i. Galactorrhea with normal prolactin level. In these cases, galactorrhea must be confirmed by presence of fat globules under the microscope in the secretions expressed from the breast. It must be differentiated from simple mucoid discharge that does have fat globules.
ii. There are patients who are known as spikers. In these patients, there are nocturnal spikes of prolactin in the first half of the cycle when CC is used for ovulation induction. The rise being transient is difficult to confirm even when repeated blood samples are taken. These patients are given bromocriptine in first half of the cycle. It is not to be continued in second half of the cycle as normal level of prolactin is required for maintenance of corpus luteum. Clinically, these patients show poor endometrium even though there is no local cause and follicle is very good and meet all parameters on B mode and color Doppler for optimum maturity. Bromocriptine can be tried in these patients and if endometrium improves, it can be continued for 3–6 months.[29]

Clomiphene citrate with hCG: Routine use of hCG for rupture of the follicle is not justified. If the follicle is mature, it will reflect as serum estradiol (E2) level of more than 150 pgm/mL. This will initiate the LH surge which in turn will cause rupture of the follicle and exogenous hCG is not required. But if the follicle is not mature and if hCG is given as a trigger for ovulation, it will lead to premature luteinization or atresia. Therefore, hCG must not be given just for ovulation.[30,31]

But hCG can be combined with clomiphene when IUI is to be done. hCG in these cases is done to time IUI as follicle ruptures at 34–36 hours after hCG injection. hCG

can also be given in patients proved to have absent, inadequate, or delayed LH surge. This is a common occurrence in endometriosis. hCG, therefore, must be used for timed intercourse, to time IUI, and in cases of delayed LH surge.

Clomiphene citrate with gonadotropin: Gonadotropins are often combined with clomiphene with the idea that it would decrease the requirement of gonadotropins. CC by its direct effect on ovary, increases the sensitivity of ovary to gonadotropins. This combination has risks of ovarian hyperstimulation and multiple pregnancies. This combination has been documented to reduce the cost of the treatment.[32] But it does not increase the pregnancy rate to our opinion. The argument is that CC increases FSH and LH both. The poor pregnancy rate with CC is because of high LH which will remain in spite of adding gonadotropins. The effect of high LH will decrease the implantation and pregnancy rate. So even though we get a follicle after adding gonadotropins, in many of these patients, the follicular quality is not good and pregnancy rates are only comparable to CC alone and much less than gonadotropins alone. Instead letrozole is a better drug to be combined with gonadotropins.

Clomiphene citrate with antagonist: This makes a good combination in thin lean PCOS patients where LH is tonically raised. It improves the quality of ovum and also the conception rates. For patients on clomiphene therapy, serum LH levels are checked on day 8–9 of the cycle. If it is more than 10 IU/mL, it is an indicator of poor pregnancy rates. These are the ideal patients to start antagonist. Regular use of antagonist is not justified because it sharply decreases LH and quality of follicle is changed and it causes regression of follicle as well as LPDs.

Clomiphene citrate with metformin: Several workers have shown that metformin is inferior to CC for ovulation induction and metformin alone should not be used for ovulation induction. But in CC resistant cases of PCOS when metformin is combined with CC, it improves ovulation four to nine times more than clomiphene alone. This shall be further discussed in the chapter of PCOS.[33,34]

Prior Treatments

i. Clomiphene after prior treatment with oral contraceptive (OC) pills: The mechanism of action of OC pills is to decrease the LH surge. High LH will cause high androgen, which in turn will cause anovulation. So OC pills when given for 2–3 months will decrease the LH and in turn and androgen and improves sensitivity to clomiphene with the same dose. Therefore, in PCOS patients prior treatment with OC pills was given routinely in CC resistant cases and did show good results.
ii. Micronized progesterone: If it is given for 5 days, it modulates LH pulsatility, reducing LH concentration, and induces more favorable environment for CC.

