Principles and Practice of Controlled Ovarian Stimulation in ART

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Foreword

The introduction of a number of assisted reproductive technologies (ARTs) during the last 35 years besides having a tremendous impact in our specialty of Obstetrics and Gynecology, also affected millions of infertile couples around the world. The starting point for this development in infertility treatments was the birth of the first IVF baby, Louise Brown, in 1978. Today, more than five million babies have been born after IVF procedures and in some countries 5 % of all births are after IVF. The accomplishment to bring IVF from animal research into the clinics was consummated by a very fruitful collaboration by Bob Edwards and Patrick Steptoe from the UK, but Professor Howard Jones from the USA also was a key player in this development.

This new and comprehensive book of the theory behind controlled ovarian stimulation in ART and the clinical guidance in this field will be an important source of information for all medical doctors and embryologists in the field of ART. Importantly, this book also covers the pathophysiology and current research on the different causes of anovulation and increased knowledge in these fields will naturally be advantageous in designing the optimal stimulation protocols for each individual patient.

Now, back to the early days of ART and the pioneers. I had the privilege to meet with Professor Dr. Howard Jones during a one-day visit in May 2015 at The Jones Institute, Norfolk, USA. Howard Jones, at that time 104 years, told me the story of a long-term friendship and collaboration with Bob Edwards, which started already in 1965. Bob Edwards had already in 1963 started his research in IVF, by attempting to build on the original finding of Benjamin Chang at Worcester Foundation, USA. He had in the early 1960s been able to fertilize rabbit oocytes in vitro and to produce offspring. Bob Edwards did initial trials of IVF in mice in 1963, 15 years before the birth of Louise Brown. In 1965

the research fellow Bob Edwards came to the research group around Howard Jones, at that time professor of Obstetrics and Gynecology at Johns Hopkins Medical School in Baltimore. Howard Jones and his team performed a large number of wedge resections for PCOS, and these tissues were valuable to extract human oocytes to be tested for fertilization. The scientific achievements of this initial collaboration are described in the paper by *Edwards*, *Donahue*, *Baramki*, *Jones*, *titled* "*Preliminary attempts to fertilize human oocytes matured in vitro*" *published in American Journal of Obstetrics and Gynecology in 1966*. The scientist Bob Edwards had a shortage of human material for research back home in the UK, but through this collaboration between a basic scientist and a science-oriented clinician much of the background research to develop conditions that would allow fertilization in vitro by human gametes were developed. In many chapters of this book, the importance of translational research to develop protocols for ovarian stimulation is also highlighted.

Another interesting fact I learned from Howard Jones was that the Norfolk group was guided in their initial attempts of IVF by the protocol behind the first IVF birth in 1978 to use natural cycle oocyte pick up, since the thought was that this would lead to better chance for fertilization and subsequent implantation. In fact, they retrieved only 19 oocytes during the natural cycle of 41 women. Thirteen of these 19 oocytes fertilized, but none of the transferred embryos established a clinical pregnancy. The breakthrough for the Norfolk group came when the wife of Howard Jones, Professor Georgeanna Segar Jones, suggested that they would start to use controlled ovarian stimulation, despite the warnings of Bob Edwards that the fertilizing capacity of the oocytes would be poorer. Preparations of hMG were used for three or four days, starting at cycle day 4. The oocytes were harvested by laparoscopy 36 h after injection of hCG, just as in modern protocols. This breakthrough of adding exogenous hormonal stimulation to boost follicular development and to time oocyte pick up would lead to up to 4–5 mature oocytes being harvested and fertilized. This protocol was also used in the specific cycle that led to the birth of the first US IVF baby, Elizabeth Jordan Carr, in 1981. Thus, the Norfolk group is the true pioneer in controlled ovarian stimulation in ART.

The magnitude of the scientific and clinical breakthrough of IVF was acknowledged by the fact that Sir Bob Edwards was awarded the Nobel Prize in Medicine and Physiology in 2012. Another major breakthrough was the invention of intra-cytoplasmic sperm injection (ICSI) by the Brussels group in the early 1990s, which when combined with IVF lead to the conclusion that most forms of male infertility were now treatable. A new ART procedure that is also a combined IVF procedure is uterus transplantation, to treat absolute uterine factor infertility. We launched the first clinical trial on IVF plus uterus transplantation in 2013 and till today four live births have been accomplished. In these patients, controlled ovarian stimulation was performed prior to transplantation for IVF and cryopreservation of embryos, to be used for transfer around one year after transplantation. An important point to make is that development in ART takes time as exemplified by the 15 years from Bob Edwards' initial trials in the mouse to the first human live birth after IVF. Concerning uterus transplantation, we published the world's first pregnancy after uterus transplantation in 2002 and this was in the mouse. After a great number of research efforts in the mouse, rat,

pig, sheep, and baboon we could at last in 2014 announce the first live birth after human uterus transplantation. This event also, like in IVF, took place 15 years after the initiation of our uterus transplantation research in 1999.

It is my hope that this book also will stimulate young clinicians, scientists, and embryologists to address clinical problems in ART and by high quality research develop our field further.

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Preface

Controlled ovarian stimulation is an integral part of infertility treatment. A clear perspective of this treatment is difficult to accumulate without practical experience, as each patient behaves differently. Individualization in ovarian stimulation is fast evolving as it important for a successful result, and in order to be able to individualize, we need to understand both the basics and the complexities of the subject. This book attempts to deal with both these issues.

The book starts with basic issues like the physiology and workup of a patient. It then touches on simple treatments with oral ovulogens, goes on to gonadotropins and their comparison, and the role of LH and androgens in ovarian stimulation. There are chapters dedicated to individualizing protocols and the economics of ovarian stimulation which is essential information for all specialists dealing with these drugs. It is not only important to give the most effective treatment, but an insight into the economics of these drugs would also help the infertility specialist in giving the most cost-effective treatment. The pharmacogenetics of ovarian stimulation is a fast evolving concept and is highlighted here. The third section being one of the most important deals with monitoring and guides the gynecologist on when and how to change drugs. The endometrium and its behavior with stimulation are of paramount importance to make the treatment yield a pregnancy. The pathologies of ovarian stimulation like premature luteinization, empty follicular syndrome, and luteinized unruptured follicle are highlighted. The sixth section discusses controlled ovarian stimulation in difficult situations like endometriosis, hyperprolactinemia, PCOS, hypogonadotropic hypogonadism, and hyporesponders. In recent years, fertility preservation has evolved as a major option for those delaying pregnancy or in cancer patients

where treatment would induce a premature menopause, and ovarian stimulation for these patients has been dealt with here. Lastly the complications of ovarian stimulation like OHSS, epigenetics, and the impact of ovarian stimulation on oocyte and embryo quality have been considered. The multifetal pregnancy reduction technique has been discussed keeping in view the fact that it is an option for which the treating infertility clinician often has to counsel.

With the concepts in this subject changing explosively, as results of extensive research unfold, this book attempts to chart therapies, defining their current application to treatment in a practical manner for the practicing infertility specialist. Debatable issues like GnRH agonist vs antagonist protocol or hCG trigger vs GnRH agonist trigger have been argued in an evidence-based manner and conclusions drawn to solve dilemmas. This book aims to introduce to the practicing infertility specialists new evidence-based protocols, at the same time initiating young gynecologists into the field of infertility. I hope it will solve the dilemmas of many and that you may enjoy reading it as much as I did putting it together!

New Delhi, India Surveen Ghumman

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Part I

Pre-stimulation Assessment and Interventions

1 Detection of Ovulation and Aetiology of Anovulation

Pratima Mittal and Navdeep Kaur Ghuman

Abstract

Ovulation comprises of two interlinked processes, folliculogenesis and oogenesis. Folliculogenesis starts soon after the formation of follicles in intra-uterine life. It is a continuous process in which a follicle passes through several stages and ultimately ruptures to release an ovum. Oogenesis is a process by which primary oocyte arrested in diplotene stage of first prophase in embryonic life attains meiotic maturation and undergoes cytoplasmic changes to be finally released as mature ovum during reproductive life, although not all primary oocytes reach the stage of mature ovum and majority of them undergo atresia along this journey.

Anovulation or oligo-ovulation comprises around 21 % of female infertility. Any factor or process that disrupts finely tuned interactions of hypothalamo-pituitary-ovarian axis can potentially lead to anovulation. WHO classifies ovulation disorders in three groups: hypothalamamic pituitary failure, hypothalamic pituitary dysregulation and ovarian failure.

Detection or confirmation of ovulation, although an integral part of infertility workup, can be quite frustrating for clinicians and patients both. Most methods or tests for detection of ovulation are retrospective and demand monitoring over a long duration. Regularity of cycles is a reasonably assuring proof of ovulation, and detection tests are advisable in women with menstrual irregularities. Combining two or more methods for ovulation detection improves efficacy, accuracy and economics involved.

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Ovulation • Folliculogenesis • Oogenesis • Anovulation • Aetiology of anovulation • Detection of ovulation

1.1 Introduction

Ovulation is the prime and most important event of menstrual cycle. By definition, it is the occurrence in the menstrual cycle by which a selected mature follicle breaks and releases a viable oocyte from the ovary. Unlike spermatogenesis, which is a continuous process throughout the life of a male, females are born with all the eggs they will ever produce. In humans, by the seventh week of intra-uterine life, primordial germ cells reach the gonadal ridge from yolk sac endoderm. Here, they divide by mitosis to reach the peak level of 6–7 million at 20 weeks. Later on, the germ cells enter the first stage of meiosis and transform into primary oocytes. From mid-gestation onwards, each primary oocyte gets surrounded by a single layer of pre-granulosa cells to form primordial follicle. Within the primordial follicles, primary oocytes remain arrested in the diplotene stage of prophase 1 of meiosis 1, and the number falls dramatically from 6 to 7 million at 20 weeks of gestation to 1–2 million at birth to few lakhs at puberty. Most of the follicles at some point of their journey from primordial follicle to pre-ovulatory follicle undergo atresia thus ultimately leading to exhaustion of pool of oocytes and ovarian senescence.

1.2 Ovulation

The process of ovulation includes two separate but closely interlinked sub-processes – folliculogenesis and oogenesis.

1.2.1 Folliculogenesis

At all times, majority of primordial follicles are in a dormant resting phase. Intra-ovarian autocrine/paracrine factors pump some of these primordial follicles into the growing follicle pool. This initial recruitment of primordial follicles is gonadotrophin independent and occurs in a continuous manner starting soon after the formation of follicles. The follicles in the growing pool undergo a long process of follicullo-genesis and carry on their journey through stages of primordial, primary, secondary (class 1), tertiary (class 2), graffian, early antral (class 3, 4, 5), antral (class 6, 7) and pre-ovulatory (class 8) follicle. These stages can be broadly divided into pre-antral [primordial, primary, secondary and tertiary] and antral [graffian, early antral, antral and pre-ovulatory] type follicles. During this journey, a follicle grows in size (mean diameter of primordial follicle is 25 μm and preovulatory follicles can grow up to 20–30 mm before ovulation), shows mitosis and stratification of granulosa cells, formation of theca cell layer, zona pellucida and development of cavity or antrum along with chromosomal and cytoplasmic changes occurring in oocyte. The pre-antral phase of growth proceeds at a slow rate because of the long doubling time (about 10 days) for the granulosa cells, and it takes 300 days for a follicle to complete it. The growth rate picks up in the antral phase, and in another 50 days an antral follicle reaches the pre-ovulatory stage (Fig. 1.1) [1]. This phase of growth and development (antral phase) is under the influence of gonadotrophins. Atresia can affect follicles at all stages beyond secondary (stage 1) follicle stage but has highest incidence in antral follicles more than 2 mm in diameter [2].

1.2.2 Oogenesis

Process of oogenesis starts with the migration of germ cells from yolk sac to gonadal ridge during intra-uterine life. By birth, all germ cells have initiated their first meiotic division (now called

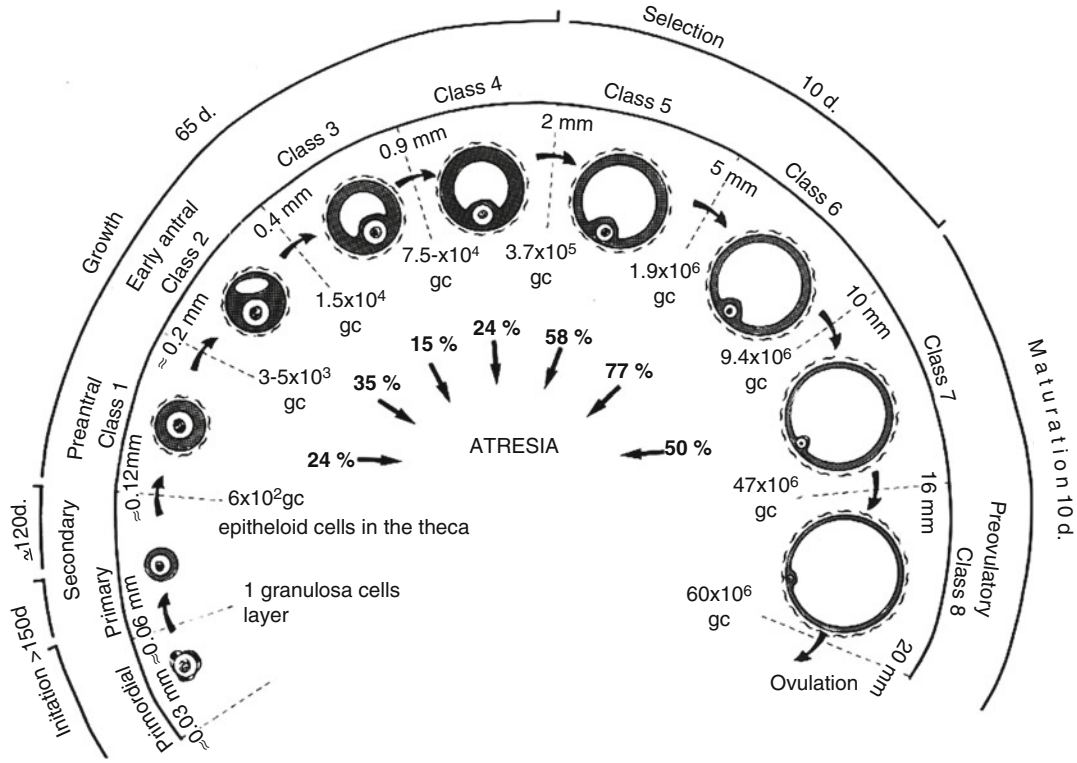

Fig. 1.1 Chronology of folliculogenesis in human ovaries. Preantral period: It takes 300 days for a recruited primordial to grow and develop to the class 2/3 (0.4 mm) or cavitation (early antrum) stage. Antral period: A class 4 (1–2 mm) follicle, if selected, requires about 50 days to grow and develop to the preovulatory stage. The dominant follicle of the cycle appears to be selected from a cohort of class five follicles, and it requires about 20 days to develop to the ovulatory stage. *gc* number of granulosa cells, *d* days (From Gougeon et al. [1]. Image courtesy of Alain Gougeon)

primary oocyte) and remain arrested in prophase stage of meiosis 1 till puberty. After puberty, each month few primary oocytes under the effect of pre-ovulatory surge of FSH and LH resume and complete their first meiotic division and result in formation of secondary oocyte and a polar body. The dominant secondary oocyte enters second meiotic division, gets arrested at second meiotic metaphase and subsequently ovulates. Fertilization triggers the resumption and completion of meiosis resulting in the formation of second polar body.

1.2.3 Physiology of Ovulation

In the luteal phase, corpus luteum is the site of estradiol and progesterone production. Corpus luteum possesses considerable capacity of self-regulation and maintains its function active for 14 days. With the demise of corpus luteum towards late luteal phase, the decreasing estradiol levels trigger rise in plasma FSH levels. This rise in FSH level recruits a cohort of class 5 follicles towards the end of luteal phase and facilitates its growth. One follicle in the recruited cohort of follicles is able to concentrate high levels of FSH in its follicular fluid and show rapid mitosis of granulosa cells to become the dominant follicle. This dominant follicle has most FSH receptors, is most sensitive to FSH and produces maximum oestrogen by FSH-mediated activation of aromatase enzyme. High concentrations of FSH in the micro-environment of dominant follicle, through gap channels between granulosa cells and oocyte, keep the concentration of cAMP and oocyte

maturation inhibitor (OMI) high, which in turn keep the oocyte in immature stage. The rising oestrogen level in turn by negative feedback mechanism lowers the plasma FSH level towards the end of the first week in follicular phase of menstrual cycle. This lowering FSH concentration is unable to sustain growth of rest of the follicles of the recruited cohort, which subsequently undergo atresia. The dominant follicle on the other hand by this time becomes less responsive to declining FSH levels and continues to grow. Moreover, the FSH-mediated induction of LH receptors on dominant follicle enables LH to take part in the growth and development of dominant follicle during later follicular phase and also in preparation of dominant follicle for upcoming LH surge. When the rising oestrogen level crosses a critical level, its negative feedback at hypothalamic-pituitary axis turns into a positive feedback giving rise to LH surge. LH surge lasts for 36–48 h. LH surge by dismantling the gap junctions between granulosa cells and oocyte inhibits the flow of maturation-inhibitory factors into ooplasm and causes drop in concentration of cAMP. Decreased concentration of cAMP in turn increases concentration of Ca and maturation-promoting factor (MPF), which are essential for the resumption of meiosis in oocyte and disruption of oocyte-cumulus complex triggering follicular rupture and ovulation about 36 h the LH surge. What enables one follicle of the cohort to concentrate FSH in its micro-environment in preference to others is still not clearly understood, but this selection leads to a single ovum being released by ovaries in each menstrual cycle [2, 3].

1.2.4 Recent Research

Recent research work has indicated the possibility of presence of renewable oogonia in the lining of female ovaries of humans, primates and mice. These studies have discovered that some mitotically active germ cells may migrate to ovaries from bone marrow and act as extra genial source of stem cells. Researchers have discovered these renewable germ cells as these were identified positive for several essential oocyte markers. If further studies support these findings, it could revolutionise treatment of infertility [4–6].

1.3 Aetiology of Anovulation

Ovulation is the result of complex and finely tuned interactions between hypothalamus, pituitary and ovary (Fig. 1.2). Any aetiology leading to the disruption of this fine tuning can cause anovulation. These can be broadly categorized as

1.3.1 Hypothalamic Factors

Hypothalamic hormones particularly gonadotrophin-releasing hormone (GnRH) are an important factor responsible for functional hypothalamo-pituitary-ovarian axis. GnRH hormone is a decapeptide which is synthesised and released by specialised neuronal endings of nucleus arcuate of hypothalamus. Any factor hindering pulsatile release of GnRH hormone leads to anovulation.

1.3.1.1 Functional Hypothalamic Dysfunction

Excessive strenuous exercise, stress, anxiety, under-nutition, eating disorders like anorexia nervosa by inhibiting normal GnRH pulsatility due to excessive release of corticotrophin-releasing hormone and stimulation of beta-endorphins can lead to amenorrhoea and anovulation. Drug abuse (cocaine, marijuana) and psychiatric disorders (schizophrenia) can also cause anovulation by suppression of GnRH.

1.3.1.2 Structural Hypothalamic Dysfunction

Infiltrative disorders of the hypothalamus (e.g. Langerhans cell granulomatosis, lymphoma, sarcoidosis, TB), tumours of hypothalamus, irradiation to the hypothalamus, chemo-toxic agents and traumatic brain injury by destruction of arcuate nucleus or distortion of hypothalamic-pituitary axis can lead to anovulation.

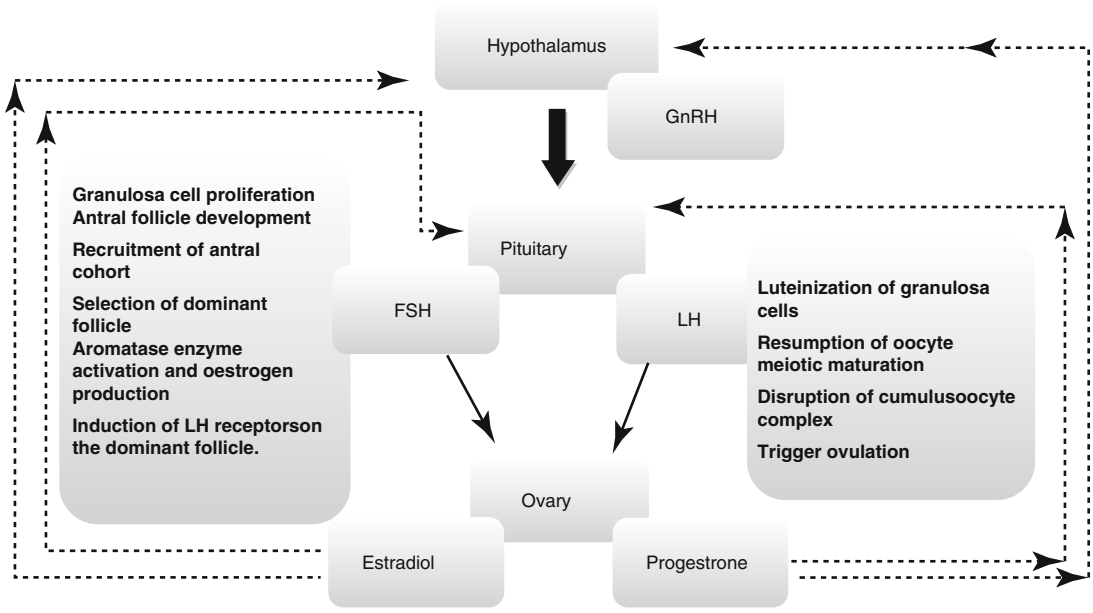

Fig. 1.2 Hormonal regulation of ovulation. *Solid arrows*: positive feedback. *Dotted arrows*: negative feedback

1.3.1.3 Genetic Disorders

Less commonly, genetic disorders like Kallmaan syndrome (defective migration of GnRH neurons), Prader-Willi syndrome and GnRH receptor gene mutation can be a cause of anovulation and infertility [7].

1.3.2 Pituitary Factors

GnRH from hypothalamus via portal circulation is transported to anterior pituitary where it leads to the release of gonadotrophins (LH and FSH). The amplitude and frequency of GnRH pulse determines the release of FSH or LH.

1.3.2.1 Structural Pituitary Dysfunction

Infiltrative conditions of pituitary (TB, sarcoidosis, hemochromatosis), space-occupying lesions of pituitary (microadenomas, macroadenomas, aneurysms), tumours of brain (meningioma, gliomas, craniopharngiomas), trauma to brain, irradiation to brain or postpartum pituitary necrosis by causing destruction of pituitary leads to anovulation.

1.3.2.2 Genetic Disorders

Idiopathic hypogonadotrophic gonadism, isolated gonadotrophin deficiency and gene mutation of beta subunit of FSH and LH are genetic disorders which can result in anovulation and infertility.

1.3.3 Ovarian Factors

The site of the final step in the process of ovulation is ovaries.

1.3.3.1 Iatrogenic Causes

Irradiation to pelvis, chemotherapy and surgical removal of ovaries are some of the iatrogenic factors that can lead to anovulation and infertility.

1.3.3.2 Genetic Factors

Chromosomal abnormalities like Turner syndrome, fragile X syndrome, idiopathic accelerated ovarian follicular atresia and gonadal dysgenesis are genetic causes of absent ovulation.

1.3.3.3 Ovarian Failure

Premature ovarian failure and resistant ovarian syndrome are other causes of anovulation [8].

1.3.4 Endocrine Causes

1.3.4.1 Polycystic Ovarian Syndrome

Polycystic ovarian syndrome is a heterogeneous group of disorders with a prevalence of 5–10 % in reproductive age-group [9]. Hyper-androgenism, oligo-ovulation or anovulation, oligo- or amenorrhoea, insulin resistance and obesity are the common clinical presentation of this syndrome complex. Abnormal endocrine environment with unopposed oestrogen and excess of LH leads to suppression of FSH release and hits the process of ovulation at the stage of follicular recruitment [10].

1.3.4.2 Hyperprolactinemia

Hyperprolactinemia of any cause can lead to anovulation by affecting the hypothalamo-pituitary axis at multiple sites. The important ones are impaired pulsatility of GnRH release and interference with the positive feedback effect of oestrogen on LH surge [7].

1.3.4.3 Hyper-androgenism

Other causes of hyper-androgenism like congenital adrenal hyperplasia, Cushing syndrome, androgen-secreting tumours and drug-induced virilization can lead to anovulatory infertility.

1.3.4.4 Thyroid Dysfunction

Severe untreated thyroid dysfunction, both hyper- or hypothyroidism, can cause menstrual irregularities and anovulatory infertility. The anovulatory effect of severe hypothyroidism is partly mediated by hyper-prolactinemia because of the fact that elevated TSH acts as a release factor for prolactin.

1.3.5 Systemic Causes

1.3.5.1 Renal Disease

Chronic and end-stage renal disease causes hypothalamic anovulation and menstrual acyclicity probably due to absence of positive feedback effect of oestrogen on hypothalamus and thus absence of LH surge. Women with uremia usually show high LH and high prolactin level [11].

1.3.5.2 Liver Disorders

Anovulatory infertility is common in women with end-stage liver disease. These women usually show decreased levels of gonadotrophins and oestrogen. These patients usually do not respond to GnRH stimulation or clomiphene, but successful liver transplant can result in restoration of ovulation.

Testicular feminising syndrome and other intersex conditions are unrelated conditions which can present with anovulatory infertility.

Anovulatory infertility accounts for 21 % of female infertility [12]. The World Health Organization classifies ovulation disorders into three groups (Table. 1.1) [13].

Table 1.1 World Health Organization Classification of ovulation disorders

Term	Definition
Group 1 Hypothalamic pituitary failure (hypogonadotrophic hypogonadism)	This group accounts for approximately 10 % of ovulatory disorders. This type is characterised by low gonadotrophins, low oestrogen and normal prolactin
Group 2 Hypothalamic pituitary dysfunction	This group includes anovulatory disorders characterised by gonadotrophin disorder and normal oestrogen. It accounts for 85 % of ovulatory disorders. Polycystic ovarian syndrome and hyperprolactinaemic amenorrhoea constitute majority of cases falling in this group
Group 3 Ovarian failure	This group is characterised by high gonadotrophins and low oestrogen. Around 5 % of women with anovulatory infertility have group 3 ovulation disorders

Adapted from Dhont [13]

1.4 Detection of Ovulation

Anovulation or ovulation disorders are the cause of infertility in 25 % of couples who have difficulty to conceive. In day-to-day clinical practice, it often becomes necessary to confirm ovulation either as a part of infertility workup or on a woman's request, who is facing difficulty in conceiving. Medical literature describes several methods to test ovulation, but majority of these tests are based on subjective symptoms and thus are not reliable or are cumbersome. The list includes

1.4.1 Regularity of Menstrual Cycle

Menstrual charting involves the recording of onset of menstruation over successive cycles. Regular menstrual cycles ranging from 26 to 36 days are usually an indicator of ovulation [9]. Longer or shorter cycles warrant further investigations to detect ovulation. Although regularity of cycles is a reasonably reassuring indicator of ovulation for a clinician, this may not be sufficient to reassure a woman facing difficulties with conceiving.

1.4.2 Pre-menstrual Symptoms

Pre-menstrual symptoms like menstrual cramps, breast tenderness, fluid retention and mood swings are useful clinical indicators of normal hormonal cyclicity and thus indirect indicators of ovulation. Though the symptoms are subjective and thus not very reliable in terms of an indicator for ovulation, their importance in a fertility workup cannot be over-emphasised.

1.4.3 Basal Body Temperature (BBT)

Van de Velde in 1904 observed the biphasic pattern of basal body temperature during menstrual cycle. Progesterone production after ovulation causes increase in basal body temperature with a minimum increase of 0.5° Fahrenheit above the follicular-phase basal temperature. This increase in basal body temperature is a retrospective indicator of ovulation. Measurement of basal body temperature is best done by specially calibrated thermometer and done as a first thing in the morning before leaving bed. Guermandi and associates in their study concluded that BBT coincides with ultrasonographic detection of ovulation in 74 % of cases with a sensitivity of 0.77 and specificity of 0.33 taking ultrasonography as standard for ovulation detection [14]. Another study has however shown that BBT agreed with ultra-sonographic ovulation only in 30.4 % cases [15]. Although a relatively inexpensive and self-administered method, studies have shown that this method is less accurate, not sufficiently reliable for detection of ovulation [16, 17]. Moreover, BBT can be affected by many factors other than hormonal changes.

1.4.4 Cervical Mucus Changes

Cervical mucus changes can be used alone or in combination with basal body temperature as an indicator of ovulation. Near the time of ovulation under the effect of oestrogen, cervical mucus becomes copious, thin and stretchy. These changes in cervical mucus do not indicate ovulation per se and are rather an index of optimum circulatory oestrogen levels before ovulation and may be seen in anovulatory cycles also. Alliende and co-workers in their study found that when adequately instructed, women can perceive ovulation by recording changes in their self-aspirated upper vaginal fluid in 76 % of cycles within ±1 day of ultrasonographic ovulation detection [18]. Other studies have quoted 48.3 % correlation with ultrasonographic detection of ovulation [15]. Insler and associates (based on quantity, spinnbarkeit, ferning and appearance of external os) [19] and Moghissi (based on amount of mucus, spinnbarkeit, ferning, viscosity and cellularity of cervical mucus) [20] had devised different cervical scoring systems. A value of 10–12 on Insler score and a value of 13–15 on Moghissi score are taken as indicator of pre-ovulatory cervical mucus. Although not very reliable, still this method is an inexpensive indicator of ovulation

and helps a woman to identify her fertile days. Presence of a vaginal and cervical infection nullifies the utility of this method as an ovulation indicator [14, 15, 21].

1.4.5 LH Surge Detection Kits

LH surge causes luteinisation of mature follicle and disruption of oocyte-cumulus complex triggering follicular rupture and ovulation. The LH surge can be measured in serum and blood and indicate imminent ovulation [22]. Urinary LH testing has the advantage of being simpler and being less affected by episodic fluctuations of LH levels than serum LH measurement. Studies have quoted sensitivity, specificity and accuracy of urinary LH test for detection of ovulation as 1.0, 0.25 and 0.97 respectively taking ultrasonographic detection of ovulation as standard [14, 15]. Several urinary LH surge detection kits are available in market which use test strips to detect changes in urinary LH levels. Urine testing is commenced 2–4 days prior to the expected ovulation and is continued till LH surge is detected. With irregular menstrual cycles, urine testing has to be timed according to the earliest and latest possible dates of ovulation. Success rate of detection of ovulation is usually quoted as 80 % with 5 days of testing and 90 % with 10 days of testing, with majority of these commercially available kits [23]. The main advantage of this test is that it can predict ovulation. On the other hand, the major disadvantage of this method is high false-negative rate which can be due to short LH surge or incorrect use of kit [24]. False positives can result in case of premature ovarian failure, peri-menopausal period and some cases of PCOS because of high basal LH level. LH surge in blood usually lasts for 36–48 h. Detection of LH surge in blood is impractical due to wide variation of normal LH levels during menstrual cycle and also is invasive and expensive. Six cohort studies evaluating the use of basal body temperature and urinary LH kits as indicators of ovulation to time intercourse did not find improvement in chance of natural conception [9].

1.4.6 Serum Progesterone Level

In practice, ovulation is confirmed retrospectively by measuring serum progesterone level in the mid-luteal phase, for example, day 21 of 28-day menstrual cycle, produced by luteinised ruptured follicle after ovulation. In women with longer cycles, the test needs to be performed later in the cycle and repeated weekly till the next menstrual bleed. Values ranging from 16 to 28 nmol/L (5–8.8 ng/ml) are taken as the lowest limit indicative of ovulation [9, 25–27]. Studies comparing the efficacy of different methods of ovulation have quoted 90 % concordance of ovulation detection by this method with ultrasonographic ovulation detection [14]. Major drawbacks of this method are lack of well-defined cutoff limits for serum ovulatory progesterone levels and the need for repeated testing especially with irregular menstrual cycles.

1.4.7 Transvaginal Ultrasonography

Follicle growth can be monitored through the menstrual cycle by using ultrasonography, ideally by transvaginal ultrasonography. Ovulation usually occurs when the follicle measures about 18–25 mm in size. Presence of free fluid in cul-de-sac, visualisation of collapsed and smaller follicle with internal echoes instead of previously visualised dominant follicle or visualisation of corpus luteum are ultrasonographic indicators of follicular rupture and ovulation. Ecochard and co-workers in their study compared different ultrasonographic indices for detection of ovulation and found the sensitivity and specificity to be 84 and 89.2 for disappearance or sudden decrease in follicle size, 61.6 and 87.1 for irregularies of follicular walls, 71 and 88.2 for free fluid in Pouch of Douglas and 38.4 and 79.7 for appearance of internal echoes in the follicle [28]. Ultrasonography is helpful in planning timed intercourse or insemination. This method is also helpful to precisely monitor follicular growth and detect multi-follicular development in women undergoing ovulation induction treatment along with providing

additional information about endometrium and pelvic organs. Although serial ultrasounds through the menstrual cycles can detect ovulation, yet this method is not very accurate in predicting ovulation as follicles can grow up to varying sizes before rupture. This is an expensive method in terms of instrument cost, requirement of skilled personnel and multiple visits required by the patient. Combining ultrasonography with other methods, for example, menstrual dating and serum hormone levels, can reduce the number of visits required making it more economical.

1.4.8 Endometrial Biopsy

Histological examination of small amounts of endometrium in late luteal phase showing progesterone-induced changes in the endometrium can provide indirect and retrospective indication of ovulation. Because of its invasive nature and risk of dislodging an implanted potential gestational sac, this method is not recommended for this purpose [23].

Conclusion

In conclusion, majority of methods used for ovulation detection are retrospective. Moreover, majority of these methods need monitoring over a long duration and therefore can be frustrating for both patient and clinician. In clinical practice, regularity of menstrual cycles is a reasonably adequate proof of ovulation, and ovulation detection tests should be resorted to in women with irregularities of cycles. Measurement of mid-luteal progesterone level is the most commonly used method in practice to detect ovulation. Transvaginal ultrasonography is useful for precise follicular growth monitoring and detection of multi-follicular development in women receiving fertility drugs especially in combination with serum hormone level measurement. Timed intercourse has been suggested to be stressful by a plethora of medical literature. Therefore, regular sexual intercourse (every 2–3 days) instead of using ovulation prediction methods for timed intercourse should be recommended. However, for minority of couples who find it difficult to have regular intercourse or couples who use some form of artificial insemination for conception, prediction of ovulation by LH kits can be useful. Also, using two or more methods in combination can improve efficacy, accuracy and economics involved.

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Evaluation Prior to Controlled Ovarian Stimulation

2

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Abstract

The couple needs to be completely evaluated before stimulation for IVF in order to assess the expected response, check fitness for pregnancy and identify correctable factors for a successful outcome. Main part of this evaluation is by various ovarian reserve tests which give a good measure of the number of expected oocytes and help to individualize the cycle. According to current evidence, antral follicle count and anti-Mullerian hormone are good markers of ovarian response and can help in defining strategy for COH.

Keywords

Ovarian reserve tests • Antral follicle count • Anti-mullerian hormone • Pelvic ultrasound • Counseling

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2.1 Definition

Controlled ovarian hyperstimulation (COH) is an integral part of assisted reproductive technologies. It traditionally involves stimulation of the ovaries with gonadotropins in combination with GnRH analogues for ovarian suppression to induce development of multiple follicles of the ovaries. Aim of COH is to achieve sufficient number of mature oocytes of good quality enabling selection of two or three good-quality embryos, at the same time avoiding undesirable outcomes like cycle cancellation or hyperstimulation. It is very important to make a complete evaluation of the female before subjecting her to COH.

2.2 Introduction

A detailed evaluation is of high clinical relevance prior to COH in order to assess the expected ovarian response, identify key factors to optimize the cycle outcome as well as do a complete pre-pregnancy evaluation.

Since there can be considerable variability in an individual response to stimulation with gonadotropins, a proper evaluation helps to tailor the cycle to fit the individual patient. That will enable clinicians to individualize ovulation induction and ovarian stimulation treatment, thereby minimizing complications and the risk of treatment failure while maximizing the chance of ongoing pregnancy.

It also helps in counselling women especially of the possible negative IVF outcomes such as cancellation of cycle, prolonged treatment, increased treatment burden and reduced pregnancy rates and perhaps reduces the number of dropouts particularly among women with an expected poor outcome. At the other end of the spectrum, identifying the expected high responders helps to reduce the burden of OHSS.

2.3 Ovarian Reserve Tests

Evaluation prior to COH is mainly by these tests. Ovarian reserve is defined as the number and quality of the follicles left in the ovary at any given time. The various tests to assess the ovarian reserve usually have good predictive value for the number of oocytes retrieved but have limited value in prediction of ongoing pregnancy.

Before a woman is subjected to any ovarian reserve testing, a complete evaluation of the couple with respect to general health and fitness must be done. This evaluation can be tabulated in simple chart form, a sample of which can be seen in Fig. 2.1. Figure 2.2 shows a sample investigation form for the infertile couple. Tests for evaluation of ovarian reserve include - antral follicle count, FSH, AMH (anti mullerian hormone), and clomiphene challege test.

2.3.1 Antral Follicle Count

Antral follicle count (AFC) is the number of antral follicles present in the ovaries and detectable by transvaginal ultrasound scan on day 2 or 3 of the period. It is commonly estimated by counting all identifiable antral follicles of 2–10 mm in diameter in both the ovaries [1]. A major technical improvement in ultrasound has been the development of three-dimensional (3D) automated follicular tracking, which can substantially decrease both intra- and inter-observer variability [2]. Antral follicle count is a good marker to predict the number of oocytes retrieved with false-positive rate of 15–20 % and can identify the expected poor, normal and high responders (Fig. 2.3).

However, there is considerable variability in agreed AFC cut-off levels used for predicting poor response. It may vary between AFC of 3 [3] and 12 [4]. A possible reason for such variability is the absence of a standardized measurement of antral follicles with different studies measuring different follicle populations 2–5, 2–9 or 5–9 mm. Most frequently reported cut-off values of AFC for prediction of poor response ranged between 5 and 7 [5]. In order to identify high responders [6], reportedly an AFC value of 16 had apparent sensitivity of 89 % and a specificity of 92 %. Other smaller prospective studies found values ranging between 9 and 14 as the most appropriate cut-off to identify hyper-responders [7, 8].

An easy-to-use algorithm to calculate the gonadotropin dose based on AFC has recently been published [9]. The nomogram calculated the

INFERTILITY PANEL

Date : .....

Name : .....

WIFE

A. Routine Tests
1. Hb+Hb Th
2. TLC/DLC/Piatelets/GBP
3. Bl Group ABO/Rh
4. Bl Sugar F/R/PP
   Glucose Tolerance Test
5. VDRL
6. HIV
7. HpB
8. H
9. Urine R/M
10. Blood Urea
11. S .. Creatine
12. L.F.T.
13. Others

B. Day 2 of Menstrual Cycle Tests
FSH/LH/PRL/TSH/E2/DHEAS
A.M. H.

C. Special investigations
TORCH Profile
APLA Profile
Karyotyping
3-D USG (TVS) for Cavity
Mock E.T.
Cx Swab {$^{C}_{S}$ & PAP's Smear
Vaginal Swab for BV & Chlamydia
Diagnostic Hysteroscopy

D. Autogen / RSA–BOH Panel

HUSBAND

A. Routine Tests
1. Hb+Hb Th
2. TLC/DLC/Piatelets/GBP
3. Bl Group ABO/Rh
4. Bl Sugar F/ R/ PP
   Glucose Tolerance Test
5. VDRL
6. HIV
7. HpB
8. HpC
9. Urine R/M
10. Blood Urea
11. S. Creatine
12. L.F.T.
13. Others

B. Semen Analysis

C. Semen {$^{c}_{s}$

D. Special Tests
1. Hormones
2. Testicular Ultrasound (Color Doppler)
3. Diagnostic TESA for Azoo
4. Karyotype for Azoo & Severeoligo spermia.

Fig. 2.1 Pre IVF evaluation and investigation chart

gonadotropin dose based on the age of the woman, Day 3 serum FSH level and AFC. For example, in a woman aged 30 years, with a Day 3 FSH of 4 IU/l and an AFC of 16, the most appropriate gonadotropin dose is 150 IU daily.

2.3.2 AMH (Anti-Mullerian Hormone)

AMH is a dimeric glycoprotein and a member of the transforming growth factor b (TGF-b) family of growth and differentiation factors. AMH is produced by granulosa cells of small growing follicles, is gonadotropin independent and remains relatively consistent in between and within the menstrual cycle. It has been shown to have inhibitory effect on primordial-to-primary follicle transition. AMH also reduces follicle sensitivity to FSH in vivo, and in vitro AMH inhibits FSH-induced pre-antral follicle growth [10]. Thus, there is clear evidence that AMH is involved in the regulation of follicle growth initiation and the threshold for FSH sensitivity. The intrafollicular concentrations of AMH in normal human antral follicles show a gradual reduction as the diameter of the follicle increases, and a sharp decline is observed around 8 mm [11]. The

INTERTILITY CHECK LIST

Pt. Name ..... **W/O** ..... **Age :** .....

Md. Life ..... **yrs** ..... **L.M.P.** .....

(A) BASIC LAB DATA INVESTIGATION

(1) Blood Group — H / W

(2) C.B.C. — H / W

(3) Th. Screen — H / W

(4) HIV1 / HIV2 — H / W

(5) Urine — A / S / M/E

(6) VDRL — H / W

(7) H & B — H / W

(8) HCV. — H / W

(9) G.T.T — H / W

(10) Chest X-Ray — H / W

H.S.G. REPORT

HYSTERCOPY REPORT

LAPROSCOPY REPORT

(B) BASAL ENDOCRINE PROFILE

(1) Sr. FSH .....

(2) Sr. LH .....

(3) Thyroid Profile .....

T3 .....

T4 .....

TSH .....

(4) TORCH Profile .....

(5) Sr.Prolaction _____

(6) Progesterone _____

(7) Estradiol _____

(8) Testosterone _____

(9) Cortisol _____

(10) DHEA-S _____

(C) SEMEN PROFILE

Total Count .....

Motility .....

RLP .....

Sperm Anti Body Test .....

P.C.T. .....

(D) FINDING CONTRIBUTING TO INFERTILITY

(E) SUGGESTED MANAGEMENT

Fig. 2.2 Infertility workup chart

rapid decline in AMH expression corresponds with the selection of follicles for dominance.

Anti-Mullerian hormone was assayed previously using primarily two different assay kits that have now been replaced by a newer assay. Evaluation of AMH levels prior to COS has several clinical utilities. There is substantial evidence in literature that AMH is superior to female age in assessing the quantitative aspects of the ovarian reserve, but its value is much more limited in the prediction of ongoing pregnancy.

Circulating anti-Müllerian hormone (AMH) can predict excessive as well as poor response to ovarian stimulation. A linear relationship exists

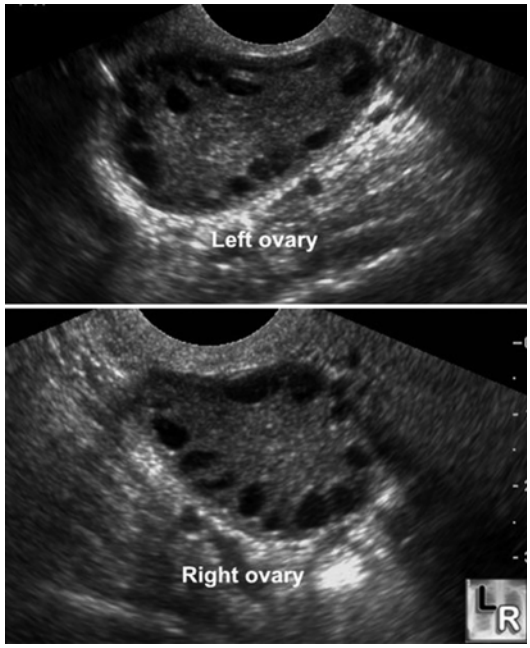

Fig. 2.3 TVS showing ovary with high AFC

between AMH and oocyte yield. At one extreme of the response, it helps to identify women at risk of ovarian hyperstimulation syndrome (OHSS) [12]. According to NICE guidelines of in vitro fertilization, an anti-Müllerian hormone level less than or equal to 5.4 pmol/l (0.8 ng/mL) predicts a low response to ovarian hyperstimulation, while a level greater than or equal to 25.0 pmol/l (3.6 ng/mL) predicts a high response [13]. Bologna's criteria for defining poor responders suggest AMH cut-off of 0.5–1.1 ng/ml [14]. But single value of low AMH especially in young women should not be used to deny treatment as even women with AMH concentrations at the limit of assay sensitivity have a significant chance of conception through IVF. AMH serves as a valuable tool in counselling the patient and may set the patient's expectations appropriately particularly at the bottom end of the spectrum where only a few oocytes may be retrieved. Nelson et al. have suggested an AMH-based strategy for deciding the protocol and gonadotropin dose for stimulation [15]. Tailoring the dosage of gonadotropin administration to AMH level has been shown to reduce the incidence of excessive response and cancelled cycles.

AMH is also useful in assessing the risk of ovarian damage secondary to chemotherapy, radiotherapy and ovarian surgery. Post-treatment AMH therefore can identify young girls and women receiving cancer therapy likely to have premature menopause or require pubertal induction, distinct from others who may be able to be reassured as to the likelihood of satisfactory ovarian function later in life.

Table 2.1 Normal AMH values

Ovarian fertility potential	ng/mL
Optimal fertility	4.0–6.8
Satisfactory fertility	2.2–4.0
Low fertility	0.3–2.2
Very low/undetectable	<0.3
High level	>6.8

AMH has been found to have sensitivity 44–97 % and specificity 41–100 % in predicting poor response to stimulation. Most of the studies have determined that cut-off level AMH >3.6 ng/ml has sensitivity and specificity of 82 % and 76 %, respectively, for prediction of OHSS (Table 2.1)

2.3.3 Basal FSH

Basal FSH levels increase on day 2, 3 or 4 of the menstrual cycle with advancing age. However, assays of FSH have significant inter- and intra-cycle variability which limit their utility. Despite its limitations, FSH is commonly used as a measure of ovarian reserve, and high values have been associated with but not necessarily predict poor response to stimulation and failure to concieve. The sensitivity of FSH in identifying poor responders varies from 10 to 80 % and decreases with increasing cut-offs. Recent study employing efficiency curves demonstrated 100 % specificity for failure to achieve live birth at levels more than 18 IU/l. A single elevated FSH level in women <40 years may not predict poor responders or failure to concieve. But clinical utility lies in being fairly certain that women with abnormally elevated FSH will have diminished reserve [16] (Table 2.2).

2.3.4 Female Age

Advanced maternal age causes decreased success rates in ovarian hyperstimulation [17]. However,

Table 2.2 Basal FSH levels and clinical implications

FSH levels IU/L	Clinical implication
<9	Reassure
9–10	Suboptimal
10–12	Decreased ovarian reserve
12–17	Markedly reduced ovarian reserve
17–20	Poor prognosis
>20	No pregnancy

a younger woman with a raised FSH would respond better than an older woman with raised FSH. Age is one of the most important determinants of ovarian response.

2.3.5 Other Ovarian Reserve Tests

2.3.5.1 Clomiphene Citrate Challenge Test

The test involves measuring baseline FSH after administering clomiphene citrate 100 mg day 5–9 of cycle typically on day 3 and 10. An elevated FSH level after clomiphene stimulation therefore suggests diminished reserve. Cycle day 10 FSH levels have a greater sensitivity but lower specificity compared to cycle day 3 FSH levels [16].

2.3.5.2 Endocrine Challenge Test (Gonadotropin-Releasing Hormone Agonist Stimulation Test)

The purpose of GAST is to evaluate changes in E2 on cycle days 2 and 3 following administration of GnRH agonist (leuprolide acetate).

2.3.5.3 Exogenous FSH Ovarian Reserve Test (EFFORT)

Originally, the test was developed to improve the predictive value of day 3 FSH values in controlled ovarian hyperstimulation for IVF. The E2 level is recorded on cycle day 3 before the administration of 300 IU of purified FSH. Another level of E2 is done 24 h after giving FSH. It was postulated that the dynamic increase in E2 of more than 30 pg/ml would be predictive of a good response in a subsequent IVF cycle.

These dynamic tests for assessing ovarian reserve are considered as too laborious for screening purposes.

2.4 History

All couples presenting with infertility should have had a detailed history and physical examination done, which should be reviewed prior to COS. This should usually include

- Menstrual history: especially in regard to cycle length and duration which might suggest anovulation, PCOS or diminished reserve
- Obstetric history: previous pregnancy outcome
- Past surgeries (procedures, indications and outcomes), serious illnesses or history of pelvic inflammatory disease or sexually transmitted infections
- Any abnormal pap smears and treatment taken
- Symptoms suggestive of other endocrine abnormalities which might be contributing to infertility
- Any medical disease contraindicating pregnancy
- Social history to evaluate for any environmental exposures or social habits (such as smoking, drinking alcohol, drug usage or extreme exercise)
- Family history of birth defects, mental retardation, early menopause or reproductive compromise
- Detailed history of male partner regarding occupational exposures, medical illness, genital surgery or infections, smoking, sexual dysfunction or difficulty in giving semen sample

2.5 Examination

The physical examination of the lady is performed to evaluate the pelvic organs and assess potential hormonal problems. It should include any thyromegaly, breast examination, signs of androgen excess, vaginal or cervical abnormality, secretions or discharge, pelvic or abdominal tenderness enlargement or masses, adenexal masses or cul-de-sac nodularity.