AROMATASE INHIBITORS: LETROZOLE

Introduction

Letrozole is an aromatase inhibitor used for ovulation induction. It is the third generation of aromatase inhibitor. Another third generation aromatase inhibitor is anastrozole. The main advantage of the drug is that it is a reversible enzyme inhibitor. It was for the first time used by Mitwally et al. in 2001 for ovulation induction.[35]

Mechanism of Induction of Ovulation

Aromatase, a cytochrome P450 dependent enzyme, acts as ultimate step in synthesis of estrogen, catalyzing the conversion of androgen to estrogen. This conversion occurs at peripheral sites such as muscle, fat, and liver. Letrozole inhibits aromatase enzyme by completely binding to the heme of cyto 450 subunits of enzyme, so that androgen is not converted to estrogen.[36,37] The end result is low estrogen causing negative feedback at hypothalamus and pituitary releasing FSH and LH releasing factors resulting in high FSH and LH causing folliculogenesis. As androgen is not converted into estrogen, there is slightly higher level of androgen, which also causes folliculogenesis. Withdrawal of estrogen centrally increases activins also, which are produced by many tissues and pituitary and stimulates FSH synthesis by direct action on gonadotropes.[38] It has no effect on estrogen receptors and acts on hypothalamus. So, it has no deleterious effect on LH surge, cervical mucus, and endometrium. As FSH and LH stimulates folliculogenesis, estrogen will rise and will cause negative feedback on FSH. This is responsible for monofollicular development unlike CC.

It is a potent aromatase inhibitor with a very specific action. After oral administration, it has almost 100% bioavailability, and aromatase inhibitors are cleared from the systemic circulation by liver and have a half life of 30–60 hours.

Indications

Letrozole is widely used in CC resistant cases. Letrozole will reduce estrogen and stimulate hypothalamus to release LHRH to secrete more FSH and induces folliculogenesis, and, therefore, is the drug of choice for patients with PCOS, but this shall be discussed in the chapter dedicated to it. It can also be used in patients with endometriosis. There is an expression of aromatase enzyme in the endometriotic tissue, which leads to progression of endometriosis by highlighting the role of estrogen.[39] Inducing ovulation in these patients with letrozole leads to lower estrogen level and, therefore, protection against progression of the disease. For the same mode of action, it is also a choice of drug for ovulation induction in the survivors of estrogen-dependent malignancies,[40] and in those with high risk of coagulation disorders.[41] It may specifically be more beneficial in patients with implantation failure, especially in women having high levels of aromatase p450 in endometrium.

Characteristics of Letrozole

It causes total suppression of estrogen (up to 97–99%) with doses up to 5 mg/day. Maximum suppression occurs between day 5 and 7. After day 7, estrogen level will rise due to folliculogenesis because of increasing FSH after negative feedback of estrogen due to letrozole. This rise of estrogen will continue for 5–7 days. This E2 peak will cause LH surge for ovulation. So LH surge occurs after 12 days unlike CC when it occurs early. Estrogen rise after 7 days will decrease FSH, which will be responsible for monofollicular development. It has no antiestrogenic effect on cervical mucus or endometrium. Temporary rise of androgen improves the sensitivity of follicle to gonadotropin. It therefore requires lower dose of gonadotropins when it is combined with letrozole.

Advantages of Letrozole

Being almost free from side effects, letrozole is well tolerated and accepted by the patients. It gives monofollicular development,[35,42] so has less chances of multiple pregnancies, has no antiestrogenic effect, and therefore may give higher pregnancy rates. Having no ill effect on the endometrium, the risk of abortion is also decreased. When combined with gonadotropins, lower doses of gonadotropins are required as the androgen is high which sensitizes the follicles to FSH.[43] It has a wide margin of safety and toxicity is rare. Moreover, it is relatively inexpensive.

Regimes

- Recommended dose is 2.5 mg from day 3 to 7.
- Some workers suggest 5 mg dose. The disadvantage of 5 mg dose as a routine is not justified as 2.5 mg can suppress 97–99% of estrogen. A dose of 5 mg may suppress more estrogen causing more FSH to be secreted. So it may not cause monofollicular development. As it is a chemotherapeutic agent, it may be toxic to ovum and embryo.
- Single dose of 20 mg on day 3: It has advantage having short half-life and reduces embryotoxic effect. But symptoms of hypo-estrinism may develop.
- It is superior to anastrozole 1 mg from day 3 to 7.

Side Effects

It may lead to mild gastrointestinal disturbances and rarely asthenia, hot flushes, headache, and back pain. It may give postmenopausal or estrogen withdrawal symptoms. It increases intrafollicular androgen, which may arrest the growth of follicle. But optimum level of androgen is not yet decided.[44-46]

Letrozole with Gonadotropins

Letrozole with recombinant FSH (rFSH) is a good combination for giving equivalent pregnancy rates as gonadotropins with advantage that it decreases the requirement of gonadotropins by sensitizing the follicles to FSH due to high androgen, gives monofollicular development, less multiple pregnancy rates, and less abortion rates.