2.5.1 Body Mass Index (BMI)

It is to identify obese or very lean individuals. Obesity is associated with higher miscarriage rates and a higher prevalence of neonatal complications, congenital anomalies and pregnancy-associated complications. Also BMI helps in deciding the starting dose of gonadotropins in association with other variables as suggested by CONSORT study [18]. In assisted reproduction, however, there are conflicting reports on the effect of obesity on oocyte quality, embryo development, lower number of mature oocytes, lower implantation and pregnancy rates. Total amount of gonadotropins used was significantly higher in patients with a BMI ≥25 kg/m [2], when compared to those with a normal BMI.

2.5.2 Male

A complete physical examination should be done including height, gynaecomastia, inguinal area, penile length, curvature and urethral meatus, testes volume and location, varicocele, epdidymal nodularity or tenderness, presence of vas deferens especially in cases of azoospermia and other male factor of infertility.

2.6 Previous Cycle Details

Details of hyperstimulation if done previously must be reviewed including type of protocol, gonadotropin dosage, days of stimulation, number of mature oocytes obtained, fertilization and cleavage rates, details of embryo transfer. This helps in planning of present cycle as well as to identify any anticipated problem.

2.7 Pelvic Ultrasound

A good baseline pelvic scan can provide invaluable information prior to starting COH and can be combined with saline hysterogram SHG examination as discussed earlier.

It helps to determine if there are any problems in the uterine musculature (fibroids or adenomyosis) (Fig. 2.4) or cavity (uterine malformations, adhesions, polyps).

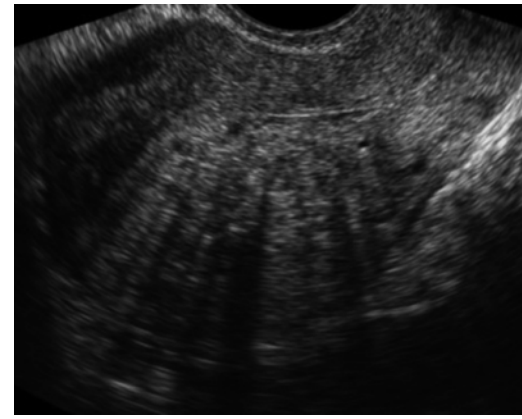

Fig. 2.4 Sagittal view of uterus with adenomyosis

Its good clinical practice to scan the endometrium in the luteal phase of preceeding cycle. A thick homogenous hyperechoic endometrium rules out presence of luteal phase defects. With addition of colour Doppler studies of uterine artery and power angio, the spiral artery and endometrial vascularization can be evaluated (Figs. 2.5 and 2.6).

Ovaries must be assessed to rule out any pathology (endometrioma, ovarian tumours) and check for accessibility during oocyte retrieval.

Measuring the volume of ovaries and antral follicle count as described earlier helps decide protocol and dosage for COH.

2.8 Serological Tests

These include HIV, hepatitis B and C, VDRL titre, blood grouping and thalassemia screen of both partners.

2.8.1 HIV

Although the risk of exposure to this virus is very low, it is good clinical practice to check the HIV status of the couple. The concern of a pregnancy in a female who is infected with the virus is that pregnancy increases the death rate dramatically in an

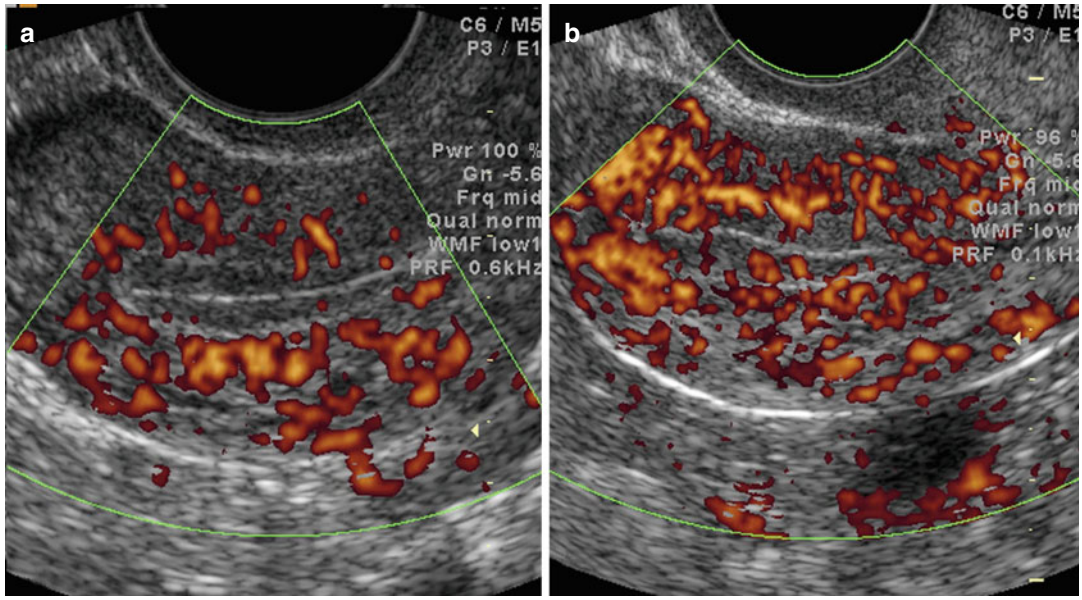

Fig. 2.5 (**a**, **b**) Power Doppler showing well-vascularized endometrium

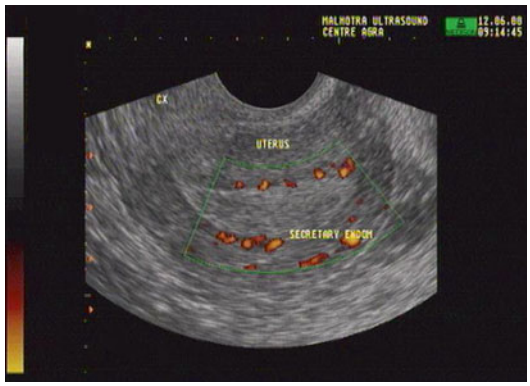

Fig. 2.6 Sagittal view of uterus showing secretory endometrium

HIV-positive woman. The second is the risk of transmitting the HIV virus to a child during childbirth.

2.9 Evaluation of Uterine Cavity

A wide range of endometrial and uterine cavity abnormalities like polyps, fibroids, intrauterine adhesions, and uterine anomalies are reported in women with subfertility, which can affect the implantation rates in an IVF cycle or cause spontaneous abortions. Also, abnormal uterine findings are reported in as many as 50 % of women with recurrent implantation failure [19]. Traditionally, HSG is used to evaluate the uterine cavity. Endometrial polyps or fibroids are shown as filling defects or uterine wall irregularities. HSG can also show intrauterine adhesions and congenital anomalies as it enables clinicians to visualize the general configuration of the cavity. Compared with hysteroscopy, HSG has a high sensitivity (60–98 %) but a low specificity (15–80 %) in detecting uterine abnormalities and is, therefore, associated with relatively high false-positive and false-negative rates [20–22].

The use of TVS in conjunction with saline infusion known as saline hysterography (SHG) or saline infusion sonography improves the delineation of the uterine cavity and is an excellent alternative to hysteroscopy for screening the uterine cavity. It has been reported to have 87.5 % sensitivity, 100 % specificity, 100 % positive predictive value and 91.6 % negative predictive value for the detection of any cavity abnormality as compared with hysteroscopy [23]. Advantages of SHG are that it can be easily performed in the office setting, is non invasive, is well tolerated and can be done as an adjunct to baseline pelvic scan prior to stimulating every IVF patient (Fig. 2.7).

However, hysteroscopy is the gold standard for the investigation of uterine cavity, particu-

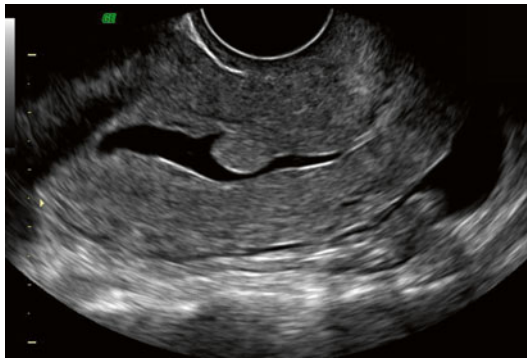

Fig. 2.7 Sonohysterogram showing endometrial polyp

larly when pathology is suspected. It permits direct visualization of the uterine cavity, revealing the nature, location, shape, size and vascular pattern of any uterine cavity abnormalities, such as polyps, submucosal fibroids, differences in endometrial thickness and adhesions. It also allows a directed biopsy and therapeutic intervention for the treatment of any pathology. Systematic review by Bosteel et al. [24] found hysteroscopy in the cycle preceding a subsequent IVF attempt nearly doubles the pregnancy rate in patients with at least two failed IVF attempts compared with starting IVF immediately. A study by Makrakis et al. [25] to estimate the effect of hysteroscopy on achieving a pregnancy in women with a history of two implantation failures following IVF 37 % had abnormal findings, 22 % of which were unsuspected, and subsequent IVF treatment showed significantly increased clinical and ongoing pregnancy rates.

2.10 Evaluation of Adnexal Structure: Endometrioma, Hydrosalpinx

2.10.1 Ovarian Cyst

Assessment of any ovarian cyst on day 2 must be made. Large cysts are aspirated before stimulation is started. Smaller ones can be ignored as long as they are not hormone producing. Estradiol and progesterone are raised in case they are hormone producing.

2.10.2 Endometrioma

Endometrioma, if less than 3 cm, can be ignored. Larger endometriomas may cause difficulty in monitoring or oocyte retrieval and hence require removal. Either a surgical removal of cyst wall is done or fulgration may be done if removal is not possible. Medical treatment is not recommended.

2.10.3 Hydrosalpinx

Ultrasound-detected hydrsalpinx is a significant finding. It is recommended that the hydrosalpinx is removed before a patient is taken up for IVF as it impairs implantation. ASRM states that the live birth rate achieved with IVF among women with hydrosalpinges is approximately one half that observed in women without hydrosalpinges. In women with hydrosalpinges, preliminary laparoscopic salpingectomy or proximal tubal occlusion improves subsequent pregnancy and live birth rates achieved with IVF. For every six women with hydrosalpinges, one more ongoing pregnancy will be achieved if salpingectomy or tubal occlusion is performed before IVF [26].

2.11 Male Evaluation

One should not forget the male partner because male factor can contribute up to 50 % to the problem of infertility. Apart from complete history and physical examination as discussed earlier, a complete semen analysis must be performed for the total sperm concentration, motility and morphology (Table 2.3) [27]. Vitality testing should be done if motility is less than 40 %. Some centres assess the teratozoospermic index and sperm deformity index in cases of male infertility. In all cases of severe oligoasthenozoospermia and non-obstructive azoospermia, additional hormonal assay (FSH, LH, testosterone) and karyotyping should be performed. Scrotal ultrasound is indicated in case of atrophic testis, ectopic testis and enlarged testis to rule out neoplasm and to confirm varicocele or epididymal abnormalities. Transrectal ultrasound (TRUS) is an invaluable tool to visualize the distal genital abnormalities

Table 2.3 World Health Organization semen parameter (2010)

Parameter	WHO 2010
Volume	1.5 ml
pH	>7.2
Viscosity	<3 Scale 1–4
Concentration	15 million/ml
Total sperm number	>39 million
Total motility (PR + NP)%	40 %
Progressive motility	32 %
Normal forms	4 %
Normal morphology WBC	<1 million/ml
MAR test	<50 %
Immunobead test	<50 % motile sperms with boundbeads
Vitality %	>58 %
Seminal zinc (μmol/ejaculate)	≥2.4
Seminal fructose (μmol/ejaculate)	>13

From WHO [27] WHO laboratory Manual for examination of human semen 2010

like vasal agenesis, absence of seminal vesicle, ejaculatory duct cyst or dilatation. In order to distinguish between obstructive and non-obstructive azoospermia, diagnostic testicular biopsy is indicated and must be combined with cryopreservation. Sperm DNA fragmentation can be assessed by various methods like sperm chromatin structure assay (SCSA), COMET and TUNEL in certain groups of patients.

2.12 Counselling

Infertility and its treatment can be a major source of stress for the couple and the family. This stress is usually intensified during treatment, especially throughout an ART cycle. While this is not a mandatory requirement, couples should be encouraged to meet with a counsellor prior to beginning treatment, as well as during and after a cycle. This helps them in pschychological adjustment, reduce stress levels and ultimately contribute towards better pregnancy rates as well as decrease the dropout rates associated with IVF.

Conclusions

As women have a fixed ovarian reserve, which diminishes with age, so complete evaluation of male, female and the couple is mandatory before the start of any controlled ovarian hyperstimulation in order to ensure successful outcome.

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Therapeutic Intervention of Endometrial Pathology Before Ovarian Stimulation

3

Lakhbir K. Dhaliwal and Shalini Gainder

Abstract

Successful pregnancy occurs only if the development of oocytes is accompanied by parallel development of endometrium which is receptive once the fertilized embryo reaches the endometrial cavity and further synchronizes with the development of the embryo by undergoing complex series of decidualization. This forms a chain of complex events taking place in the endometrium. Knowledge about endometrial receptivity is still limited, and where needed, interventions can improve infertility outcome. This may be in the form of removing endometrial polyps, resection of sub-mucous fibroids, treatment of endometritis, resection of uterine septum and uterine adhesions and hormonal manipulation of the endometrial milieu using estrogen therapy, sildenafil, aspirin, pentoxifyline and vitamin E. Women with polycystic ovaries also need treatment of endometrium, which may be hostile under the influence of high androgens or due to excessive stimulation under estrogen therapy and harbour hyperplasia or endometrial carcinoma. Similarly, women with endometriosis have luteal phase deficit and therefore may have irregular bleeding. Stem cell therapy is emerging as a new hope for women with damaged endometrium as a result of Asherman syndrome.

Keywords

Endometrium • Polyps • Submucous fibroids • Asherman syndrome • Stem cell

3.1 Introduction

Embryo implantation is dependent on multiple interactions that take place in the endometrium which are regulated by complex endocrine and paracrine–autocrine interactions; endometrial receptivity may undergo inter-cycle

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and inter-individual variations. Over the previous decades, a lot of research has benefited in improving the ovarian stimulation protocols, but pregnancy is difficult to achieve if a good receptive endometrium does not parallel the oocyte maturation. Various pathologies can affect the endometrium directly, and different infertility causes can have indirect effect on the endometrium. Therefore before ovulation induction, one needs to ascertain that the endometrium would be receptive for the embryo. Drugs being used for induction of ovulation also may influence the endometrium, therefore an infertility expert has to balance the hormonal milieu to obtain optimal endometrial lining in various stimulation protocols for controlled stimulation as well as when stimulation is for fresh IVF cycles or endometrium is prepared for *frozen IVF cycles.*

3.2 Various Endometrial Pathologies Affecting the Endometrium

3.2.1 Infections

Chronic endometritis may be found in infertile patients and would require a course of antibiotics. Besides that in India, tuberculosis forms an important cause of infertility, and endometrium is often involved. Clamydia may also be present.

3.2.2 Anatomical Malformations

Intrauterine synechia due to old infections, fibrosis and adhesions may cause a distorted endometrial cavity with decreased blood flow. Mullerian anomalies like uterine septum or bicornuate uterus may be present.

3.2.3 Fibroids

Fibroid in uterus or endometrial polyps may be present. It can cause an impaired blood flow to endometrium if it is a sub-mucous fibroid.

3.2.4 Foreign Body

Foreign bodies like bone fragments, old products of conception or intrauterine devices may be present.

3.2.5 Pathologies That Indirectly Affect the Endometrium

3.2.5.1 Polycystic Ovarian Syndrome

Oestrogenic effect leads to thick endometrium, endometrial hyperplasia and endometrial carcinoma. Androgenic effect leads to thin endometrium.

3.2.5.2 Luteal Phase Defect

Luteal phase defect may be seen in endometriosis or women with prolactin disorders, thyroid disorders and ovarian ageing. Irregular shedding leading to premenstrual spotting may occur.

3.2.5.3 Poor Hormonal Environment

Insufficient oestrogen, progesterone or high androgens could be responsible for poor endometrial development.

3.2.6 Iatrogenic

Excessive curettage could damage endometrium and lead to intrauterine adhesions. Use of clomiphene citrate in some cases leads to anti-oestrogenic effects on endometrium preventing its development in mid-cycle.

3.3 Evaluation of Endometrium Prior to Controlled Ovarian Stimulation

3.3.1 Clinical History

Knowledge of the menstrual cycle, amount of bleeding, number of days for which the woman bleeds and cycle length is vital as it gives an insight of the endometrium. Women may complain of premenstrual spotting in presence of luteal phase defect, especially if the oocyte is

of poor quality, which may occur in endometriosis, prolactin disorders or in ageing women. Periods of amenorrhoea followed by excessive bleeding should make the physician consider anovulation-associated endometrial hyperplasia, which can be present in young women with PCOS. Scanty menstruation may be present in women having genital tuberculosis or Asherman syndrome or because of clomiphene therapy.

3.3.2 Ultrasound

A baseline trans-vaginal ultrasonography helps in imaging of the endometrium to rule out the presence of any endometrial polyps or fibroid which may be polypoidal projecting into the cavity or sub-endometrial in location. Any collection inside the endometrial cavity can be seen, or thick echogenic endometrium may suggest endometrial proliferation or hyperplasia. Endometrial adhesions may be suggested by thin irregular endometrium with areas of echogenicity. Sometimes, uterine septa or other anatomical malformations can be suspected on performing USG.

3.3.3 Colour Doppler

In recent years, the role of colour Doppler to assess endometrial vascularity has been introduced. Endometrial and sub-endometrial blood flows can assess the angiogenesis that takes place during the cyclical growth of endometrium.

3.3.4 3D Ultrasonography

A 3D sonography may at times give insight into the shape of the endometrial cavity in cases of malformations and help to distinguish between septate and bicornuate uterus. Endometrial polyps and fibroid location can be more defined; however, if a patient is anyway planned for hysteroscopy, then this test may only add to the cost of patient treatment.

3.3.5 Endometrial Biopsy/ Aspiration

Endometrial biopsy/aspiration for endometrial sampling is not a mandatory evaluation in the developed countries; however, its importance is unmatched to any other investigation as it helps to pick up about 8–15 % of cases of genital tuberculosis either by the microscopic examination of simple concentrated smear by Ziehl-Neilsen stain to identify the acid-fast bacilli or by identification from various short-term cultures like BACTEC, MGIT 460 or by conventional culture. Histopathology also aids in diagnosing granulomas, or features of chronic inflammation along with detailed dating of the endometrium. Cases of hyperplasia both simple and complex as well as early cases of endometrial carcinoma have been diagnosed while evaluating endometrium for infertility at our centre, which thereby changes the management of these women.

Endometrial aspiration is usually performed in the premenstrual phase, and the aspirate is divided; a part of it is taken in saline and forwarded for smear, culture and PCR for tuberculosis, whereas a part of the sample is forwarded in formalin for histopathology examination.

3.3.6 Hysterosalpingography

This is a simple outpatient procedure used mainly for demonstrating the tubal patency; however, it gives a real insight into the shape of the endometrial cavity and can document any anatomical distortion either due to anomaly or due to presence of polyps, synechia or adhesions.

3.3.7 Saline Infusion Sono-Hysterography

It is done by instilling saline in the uterine cavity and distending the cavity, which helps in identifying the presence of any polyps in the cavity and distinguishing them from sessile fibroids. This helps in deciding whether they can be adequately managed by hysteroscopy. This technique is safe,

low cost, well tolerated and feasible in most outpatient infertility clinics. Saline infusion sonohysterography has been demonstrated to be superior to TVS and/or HSG also for the diagnosis of uterine malformations. Soares et al. [1] found that SIS had a higher sensitivity (77.8 %) compared with TVS and HSG (44.4 %). Alborzi et al. [2] showed in a study of 20 patients with a history of recurrent pregnancy loss and an HSG diagnosis of septate/bicornuate uterus that SIS was better than HSG for differentiating a septate from a bicornuate uterus [3].

3.3.8 Hysteroscopy

If any endometrial pathology is suspected, then a hysteroscopy becomes mandatory, as a directed intervention is possible along with confirmation of the diagnosis. When a polyp is seen, then a polyp removal or resection of sub-mucous myoma is possible. Operative hysteroscopy can be used to remove adhesions and perform septum resection thereby improving the uterine capacity. In all women where any endometrial pathology is suspected, hysteroscopy can help diagnose and treat and thereby improve the subsequent implantation and pregnancies. In women with unexplained infertility, hysteroscopy can at times help in finding small adhesions which can be resected simultaneously and visualize areas of endometritis, calcifications or granulomas. It is not rare to find women having remnant bone chips in the endometrial cavity from a previous abortion which prevent implantation and pregnancy thereby warranting removal. A hysteroscopic visualization and removal of all the bone fragments improve the likelihood of having pregnancy in future.

Hysteroscopy along with laparoscopy also help in diagnosing the Mullerian anomaly present and distinguish presence of septum in the cavity from bicornuate or didelphus uterus. A metroplasty or septum resection may improve the chances of pregnancy after surgery.

A systematic review and meta-analysis investigated the use of routine hysteroscopy prior to starting the first IVF cycle on treatment outcome in asymptomatic women. One randomized and five non-randomized controlled studies including a total of 3179 participants were included comparing hysteroscopy with no intervention in the cycle proceeding the first IVF cycle. There was a significantly higher clinical pregnancy rate (relative risk, RR, 1.44, 95 % CI 1.08–1.92, $P=0.01$) and LBR (RR 1.30, 95 % CI 1.00–1.67, $P=0.05$) in the subsequent IVF cycle in the hysteroscopy group. Hysteroscopy in asymptomatic women prior to their first IVF cycle could improve treatment outcome when performed just before commencing the IVF cycle [4]. Recommendations regarding the efficacy of routine use of hysteroscopy prior to starting the first IVF treatment cycle are lacking.

3.4 Endometrial Pathology

3.4.1 Endometrial Polyps

Polyps diagnosed prior to commencement of controlled ovarian stimulation (COS) for in vitro fertilization (IVF) should be removed. The management of polyps seen during the course of COS for IVF should be individualized given the number of embryos created, the previous reproductive history of the patient and the individual clinics' success rates for their frozen embryo programme. Polyps when present especially near the cornua may inhibit the transport of sperms and thereby interfere with fertility [5].

3.4.2 Fibroids

Submucosal myomas and intramural myomas that distort the endometrial cavity are associated with lower pregnancy, implantation and delivery rates in women undergoing IVF compared with infertile women without myomas [6, 7]. Reproductive outcomes improve after myomectomy for a submucosal myoma, and the difference is more pronounced if the myoma was the only identifiable aetiology. Recent studies demonstrate that leiomyomas may adversely affect the overlying endometrium and impair endometrial receptivity by altered expression of

HOXA-10 in endometrial stromal cells during the window of implantation in 69 % of patients with uterine leiomyomas [8].

3.4.3 Mullerian Anomaly/Septate Uterus

The septum in the uterus has poor vascular supply and therefore lowers fecundity. The role of metroplasty or hysteroscopic septum resection in patients with primary infertility remains controversial especially done prophylactically during infertility treatment when it is only proposed to hinder fertility, and its impact on pregnancy is unknown. Hysteroscopic incision of the septum has been shown to be a safe, simple, and efficient method of treating septate uteri [9]. Many IVF centres recommend removal of incomplete uterine septa before IVF to reduce the possibility of miscarriage and improve the pregnancy outcome [9, 10].

3.4.4 Genital Tuberculosis

The definitive diagnosis of genital tuberculosis is in most cases based on the microbiology report or the histopathology of the endometrial sampling done prior to treatment of infertility. Once a diagnosis of tuberculosis is established, then it is mandatory to treat them for the disease else it progresses to damage the endometrium to the extent when treatment is impossible or the scarring is produced. The treatment is in the form of anti-tubercular drugs given for duration of 12 months. It is advisable to repeat the biopsy at 6 months after treatment and confirm treatment. However if the bacilli still persist, then she may be a candidate having multidrug-resistant form of tuberculosis where second-line therapy or category II drugs are needed [11].

Once the treatment is completed, then a hysteroscopy and laparoscopy should be done to see the effect of tuberculosis on the genital tract. If any adhesions or synechia are seen, they should be cut, and the anatomical correction may benefit in achieving conception.

3.4.5 Endometriosis

The decrease in implantation rates in endometriotic patients is still a matter of debate. The meta-analysis of Barnhart [12, 13], which evaluated the impact of endometriosis on IVF outcome, showed a lower pregnancy rate, with a particular impact of endometriosis on implantation (OR = 0.86 IC = 0.85–0.87). The sub-group analysis according to the stage of endometriosis highlighted a weaker implantation in the event of severe endometriosis. This effect is mediated through a decrease in embryo quality, probably consequent to a decrease in the number of embryos available. More recent studies with long GnRH agonist protocols of stimulation did not find these results: Hickman studied 149 cycles, comparing patients with endometriosis with those having a tubal infertility; the rate of implantation was comparable between groups (of 28.0 % versus 29.8 %, respectively), thereby suggesting that suppression of endometriosis by GnRH analogues does seem to have an overall benefit in treatment of endometriosis [14]. The decreased expression of biomarkers of implantation such as glycodelin A (GdA), osteopontin (OPN), lysophosphatidic acid receptor 3 (LPA3) and HOXA10 may indicate impaired endometrial receptivity in patients with endometriosis [15].

3.4.6 Hypogonadotropic Hypogonadism

Women having diagnosis of hypogonadotropic hypogonadism should receive cyclical oestrogen followed by progesterone which helps in priming the endometrium for the future when controlled ovarian stimulation would be done. These women may not respond to combined oestrogen progesterone pills and therefore should receive oestrogen followed by progesterone in a more physiological pattern.

3.4.7 Polycystic Ovarian Syndrome

PCOS women have oligo or anovulatory cycles, and therefore there is suboptimal long-standing

unopposed oestrogen leading to periods of amenorrhoea followed by heavy bleeding [16]. The regulatory role of progesterone is suboptimal or absent. The endometrial growth and differentiation in women with PCOS is influenced by androgens and insulin also. Under this hormonal milieu, the endometrium does not undergo a secretory transformation, and there continues a constant mitogenic effect of estradiol which may lead to endometrial overgrowth, unpredictable bleeding patterns, hyperplasia and endometrial carcinoma. The endometrium of women with PCOS is considered a model of dysfunctional endometrium, demonstrating over-expression of androgen receptors and failing to regulate oestrogen receptors (ERs), when compared to normal women. Studies carried out in PCOS have shown differences in complements of steroid receptors and co-activators, when compared to fertile women. The endometrium, in this case, over-expresses androgen receptors and fails to regulate the ER-α (oestrogen receptor, α) in the window of implantation [17–19]. Women with simple hyperplasia should be treated with progesterone cyclical for 3 months and then ovulation induction where luteal phase support with dihydroprogesterone 10 mgs is given for 10–12 days. Women with complex hyperplasia or diagnosis of early-stage carcinoma should be treated as per guidelines prior to ovulation induction. Giving high dose of megesterol (80–120 mgs/day) or medroxy progesterone acetate (200 mg/day) with or without levonorgestral containing intrauterine device, hysteroscopic resection of growth and re-evaluation after 3 months have been suggested. After 6 months of treatment, IVF can be advised in such women when regression is documented.

3.4.8 Hydrosalpinx

Women having hydrosalpinx have poor implantation which may be due to the toxic effect of the inflammatory fluid or due to the backflow of the fluid into the endometrial cavity effecting the implantation. Meta-analyses have shown that women with hydrosalpinx have lower implantation rates [20]. This can be treated prior to stimulation for IVF by removing the damaged tubes or by clipping the tubes or by hysteroscopic closure of hydrosalpinx using the Essure device.

3.4.9 Asherman Syndrome and Uterine Synechia

Any form of uterine infection causes endometritis leading to inflammation and destruction of the endometrium, its basal layer and myometrium and leads to adhesion formation called synechia which can sometimes follow the iatrogenic injury like excessive endometrial curettage. In Asherman syndrome, there is complete obliteration of the uterine cavity with adhesions resulting in amenorrhoea and infertility. The endometrial cavity may be replaced by adhesion and fibrosis. Symptoms relate to the degree and location of adhesions and include irregular bleeding ranging from hypomenorrhoea to amenorrhoea, infertility and pregnancy loss [10].

Treatment usually involves hysteroscopic resection of adhesions followed by oestrogen progesterone therapy. The management of moderate to severe disease still poses a challenge, and the prognosis of severe disease remains poor. Repeat surgery may be necessary in some cases but may not always produce the desired outcome. Stem cell therapy as described in the following section is an emerging treatment option. Future research should focus on the cellular and molecular aspects of endometrial tissue regeneration as well as the prevention of postsurgical adhesion formation and reformation.

3.4.10 Thin Endometrium

A thin endometrium is one of the most difficult problems encountered in assisted reproduction everyday practice. It is the single factor which has a significant impact on the pregnancy rate. Usually, there is no pregnancy when the endometrium remains below 7 mm, although pregnancy has been reported at endometrial thickness of 3.7 mm also, but these pregnancies can have poor outcome resulting in missed

abortion and preterm labour [21]. Various interventions have been tried to improve the endometrial lining, still most are ineffective.

3.5 Therapeutic Interventions to Improve Endometrium and Implantation

3.5.1 Extended Oestrogen Therapy

Women with thin endometrium either due to previous iatrogenic causes like excessive curettage or endometritis having secondary amenorrhoea which fail to respond to cyclical oestrogen progesterones. Vaginal E2 gives maximum levels at uterus. Endometrium may still be irresponsive to oestrogen therapy when there is receptor defect in women where the receptors are depleted due to damaged endometrium or there may be a genetic defect rarely [22].

Women who have amenorrhoea due to end-organ damage not responding to cyclical oestrogen therapy may be put on continuous high oestrogen therapy either orally or vaginally to cause regeneration of endometrium given for 4–6 weeks before progesterone is supplemented. Tourgeman et al. [23] compared vaginal administration of E2 with oral E2 administration in recipients of donor oocytes. They observed an increase in endometrial thickness to 7 mm and an ongoing pregnancy rate of 70 % with vaginal E2 administration extended to 4–6 weeks before the addition of progesterone.

3.5.2 Role of Aspirin

Numerous pharmacological interventions have been studied as adjuvant therapy over the years [24, 25]. Aspirin (acetylsalicylic acid) is a widely used vasoactive substance that exerts its effects by inhibiting the enzyme cyclo-oxygenase in platelets. In low doses, it inhibits synthesis of thromboxane A2 (a vasoconstrictor and promoter of platelet aggregation) more than that of prostacyclin (a vasodilator). Due to these antithrombotic and vasodilatory effects, aspirin has been one of the agents studied in several trials to evaluate its potential role in increasing IVF success rate through improving either ovarian blood flow, folliculo-genesis and ovarian responsiveness or uterine vascularity and receptiveness, or both [26, 27]. Some authors have recommended starting low-dose aspirin in the preceding cycle or on day 15 or 21 continuing till pregnancy is achieved whereas others have considered aspirin from day 2 of the cycle. Till date, only conflicting results are available, and it is not recommended but can be considered under clinical trials only [28].

3.5.3 Sildenafil

Nitric oxide (NO) relaxes vascular smooth muscle through a cGMP-mediated pathway, and NO synthase isoforms have been identified in the uterus. Sildenafil citrate (Viagra), a type-5-specific phosphodiesterase inhibitor, augments the vasodilatory effects of NO by preventing the degradation of cGMP. The use of vaginal sildenafil helps in improving uterine artery blood flow and sonographic endometrial appearance, and women with prior failed assisted reproductive cycles due to poor endometrial response have conceived following its use [29].

3.5.4 Endometrial Injury to Improve Endometrial Receptivity

Implantation is a process of embryonic attachment to the endometrium and subsequent invasion into the stroma of the uterine wall. It is a multistage process involving several cytokines and growth factors. One of the most promising methods of improving implantation is local injury to the endometrium. In 2003, Barash et al. [30] reported that endometrial injury before in vitro fertilization (IVF) among women with repeated implantation failure was associated with increased rates of implantation, clinical pregnancy and live birth. The findings were supported by two other studies [31, 32]. Endometrial injury results in decidualization. Massive release of cytokines and growth factors from injured

endometrium has been suggested as an underlying process [33].

Friedler et al. [34] reported patients with repeated implantation failure who were treated by a special protocol including hysteroscopy, dilation and curettage, triple antibiotics and oestrogen. Six of fourteen patients conceived (pregnancy rate 43 %) in the subsequent IVF-ET cycle with implantation rate of 24 %. The authors postulated that implantation failure could have been caused by endometritis that was treated with antibiotics. In addition, the endometrial flow was improved by oestrogen administration. Because dilation and curettage was part of this protocol, it is also possible that endometrial injury by curettage plays a role in their improved results.

Basic scientific studies regarding the pathophysiology of local injury and improved implantation are lacking. Endometrium in IVF cycles is ahead of that of natural cycles by 2–4 days. It is possible that repeated IVF-ET implantation failure is related to asynchrony of the endometrium with the embryo stage [35–37]. Zhou et al. [32] postulated that local endometrial injury in stimulated cycle delays the endometrial development because of wound repair processes correcting the asynchrony between endometrial and embryo stages.

3.5.5 Prolonged Use of Pentoxifyline and Vitamin E

A combination of pentoxyfylline and tocopherol may improve endometrial growth in resistant cases by reducing fibro-atrophic uterine lesions. It thus improves the uterine response to HRTs that are unresponsive to conventional therapy. Endometrial thickness, myometrial dimensions and diastolic uterine artery flow improved significantly. A study by Acharya et al. where women were given 800 mg of PTX and 1,000 IU of Vit E daily for up to 8 months showed improvement in endometrial thickness. The mean thickness of endometrium before and after treatment was 4.37 and 6.05 mm, respectively. Pregnancy occurred in 40 % of the cases [38].

3.5.6 Stem Cell Therapy

Human endometrium is a highly regenerative tissue, undergoing more than 400 cycles of growth, differentiation and shedding during a woman's reproductive years. Adult stem cells are identified in the endometrium which can reconstruct endometrial tissue in vivo suggesting their possible use in treating disorders associated with inadequate endometrium. In scarred endometrium or endometrium obliterated with adhesions, functional endometrial stem/progenitor cells likely are lacking, as scant functional endometrium is present in the uterine cavity.

From adult autologous stem cells, endometrial angiogenic stem cells can be separated. These cells are placed in the endometrial cavity under ultrasound guidance after curettage. Patients can then be given cyclical hormonal therapy. On development of endometrium with a thickness of 8 mm and good vascularity, in vitro fertilization and embryo transfer was done. This has resulted in pregnancy. The bone marrow cells act by production of trophic factors promoting angiogenesis and tissue growth to regenerate endometrial tissue. Various other adult cells like endometrial epithelial progenitor, menstrual, bone marrow or embryonic stem cells can be used for generation of endometrium stem cells [39].

3.5.7 Granulocyte Colony-Stimulating Factor

Recently, the use of granulocyte colony-stimulating factor in women with inadequate endometrial growth has been tried, but its use is during stimulation cycles at the time of receiving HCG.

3.6 Scope for Research

The understanding of endometrial receptivity still needs a clear understanding, and the research in stem cell therapy and gene therapy gives hope of treating damaged endometrium. To summarize, various interventions for the endometrium

may help in achieving pregnancy when the defect lies in it, although this is the most difficult at times when endometrium fails to respond to all treatment options.

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Obesity and Its Impact on Ovarian Stimulation

4

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Abstract

Increased body mass index (BMI) has effect on various aspects of infertility treatment and assisted reproductive technology (ART) procedures. The extent of this influence is, however, conflicting in literature. Overweight and obese women require a higher dose of gonadotropin with greater number of days of stimulation and yet have lower peak oestradiol levels with an increased risk of cycle cancellation due to poor follicular development. On controlled ovarian stimulation, there is less number and poor quality of oocytes reported in these women. They have lower fertilization and pregnancy rates. The effect of obesity upon implantation rate has also been inconsistently reported. Some studies have identified a reduction in implantation rates among the obese women. Weight loss results in regularization of the menstrual pattern, a decrease in cancellation rates, an increase in the number of embryos available for transfer, a reduction in the number of ART cycles required to achieve pregnancy and a decrease in miscarriage rates. There are higher obstetric complications with a lower live birth rate in these women. Obesity is a modifiable risk factor. It has an effect on fertility, its treatment and obstetrics outcome. Women with increased BMI should be first encouraged to reduce weight before starting any treatment for infertility or planning conception.

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4.1 Incidence

In the last few decades, there has been a change in lifestyle and socioeconomic conditions globally. This has led to rise in obesity. Obesity has now become a major epidemic. Rising incidence of obesity is seen both in developed and developing countries. In England, it is seen that 56 % of women are either overweight or obese [1]. More than 30 % of women in the United States are found to be obese [2]. Prevalence of obesity is rising even in developing countries. Over the last 20 years, the obesity rates have tripled in the developing world, and now it is seen that 10 % of all children across the world are overweight or obese [3].

The World Health Organization has defined obesity as body mass index (BMI) more than or equal to 30 kg/m^2 [4]. Obesity poses a major health challenge, as it is a risk factor for cardiovascular disease, diabetes mellitus, arthritis and cancers of oesophagus, colon and endometrium. In addition to this, in women of child-bearing age it is associated with infertility and increased complication during pregnancy [5].

4.2 Obesity and Its Impact on Fertility

Infertility issues are more commonly seen in obese women. Thirty to seventy-five percent of women with polycystic ovary syndrome (PCOS) are also found to be obese [6]. Anovulation is a major cause of infertility among obese women, the relative risk being 3.1 (95 % confidence interval (CI) 2.2–4.4) when compared to those with a normal BMI [7]. Body fat distribution in women of reproductive age also has an impact on fertility in addition to age or obesity. Zaadstra observed that a 0.1 unit increase in waist-hip ratio (WHR) leads to a 30 % decrease in probability of conception per cycle (hazard ratio 0.706; 95 % CI 0.562–0.887) [8].

Obesity is found to decrease successful pregnancy rates in both natural and assisted conception cycles [9]. Ovulation and conception requires a fine complex balance of hormones released by reproductive organs. Obesity interferes with this in many ways. Insulin resistance is commonly seen in most obese women. Elevated levels of insulin lead to a reduction in hepatic synthesis of sex hormone-binding globulin (SHBG), an elevated level of circulating free androgens and high levels of free insulin-like growth factor 1 [10]. This relative hyperandrogenemia found in obese women has undesirable effects on ovarian function and contributes towards oligo-anovulation. Insulin also stimulates LH receptor expression on theca and granulosa cells resulting in LH hypersecretion and unfavourable folliculo-genesis.

Increased levels of circulating leptin are found in obese women. Leptin plays a regulatory role at hypothalamus-pituitary-ovarian axis and, influences ovarian folliculo-genesis and endometrium development. A high level of leptin leads to subfertility in them. Low levels of adiponectin and altered levels of TNF, plasminogen activator inhibitor (PAI) and type-1 interleukin-6 (IL6) are found in obese women, which reduces conception rate. Other potential mechanisms of infertility in the obese include poor-quality oocytes and a defect in endometrial receptivity.

It is seen that a weight loss of 5 % or more in obese women leads to a decrease in insulin and IGF levels and increase in SHBG levels, resulting in regular menstruation in women with PCOS [11]. Weight loss leads to reduction in insulin resistance and central adiposity [12]. Also, obese women who lose weight tend to have spontaneous ovulation and improved response to ovarian stimulation in infertility treatment. It is therefore advocated that for overweight and obese infertile

women after initial assessment for infertility, weight management interventions like lifestyle change, diet, exercise or drug to decrease weight should be recommended first before embarking on any treatment modality.

4.3 Impact of Obesity in Infertility Treatment

Increased body mass index (BMI) has effects on various aspects of infertility treatment and assisted reproductive technology (ART) procedures. The extent of this influence is, however, conflicting in literature.

4.3.1 Ovulation Induction

Clomiphene citrate is commonly used as first line of ovulation induction drug in obese women. It is, however, found to be associated with low ovulation and pregnancy rates [13].

Obesity and insulin resistance have been implicated to lead to suboptimal response even when gonadotropins were used for ovulation induction. Studies have shown that women with high BMI need higher total doses of FSH to achieve ovulation [weighted mean difference 771 IU (95 % CI, 700–842)]. These women also face a higher risk of cycle cancellation [OR 1.86 (95 % CI: 1.13–3.06)] and are less likely to ovulate [OR 0.44 (95 % CI: 0.31–0.61)] [14]. A multicentre randomized controlled trial also showed that with increasing BMI, a higher threshold dose of gonadotropins was required with more days of stimulation; however, despite greater gonadotropin requirements, no difference was seen in overall outcome of ovulation induction and clinical pregnancy in women with anovulatory polycystic ovary syndrome and a BMI of less than 35 kg/m^2 [15].

Insulin sensitizers have been frequently used in obese women with PCOS. Tang et al. updated the Cochrane review on the role of metformin for women with PCOS. They concluded that metformin is beneficial in improving clinical pregnancy and ovulation rates. However, there is no evidence that metformin improves live birth rates whether it is used alone or in combination with clomiphene or when compared with clomiphene, and hence it has limited role in PCOS [16]. Neil P. Johnson observed that in women with PCOS undergoing IVF metformin when added reduces the risk of ovarian hyperstimulation syndrome [17].

Rosiglitazone has been found to be effective. Short-term rosiglitazone therapy enhances both spontaneous and clomiphene-induced ovulation in overweight and obese women with PCOS. Rosiglitazone therapy improves insulin sensitivity and decreases hyperandrogenemia primarily through increase in SHBG but is hepatotoxic [18]. Pioglitazone appears to be effective as well; however, the study is still limited. Since foetal safety for both these drugs has not been established (pregnancy category C of the US FDA guidelines), these drugs when used should be discontinued as soon as pregnancy has been established.

4.3.2 Impact of Obesity on ART

Obesity has influence on all aspects of ART. There is inconsistent evidence regarding the effect of raised BMI on the outcome of assisted reproductive technology.

4.3.2.1 Ovarian Stimulation

As already stated, overweight and obese women require a higher dose of gonadotropin with greater number of days of stimulation and yet have lower peak oestradiol levels, with an increased risk of cycle cancellation due to poor follicular development [19]. The dose of gonadotropins was higher in women with BMI of ≥25 (WMD 210.08, 95 % CI: 149.12, 271.05) in comparison with those with BMI of <25 and in obese women (BMI ≥30 versus BMI <30) (WMD 361.94, 95 % CI: 156.47, 567.40) [20].

4.3.2.2 Oocyte Recovery

Ovum pickup in obese women can at times be technically more difficult to perform. Also general anaesthesia in them poses challenges like difficult endotracheal intubation due to excessive tissue and oedema and hypoxia from failed or difficult intubation.

4.3.2.3 Oocyte Number and Quality

The maturing oocyte is very vulnerable to changes in its micro-environment, the follicular fluid. Valckx et al. showed that differences in BMI are associated with alterations in the fatty acid composition of the follicular fluid. This variation possibly affects granulosa cell viability, oocyte development and subsequent embryo quality, possibly explaining differences in oocyte quality in obese patients [21].

Increased LH and altered LH: FSH ratio affects ovulation and the resumption of oocyte maturation in obese women. Frequently, obese women require greater amounts of gonadotropins for IVF and a longer period of stimulation leading to alterations in oocyte development [22]. Obesity affects oocyte competence and maturation through alterations in various hormones, particularly those hormones that trigger oocyte maturation [23].

Esinler et al. in their study found that in women with BMI >30 the number of cumulus-oocyte complexes collected was lower and stage of oocyte maturation delayed. Fertilization rate, embryos transferred, implantation and pregnancy rates, however, were not influenced by obesity. The number of cycles with good-quality cryo-preservable embryos was significantly lower in them ($P<0.05$) [24]. Carrell et al. in their study have shown that there is impairment in oocyte maturation with increasing BMI [25]. Ronit et al. studied the characteristics of failed fertilized oocytes. They found that compared to women with normal BMI, severely obese women had a greater prevalence of spindle anomalies and non-aligned chromosomes in failed fertilized oocytes [26].

Zang et al. also showed that the number of oocytes obtained by obese women was significantly lower than normal-weight women (oocytes retrieved 2.98 ± 6.91 vs. 14.49 ± 7.96 respectively, $P<0.001$) [27]. This result was supplemented by another systematic review where the weighted mean difference (WMD) of the number of oocytes recovered in women with BMI >25 kg/m^2 was 0.58 (95 % CI: 0.22, 0.94) in comparison with women with BMI <25 kg/m^2 [20]. A study done by Metwally et al., however, reported that oocyte quality assessed by number of oocytes considered suitable for injection or the number that fertilized was unaffected by BMI [28].

4.3.2.4 Oocyte Fertilization and Embryo Quality

Oocyte fertilization rates have been shown to be lower in morbidly obese women (59 % vs. 69 %; $P<0.03$) [29]. In a large cohort study, it was seen that in comparison with women of normal weight, overweight women (BMI >25<30 kg/m^2) have lower fertilization rates (60.8 ± 23.3 vs. 61.1 ± 23.0, $P<0.001$), fewer cleaved embryos (7.55 ± 4.86 vs. 8.67 ± 5.90, $P<0.001$), fewer high-grade embryos (4.65 ± 3.96 vs. 5.59 ± 4.81, $P<0.001$) and fewer cryo-preserved embryos (4.44 ± 4.55 vs. 5.49 ± 5.55, $P<0.001$) [27]. A conflicting report was found by Bellver et al. in their retrospective study on 6,500 IVF/ICSI cycles. They concluded that the fertilization rate or embryo quality was not impaired in overweight and obese women. However, implantation, pregnancy and live birth rates were poorer in obese women. In fact, pregnancy and live birth rates were reduced progressively with each unit of BMI with a significant odds ratio of 0.984 (95 % confidence interval 0.972–0.997) and 0.981 (95 % confidence interval 0.967–0.995), respectively. In addition, the cumulative pregnancy rate after four IVF cycles was reduced as BMI increased [30].

4.3.2.5 Cycle Cancellation

Several studies have looked at the cycle cancellations in overweight and obese women. Maheshwari et al. in their systematic review suggest that the odds of cycle cancellation in women with BMI of >25 kg/m^2 were 1.83 (95 % CI: 1.36, 2.45) as compared to women with BMI <25 kg/m^2. However, there was significant statistical heterogeneity ($P<0.05$) in the pooled data, and hence results were inconclusive [20].

4.3.2.6 Ovarian Hyperstimulation Syndrome

Ovarian hyperstimulation syndrome (OHSS) is an avoidable complication of ovarian stimulation. In a review of women with BMI of ≥25, the odds

of OHSS were 1.12 (95 % CI: 0.74, 1.68), and with BMI of ≥30, the odds of OHSS were 1.16 (95 % CI: 0.69, 1.96) [20]. The higher incidence of ovarian hyperstimulation may be due to the increased incidence of PCOS in overweight women. In another review, none of the studies included found any significant difference between the risk of OHSS in normal and overweight women. The pooled OR for overweight women was 1.0 (95 % CI 0.77–1.3) [31].

4.3.2.7 Implantation, Pregnancy and Live Birth Rates

The end result of ovarian stimulation is implantation, pregnancy and live birth rates. The effect of obesity upon implantation rate has also been inconsistently reported. Some authors have identified a reduction in implantation rates among the obese women [32, 33]. Bellever et al. reported that implantation, pregnancy, twin pregnancy and live birth rates were significantly reduced as BMI increased [30]. An unfavourable intrauterine milieu, compromised oocyte quality and impaired endometrial receptivity may be contributing factors for this.

Meta-analyses have been done by various authors to find the association between increased BMI and pregnancy or live birth rate following assisted ART treatment. Rittenberg et al. reviewed 33 studies including 47,967 treatment cycles to evaluate the effect of raised BMI on treatment outcome following IVF/ICSI treatment. They concluded that women who were overweight or obese (BMI ≥25) had significantly lower clinical pregnancy (RR = 0.90, $P < 0.0001$) and live birth rates (RR = 0.84, $P = 0.0002$). Rittenberg et al. found that the probability of live birth after ART was reduced by 9 % (95 % CI: 2–15 %) in overweight women compared with a reduction of 20 % (95 % CI: 12–29 %) in the obese group [34].

Koning et al. analyzed 27 studies. They reported that clinical pregnancy rates were not different for overweight and normal-weight women [OR 0.94 (95 % CI 0.69–1.3)] or for obese women when compared with normal-weight women [OR 0.97 (95 % CI 0.59–1.6)]. However, overweight women undergoing ART had a significant lower live birth rate after ART than women with a normal weight [OR 0.90 (95 % CI 0.82–1.0)]. They found an OR of 0.90 for the association between overweight and live birth, indicating a 10 % reduction in the success rates of IVF in overweight women [31]. Similarly, Maheshwari et al. analyzed 37 studies and found lower pregnancy (odds ratio [OR]: 0.71; 95 % CI: 0.62–0.81) [20]. Another retrospective cohort study was done of 1,721 women to study effects of BMI in women undergoing IVF. They found that the odds of clinical pregnancy (odds ratio [OR] 0.50, 95 % confidence interval [CI] 0.31–0.82) and live birth (OR 0.51, 95 % CI 0.29–0.87) were 50 % lower in women with class III obesity as compared with women of normal BMI [35].