GONADOTROPINS

Introduction

Since 1958, human pituitary gonadotropin preparations have been used for ovulation induction.[47,48] Lunenfeld et al. first reported the use of HMG, produced from menopausal urine, followed by pregnancy in 1963.[49,50] Now purified preparations and recombinant preparations of gonadotropins are also available.

Physiology of Ovulation and Gonadotropin-induced Ovulation Induction

Ovary has a pool of primordial follicles. Primordial follicles are independent of gonadotropins. Primordial follicles

grow into preantral follicles and primary antral follicles. Under the influence of androgen, preantral follicles grow into primary antral and secondary antral follicles. FSH rise in the late luteal phase of previous cycle decides the dominant follicle. Androgen sensitizes the follicles to FSH. For follicular cohort, minimum level of FSH is required and this is called FSH threshold. Large antral follicles can respond to gonadotropin stimulation. As the sensitivity of follicle to FSH increases with development, lower amount of FSH is required. As the amount of FSH decreases, it stops growth of other follicles except the dominant follicle. The process of selection of follicle is completed in 7 days. But when gonadotropins are given from outside, there will be larger number of follicles to grow and ovulate. FSH window means number of days the FSH levels are above threshold. FSH stimulates the follicle to grow. Follicle secretes estrogen which has negative feedback on hypothalamus to secrete less FSH. All oocytes are arrested in prophase stage in meiosis in preantral follicles. When follicle is mature and estrogen secretion is at its peak, pituitary receives a positive feedback for LH surge by the short loop. LH surge causes ovulation to occur. This inhibition is removed by LH surge and first meiotic division will be complete with extrusion of first polar body. Oocytes should be fertilized within 48–72 hours and second meiotic division occurs otherwise oocyte dies. Mature follicle is 16–18 mm and estrogen secretion from such a follicle is 150 pgm/mL/follicle.

Principles of Gonadotropin Therapy

The basic principles were suggested by Insler and Lunenfeld in 1974.[51]

1. Effective dose: Ovarian response is seen when certain dose of FSH is given. Below this dose, the ovaries do not respond.
2. Latent phase: This phase lasts for 3–7 days. It starts with onset of stimulation, but there is no estrogen rise or ultrasound demonstrable follicular growth during this phase.
3. Active phase: This phase starts with ultrasound demonstrable follicle growth to ovulation and it lasts for 4–6 days.

Follicle-stimulating Hormone Preparations Available

1. HMG 75 IU: 75 IU of FSH and 75 IU of LH
2. HMG-HP (highly purified): 75 IU of FSH and 75 IU of LH with less than 5% of urinary proteins
3. Urinary FSH: 75 IU of FSH and 1 IU of LH
4. FSH-HP: 75 IU of FSH with less than 0.1 IU of LH and less than 5% of urinary proteins
5. rFSH: 75 IU of FSH and no LH activity
6. rFSH + rLH combination

The urinary products are prepared from human menopausal urine and are called menotropins. These contain a lot of potentially allergic urinary proteins. These are orally inactive and are to be administered intramuscularly. These menotropins are processed and purified to remove these allergens in highly purified products. These can be given subcutaneously. These products may be called urofollitropins. These have only 1% of LH activity.[52] But these are still contaminated with 95% nongonadotropin related proteins. In 1990s, recombinant gonadotropins were produced by immunochromatography with monoclonal antibodies against gonadotropins. After development of rFSH, rLH was developed in 1994.[53] Where urinary products have the least bioactivity for a particular hormone, it is highest for the recombinant products.

Different Regimes of Gonadotropin Therapy

1. Step up protocol
2. Step down protocol
3. Chronic low dose protocol

Indications of Gonadotropins Therapy

A. Hypogonadotropic hypogonadism (substitutional therapy)
B. Hypothalamo-pituitary dysfunction:
   1. Clomiphene resistant patients
   2. Clomiphene failure patients
   3. Superovulation combined with IUI
   4. Gonadotropin as first line of treatment
   5. Controlled ovarian hyperstimulation (COH) in ART cycles

Step-up Protocol

Baseline scan, done on day 2–3 of the menstrual cycle, before starting stimulation, guides to decide the stimulating dose. Conventionally, in normal reserve, normal responding ovary, 1 amp, i.e. 75 IU of FSH is started from day 5 for 5 days. Ultrasound scan is done on day 5 of stimulation. If this scan shows a follicle of 10–12 mm and/or there is increase in endometrial thickness, the same dose

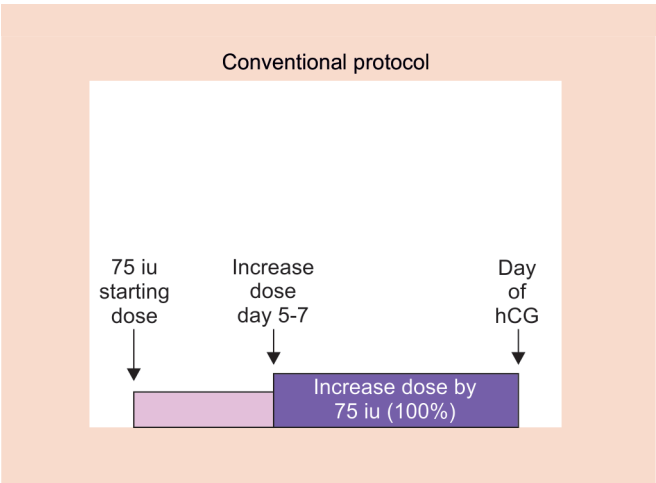

Fig. 4.1: Conventional protocol of gonadotropins in IUI cycles.

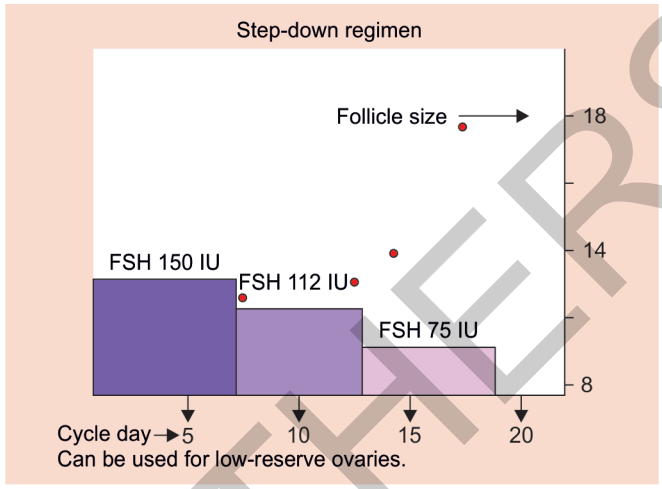

Fig. 4.2: Step-down protocol regimen of gonadotropins for IUI.

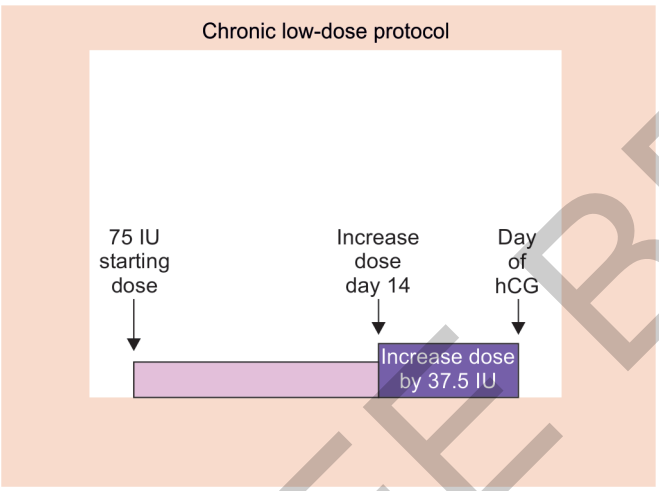

Fig. 4.3: Chronic low-dose protocol of gonadotropins in IUI cycles.

is continued till follicle and endometrium become mature. If there is no increase in the size of the follicle or in the endometrial thickness, the dose is doubled and scan is repeated after 3 days. If the follicle or endometrium grows, the same dose is continued till follicle and endometrium are mature, otherwise dose is further increased by 75 IU/day (Fig. 4.1). But this is rarely the case now as each patient is assessed on day 2–3 of period (baseline scan), and this scan diagnosis poorly responding ovaries, and in these patients stimulation is to be started with higher doses.

This protocol has been blamed for higher rates of multiple pregnancies and ovarian hyperstimulation syndrome (OHSS).[54] Conventional protocol gives excellent pregnancy rates in normal responding ovary. Good ultrasound monitoring can replace hormonal (E_2—estradiol) estimations.