Luke et al. found that maternal obesity is associated with lower live birth rates in women receiving ART treatments. This was seen with use of both the autologous oocyte as well as donor oocytes; however, the effect was most severe among treatments using autologous oocytes suggesting a detrimental effect of obesity on the foetal environment [36]. Data from another study suggests that obesity does not affect IVF outcomes in women using donor oocytes. Oocyte quality rather than endometrial receptivity may be the overriding factor influencing IVF outcomes in obese women using autologous oocytes [37].

Overall, studies indicate that overweight or obesity result in poorer ART outcomes.

4.3.3 Obesity and Its Impact on Pregnancy

Overweight and obese women have increased risk of miscarriage in all types of conception, be it in spontaneous cycle, induced cycle, IVF or oocyte donation cycle. A systematic review of literature done by Maheswari et al. showed that when compared with women of BMI <25, the odds of miscarriage in women with BMI of ≥25 kg/m^2 were 1.33 (95 % CI: 1.06–1.638) and 1.53 (95 % CI: 1.27–1.84) in women with BMI ≥30 kg/m^2 when compared to women of BMI <30 kg/m^2. The results, however, showed evidence of statistical heterogeneity. Koning also

found a significantly higher miscarriage rate in overweight women (RR = 1.31, $P < 0.0001$) compared to women with BMI <25. This has been supported by few others [38, 39].

Another recent meta-analysis of the available evidence suggested that there was a significant increase in the odds of miscarriage in women with a BMI of ≥25 kg/m^2 (OR 1.67; 95 % CI, 1.25–2.25) following spontaneously conceived pregnancies as well as following ovulation induction (OR, 5.11; 95 % CI, 1.76–14.83) but not in women who underwent IVF/ICSI (OR, 1.52, 95 % CI, 0.88–2.61). This may be due to differences in the nature of the included studies and the type of denominator used to calculate the prevalence of miscarriage [40].

Relation between BMI and multiple pregnancies for women undergoing ART was reviewed by Koning. The pooled OR expressing the association between overweight and the risk of multiple pregnancies was 0.97 (95 % CI 0.91–1.04). Only retrospective cohort study a significantly higher risk of multiple pregnancies was observed in women with normal weight following ART, whereas the six other studies did not. They also studied the association between BMI and ectopic pregnancies but found no significant difference in risk of ectopic pregnancy [31].

However, obese and morbidly obese subjects have a significantly higher risk for obstetric complications during pregnancy like gestational diabetes mellitus, pre-eclampsia, thromboembolism, caesarean delivery and foetal anomaly [41].

Age is an important factor that has a bearing on live birth rates following IVF in relation to female age for women with a BMI of <25 and >25 kg/m^2. It is observed that live birth rates decrease from 26 % for younger women to 10 % for women aged 40 [20]. Koning stated that the profound effect of age is much stronger when compared with the moderate effect of excessive weight on the live birth rate following IVF [31].

IVF treatment in women with increased BMI has reduced live pregnancy rate. Cost is a major factor involved in infertility treatment. Due to this risk, there are guidelines that regulate access to fertility care for overweight and obese women in various countries. In New Zealand, women with a BMI of >32 kg/m^2 are excluded from any fertility treatment under public IVF funding. RANZCOG considers a BMI >35 kg/m^2 to be a contraindication to assisted reproduction. The British Fertility Society recommends deferring IVF treatment for BMI of >35 [42]. Current 2013 NICE guidelines also recommend an ideal BMI between 19 and 30 kg/m^2 before commencing IVF treatment.

4.4 Weight Loss and Fertility

Since obesity has a detrimental effect on reproductive outcome, studies have evaluated the impact of weight loss on fertility. A systematic review was done by Sim et al. to assess the effect of weight loss achieved by various modalities like diet, lifestyle changes, non-surgical medical interventions and bariatric surgery. They found that weight loss in the obese and overweight did significantly increase pregnancy rates and live birth rates. In addition to this, weight loss also resulted in regularization of the menstrual pattern, a decrease in cancellation rates, an increase in the number of embryos available for transfer, a reduction in the number of ART cycles required to achieve pregnancy and a decrease in miscarriage rates. They also reported a number of natural conceptions with weight loss [43].

As little as 5–10 % weight loss can improve fertility outcomes and lead to an improvement in endocrine parameters, such as decrease in free testosterone, lower fasting insulin levels and increased frequency of ovulation [11, 12]. In addition, weight loss causes a significant reduction in central fat deposits (11 %) and serum luteinizing hormone levels [44].

Drugs have been used to bring about change in weight. In a large randomized controlled trial with metformin, there were no significant changes in insulin sensitivity or lipid profiles; however, a significant reduction in waist circumference and free androgen index was seen with metformin. An improvement in menstruation was only seen when there was associated weight loss [45]. Orlistat, in a small prospective trail, has

been found to be effective in the obese both with and without PCOS [46].

Bariatric surgery has maximum effect of weight loss. In a study done, it was found that post bariatric surgery there was a significant improvement in the number of follicles seen and number of oocytes retrieved [47]. Weight loss in women with increased BMI improves their reproductive outcomes; however, in order for this to be effective, it has to be gradual and sustained.

Conclusion

Obesity is a modifiable risk factor. It has an effect on fertility, its treatment and obstetrics outcome. Women with increased BMI should be first encouraged to reduce weight before starting any treatment for infertility or planning conception.

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Part II

Controlled Ovarian Stimulation: Protocols

Oral Ovulogens in IUI and IVF

5

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Abstract

Oral ovulogens constitute the most commonly used intervention in a subfertile couple. They have the advantage over the gonadotropins of being of lower cost, safer and easy to administer. The main ovulogens utilized are clomiphene, letrozole and metformin. Trials have failed to show any conclusive advantage of clomiphene and letrozole over one another in women with anovulatory PCOS. Ovulation rates with both are in range of 70–80 % and pregnancy rate per cycle around 20–25 %. Clomiphene also constitutes an important place in mild stimulation for IVF since it possesses properties to prevent premature LH surge. Major problems with clomiphene are the negative effects on endometrium and cervical mucus which can be resistant and repetitive necessitating alternative therapies. Letrozole also has been used sparingly in IVF with reasonable success. Its further usage is marred by unfounded fears about foetal anomalies based on a flawed study leading to the drug getting prohibited in many countries. Metformin constituted the standard of care for PCOS not too long ago. Now, enough evidence exists to render it useful only in very small subsets of women, including mainly the ones with glucose intolerance and sometimes in obese and clomiphene-resistant cases. Also, the adverse effect profile of metformin does not augur well for it. Further research might open more gates in this important aspect of care with advent of safer and more efficacious alternatives including orally active gonadotropins.

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5.1 Introduction

The number of couples with subfertility is on a steep rise even with the most conservative estimates. Be it male factor, tubal factor, endometriosis or PCOS, the rise is pretty much eminent with few expected geographic variations in the aetiology. This has brought ovulation-inducing medicines, especially oral ovulogens, into the forefront as a first-line therapy in many of these cases.

The main group catered with oral ovulogens is undoubtedly of that with anovulation. It affects approximately 21 % of the subfertile population.

There are three classes of anovulatory subfertility as per WHO [1]:

- WHO 1: hypogonadotropic hypoestrogenic (10 %)
- WHO 2: normogonadotropic normoestrogenic (80 %, majority being PCOS)
- WHO 3: hypergonadotropic hypoestrogenic (10 %)

The oral ovulogens have a role primarily in WHO 2 anovulation, while the WHO 1 and 3 have to be targeted with injectables as discussed elsewhere.

5.2 Definition of Ovulation Induction (OI) and Controlled Ovarian Stimulation (COS)

In the strict sense of the term, OI refers to the triggering of ovulation.

In the clinical context, it refers to the type of ovarian stimulation for anovulatory women aimed at restoring normal fertility by generating normo-ovulatory (mono-ovulatory) cycles.

COS for IUI aims to induce development of two to three follicles in order to improve chances for conception.

OI is primarily aimed at women where the cause of subfertility is anovulation or male factor, while COS is for those with unexplained subfertility where the aim is to increase the number of gametes available for fertilization.

5.3 Physiology of COS

5.3.1 FSH Threshold

FSH level starts rising during the luteo-follicular transition in a woman with regular menstrual cycles. This kick-starts growth of the antral follicles once the FSH level rises above the threshold level. The threshold level differs for different follicles as it is dependent on the sensitivity of the follicles to FSH which in turn is dependent upon the number of FSH receptors in a given follicle. This is termed as FSH threshold concept.

5.3.2 FSH Window

The duration of the time for which FSH remains above this critical threshold determines the number of dominant follicles selected from the recruited cohort for preferential growth. This is known as FSH window, during which the follicles whose threshold is crossed keep on getting recruited for growth. Longer the window remains open, greater the number of follicles recruited for growth.

All the ovulogens, whether oral or injectable, work on this principle. They push the FSH above the threshold and keep the window open for a longer amount of time allowing the follicles to get recruited. In a normal cycle, as the follicle grows, the increase in oestradiol and inhibin from the growing follicle reduces the FSH level through the negative feedback on hypothalamus and pituitary leading to a fall in FSH secretion. The fall of FSH below the threshold then closes

the FSH window and causes the atresia of the follicles other than the dominant follicle.

5.4 Couple Evaluation Before Administering Ovulation-Inducing Medicines

It is mandatory to do a complete evaluation of the couple before OI/COS to increase its effectiveness and to reduce the risks. This should include a semen analysis along with physical examination for the male and an ultrasound to check the ovarian reserve apart from physical examination for the female. Underlying pathologies like thyroid and prolactin disorders, diabetes, hyperandrogenism, overweight and obesity should be screened and corrected before embarking for OI/COS.

5.5 Monotoring of an OI/COS and IUI Cycle

A baseline ultrasound should be done to rule out any ovarian or uterine pathology (Fig. 5.1). The first cycle should be monitored with ultrasound to note the number of growing follicles more than 12 mm in size, endometrial thickness, cervical mucous thickness and confirmation of ovulation (see Fig. 5.1). Patients with more than two growing follicles should be counselled for risk of multiple pregnancy and mild hyperstimulation. These patients should be given lower dose of medicine in the next cycle. Patients with less than 7 mm endometrium and thin layer of cervical mucous need oestrogen supplementation and, if possible, dose reduction in next cycle. Although majority of patients with CC (47.2 %) have endometrium of more than 9 mm but approximately 10 % patients have ET less than 6 mm^2 [2].

5.6 Classes of Oral Ovulogens (Table 5.1)

1. Anti-oestrogens: clomophene citrate and tamoxiphen (Figs. 5.1 and 5.2)
2. Aromatase inhibitors: letrozole and anastrazole
3. Insulin sensitizers; metformin, pioglitazone, rosiglitazone

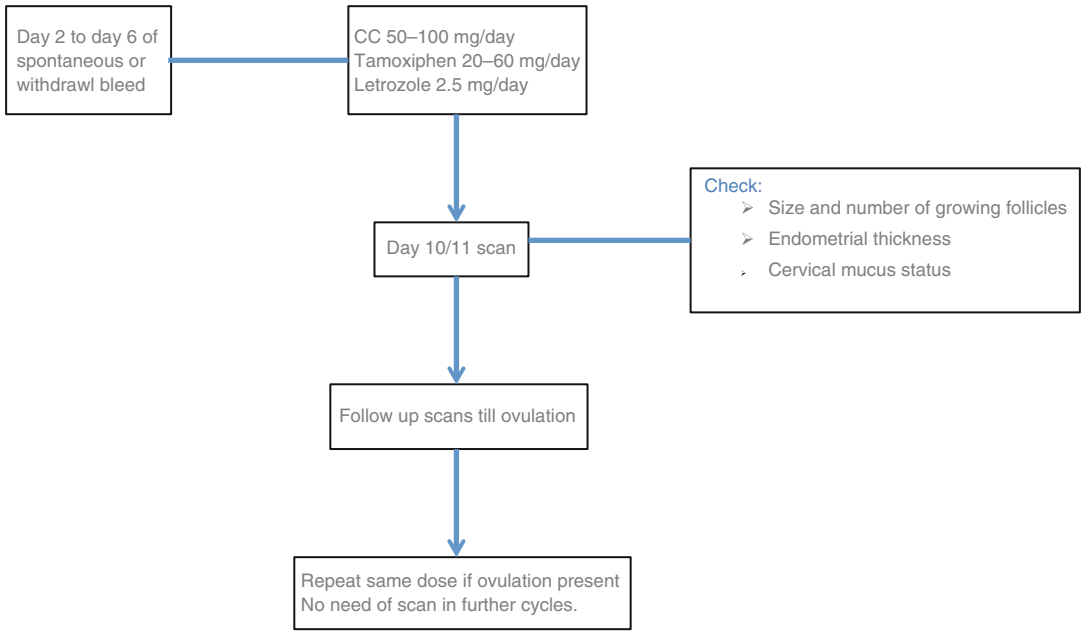

Fig. 5.1 Standard plan of OI and follicular monitoring in first cycle

Table 5.1 Oral ovulogens: mechanism, dosage, success, and adverse effects

Ovulogen	MOA	Dosage	Ovulation rate	Pregnancy rate per cycle	Adverse effects
Clomiphene	Antiestrogen	50–100 mg/day	70–80 %	22 %	Hot flushes Antiestrogenic effects on endometrium and cervical mucus
Tamoxiphene	Antiestrogen	10–40 mg/day	Nearly same as CC	Nearly same as CC	Hot flushes
Letrozole	Aromatase inhibitor	2.5–5 mg/day	70–80 %	20–27 %	Hot flushes, leg cramps and gastrointestinal disturbances
Metformin	Insulin sensitizer	1.5–2 g/day			G.I upset Lactic acidosis

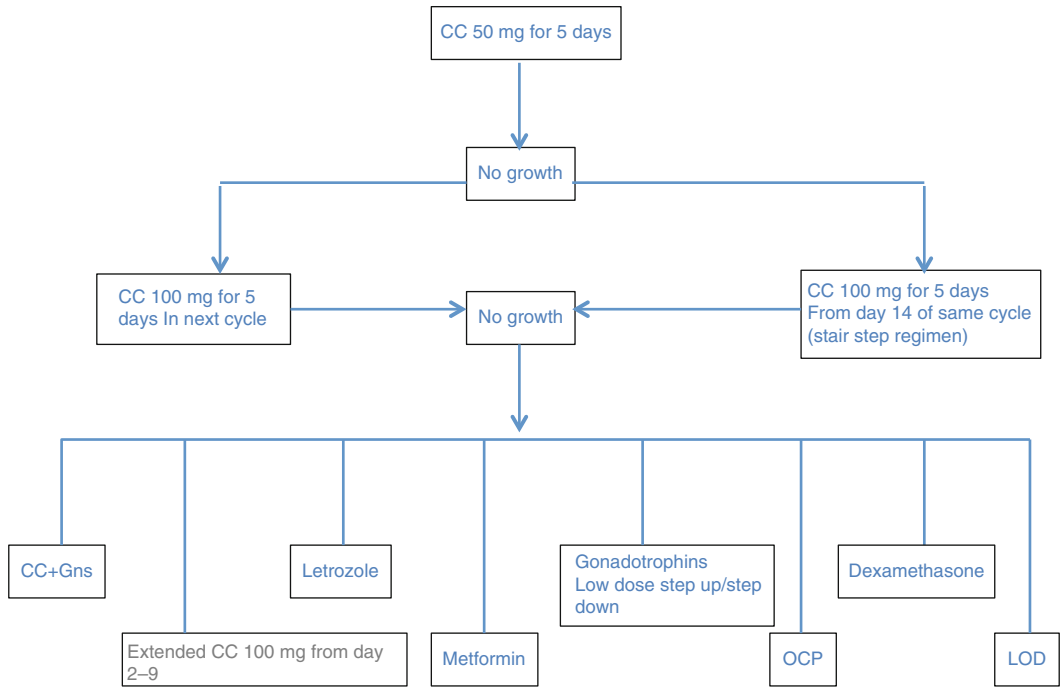

Fig. 5.2 Action plan of OI with clomiphene resistance in PCOS. *OCP* Oral contraceptive pill, *LOD* Laparoscopic ovarian drilling

5.6.1 Clomiphene

Clomiphene is the oldest and most commonly used oral ovulogen. It still has a place in ovulation induction and is now also being used in minimal stimulation protocols in IVF. These are nonsteroidal triphenylethylene derivative which acts as selective estrogen receptor modulators(SERM), thus having both estrogen agonist and antagonistic actions [3].

5.6.1.1 Relevant Pharmacology and Mechanism of Action (MOA)

Oral dose is absorbed fast. Serum levels of zuclomiphene peak 6 h after oral intake. It is metabolized in liver and excreted in intestine. Final serum levels of zu- and enclomiphene are determined by its metabolism and weight of the women. It is generally agreed that enclomiphene is the more potent is omer and it is rap-

idly cleared from serum, making it theoreticlly more appealing as an ovulogen [4]. Females with higher BMI have larger plasma volume and thus have lower serum levels of clomiphene. Both isomers have long half-life. Serum half-life of zuclomiphene is 14.2–33.4 days and enclomiphene about 2.5–11.8 days [5].

Even after discontinuation of the drug, 50 % of dose can be detected in serum after 5 days, and its metabolites can be found up to 6 weeks. Zuclomiphene tends to accumulate over consecutive cycles with 50 % rise in serum level in next cycle [6].

Clomiphene acts as a competitive antagonist to 17 ß oestradiol at the level of nuclear receptor complex as the binding of clomiphene to the oestrogen receptor is more prolonged than oestrogen [7], which results in a pseudo-hypo-oestrogenic state. However, in hypo-oestrogenic condition clomiphene may show oestrogen agonistic action. Its primary site of action is hypothalamus as an oestrogen antagonist and secondarily at pituitary as a weak agonist, increasing the sensitivity of pituitary to GnRH. Serum LH levels start rising 2–3 days after admnistration by its direct action on pituitary [8].

Anovulatory PCOS patients have high GnRH pulse frequency. In these patients CC increases gonadotropin secretion by increasing GnRH pulse amplitude [9], while in ovulatory patients with normal GnRH pulse it acts by increasing pulse frequency [10]. It causes moderate rise in gonadotropin levels. In anovulatory patients gonadotropins rise by 50 % [7]. LH surge usually occurs 5–12 days after last clomiphene tablet.

5.6.1.2 Dosage and Effectiveness

CC can be started any day from cycle day 2 to 5 [11]. One study has reported lower ovulation rates when CC was started immediately after spontaneous or induced bleed [12].

Usual starting dose is 50 mg/day for 5 days in women less than 50 kg, while in women with weight more than 75 kg,100 mg/day can be started. Approximately 46–52 % patients ovulate with 50 mg/day, 21–22 % ovulate with 100 mg/day and 8–12 % will need 150 mg/day [13]. Dose higher than 100 mg/day is not approved by US FDA. Patients hypersensitive to CC can be started with 25 mg/day. Doses higher than 150 mg/day are not used due to high LH level and anti-oestrogenic action. Approximately 15–20 % anovulatory patients do not ovulate with these doses [14].

Lower ovulation rates are seen in women with higher BMI, higher free androgen index, insulin resistance and large ovarian volume [15]. There is no reliable predictor of ovarian response to CC [16, 17]. Failure to ovulate with CC for 6 months is termed as clomiphene resistance. An overall cumulative pregnancy rate of 55–73 % is seen in patients treated with CC [18]. Despite high ovulation rates, PR is low, and this is due to anti-oestrogenic action of CC on endometrium causing several dysfunctions and making cervical mucus thick and hostile for sperm penetration [19–21].

Lower pregnancy rates are seen in older women, hyperandrogenic PCOS with severe cycle disorder [22] and obese women. The landmark study by Legro et al. reported 28 % LBR in women with less than 30 kg/m^2 BMI vs. 16 % LBR in women with more than 35 kg/m [23].

Up to 71–87.5 % of pregnancies occur in first three cycles. The cumulative PR among anovulatory patients who ovulate with CC 50 mg/day, 100 mg/day and 150 mg/day for 3 months are 50 %, 45 % and 33 %, respectively, and further at 6 months are 62 %, 66 % and 38 %, respectively [18]. Since most of the pregnancies occur in first three to six cycles, treatment beyond six cycles is not recommended. Failure to conceive despite ovulation with clomiphene citrate is termed as CC failure.

Miscarriage Rate

There is 23 % miscarriage rate in CC conceptions probably due to hypersecretion of LH causing early resumption of meiosis. In an analysis of CC conceived patients,75 % patients who aborted had LH more than 10 IU/L as compared to 37 % in ongoing pregnancy group [24].

Multiple Pregnancy

As CC can result in multifollicular growth, the rate of multiple pregnancy is 6–8 % in anovulatory patients and 2.6–7.4 % in unexplained infertility patients [25], with majority being twins. Rarely it results in triplet (1 %), quadruplet (0.3 %) or quintuplet pregnancy (0.1 %) in all CC-induced pregnancies.

In a meta-analysis of 11,599 IUI cycles, during monofollicular growth the absolute pregnancy rate was 8.4 with 0.3 % multiple pregnancy, while after multifollicular growth the absolute pregnancy rate was 15 % with 2.8 % multiple PR. The pooled OR for multiple pregnancies after two follicles was 1.7 (99 % CI 0.8–3.6) and increased significantly for three and four follicles (2.8 and 2.3, respectively) [26].

5.6.1.3 Clomiphene and IUI

CC is the most common protocol for IUI and results in 5–7 % pregnancy rate per cycle even after seven cycles [27]. Prior to using IVF, IUI with clomiphene ovarian stimulation is relatively cheap, and many couples will conceive and not require IVF [28].

Anovulatory patients do not need IUI [29], unless cervical or male factor is abnormal. However, for patients with unexplained infertility, CC with IUI is superior to expectant management. But CC without IUI is not superior to expectant management. A recent Cochrane review found that CC was not superior to expectant management or placebo for live birth (odds ratio [OR] 0.79, 95 % CI 0.45–1.38; $p=0.41$) or for clinical pregnancy per woman randomized both with intrauterine insemination (IUI) (OR 2.40, 95 % CI 0.70–8.19; $p=0.16$), without IUI (OR 1.03, 95 % CI 0.64–1.66; $p=0.91$) and without IUI but using human chorionic gonadotropin (hCG) (OR 1.66, 95 % CI 0.56–4.80; $p=0.35$) [30].

LH surge monitoring or ovulation trigger is needed to time IUI. Traditionally, ovulation trigger is usually given with urinary hCG 5,000 IU s/c once the follicle reaches 18 mm and IUI performed 36 h post trigger or after ovulation is detected on ultrasound. A recent study challenged this practice and showed that ovulation trigger at follicular diameter of 24 mm was associated with thicker endometrial thickness (9 mm) and higher probability of pregnancy [31].

5.6.1.4 Clomiphene in IVF

The recent interest in mild/minimal stimulation and low-cost IVF has made CC an important weapon in the arsenal of a reproductive medicine practioner. It is started from day 2–3 of periods and can be either given for 5 days as in OI with addition of antagonist taking care of premature LH surge or till a day prior to trigger injection. The latter, apart from follicular growth, helps prevent premature LH surge due to its antagonistic action on the oestrogen receptor in the hypothalamus strongly inhibiting the positive feedback of the rising oestradiol level [32].

The largest study done till date has been of 43,433 cycles from Japan. CC was started from day 3 at 50 mg/day and was continued until the day before maturation trigger. If the ultrasound on day 8 suggested good number of follicles growing, 150 units of HMG or FSH were added. Emergency oocyte retrieval due to the premature LH surge was required in 3.5 % of cases. The ovulation rate was between 2 and 3 % confirming the efficacy of CC in preventing premature LH surge. The oocyte retrieval rate and embryo cleavage rate were 83 and 64 % respectively. The live birth rate in the study was 11.1 % [33].

A recent meta-analysis exploring the efficacy of CC-antagonist protocol vs. the conventional non-CC protocol evaluated seven trials with total of 702 women. There was no significant difference in the parameters of live birth ($p=0.26$), clinical pregnancy ($p=0.12$) and number of oocytes retrieved. Importantly, significant reduction in OHSS (1/216 = 0.5 % vs. 9/217 = 4.1 %, $p=0.01$), consumption of gonadotropins and duration of COH were seen [34].

In our centre, we have been using the continuous CC protocol by starting CC on day 2 till the day prior to trigger. We add FSH 75–150 IU from day 4 of CC to recruit more follicles. Of 440 cycles with this protocol, the clinical pregnancy rate per started cycle is 29.8 % (unpublished data).

5.6.1.5 Unconventional Regimens for CC Resistance (Fig. 5.2)

In case there is clomiphene resistance, the woman can be put on extended regimes with clomiphene. However, adverse effects of clomiphene on the endometrium must be kept in mind.

Extended Clomiphene

CC given for more than 5 days can achieve acceptable ovulation rate. In an RCT of women with CC resistance, hMG 75 IU from day 3–7 was compared with extended CC group, which

was given 100 mg CC from day 2 to 9. The gonadotropin group had significantly higher ovulation rates (57.6 versus 28.1 %; $p<0.001$) and pregnancy rates (20.2 % vs. 11.4; $p=0.03$) when compared to extended CC [35].