Step-down Protocol

This protocol is very useful for the poor responding ovaries or low reserve ovaries. According to this protocol, stimulation is started with higher dose, i.e. 150–225 IU FSH from day 5 for 5 days. Once the follicle grows to 10–12 mm, the dose is reduced to 75–150 IU FSH; once the follicle size reaches 14 mm, the dose is further reduced, if it was 150 IU, or is continued with 75 IU FSH till follicle and endometrium mature (Fig. 4.2).

Chronic Low-dose Protocol

This protocol is very useful in PCOS patients. PCO contains twice the number of available FSH sensitive antral follicles in cohort as compared to normal ovaries. This protocol gives good pregnancy rates with very little risk of OHSS or multiple pregnancies. But it is essential that PCOS is confirmed on baseline ultrasound scan. In this protocol, stimulation is started with 75 IU of FSH and same dose is continued for 14 days. If follicle or endometrium grows, the same dose is continued till mature follicle and endometrium are achieved otherwise the dose is increased by 37.5 IU for other 7 days. (Fig. 4.3) Almost all the patients develop good follicle and endometrium and incidence of multiple pregnancy and OHSS is extremely low.[55] Majority of the patients develop good follicle within 14 days and we should strongly resist ourselves to increase the dose till then. There may be spotting or some bleeding during this period which should be ignored. With chronic low dose protocol, there is monofollicular development in 70% of cycles, OHSS can be almost totally eliminated and multiple pregnancy rate less than 6% with same pregnancy rate as conventional protocol.[56]

RECOMBINANT FOLLICLE-STIMULATING HORMONE

The invent of rFSH has changed the management of infertile patients. In 1990, 6 lakhs urine donors were required for 120 million liters of urine for urinary gonadotropin production. This was becoming extremely difficult as with developing techniques in ART, the need for gonadotropins were further increasing. Therefore, there was an urgent need for FSH by alternate route. The first rFSH was introduced in 1995. But it was 1992 that Prof Paul Devroey and Germond reported the clinical utility of rFSH.[57] rFSH has highest bioactivity and has no proteins or LH/hCG like activity and best batch-to-batch consistency. As it has no proteins, it is never allergic and also does not produce antibodies against FSH. It has low level of oxidation and/or degradation, i.e.10% compared to 40% with uFSH.

How is Recombinant Follicle-stimulating Hormone Produced?

The objective of recombinant DNA technology is to insert genes in the cell to produce desired proteins. The gene that is coded for desired protein is isolated enzymatically from DNA chain. The isolated gene is put in vector (a large segment of DNA). This is known as rDNA in host cell. This rDNA produces desired proteins-rFSH. Therefore, human DNA genes into a mammalian cell line produces rFSH which is 99.9% pure without LH activity. rFSH has bioactivity of 10,000 IU/mg of protein (Table 4.1).

Preparations

Follitropin α is produced by transfecting Chinese hamster ovary cells with genes for the α and β subunits of human FSH.

The secretory products of these cells undergo six step purification process to get a final preparation which is highly pure biochemically (>99% FSH).

Table 4.1: Specific bioactivity of different gonadotropins.

Product	*Specific bioactivity (IU/mg protein)*
HMG (1950)	8
uFSH (1980)	100–150
uHMG-HP	2,000
uFSH-HP	9,000
rFSH (1995)	10,000

Comparison of Recombinant Follicle-stimulating Hormone with Urinary Follicle-stimulating Hormone

The study conducted by Hedan B clearly indicates superiority of rFSH (Table 4.2).[58]

The meta-analysis conducted by Salim Daya and Gunby of 12 randomized controlled trials has proved that rFSH is 20% more likely to achieve pregnancy than uFSH. The amount of FSH used was 400 IU less.[59]

The meta-analysis data was summarized by the author as "The overall conclusion from this meta-analysis is that rFSH is more effective than uFSH because of the higher rates of clinical pregnancy per cycle started and is more efficient because the total dose of gonadotropin required was lower."[59]

Prospective meta-analysis has proved that clinical pregnancy rate is 5% higher in rFSH and if cryopreserved embryos are taken into account, pregnancy rate is 6.4% higher.[60]

Though a recently published Cochrane review has shown no difference in pregnancy outcomes with fresh embryo transfers when rFSH and urinary FSH were compared. It was concluded that "all available gonadotropins in clinical practice are equally effective and safe" and the choice is based on availability, convenience, and cost.[61]