Stair Step Protocol

In this protocol CC 50 mg for 5 days is given. If there is no dominant follicle (DF) on cycle day 14 (i.e. DF more than 11 mm), patients are given CC 100 mg for 5 days followed by repeat scan starting on day 19. If still there is no DF on day 23, then cycle was cancelled. This regimen acheives ovulation faster without the need to induce withdrawal bleed between treatment cycles, while the control group underwent stimulation with 100 mg in the next cycle after withdrawal bleed. When comparing the outcome, the ovulation rates/cycle were 43.3 % vs. 33.3 %, cycle cancellation rates were 50 % vs. 56.6, clinical pregnancy rates/cycle were 16.7 % vs. 10 % and endometrial thicknesses on hCG day (mm) were 8.3 ± 2.1 mm vs. 9.3 ± 2.4 mm in stair step vs. control group [36].

5.6.1.6 Side Effects

Side effects are mostly caused due to pesudo-hypo-oestrogenic environment. About 64–78 % women complained of mood swings, while 10 % women complained of hot flushes during the course of medication. Breast tenderness, nausea and pelvic discomfort are noted in 2–5 % women. Visual disturbances in the form of blurring of vision, scotoma, diplopia and light sensitivity are rare (<2 %). Visual symptoms are usually reversible but warrant discontinuation of further treatment with CC. Some clinicians suggest lower dose in next cycle. Optic neuropathy is very rare. Mild OHSS is found in 13 % patients, but severe OHSS is rare [37].

5.6.1.7 Contraindication

As clomiphene is metabolized in liver, it is contraindicated in patients with chronic liver disease. Functional ovarian cysts may become larger with clomiphene. History of blurring of vision or scotoma with use of clomiphene contradicts its use. Although it does not increase the risk of congenital anomalies [38], still it should be avoided during pregnancy. There is no cause/effect relationship between any OI agent and invasive ovarian neoplasia even when used for more than 12 months [39].

5.7 Alternatives/Adjuncts to Clomiphene in Case of Resistance or Failure

In case of clomiphene resistance or failure, there are other oral alternatives and adjuncts to clomiphene like tamoxifen, aromatase inhibitors, insulin sensitizers and dexamethasone. Before going on to other alternatives if the woman is overweight she must be asked to lose weight as her response to ovulation improves with weight loss. Besides oral alternatives gonadotropins can be tried. Gonadotropins are either overlapped with clomiphene or given as a sequential regime. In case of a PCOS woman laparoscopic ovarian drilling may be performed to improve response to clomiphene.

5.7.1 Clomiphene vs. Tamoxiphen

Tamoxiphen is also a SERM with anti-oestrogenic action at hypothalamus but oestrogenic action at endometrium and vagina, while its action on cervical mucosa is controversial. It is given 20–60 mg/day for 5 days. A meta-analysis comparing CC and tamoxiphen found similar results [odds ratio (OR) 0.755, 95 % confidence interval (CI) 0.513–1.111]. Despite theoretical superiority of tamoxiphen pregnancy rates per cycle (OR 1.056, 95 % CI 0.583–1.912) or per ovulatory cycle (OR 1.162, 95 % CI 0.632–2.134) were not significantly different although there was a trend towards higher PR per ovulatory cycle with tamoxiphen [40].

5.7.2 Clomiphene with Dexamethasone

Dexamethasone reduces circulating DHEAS, testosterone and LH levels. Additionally, it may act directly on pituitary to suppress the

Table 5.2 Putative actions of dexamethasone

Increases endometrial thickness
Increasing serum GH
Increasing serum IGF-1
Affecting LH activity and ovarian steroidogenesis
Elevation in serum FSH levels by direct action on pituitary
Inhibiting adrenal androgens secretion

action of oestradiol [41] (Table 5.2). It may also act directly on follicles or indirectly through GH or IGF −1. In a prospective, double-blind, placebo-controlled, randomized study of 230 CC-resistant PCOS women with normal DHEAS level, addition of 2 mg dexamethasone from day 5 to 14 of period increased both the ovulation rate from 20 to 88 % and pregnancy rate from 4.2 to 40.5 % [42].

5.7.3 Aromatase Inhibitors (AIs)

AIs are highly potent and highly selective inhibitors of aromatase enzyme. It has been more than half a century since AIs entered the market. However, the first two generations were associated with significant adverse effects. Things have improved immensely since the introduction of third generation of AIs including letrozole and anastrozole. Though these medicines were primarily introduced for women with oestrogen-dependent malignancies, especially breast cancer [43], their usage for ovulation induction have opened a new avenue of research. The two molecules widely researched include letrozole and anastrozole with more focus on the former.

The first reported use for infertility treatment in anovulatory women was in 2001 in women who failed to ovulate with clomiphene citrate. They were given 2.5 mg of letrozole on days 3–7 of the menstrual cycle. With letrozole use, 9 out of 12 women ovulated and 25 % became pregnant [44].

5.7.3.1 Relevant Pharmacolgy and Mechanism of Action (MOA)

Letrozole is discussed in detail as it is the most researched of the aromatase group. Letrozole is 100 % bioavailable following oral administration and has a terminal half-life of around 45 h.

The rate-limiting step in oestrogen synthesis is the conversion of androgens (androstenedione and testosterone) into oestrogens (oestrone and oestradiol, respectively) and is catalyzed by aromatase or oestrogen synthetase enzyme.

Aromatase is a microsomal cytochrome P450 hemoprotein-containing enzyme (a product of the CYP19 gene). This enzyme is expressed in various tissues in the human body including ovaries, uterus, brain, breast and adipose tissues. Aromatase inhibitors selectively target the aromatase enzyme, which is the last in the cascade of steroidogenesis, thus depleting the oestrogen levels in target tissues.

Due to the presence of the aromatase enzyme in brain as well as ovaries, it is likely that the fertility effects of AIs are the result of dual actions at central and peripheral levels.

At the central level, lowered circulating oestrogens as a result of blocked oestrogen synthesis in brain and other tissues would release the hypothalamus and/or pituitary from the oestrogen-negative feedback on the production and release of gonadotropins. This action differs immensely from that of clomiphene, as there is no depletion of oestrogen receptors. This leads to an increase in gonadotropin secretion, which stimulates the growth of the ovarian follicles. It also increases the level of activins, which further stimulates synthesis of FSH by a direct action on the gonadotropes [45].

At the peripheral level, aromatase inhibition leads to temporary accumulation of intraovarian androgens since the conversion of the androgen to oestrogens is blocked by inhibition of the aromatase enzyme. This can lead to an increase in responsiveness of the ovarian follicle to FSH as a result of either a direct action of testosterone on the augmentation of FSH receptor expression [46] or indirectly by increasing the IGF-1 levels [47].

The chances of having a monofollicular growth is higher with AIs compared to clomiphene as the increasing oestradiol level towards mid and late follicular phase reduces the FSH levels, thus allowing only the follicles with highest number of FSH receptors to sustain growth while the others undergo atresia. In case of clomiphene, the longer half-life of clomiphene allows the oestrogen receptors in the hypothalamus and pituitary to be blocked for longer duration leading to growth of more than one follicles in many women.

The oestradiol level per growing follicle is 40–60 % lesser in cycles where AIs are utilized for ovulation induction or COS. This is in line with the reduced functioning of the intraovarian machinery responsible for converting androgens to oestrogens. Theoretically, these reduced levels can be of help during IVF cycles as supraphysiological steroid levels are the major factors leading to advancement of the endometrial maturity. Though appealing, it has not been proven yet.

5.7.3.2 Dosage and Effectiveness in Ovulation Induction

Ovulation induction with letrozole is shown to have an ovulation rate of 70–84 % and a pregnancy rate of 20–27 % per cycle [48].

The indication most widely studied for letrozole usage has been that of PCOS, including ones with clomiphene resistance. There has been no formal dose-finding study for OI or COS. In fact, the doses used have been directly extrapolated from that used for breast cancer. The commonest dose of letrozole used is 2.5 mg for 5 days from day 2 to 3 of spontaneous or withdrawal bleed. Though dosage schedules of 5 and 7.5 mg have also been used, they did not incur any significant advantage in terms of pregnancy rates, though the total number of follicles growing were higher with higher dosage [49].

As for the dosage, different length of letrozole supplementation has been studied as well. Extended letrozole therapy for 10 days was tried in 218 patients who had previously failed clomiphene citrate at 100 mg for 5 days. They were randomized to receive either 5 mg of letrozole for 5 days or 2.5 mg for 10 days, both starting on day 1 of the menstrual cycle. Ovulation rates were similar at 65.7 % for the extended versus 61.8 % for the short course while a mean of three follicles more than18 mm were seen in the extended regimen compared to 1.8 in the short regimen. Pregnancy rates with the short and extended regimens were 12.4 % and 17.4 % respectively [50].

AIs with added gonadotropins can be used if more than one follicle are required to be grown as in case of unexplained infertility. This obviously will have attendant risk of OHSS and multifoetal gestation, so it should be used cautiously. Letrozole and clomiphene have been tried in unexplained infertility in many studies, the former at dosage between 2.5 and 7.5 mg/day and the latter at 100-mg/day dosage. A recent meta-analysis showed equivalence in terms of pregnancy rate in between both ovulogens, though the numbers of growing follicles were lesser with letrozole [51].

There have been many studies done in PCOS comparing clomiphene with letrozole, and the results have been mixed. But in the recent Cochrane review on the role of aromatase inhibitors in anovulatory PCOS comprising of 26 RCTs (5,560 women), it was shown that letrozole when compared to clomiphene had significantly better live birth rate (OR 1.63, 95 % CI 1.31–2.03, $n=1,783$) and clinical pregnancy rate (OR 1.32, 95 % CI 1.09–1.60, $n=2,066$) [52]. The reviewers advised caution in interpreting the results, as the quality of evidence was low. Nevertheless, it means that letrozole is at least as effective, if not better than clomiphene in this group of women. In clomiphene-resistant PCOS, when compared with placebo, letrozole was shown to have 33.3 % ovulation rate compared with nil in the placebo group [53].

5.7.3.3 Aromatase Inhibitors and IVF

It has been nearly a decade since AIs have been used in IVF, and they are yet to find a defined place. A significant number of trials are conducted studying their role in IVF. The only randomized trial conducted in normo-responders evaluating the addition of letrozole in patients with normal

ovarian response undergoing IVF or ICSI with antagonist protocol showed a higher trend for both implantation and ongoing pregnancy rates in the letrozole group, though it did not reach statistical significance. Most of the trials done for use of letrozole in IVF have been for poor responders. Since the earlier trials were done before Bologna criteria came up, they have varying inclusion criteria. One recent RCT done on 55 women with poor response as per Bologna criteria comparing microdose flare protocol with GnRH antagonist/letrozole protocol showed no statistically significant difference in total number of oocytes retrieved and pregnancy rate but showed a higher cycle cancellation in letrozole group ($p<0.001$) [54].

In contrast, a previously reported prospective non-randomized study of 147 poor responders stimulated with antagonist protocol with or without letrozole 2.5 mg for first 5 days of stimulation showed that the letrozole group had higher number of oocytes retrieved and higher implantation rate compared to the control group [55]. AIs have been shown to reduce the dose of FSH when added, thus reducing the cost of stimulation [56].

Letrozole has emerged as a preferred agent for stimulation in women with breast cancer as it is associated with significantly lesser oestradiol levels while having the similar success [57]. We would require larger RCTs before we can conclude firmly about the role of AIs in controlled ovarian stimulation for IVF.

5.7.3.4 Side Effects

The side effects extrapolated from trials in women with breast cancer include hot flushes, leg cramps and gastrointestinal disturbances [57]. Safety concerns pertaining to pregnancy have come into the forefront in the recent years. The concern mainly stems from an American Society for Reproductive Medicine (ASRM) abstract presented in 2005 comparing the outcome of letrozole conceptions with that of natural conceptions, which concluded that the use of letrozole for infertility treatment might be associated with a higher risk of congenital cardiac and bone malformations in the newborns [58]. This led to a major stir in the world of reproductive medicine and was widely covered in the media as the bad news usually spreads like wildfire. The manufacturer of the letrozole, Novartis Pharmaceutical, following the stir, issued a statement [59], advising that the usage of letrozole in premenopausal women, specifically for ovulation induction, is contraindicated. This was one of the biggest body blows to the field of reproductive medicine.

Subsequently, the Indian government has prohibited letrozole usage as ovulation-inducing agent [60]. If the above-mentioned abstract presentation and other following studies are evaluated in detail, it can be clearly seen that the fears are largely unfounded.

1. The abstract compared 150 infants (20 out of 170 were lost to follow-up) born of letrozole to 36,050 infants born to low-risk mothers in a community hospital. This comparison is flawed not only by disproportionate numbers but also by the fact that subfertility per se confers a pregnancy as a high risk [61] and comparing them with low risk is akin to comparing apples with oranges.
2. The mean age of the women in the control population was lesser (mean age (SD) 30.5 ± 1.2 years) than the letrozole group (35.2 ± 4.7 years), which again points towards methodological flaws.
3. Cardiac and skeletal anomalies are usually diagnosed in the antenatal period by ultrasound and referred to tertiary care centres for further management. There is no logical reason to believe that these anomalies would have been correctly represented by the 36,050 infants born in a community hospital due to these referrals making the relative risk calculation in letrozole group skewed.
4. The number of babies with cardiac anomalies in the control group was not commented upon in the abstract. If the incidence of congenital cardiac malformation in the general population is taken to be 5 per 1,000 babies, then by mathematical calculation, out of 36,050 babies born, 180 babies would have had congenital heart malformation in the control group. Compared with the incidence in the

letrozole group, this will not be statistically different (95 % CI, 0.3–10.0) [62].

5. The biological plausibility of an association between letrozole and teratogenicity is weak to say the least. It is a known fact that for a drug to be a teratogen, it should be present at the time of organogenesis [63]. Since the half-life of letrozole is approximately 45 h (range 30–60 h), considerably shorter than that of clomiphene citrate (5–7 days), it should be cleared from the body completely by the time of embryo implantation.
6. Larger and well-planned studies from other authors have refuted the association of letrozole with the said anomalies. One of the biggest studies to date done at five fertility centres of Canada evaluated the outcomes of 911 infants born out of CC or letrozole. They found that though the major malformation rate between clomiphene and letrozole was not significantly different (3.0 % (12/397) and 1.2 % (6/514) respectively), congenital cardiac anomalies were found to be significantly higher ($p = 0.02$) in the CC group (1.8 %) compared to the letrozole group (0.2 %) [64].

5.7.4 Insulin Sensitizers

The major insulin sensitizers available include metformin and thiazolidinediones.

Metformin is an oral biguanide drug primarily used for the treatment of type 2 diabetes mellitus and belongs to the Food and Drug Administration (FDA) pregnancy category B classification. It is the most studied of the insulin sensitizers. It reduces the peripheral insulin resistance, serum insulin and blood glucose levels. Thiazolidinedione serves as a selective ligand of the nuclear transcription factor peroxisome proliferator-activated receptor. Troglitazone was withdrawn from the market in the year 2000 due to severe hepatotoxicity. The newer agents rosiglitazone and pioglitazone are safer but still are category C drugs as per FDA due to their potential risk of causing foetal growth restriction in animal experiments [65].

A decade ago, management of PCOS was synonymous with prescribing metformin for the women. This has changed since 2007 due to emergence of some robust trials and evidence coming to the contrary. Insulin resistance (IR) seems to have a role in the pathogenesis of PCOS, but whether it has a central role or is present only as a contributory factor to central dysfunction involving hyperandrogenism is far from clear. Decades have gone elucidating the pathogenesis, but no conclusive evidence has been brought forth.

5.7.4.1 Relevant Pharmacolgy and Mechanism of Action (MOA)

The oral bioavailability of metformin is 50–60 % under fasting conditions and is absorbed slowly [66]. Pertaining to its ovarian action, metformin can have either direct effect on the ovary or indirect effect by improving the insulin sensitivity. By improving insulin sensitivity, metformin reduces CYP17 activity in the ovary. As a part of direct action, metformin suppresses androstenedione production from ovarian theca cells and decreases FSH-stimulated 3β-HSD, StAR, CYP11A1 and aromatase activities in granulosa cells leading to reduced basal and FSH-stimulated progesterone and oestradiol levels [67].

5.7.4.2 Dosage and Effectiveness

The usual dosage of metformin is 1.5–2 g/day in divided doses. The start of metformin usually predates the ovulation induction in order to get the maximum effect of the medicine. Peak plasma concentrations (C_{max}) are reached within 1–3 h and 4–8 h after taking immediate-release and extended-release formulations respectively [68]. Metformin is not metabolized in the body and is excreted unchanged in the urine, with a half-life of around 5 h [69, 70]. It is always better to start with a low dose before escalating to avoid the side effect especially on the G.I. tract. Though it has theoretically appealing effects, these have not transformed proportionately into clinical benefits. There have been umpteen numbers of trials evaluating the role of metformin in subfertility

associated with PCOS. We will review the use of metformin in PCOS in varying roles:

- As a first-line monotherapy compared to a placebo or no treatment
- As a first-line monotherapy compared to clomiphene
- As an adjunct added to clomiphene compared to clomiphene alone

As a Monotherapy First-Line Therapy Compared to a Placebo or no Treatment

The earlier studies favoured metformin as an effective drug for restoring menstrual cyclicity and for inducing ovulation in anovulatory PCOS women. Meta-analysis by Lord et al. showed ovulatory cycles being doubled with metformin compared to placebo with a number needed to treat (NNT) of 4.4 [71]. The recently updated Cochrane review also suggests that the ovulation rate is significantly higher in metformin group (16 RCTs, 1,208 participants; OR 1.81, 95 % CI 1.13–2.93) though with moderately high heterogeneity. Interestingly, in the subgroup analysis, neither the non-obese group (5 RCTs, 441 participants; OR 2.94, 95 % CI 0.81–10.61) nor the obese group (11 RCTs, 767 participants; OR 1.50, 95 % CI 0.95–2.37) was found to benefit from using metformin. The clinical pregnancy rate was higher in metformin compared with placebo (8 RCTs, 707 participants; OR 2.31, 95 % CI 1.52–3.51). In the subgroup analysis the benefit was found confined to the non-obese group only and that too with significant heterogeneity (I2 = 75 %). Importantly, there was no difference in the live birth rates between the two groups (OR 1.80, 95 %CI 0.52–6.16) [72]. As per available high-quality evidence, metformin as a monotherapy has not been shown to confer any significant benefit in terms of improving reproductive outcome compared to placebo.

As a First-Line Monotherapy Compared to Clomiphene

As per the Cochrane review, in the non-obese group, ovulation rates in both groups were similar (2 RCTs, number of cycles = 497; OR 0.87, 95 % CI0.60–1.26; I2 = 0 %) while in the obese group, clomiphene had a better ovulation rate (2 RCTs, number of cycles = 2,044; OR 0.43, 95 % CI 0.36–0.51; I2 = 0 %). The clinical pregnancy rate had opposite effects in obese vs. non-obese women, so it was non-conclusive. The live birth rate in obese women favoured clomiphene (OR 0.30, 95 % CI 0.17–0.52; I2 = 0 %), while that in the non-obese was neutral [72]. One recent systematic review undertaken to answer the role of metformin as the primary ovulation agent in PCOS studied 14 trials and concluded that compared with CC alone, patients treated only with metformin had a reduction in the live birth rate (OR = 0.48, 95 % CI 0.31–0.73, $p = 0.0006$) [73].

As an Adjunct Added to Clomiphene Compared to Clomiphene Alone

A higher ovulation rate was seen in the Cochrane review with a moderate degree of heterogeneity (I2 = 62 %) when combined therapy was given compared with clomiphene alone (18 RCTs, OR 1.74, 95 % CI 1.50–2.00). Clinical pregnancy was also higher with combined therapy (11 RCTs, 1,208 participants; OR 1.51, 95 % CI 1.17–1.96), though the benefits appeared confined to the obese group. There was no evidence that combined therapy improved the all-important live births compared with clomiphene alone (7 RCTs, 907 participants; OR 1.16, 95 % CI 0.85–1.56) [72].

Overall, the only universal indication of metformin in PCOS is when there is an impaired glycemic control. Otherwise, the evidence is not yet in favour of metformin. It can be argued that metformin can be used in obese PCOS and women with CC resistance in view of mixed evidence at least on ovulation and clinical pregnancy rates if not on live births. The advantage here of metformin would be low cost and lesser chances of OHSS compared to addition of gonadotropins. This needs to be weighed against the significantly increased gastrointestinal side effects of metformin. A recent RCT on 250 CC-resistant women, randomized to either 2.5 mg of letrozole daily or combined metformin–CC for three treatment cycles, showed no

difference in the pregnancy rates between the two groups (14.7 % vs. 14.4 %), though the number of ovulatory follicles was higher in the metformin-CC group [74].

5.7.4.3 Side Effects

The commonest side effects are related to the G.I. tract and include diarrhoea, cramps, nausea and vomiting. The most serious, but rare, side effect is lactic acidosis.

Conclusion

Ovulation induction constitutes the most utilized intervention in the treatment of a subfertile couple. A stepwise approach starting with oral ovulogens, then adjuncts and finally injectables or LOD would be the one to be followed, as it would be safer and more cost effective. Clomiphene is, was and will be, at least in the near future, the drug of choice for OI due to its proven track record and absence of valid drug controller-approved medications. It is of utmost importance to assess a given couple individually and plan for targeted treatment rather that a blanket one.

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Ovarian Stimulation in IUI

6

Manju Puri and Richa Aggarwal

Abstract

Worldwide, intrauterine insemination (IUI) is one of the most frequently used fertility treatments for couples with unexplained or male subfertility. IUI is widely used, often as an empirical treatment, for a broad range of indications. Contrary to IVF/ICSI methods, IUI is easy to perform and inexpensive and offers particular advantages such as the minimal equipment required, an easy to learn technique, and being less invasive when compared to IVF/ICSI. IUI can be carried out in natural cycles or in combination of ovarian stimulation. The rationale for the use of ovarian stimulation in IUI is to increase the likelihood and efficiency of ovulation and to increase the number of oocytes available for fertilization. Ovarian stimulation helps overcome subtle defect in ovulatory function and luteal phase as well as enhances steroid production, which may improve the chances of fertilization and embryo implantation. However, excessive follicular development is usually associated with very high estradiol levels which may lead to two important iatrogenic complications: ovarian hyperstimulation syndrome (OHSS) and multifetal pregnancy. Therefore the optimal ovarian stimulation protocol should maximize the probability of conception and in the mean time minimize the risk of multiple pregnancies and the occurrence of OHSS. Clomiphene citrate and gonadotropins are the two commonly used drugs for ovarian stimulation in IUI. In couples with unexplained infertility, ovarian stimulation with IUI has shown higher conception rates compared to IUI alone. In couples with male subfertility or cervical factor infertility, studies show no significant difference in the pregnancy rates with IUI alone and IUI with ovarian stimulation.

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Ovarian stimulation • Clomiphene citrate • Gonadotropins • GnRH antagonists • Intrauterine insemination • Unexplained infertility • Male infertility

6.1 Introduction

Intrauterine insemination (IUI) is one of the most frequently used fertility treatments for couples with unexplained or male subfertility [1]. Ever since the first paper entitled intrauterine insemination (IUI) was published in 1962 [2], there has been a constant effort to improve the outcome of IUI by modifications in sperm preparation techniques, combining IUI with ovarian stimulation and optimizing the timing of IUI.

The rationale of IUI is to facilitate availability of an increased number of motile spermatozoa for fertilization of the oocyte close to the site of fertilization bypassing the cervix. Combining IUI with ovarian stimulation is based on the rationale that ovarian stimulation will further increase the number of available oocytes for fertilization with resultant increase in the probability of conception. IUI needs to be timed accurately with ovulation so that the oocyte is available when insemination is carried out. As the cervix is bypassed in IUI, the advantage of storage of spermatozoa in the cervix for 3–5 days with vaginal insemination is lost. Ovarian stimulation facilitates optimal timing of intrauterine insemination. It is also assumed that ovarian stimulation will overcome any subtle disturbance in the follicular growth or luteal phase of the menstrual cycle contributing to unexplained infertility.

Ovarian stimulation is associated with complications like ovarian hyperstimulation syndrome (OHSS) and multiple pregnancies. Hence the advantages of combining ovarian stimulation with IUI need to be weighed against the potential complications and related morbidity. Milder stimulation protocols are preferred to achieve these objectives.

The goal of ovarian stimulation in anovulatory women and that undergoing in vitro fertilization (IVF-ET) is different from those planned for IUI. In anovulatory women, the aim is to induce a monofollicular growth, whereas in women undergoing IVF-ET, it is multifollicular growth so that more oocytes are available for in vitro fertilization and cryopreservation of embryos. In women undergoing IUI, the desired end point of ovarian stimulation is to have two dominant follicles so as to increase the probability of conception with a small risk of multiple pregnancy and ovarian hyperstimulation syndrome [3].

6.2 Basic Concepts of Gonadotropic Control of Ovarian Function

For proper understanding of the ovarian stimulation protocols for IUI, it is essential to have the basic knowledge of the gonadotropic control of the ovarian function. Follicular responsiveness to FSH and LH is developmentally regulated. FSH plays a crucial part in recruitment, selection, and dominance of the follicle, while LH contributes to dominance, final maturation, and ovulation.

At the beginning of each menstrual cycle, a cohort of follicles are recruited under the influence of increasing serum levels of FSH usually in the first 2–3 days of the menstrual cycle. The granulosa cells of these follicles undergo proliferation with expression of LH receptors. Steady serum LH levels in this phase are essential to maintain intrafollicular androgen synthesis. At a follicle size of 10 mm around day 8 of the cycle, the selection of the dominant follicle takes place. This is the follicle destined to ovulate, and this follicle has the maximum sensitivity or the lowest threshold to circulating FSH levels. This follicle starts secreting estradiol with a resultant fall in serum FSH levels due to negative feedback of the hypothalamic-pituitary axis. The growing follicles except the dominant follicle are unable to sustain their growth and undergo atresia (Fig. 6.1). The dominant follicle sustains its

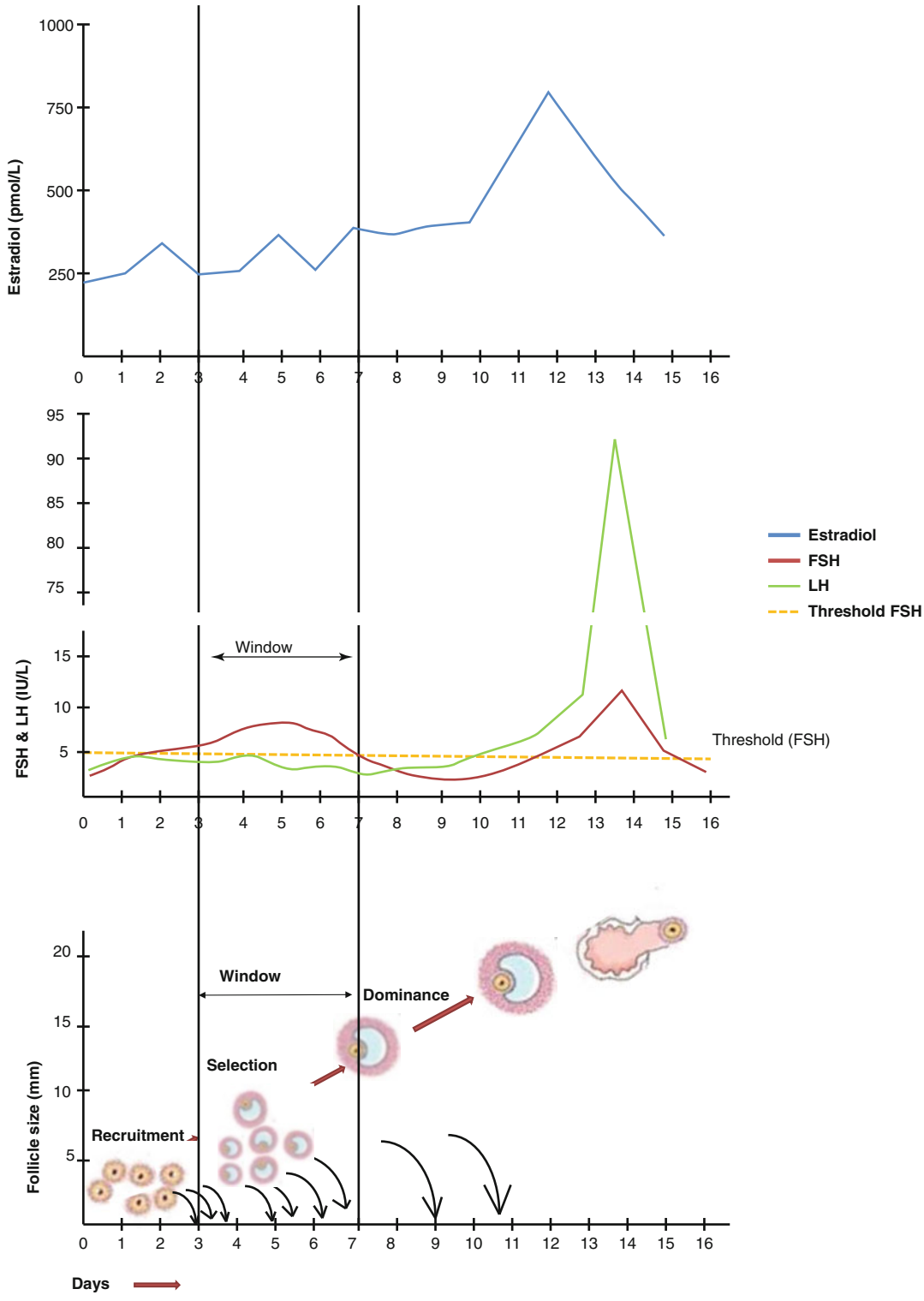

Fig. 6.1 The normal FSH window, *FSH* follicle stimulating hormone, *LH* luteining hormone

growth due to its reduced dependence on circulating serum FSH consequent to increased expression of LH receptors on it and its increased sensitivity to both FSH and LH. The dominant follicle continues to grow and secrete estradiol [4, 5]. Thus contrary to the conventional role of LH in triggering ovulation and supporting the corpus luteum, LH plays an important role in maturation of the dominant follicle in late follicular phase. It stimulates androgen production from the theca cells that in turn is converted in to estrogen by the FSH-stimulated aromatase activity of the granulosa cells [4, 5]. Finally when the estradiol levels reach a threshold, there is an LH surge and ovulation follows 12–24 h after LH surge.

Exposure to excess of LH in early phases of follicular development can adversely affect the growing follicles. There is an LH ceiling effect, that is, high levels of circulating LH as in women with PCOS can cause atresia of the follicles and premature luteinization [4, 6]. At the same time, circulating levels of LH below the LH threshold levels also interfere with the late follicular growth and maturation of oocyte. Thus the maintenance of optimum levels of LH during ovarian stimulation is important [4, 6].

In women undergoing ovarian stimulation for IUI, the aim is to have more than one dominant follicle so that the chances of conception are increased. To achieve this, one needs to maintain FSH levels above the threshold level for a longer period than in a spontaneous cycle, that is, widen the FSH window in the recruitment phase so that more than one dominant follicle is selected (Fig. 6.2). The number of dominant follicles selected depends upon the dose of drug, that is, clomiphene, FSH, or HMG used for ovarian stimulation, and the number of days the circulating level of FSH is maintained above the threshold. As ideally ovarian stimulation in IUI aims at only two dominant follicles, the FSH levels are kept above the threshold level for a shorter period till two to three dominant follicles are selected. The chances of premature LH surge are increased if more follicles are selected as each follicle adds on to the circulating levels of estradiol, which can then reach a threshold and trigger premature LH

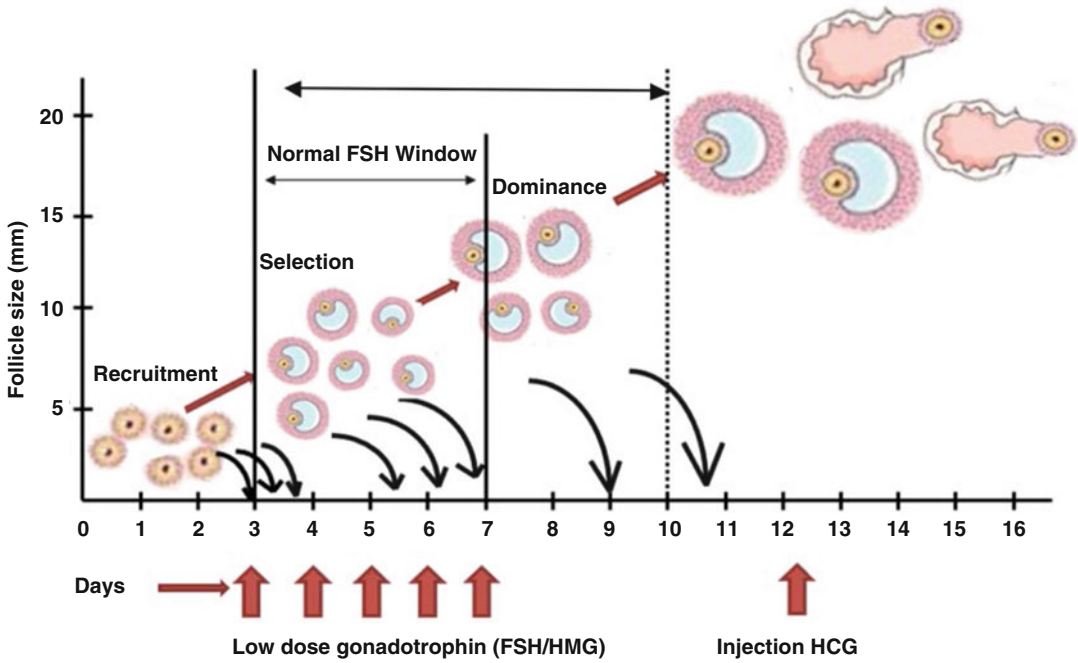

Fig. 6.2 The extended FSH window, *FSH* follicle stimulating hormone, *HMG* human menopausal gonadotropin

surge even when the follicles are not yet mature. Hence the role of GnRH antagonists in late follicular phase to prevent premature LH surge and allow the follicles to mature before LH surge is triggered.

6.3 IUI in Natural Cycle Versus Stimulated Cycle

IUI is the preferred first-line treatment for infertility due to cervical factor, mild to moderate male factor and unexplained infertility, and minimal to mild endometriosis. IUI can be carried out in natural cycles or in combination with ovarian stimulation. As ovarian stimulation is associated with an increased risk of ovarian hyperstimulation syndrome and multiple pregnancies with related maternal and perinatal morbidity and mortality, combining IUI with ovarian stimulation is justified only if it is effective. Goverde et al. found IUI combined with ovarian stimulation to result in higher pregnancy rates as compared to IUI in natural cycle [7]. A systematic review by Verhulst et al. suggests that IUI combined with ovarian stimulation is more likely to result in a live birth than IUI in natural cycle (OR 2.0, 95 % CI 2.0–3.5) [8]. In another systematic review of randomized controlled trials conducted by Bensdrop et al., IUI combined with ovarian stimulation has shown to result in higher pregnancy rate as compared to IUI done in natural cycle (OR 1.47, 95 % CI 0.91–2.38) [9]. In couples with unexplained infertility and infertility due to mild or minimal endometriosis, IUI combined with controlled ovarian stimulation either by clomiphene citrate, letrozole, or gonadotropins results in significantly higher conception rate compared to IUI alone [10]. There is no robust evidence supporting ovarian stimulation with IUI to be more effective than IUI alone for male subfertility [11]. In isolated cervical factor infertility, IUI in natural cycles is associated with a significant increase in probability of conception; hence there appears to be no added advantage of combining ovarian stimulation to it [12].

6.4 Therapeutic Options for Ovarian Stimulation for IUI

The various therapeutic options available for ovarian stimulation for IUI include clomiphene citrate, aromatase inhibitors, and gonadotropins.

6.4.1 Clomiphene Citrate

6.4.1.1 Mechanism of Action

Clomiphene citrate is a nonsteroidal selective estrogen receptor modulator (SERM) having both estrogen agonist and antagonist properties. Structural similarity to estrogen allows clomiphene to compete with endogenous estrogen for nuclear estrogen receptors at sites throughout the reproductive system. However, unlike estrogen, clomiphene binds to nuclear estrogen receptors for an extended interval of time, thereby depleting receptor concentrations. Reduced negative estrogen feedback triggers normal compensatory mechanisms that alter the pattern of gonadotropin-releasing hormone (GnRH) secretion and stimulate increased pituitary gonadotropin release, which in turn drives ovarian follicular development.

6.4.1.2 Indications

Clomiphene citrate is the traditional drug of choice for ovulation induction in anovulatory infertile women with normal thyroid function, normal serum prolactin levels, and normal endogenous estrogen levels. Clomiphene citrate is not effective in women with hypogonadotropic hypogonadism. The efficacy of clomiphene treatment in women with unexplained infertility can be attributed to optimizing follicular development or to the superovulation of more than a single ovum.

6.4.1.3 Side Effects

Clomiphene citrate is generally well tolerated though minor side effects include transient hot flashes, mood swings, headache, breast tenderness, pelvic pressure, nausea, and visual disturbances like blurring, scotoma, and light sensitivity.

6.4.2 Aromatase Inhibitors

6.4.2.1 Mechanism of Action

Aromatase inhibitors act as potent, competitive, nonsteroidal inhibitors of aromatase, the enzyme catalyzing the rate-limiting step in estrogen production. They block estrogen production both in the periphery and in the brain, resulting in a compensatory increase in pituitary gonadotropin secretion that stimulates ovarian follicular development. Aromatase inhibitors do not interfere with actions of estrogen on cervical mucus and endometrial proliferation unlike clomiphene citrate.

6.4.2.2 Treatment Schedule

After a spontaneous or progestin-induced menses, letrozole is started on day 3 and continued for 5 days with a daily dose of 2.5 mg. However the use of this drug has been prohibited for ovulation induction in India.

6.4.2.3 Complications

Use of aromatase inhibitors for ovulation is controversial because of its possible teratogenicity as observed in animal models. A case series comparing the incidence of congenital malformations in 911 newborns of women who conceived after treatment with letrozole or clomiphene found no difference [13].

6.4.3 Gonadotropins

6.4.3.1 Mechanism of Action

Exogenous gonadotropins directly stimulate the ovaries leading to ovulation.

6.4.3.2 Indications

Women with hypogonadotropic hypogonadism (WHO group I) are the most obvious candidates for ovulation induction with exogenous gonadotropins. In PCOS when clomiphene citrate treatment fails to achieve ovulation, exogenous gonadotropins are used. Exogenous gonadotropins are used intentionally to stimulate the development and ovulation of more than one mature ovum in order to increase cycle fecundity in older subfertile women and those with unexplained infertility.

6.4.3.3 Side Effects

Although superovulation is intended, careful monitoring is required to avoid excessive stimulation. They are highly effective but are very costly and associated with substantial risks including multiple pregnancy and ovarian hyperstimulation syndrome. Therefore, exogenous gonadotropins should be used only by clinicians having the training and experience necessary to provide safe and effective treatment.

6.4.4 Gonadotropin-Releasing Hormone Antagonist

6.4.4.1 Mechanism of Action

GnRH antagonists act by competitive inhibition of GnRH receptors, which results in rapid decline in FSH/LH levels, thus preventing premature LH surge.

6.4.4.2 Advantages of GnRH Antagonist

Use of GnRH antagonists offers a number of potential advantages over agonists. Prolonged pretreatment to achieve pituitary downregulation is not required. Since its only purpose is to prevent a premature endogenous LH surge and its effects are immediate, antagonist treatment can be postponed until later in follicular development (after 5–6 days of gonadotropin stimulation), after estradiol levels are already elevated, thereby eliminating the estrogen deficiency symptoms that may emerge in women treated with agonists. The total dose and duration of gonadotropin stimulation required is decreased since any suppressive effects of agonists on the ovarian stimulation by gonadotropins are eliminated. By eliminating the flare effect of agonists, GnRH antagonists avoid the risk of stimulating development of a follicular cyst and OHSS. Use of antagonists allows the manipulation of follicular development so that IUI can be avoided at

weekends or inconvenient timing without any detrimental effect on pregnancy rate.

6.4.4.3 Treatment Schedule

The two GnRH antagonists available for clinical use are ganirelix and cetrorelix. For both, the minimum effective dose to prevent a premature LH surge is 0.25 mg daily, administered subcutaneously. The treatment protocol may be fixed and begin after 5–6 days of gonadotropin stimulation or tailored to individual response, starting treatment when the lead follicle reaches approximately 13–14 mm in diameter.

6.4.4.4 Side Effects

The common side effects observed are injection site reactions and possibly malaise, headache, fatigue, and nausea.

6.5 Ovarian Stimulation Protocols for IUI

Ovarian stimulation protocols IUI would include stimulation with clomiphene, tamoxifen, and gonadotropins.

6.5.1 Clomiphene Citrate

This is usually the first-line ovarian stimulation protocol used in combination with IUI as this can be administered orally; it is convenient and cost effective. The risk of complications like higher-order pregnancies and OHSS is lesser compared to gonadotropins, but it is associated with adverse effects like hot flushes, visual disturbances, and antiestrogenic effect on the endometrium and cervical mucus.

Clomiphene citrate is administered orally starting on day 2–5 of onset of a spontaneous or induced cycle, usually on day 3, in a dose of 50–100 mg once daily for 5 days. A baseline scan is performed on day 2–3 to determine the antral follicle count and endometrial thickness and look for presence of any residual cyst or endometrioma. The dose of clomiphene citrate is arbitrarily decided based on antral follicle count on baseline scan. Women with a count of less than 8–10 per ovary can be started on 100 mg clomiphene citrate per day, and those with a higher count are started on 50 mg per day. If a cyst is present on the baseline scan, serum progesterone is estimated and ovarian stimulation is initiated if the serum progesterone level is <1 ng/ml. The endometrial thickness should be <6 mm on baseline scan prior to initiating stimulation protocol. Serial transvaginal ultrasound scan is performed starting on day 10 of the cycle, and 5,000–10,000 IU of human chorionic gonadotropin (hCG) or 250 μg of recombinant hCG is administered intramuscularly at a follicle size of 18 mm for triggering ovulation. IUI is done after 32–36 h of injection hCG. The couple is advised to have sexual intercourse on alternate day from 5 days after the last tablet, that is, usually from day 10 of the cycle for 10 days.

The cycle is canceled if there are more than three follicles of ≥14 mm present at the time of trigger to avoid multiple pregnancies. The couple is advised to use barrier contraception for 7–10 days. The other option is aspiration of extra follicles with or without escape in vitro fertilization (IVF-ET). This option is usually offered when ovarian stimulation is done with gonadotropins.

6.5.2 Tamoxifen

This is administered in the same way as clomiphene citrate in a dose ranging between 20 and 60 mg per day for 5 days. Unlike clomiphene citrate, tamoxifen has the advantage of absence of antiestrogenic effect on the cervical mucus and endometrium, hence preferred in women where the endometrial response is not satisfactory with clomiphene citrate.

6.5.3 Letrozole

After a spontaneous or progestin-induced menses, letrozole is started on day 3 and continued for 5 days with a daily dose of 2.5 mg. However the use of this drug has been prohibited for ovulation induction in India.

6.5.4 Gonadotropins

As the aim of ovarian stimulation in women undergoing IUI is to have two dominant follicles, mild stimulation protocol is preferred [12]. The duration and extent of FSH above threshold level determines the number of follicles that are capable of ovulation. HMG or highly purified FSH is used in low dose of 37.5–75 IU intramuscular per day for 5 days, starting on day 3 of the onset of spontaneous or induced cycle. Close serial monitoring of the cycle is mandatory starting from day 8–10 of cycle. At a follicle size of 18 mm, 5,000–10,000 IU of hCG is administered intramuscularly to trigger ovulation and IUI is done after 32–36 h of injection hCG. As spontaneous LH surge is common in gonadotropin cycles, serum LH measurement or urinary LH detection is done to predict a natural LH surge in which case gonadotropin antagonists may be added and/or both the trigger and IUI can be preponed. Discordancy between the follicular growth and endometrial thickness and differentiation may be another indication for adding gonadotropin antagonists. The cycle has to be canceled if there are more than three follicles of ≥14 mm in diameter present at the time of trigger.

6.6 Comparison Between the Various Ovarian Stimulation Protocols for IUI

Balasch et al. [14] and Matorras et al. [15] in their respective randomized controlled trials found higher pregnancy rate when IUI was done in cycles stimulated with gonadotropins as compared to IUI done in cycles stimulated with clomiphene citrate. Cochrane review of randomized controlled trials found that there is a significant increase in the pregnancy rate per couple in women undergoing IUI and ovarian stimulation with gonadotropins compared to that with antiestrogens [10] (OR 1.76, 95 % CI 1.16–2.66). A meta-analysis by Hughes concluded that gonadotropins seem to be more effective compared to clomiphene citrate [11]. Costello reviewed studies comparing clomiphene citrate with gonadotropins both combined with IUI and found a significantly higher pregnancy per cycle when treated with gonadotropins [16]. Similarly, Eccochard et al. found IUI combined with ovarian stimulation with gonadotropin to result in higher pregnancy rate as compared to IUI combined with clomiphene stimulation [17]. However, Dankert et al. [18] and Raslan [19] did not find any significant difference in the pregnancy rates with gonadotropin and clomiphene citrate stimulation in their respective studies.

A recent study by Mitwally and Casper showed that letrozole is as effective as clomiphene citrate in terms of pregnancy rates with the advantage of lower incidence of multiple pregnancy [20]. A systemic review of randomized controlled trials comparing the two drugs found no significant difference in the pregnancy rates [10].

According to available evidence, there seems no place for GnRH agonist or antagonists in IUI programs both as regards pregnancy rate per cycle and cost-effectiveness [10]. Eskander et al. studied 500 cases and found no significant increase in the pregnancy rate on adding GnRH agonist to cycles stimulated with gonadotropins (OR 0.67, 95 % CI 0.43–1.05) [21]. Similarly, Bellver et al. did not find any significant increase in the pregnancy rates on adding GnRH agonist to gonadotropin-stimulated cycles [22]. Premature LH surge occurs in 25–30 % of stimulated IUI cycles [23] and may interfere with timing of the IUI or result in cancelation and higher treatment failures. Although administration of a GnRH antagonist almost abolishes premature luteinization, it does not seem to considerably improve the pregnancy rate. Routine use of GnRH antagonists in women undergoing IUI and mild ovarian stimulation with gonadotropins is not warranted. In some randomized controlled trials, the average ongoing pregnancy rate was only 5.3 % greater with GnRH antagonist treatment, implying that it would take 20 cycles of GnRH antagonist administration to have one pregnancy more than without GnRH antagonist treatment [23–27].

Conclusion

Treatment decisions as regards IUI should be considered against the likelihood of pregnancy without treatment in a given couple, which is usually underestimated. The treatment decision depends on whether a reasonably increased chance of pregnancy with IUI in stimulated cycles justifies the increased cost of treatment, inconvenience to the patient, and the risk of complications such as multiple pregnancy and OHSS. Stimulated IUI is ineffective in male infertility and the effect on other diagnoses is small. With clomiphene citrate and IUI, the most common IUI protocol, pregnancy rates average 7 % per cycle. FSH ovarian stimulation and IUI treatment is only modestly better with a pregnancy rate of 12 % but multiple birth rates averaging 13 %. Mildly stimulated (one to two follicles) cycles might reduce the cost of treatment and multiple birth rates but may require more cycles of treatment [10]. One should aim for a maximum of two dominant follicles in order to avoid high-order multiple pregnancies [28]. Ovarian stimulation should be mild, and clomiphene citrate (50–100 mg per day for 5 days) remains the first-choice drug to use as it is easily available, easy to use, and less costly. If indicated hMG or recombinant FSH can be used in dosages of 50–75 IU per day. Strict ultrasound monitoring of each stimulated cycle is mandatory. One should strive for two dominant follicles larger than 15 mm, but all follicles larger than 10 mm should be measured and taken into account when defining cancelation criteria.

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Protocols for Gonadotropin Use

7

Fessy Louis Thalakottoor

Abstract

Controlled ovarian stimulation (COH) is a necessary prerequisite for the success of in vitro fertilization (IVF). Gonadotropins play a pivotal role in ovulation. Follicle-stimulating hormone (FSH) and luteinizing hormones (LH) are the main gonadotropins. Gonadotropins are inactive orally and, therefore, must be given parenterally. In routine ovulation induction, the goal is to promote the growth and development of a single mature follicle. But in controlled ovarian hyperstimulation, the aim is to obtain around 10–15 follicles so that we can select the best embryos formed, and the excess can be cryopreserved, but OHSS must not occur. There are different regimens for gonadotropins like fixed dose regime, step-up protocol, step-down protocol, chronic low-dose step-up regime, sequential regime, and combined therapy with other drugs like clomiphene citrate and tamoxifen. In controlled ovarian hyperstimulation (COH) with GnRh agonist, there is long protocol with GnRH agonist that is starting in the midluteal phase and gonadotropin treatment starting following menstruation. In short or flare-up protocol, GnRH agonist is started in the early follicular phase (day 2 of menses), and gonadotropins are started on the same day or on the following day (day 2/3). In the GnRH-antagonist protocol, the gonadotropins are started on day 2 of the cycle, and GnRH antagonist is added in the mid-follicular phase to prevent the premature LH surge. Even though long protocol is considered the 'gold standard' in IVF cycles, in

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future the use of antagonist for pituitary suppression and agonist for ovulation trigger would eliminate OHSS making ART protocols simpler and patient friendly.