Comparison of Recombinant Follicle-stimulating Hormone with Urinary Follicle-stimulating Hormone (Highly Purified)

Recombinant FSH is more effective than highly purified FSH in multiple follicular development (Table 4.3).[62]

Frydman and associates also published the data that rFSH gives more number of oocytes, more embryos with lesser number of injections.[63]

Table 4.2: Comparison of recombinant and urinary FSH in terms of doses used and cycle outcome.

	rFSH	*uFSH*
Higher number of oocytes	10.8	8.9
Lower dose of FSH	2,138 IU	2,385 IU
Shorter period	10.7	11.3
High-quality embryos	3.1	2.6
Pregnancy rate with cryopreservation	25.5%	20.4%

Table 4.3: Comparison of recombinant and urinary FSH in ART cycles.

	rFSH	*uFSH*	*P value*
No. of ampoules	21.9 ± 5.1	31.9 ± 13.4	<0.0001
FSH days	11 ± 1.6	13.5 ± 3.7	<0.001
Oocytes retrieved	12.2 ± 5.5	7.6 ± 4.4	<0.0001
Embryos transferred	2 ± 0.19	1.9 ± 0.4	NS
Pregnancy rates	53/119 (45%)	42/114 (37%)	NS
Implantation	32	31	NS

Gearon and Abdalla from Lister hospital, London in an unpublished study have reported for the first time improvement in quality of oocytes in older women treated with rFSH instead of uFSH-HP. Another randomized single blind, multicentric multinational study by Schats et al. also confirmed the same findings.

Recombinant FSH gives more embryos with lesser ampoules. More than four embryos in culture has higher chance of achieving pregnancy.[64] The chances of blastocyst transfer is increased, if more than four oocytes are retrieved.[65]

Recombinant FSH was associated with higher per cycle pregnancy rate than uFSH-HP, when used at the same dose, whereas the pregnancy rates were similar when the dose of r-FSH was 50% lower.[66]

Recombinant Follicle-stimulating Hormone versus Human Menopausal Gonadotropin

Meta-analysis of eight randomized controlled trials comparing rFSH with HMG has shown that rFSH has 50% higher pregnancy rates.[67] The FIVNAT report 1999 showed that the pregnancy rates were higher with rFSH than HMG.

Prospective analysis of 24,000 ART cycles from Germany also showed higher birth rate and lower dosage with rFSH than HMG.[68]

Meta-analysis including 400,000 IVF cycles showed that compared to rFSH, HMG resulted in fewer oocytes and higher doses.[69]

Facts about Highly Purified Human Menopausal Gonadotropin

1. The process is partially successful with specific activity of 2,000 IU/mg of protein.
2. In many cases, LH activity has to be added using hCG which has longer half-life.
3. Presence of variable amount of hCG may further increase variation between different batches of product and result in follicular atresia.[70]
4. HMG-HP contains 30% extraneous proteins including leukocyte elastase inhibitor protein C inhibitor and Zn-α2 glycoprotein apart from hCG.

Different Types of Recombinant Follicle-stimulating Hormone

1. Follitropin α 1995
2. Follitropin β 1996
3. Immunopotency, biopotency, and internal carbohydrate complexity are same for both but follitropin α contains higher proportion of acidic glycoforms.
4. Follitropin α has a specific activity of 13,645 IU/mg whereas that for follitropin β is 9,396 IU/mg.

There is a difference in tolerability between rFSH preparations favoring follitropin α over beta.[71] Though follitropin α has a higher incidence of OHSS as compared to follitropin β (4.1% as compared to 2.7%).[72]

Fill by Mass Concept

Dose of gonadotropin is expressed in international units (IU). But recent fill by mass (FbM) technique reflects a constant relationship between mass and bioactivity and guarantees consistency of dose from batch-to-batch. So drawbacks of vivo bioassay can be avoided. Bioassay of FSH preparation is 50 years old. Steelman-Pohley bioassay which is cumbersome and requires large number of animals and variation can be 10–20%. But FbM is by liquid chromatography supported by glycine mapping isoelectric focusing that demonstrates physicochemical consistency of the product.

Duration of stimulation was significantly shorter in FbM group, and embryo quality and implantation rates were significantly higher—28.6% in FbM versus 18.6% in Filled by International Units (FbIU).[73,74]

Clinical Benefits of Recombinant Follicle-stimulating Hormone[75]

- Improved logistics of pharmaceutical process
- Controlled manufacturing
- Reduced batch-to-batch variability
- Potentially unlimited supply—no shortage
- Not reliant on the urine supply
- No risk of infection

- No risk of contamination with drugs or metabolites
- No seroconversion to antigonadotropin antibodies
- Effective, safe, and less traumatic subcutaneous administration
- Greater purity and specific activity
- Smaller doses required
- More predictable response
- Cost benefit

PROTOCOLS FOR ASSISTED REPRODUCTIVE TECHNIQUE

Gonadotropins in Assisted Reproductive Technique

In ART cycles also, stimulation protocol is decided by baseline scan.

Long Agonist Protocol

Downregulation from 21st day of previous cycle as nasal spray or subcutaneous injections of GnRH agonist (leuprolide acetate 0.5 mg or decapeptyl 0.1 mg) and is continued till periods. The dose is halved from the day of period. Depot preparations of agonist can be used instead of daily agonist injections on day 21 of the previous cycle. The most common starting dose for COS with rFSH is 150–225/day. Increasing the dosage in elderly women does not result in increased oocyte yield.[76] It has been found that 150 IU of rFSH is equivalent to 225 IU of uFSH for follicular growth. The dose of 150 IU is optimum,[74] rather than 100 IU that has been mentioned in a comparative study for IVF patients.

Short Protocol

This protocol exploits the flare-up effect of agonist during the ovarian stimulation. Agonist is started with the beginning of the menstrual cycle and flare-up provides the surge of gonadotropins secretion. A few days later, exogenous gonadotropins is administrated to supplement the flare. Both agonist and gonadotropins are continued till the day of hCG. This protocol is preferred for poor responders.

Ultrashort Protocol

Agonist is given on day 2, 3, 4 of the stimulation cycle in the dose of 500–1,000 μg per day and then gonadotropins are given subsequently till day of hCG.

Antagonist Protocol

High resistance, low velocity stromal flow, fewer antral follicles, smaller ovarian volume, high BMI, and age more than 35 years are the parameters which decide higher doses for stimulation with antagonist protocol; whereas, low resistance, high velocity stromal flow, polycystic ovaries, low BMI, and age less than 24 years are the parameters which would be in favor of lower doses with antagonist protocol. Antagonist is started from day 6 and continued till the day of hCG. In these patients, GnRH agonist can be used for ovulation trigger, instead of hCG. This protocol reduces the incidence of OHSS significantly. This is known as fixed antagonist protocol. Antagonist can also be used as flexible protocol, when antagonist is started when at least one follicle is 14 mm in diameter.[77] Antagonist protocol has high cumulative pregnancy rate in both groups, high AMH (40.1–63.6%), and low AMH (11.1–18.7%).[78] A meta-analysis of 45 randomized controlled trials involving 7,511 women comparing GnRH antagonist protocol and long agonist protocol for COS in ART has found no significant differences in live birth or ongoing pregnancy rates, but marked decrease in OHSS with antagonist protocols.[79] Another meta-analysis comparing agonist and antagonist protocols for poor responders has shown advantage in terms of FSH stimulation, but no statistically significant difference in terms of oocytes retrieved or clinical pregnancy rates.[80,81]

Antagonist–Agonist Protocol

This protocol is especially used for PCOS patients. It is known as OHSS free protocol. Fixed antagonist protocol is used for downregulation and then agonist is used for ovulation trigger.

Antagonist–Agonist Protocol with hCG as Luteal Support

The above mentioned protocol is very likely to lead to LPD. It is therefore recommended to supplement the luteal phase with 1,500 IU of hCG on day 3-7-10 of ovum pick-up.[82]

Predictors of Ovarian Response

These are age, BMI, baseline FSH, AMH, and AFC. A nomogram can be prepared using these predictors. *Howles and colleagues have prepared a manuscript on the same.*[78] The cost effectivity has been mentioned in the results presented here. These are the results of computer modeling study (Tables 4.4 and 4.5).

Table 4.4: Comparison of average cost per cycle with recombinant and urinary FSH in different countries.

	rFSH	*uFSH*
UK	5,906 pounds	6,060 pounds
US (Social) (Insurance)	40,688 $ 28,481 $	47,096 $ 32,967 $
Spain (Public insurance)	12,791 €	13,007 €
Spain (Private)	19,739 €	20,467 €
Germany	21,686 €	22,189 €

Table 4.5: Individualized dose calculation for COS.[83]

AMH level	*Predicted response to COS*	*Treatment strategies*
15 pmol/L	High responders (150 IU)	GnRH antagonist protocol Normal oocyte yield, very low excess response, low embryo cryopreservation, high/maintained fresh CPR
5–15 pmol/L	Normal responders (225–300 IU)	GnRH agonist protocol: very low cancellation of oocyte pick-up and OHSS
<5 pmol/L	Poor responders (375 IU)	High-dose FSH: long stimulation, high cancellation Antagonist strategy: short stimulation, moderate cancellation

LUTEINIZING HORMONE SUPPLEMENTATION: FOR WHOM, WHEN, AND WHY?

Two-cell Two-gonadotropins Theory

Both FSH and LH are required for estradiol synthesis. LH binds to theca cells to induce androgen synthesis. Androgen diffuses into granulosa cells, where FSH-stimulated aromatization leads to estrogen synthesis.[83] LH in follicular phase helps conversion of progesterone to androgen to reduce follicular phase progesterone, which is detrimental to endometrial receptivity.[84] A combination of rFSH and rLH in a ratio of 2:1 is indicated for women with severe gonadotropin deficiency—hypogonadotropic hypogonadism.[85]

Luteinizing Hormone Threshold and Luteinizing Hormone Ceiling Effect

The LH is required for terminal stages of follicular maturation, for meiosis, and ovulation. But high LH causes follicular atresia, premature luteinization, and oocyte quality may be compromised.[86] In patients over-responding to FSH, adding 30 μg rLH leads to monofollicular development.[87] This indicates LH ceiling effect.

CURRENT OPINION

1. Current opinion is that there is no absolute requirement for LH supplementation in unselected patient population.[88]
2. However, LH may have benefit in women aged more than 35 years and those who are poor responders to COS.[89] Though Cochrane review[88] and studies subsequently published have shown that LH supplementation—including for women with advanced age—has no benefit on ongoing pregnancy, with an adjusted OR of 0.99 (CI 0.76—1.29).[88]
3. LH supplementation may improve outcomes in patients with suboptimal response to FSH stimulation.[90]
4. No LH cut-off value is known to identify a female requiring LH supplementation and LH assessment is therefore of no use.[91]
5. In antagonist cycle, LH supplementation is controversial. Though some studies have shown that it improves results in selected groups.
6. LH estimation may not be a reliable guide to decide for LH supplementation because of variable bioactivity and polymorphism.

SUMMARY

Adding LH increases the number of developmentally competent oocytes in woman with endogenous LH suppressed below 1 IU/L at the start of stimulation. However, with LH more than 1 IU/L, adding LH is associated with significant lower embryo quality and implantation rate compared with rFSH alone.[92] Recombinant human LH (r-hLH) alone can trigger follicular growth arrest in a significant number of patients supporting the existence of LH ceiling during late follicular maturation.[93] rFSH with r-hLH 150 IU from day 6 might be beneficial in subgroup women in older reproductive age, having LH polymorphism and less sensitivity to LH.

The results of analysis by age showed a trend toward higher implantation rate with rFSH alone in patients aged less than 35 years. In contrast, the group aged more than 35 years had numerically higher implantation rate in the rFSH plus r-hLH group.[94,95] r-hLH supplementation on day 8 in patients having E2 less than 180 pgm/mL and no follicles more than 10 mm, significantly increases the number

of oocytes retrieved and requirement of rFSH was also decreased.[96]

CARRY-HOME MESSAGE

1. CC should be used for 3–6 months, 100 mg/day starting from day 5.
2. CC with estrogen has no role.
3. CC with dexamethasone is useful in chronic anovulatory patient and DHEAS is high.
4. CC with bromocriptine is used in patients with normal prolactin levels with galactorrhea and spikers when prolactin is high in first half of the cycle.
5. Routine use of hCG is not justified.
6. CC with GnRHa for downregulation has no role in IUI cycles.
7. CC with gonadotropins do not improve pregnancy rates.
8. CC with metformin can be used in CC resistant PCOS patients.
9. Metformin, OC pills, and progesterone are useful prior to CC.
10. Gonadotropins give better pregnancy rates than CC.
11. Step up protocol is used for normal responding ovaries.
12. Chronic low dose protocol is best for PCO.
13. r-FSH requires lower dosage with better efficacy.
14. rFSH is better than all urinary preparation.
15. rLH is used in poor responders and patients of age more than 35 years.

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