Keywords

Gonadotropins • Controlled ovarian hyperstimulation • Follicle-stimulating hormone • Luteinizing hormone • Step-up protocol • Step-down protocol • GnRh agonist • Long protocol • Short or flare-up protocol • GnRH antagonist • Ovarian hyperstimulation syndrome

7.1 Introduction

Controlled ovarian stimulation (COH) is a necessary prerequisite for the success of in vitro fertilization (IVF), because it enables the recruitment of multiple healthy fertilizable oocytes. The ovarian stimulation protocol most commonly used in the past 20 years was the GnRH-agonist "long protocol." Pituitary desensitization with GnRH analogues and urinary or recombinant gonadotropins is used to promote multifollicular growth [1]. Ovarian stimulation with FSH is a central component of the success of assisted reproduction technologies. Using daily injections of recombinant human FSH (r-FSH), FSH concentrations are maintained above the threshold for single follicle development for several days, allowing multiple follicles to mature and, consequently, multiple oocytes to be retrieved [2].

In the early ages of human in vitro fertilization (IVF), some of the women subjected to several days of gonadotropin treatments could not reach oocyte retrieval because of an unpredicted rise in luteinizing hormone (LH), which could lead to premature luteinization, asynchrony of oocytes maturation, and follicle maturation arrest [3]. This was accompanied by ovulation before oocyte pickup or by retrieval of postmature oocytes that were incapable of fertilization [4]. GnRH agonists were introduced in ovarian stimulation for IVF to inhibit the premature surge of LH. However, their use is not without disadvantages. The GnRH-agonist long protocol needs at least 2 weeks for desensitization with relatively high costs due to the increased requirement of gonadotropin injections. The inhibition of a premature LH surge by GnRH agonists requires at least 7 days, as it is accompanied by an initial stimulation of GnRH receptors before gonadotropin desensitization is achieved. On the other hand, GnRH antagonists compete directly with endogenous GnRH for receptor binding and therefore rapidly suppress the secretion of gonadotropins and steroid hormones [5].

7.2 Gonadotropins

Gonadotropins also exist naturally in the body, playing a pivotal role in ovulation. Gonadotropins are hormones synthesized and released by the anterior pituitary and act on the gonads (testes and ovaries) to promote production of sex hormones and stimulate production of either sperm or ova. Follicle-stimulating hormone (FSH) and luteinizing hormones (LH) are the main gonadotropins. Human chorionic gonadotropin is a gonadotropin that is only produced during pregnancy by the placenta. The effects of gonadotropins differ in males and females. Gonadotropins are used in fertility treatment to produce mature follicles and ovulation induction, in women. Gonadotropin production is controlled by gonadotropin-releasing hormone, which is released by the hypothalamus. Gonadotropins are clinically used to stimulate ovulation. They are administered by injection only. They contain follicle-stimulating hormone (FSH), luteinizing hormone (LH), or a combination of the two. There are two basic types of gonadotropins, recombinant gonadotropins and urinary-extracted gonadotropins (which include human menopausal

gonadotropins (hMG), purified FSH, and highly purified FSH). Recombinant gonadotropins are created in a laboratory using recombinant DNA technology, while urinary-extracted gonadotropins are extracted and purified from the urine of postmenopausal women.

Gonadotropins are inactive orally and, therefore, must be given parenterally; the heavy protein content of the urinary preparation requires intramuscular injections. For over 30 years, the only preparation used for gonadotropin treatment consisted of human menopausal gonadotropins, a preparation of gonadotropins extracted from the urine of postmenopausal women. The commercial preparations available are with either 75 units of FSH and 75 units of LH per ampoule or in an ampoule with twice the amount, 150 units of each gonadotropin. A more purified urinary preparation of FSH became available by removing most of the LH in the urinary product. This product still requires intramuscular injection. A more highly purified form is available that can be administered subcutaneously. Recombinant FSH is now produced in Chinese hamster ovary cells transfected with the human FSH subunit genes. Recombinant FSH is homogeneous and free of contamination by proteins (characteristic of menopausal gonadotropins from urinary extracts), and this allows simpler subcutaneous administration [6].

Indications for gonadotropin usage are:

1. Substitution therapy: hMG is used in cases of WHO group I anovulatory disorders (hypothalamo-pituitary insufficiency).
2. Addition or assistance therapy: In clomiphene failures and clomiphene resistance, as happens in certain cases of hypothalamo-pituitary dysfunction with or without associated hyperandrogenism (PCOS).
3. Gonadotropins are also used for controlled ovarian hyperstimulation in conjunction with IVF in ovulatory women treated for unexplained, tubal factor or mild male factor infertility [7].
4. Our goal is to have mono-follicular development for a non-ART cycle and multifollicular development for an ART cycle, which then requires specific protocols and stringent monitoring. Premature ovarian failure (POF), also known as hypergonadotropic hypogonadism or WHO group III, is not responsive to exogenous gonadotropins and must be excluded.

7.2.1 Different Regimes for Gonadotropin Therapy

Exogenous FSH stimulates proliferation of granulosa cells and follicular growth. In routine ovulation induction, the goal is to promote the growth and development of a single mature follicle. But in controlled ovarian hyperstimulation, the aim is to obtain around 10–15 follicles so that we can select the best embryos formed, and the excess can be cryopreserved, but OHSS must not occur.

7.2.1.1 Fixed Dose Regime

A constant daily dose of 75–150 IU of gonadotropins is started from day 2 or day 3. Monitoring USG and E2 levels guides as to till when the injections are continued. In the fixed dose regimen, the gonadotropin dose is kept constant throughout the stimulation. If the optimal starting dose has been determined, this protocol is simple to follow and results in good outcomes.

7.2.1.2 Individually Adjusted Regimes

There are regimes that are individually adjusted as guided by the TVS follicular scan and serum E2 levels.

Step-Up Protocol

This protocol is designed to maintain FSH levels at the minimum dosage required early in the cycle, when multiple follicular recruitment is most likely to occur (Fig. 7.1). It is typically begun with 75–150 IU of hMG or FSH on day 2 or 3 of the cycle and continued with that dose for 5–7 days. If the follicular and estradiol response are deemed inadequate, the dose is increased by 37.5–75 IU for another 5–7 days at which point the patient returns for monitoring. If necessary, another 37.5 IU incremental increase can be used until an appropriate response occurs. This protocol is rarely used in our practice as a first-line

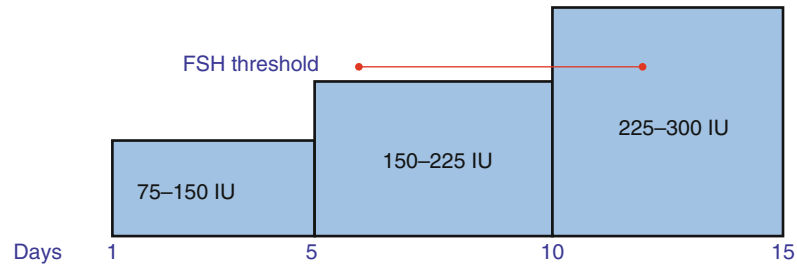

Fig. 7.1 Conventional step-up protocol

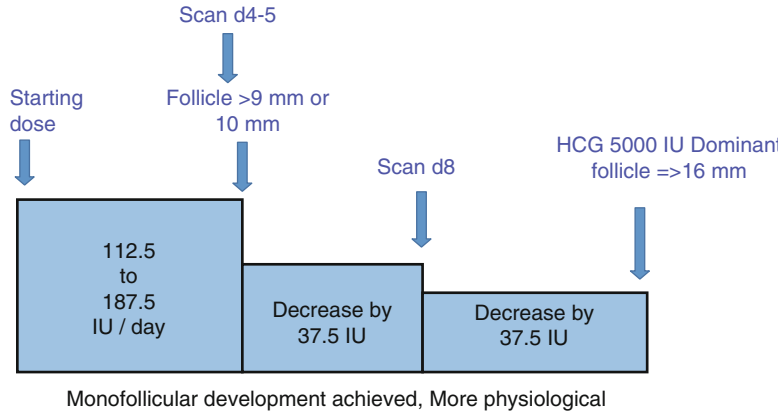

Fig. 7.2 Step-down protocol

therapy and reserved for patients who hyperstimulate with conventional low-dose regimen. The main problem with this protocol is that the stimulation gets prolonged into many days, and both the patient and the doctor can get impatient [8].

Step-Down Protocol

This regimen attempts to reproduce the normal physiological negative feedback of FSH where the development of a dominant follicle results in the rising of mid-follicular estradiol concentrations and the suppression of FSH levels and non-dominant follicles become atretic (Fig. 7.2). One such regimen begins with 150 IU on day 2 or 3 of menses, which is continued for 2 or 3 days and then reduced to 75 IU for another 3 days, after which the patient undergoes follicular monitoring and serum estradiol measurement. If follicles >10 mm are observed on TVS, the dose is decreased in decedents in two steps. The last dose is then continued till the day of the hCG injection. The step-down regimen is intended to reduce the incidence of OHSS, but the long half-life of gonadotropin preparations makes it difficult to judge the proper dosage for maintenance of a lead follicle without risk of OHSS [9].

Chronic Low-Dose Step-Up Regime

The principle behind this regimen is to find the "threshold" level of FSH which will lead to the development of a single preovulatory follicle (Fig. 7.3). This regime was proposed mainly by the ESHRE and ASRM joint consensus Thessaloniki group to prevent the OHSS. The key feature of this regimen is the low starting dose (37.5–75 units/day) of drug and a stepwise increase in subsequent doses, if necessary with the aim of achieving the development of a single dominant follicle rather than the development of many large follicles, so as to avoid the complications of OHSS and multiple pregnancy. Serum E2 levels are measured and USG is performed on day 7. If Serum E2 is >200 pg/ml or follicle size is above 10 mm, the same dose is continued. Otherwise, if the parameters are less than the above described, the daily dose is increased by an increment of 37.5 units every week, till the serum E2 level rises adequately [10].

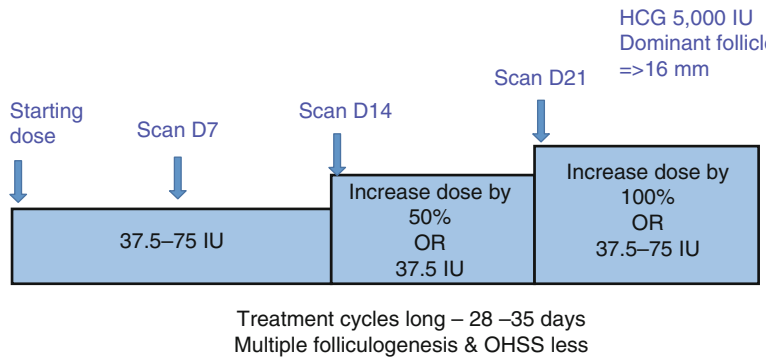

Fig. 7.3 Chronic low-dose step-up protocol

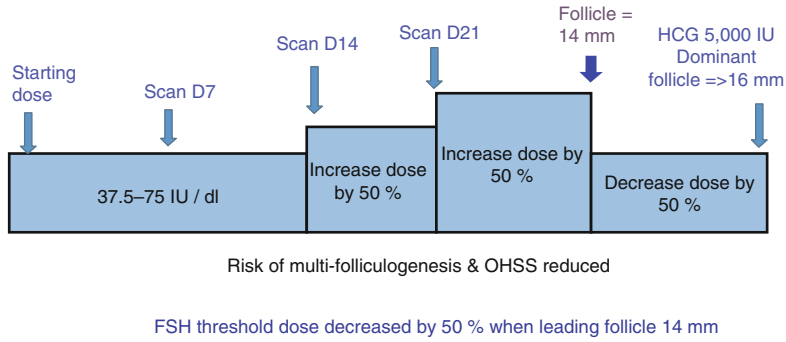

Fig. 7.4 Sequential protocol

Sequential Regime

The principle for using the sequential protocol is that FSH dependence of leading follicle decreases as follicle grows (Fig. 7.4). The decrease in FSH threshold contributes to the escape of the leading follicle from atresia when FSH concentrations start to decrease due to negative feedback of rising E2. Start stimulation with low (37.5–75 IU/day) FSH dose, which is increased by 50 % or 37.5 IU after 14 days if no ovarian response. Thereafter, any further FSH increment is made by 37.5–75 IU at weekly intervals to a maximum of 225 IU/day. Once dominant follicle emerges and reaches a diameter of 14 mm, the dose is reduced by 50 %.

7.2.1.3 Combined Therapy with Other Drugs

Clomiphene citrate or tamoxifen with gonadotropins: CC 100 mg or tamoxifen 20 mg is administered from day 2 to day 6, and injectable FSH/hMG 75/150 units is given on days 7, 8, and 9. Transvaginal sonography is done from day 10 onward, and in case the follicle growth or number is inadequate, additional FSH/hMG injections are administered. The combination of clomiphene and gonadotropin was explored in order to minimize the amount and the cost of gonadotropin alone. This method may decrease the amount of gonadotropin required by approximately 50 %; however, the same risks of multiple pregnancy and hyperstimulation can be expected. This reduced requirement for gonadotropin is found only in those patients who demonstrate a positive withdrawal bleeding following progestin medication or who have spontaneous menses [11].

7.3 Controlled Ovarian Hyperstimulation

Controlled ovarian hyperstimulation (COH) is a principal step of IVF therapy. The first IVF baby was born during a natural (unstimulated) IVF cycle. However, it was soon recognized that the success rate of IVF in natural cycles was low, primarily due to the low number of oocytes retrieved.

Ovarian stimulation using urinary gonadotropins was adopted to deal with this problem, resulting in a significant increase in both the number of eggs retrieved and the success rate of IVF. With the increasing use of stimulation in IVF cycles, various problems were recognized. Premature luteinization and failure of synchronous follicular recruitment due to early dominant follicle selection were the two main problems resulting in reduced success rates. Also, ovulation could occur at any time of the day necessitating intensive monitoring and oocyte retrieval at inconvenient times of the day.

7.3.1 GnRh-Agonist Protocols

Several different GnRH agonists, buserelin, leuprorelin, nafarelin, and triptorelin, are routinely used in ART. The preparations differ in their potency and route of administration. Nafarelin and buserelin are available as a nasal spray, which needs to be given two to six times a day, while buserelin, leuprorelin, and triptorelin are given as subcutaneous injections once a day. With the intranasal route, the absorption of the GnRH agonist fluctuates resulting in an unpredictable response. Nevertheless, in most patients it is sufficient to prevent the spontaneous LH surge. Single injection of GnRh-agonist depot preparations is being tested with good results [12].

7.3.1.1 Long Protocol

Gonadotropin-releasing hormone agonists (GnRHa) were demonstrated to result in pituitary desensitization and successfully dealt with these problems, becoming the next major breakthrough in IVF treatment. In the late 1980s, gonadotropin-releasing hormone agonists (GnRH agonists) were introduced as a means of downregulating the pituitary to prevent premature ovulation, which in the past had necessitated canceling approximately 15 % of IVF cycles prior to egg retrieval. Since their introduction, pregnancy rates have increased because of the opportunity to retrieve cycles that would have been lost to early ovulation and because of the increase in the number of oocytes obtained in GnRH-agonist cycles [13].

The long protocol is the oldest and still the most commonly used regimen for ovarian stimulation. Most commonly, the GnRH agonist is started in the midluteal phase, and gonadotropin treatment is started following menstruation. The downregulating effects of GnRH agonists, as opposed to the stimulatory effects of GnRH, are related to the frequency of administration and the prolonged occupation of GnRH receptors by the agonists. GnRH agonist is being administered daily subcutaneously or by depot preparation. Criteria for downregulation to complete and start stimulation after getting menstruation are estradiol (E2) levels below 180 pmol/L, luteinizing hormone (LH) below 2 IU/L, and P4 below 2 nmol/L. Ultrasonography is used prior to initiation of gonadotropin treatment to rule out the presence of an ovarian cyst larger than 15 mm. Ovarian cysts form in approximately 10 % of women when the GnRH agonist is started in the midluteal phase, but these cysts almost always regress spontaneously in 1–3 weeks. Gonadotropin treatment is postponed until the cysts disappear or decrease to less than 15 mm in size. GnRH-agonist administration is continued for the duration of gonadotropin treatment (Fig. 7.5) [14].

Once downregulation is achieved, gonadotropins are administered to stimulate follicular growth with the GnRH agonist being continued at a lower dose. The initial dose of gonadotropin is usually 150–300 IU/daily, except in young women or those with polycystic ovarian disease where a lower dose (75–150 IU/daily) is appropriate. Intramuscular injections of urinary

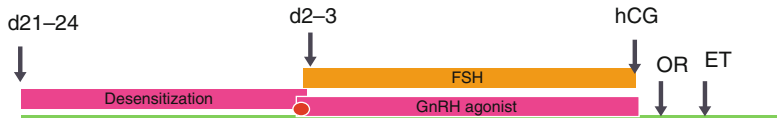

Fig. 7.5 GnRH-agonist long protocol. *OR* oocyte retrieval, *ET* embryo transfer

menopausal gonadotropins containing both FSH and LH were the mainstays of treatment until the development of a urinary gonadotropin that contained primarily FSH. Still newer, highly purified urinary FSH products introduced the advantage of being effective with subcutaneous administration. Recombinant FSH also allows subcutaneous administration. The hMG/FSH dose is subsequently adjusted according to follicular growth, as monitored by serum E2 levels and/or transvaginal ultrasonography. Human chorionic gonadotropin (hCG), either urinary or recombinant, is given once the follicular cohort consists of at least two follicles more than 18 mm in diameter. Oocyte retrieval follows 35–36 h later.

This protocol provides excellent cycle control making it the protocol of choice for first-time patients as well as for those with previous normal response. The main disadvantages of the long protocol are uncertainty of pregnancy at the start of GnRH-agonist treatment, longer duration of treatment, greater consumption of gonadotropins, and higher cost. In GnRH-agonist step-down regimen study by Olivennes et al. using leuprolide 0.1 mg/day, s.c., from day 21 and reducing it to 0.05 mg/day on stimulation, the cancelation rate remained high, and the pregnancy rate was relatively low [15].

Another study of the same step-down fashion of leuprolide (from 0.1 to 0.05 mg/day) showed higher number of oocytes and improved pregnancy rates [16]. In a Cochrane review, a single-dose depot of GnRH-agonist preparation (leuprolide 3.75 mg) was administered on day 21 of a previous cycle. It was observed that there was no evidence for differences between the long protocols using depot or daily GnRH agonist for IVF cycles. The use of depot GnRH agonist was associated with increased requirements for gonadotropins and a longer time for ovarian stimulation [17].

7.3.1.2 Short or Flare-Up Protocol

The administration of a GnRH agonist to a woman who has menstrual function will initially produce a stimulatory response, known as the "flare." The magnitude of the flare response depends upon when in the cycle the agonist is administered. In the short protocol, the administration of GnRH agonist is started in the early follicular phase (day 2 of menses), and gonadotropins are started on the same day or on the following day (day 2/3). The monitoring, timing of hCG injection, and oocyte retrieval are for the long protocol. This protocol tries to derive benefit from an initial "flare-up" response due to endogenous FSH release from the pituitary gland that usually occurs in the first few days of GnRH-agonist administration. As there is no preceding pituitary suppression, this protocol results in a better response than the long protocol. However, the most important disadvantage is the high progesterone level during the early follicular phase, likely caused by the rescue of the preceding corpus luteum. Studies have confirmed a lower pregnancy rate using this protocol compared to the long protocol. Hence, in practice it is used only in patients with poor ovarian reserve or those with a previous poor response in the long protocol cycle (Fig. 7.6) [18, 19].

7.3.2 GnRH-Antagonist Protocols

Although GnRH-agonist treatment is very effective, it has several pitfalls. There is an initial stimulation of GnRH receptors before pituitary desensitization is achieved. Hence, 7–14 days are required for adequate downregulation, menopausal symptoms are not unusual, and, unless a depot preparation is used, daily injections or multiple daily intranasal administrations

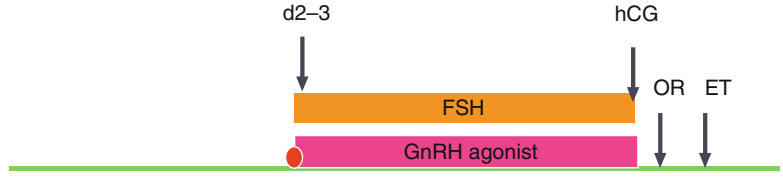

Fig. 7.6 GnRH-agonist short or flare protocol. *FSH* follicle stimulating hormone, *OR* oocyte retrieval, *ET* embryo transfer

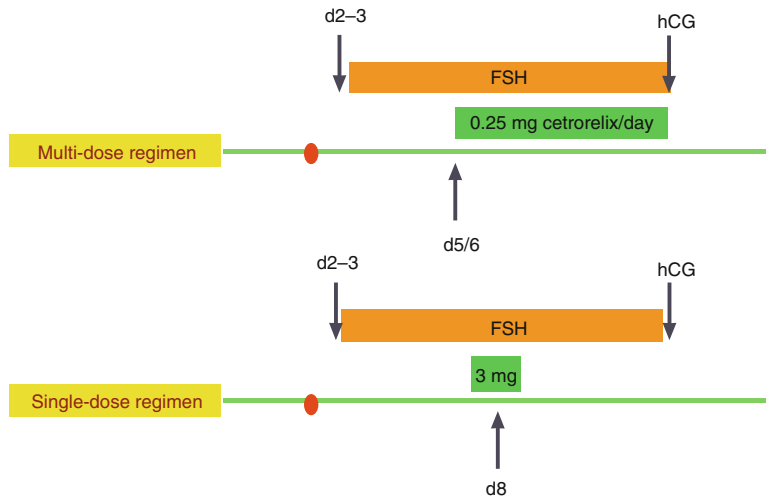

Fig. 7.7 GnRH-antagonist protocol. *FSH* follicle stimulating hormone

are required for 2–4 weeks. In contrast, GnRH antagonists, being competitive inhibitors of endogenous GnRH due to their receptor binding property, rapidly inhibit secretion of gonadotropin and steroid hormones with a reduction of FSH and LH secretion within 8 h after administration, a potential advantage over GnRH agonists. In the GnRH-antagonist protocol, the gonadotropins are started on day 2 of the cycle, and GnRH antagonist is added in the mid-follicular phase to prevent the premature LH surge (Fig. 7.7) [20, 21].

Two different compounds, cetrorelix and ganirelix, are available and are equally efficacious. Gonadotropin-releasing hormone antagonists are typically initiated either in a flexible protocol when the lead follicle is 14 mm in mean diameter or in a fixed protocol on stimulation days 5–6. They can be used in two different protocols, the single- and multiple-dose protocol. The multiple-dose GnRH-antagonist protocol involves the daily subcutaneous injections of 0.25 mg of either cetrorelix or ganirelix from day 5 or 6 of stimulation (the fixed start) until administration of human chorionic gonadotropin (hCG). The single-dose protocol involves a single subcutaneous injection of 3 mg GnRH antagonist on day 7 or 8 of stimulation. This single dose provides 4 days of pituitary suppression. If the patient needs more days of stimulation, the daily 0.25 mg of GnRH-antagonist injections are required until the hCG trigger. The monitoring, criteria for hCG administration, and oocyte retrieval are similar to the agonist protocols [22].

The treatment cycle is significantly shorter with GnRH antagonist than with GnRH-agonist treatment. GnRH antagonists are associated with simpler stimulation protocols, lower gonadotropin requirements, reduced costs, shorter duration of injectable drug treatment, absence of vasomotor symptoms, less risk of inadvertent administration during early pregnancy, avoidance of ovarian cyst formation, a significantly smaller dose of gonadotropin, and shorter intervals between successive cycles [23, 24]. Despite an initial trend toward a lower pregnancy rate with GnRH antagonists compared with agonists in a number of early randomized controlled studies, a recent meta-analysis found no significant difference in the probability of live birth rates with the use of either a GnRH-agonist or GnRH-antagonist protocol [25, 26]. As an effective alternative to hCG-induced ovulation, GnRH agonists induce a sustained release of LH (and FSH) from the pituitary that effectively induces oocyte maturation and ovulation in antagonist cycle.

Another possible advantage of antagonist cycle is that we can use GnRH agonist for trigger in comparison with hCG. GnRH-agonist triggering, however, results in a shorter endogenous LH surge that leads to a defective corpus luteum formation and an inadequate luteal phase.

The profound luteolysis observed after GnRH-agonist triggering in contrast to the prolonged luteotropic effect often seen after triggering with hCG has been shown to almost completely eliminate the risk of OHSS in high responders. When we use standard luteal phase support after GnRH-agonist trigger, there is inadequate luteal phase, and it results in lower conception and higher miscarriage rates [27–29].

7.4 Protocol for Poor Responders

This group of women has the poorest prognosis for COH results and IVF pregnancy outcome. The definition of poor responder has differed widely in the literature and has included the woman's age, basal hormonal status (high FSH), previous cancelation, and/or a poor response in a previous cycle with less than five oocytes retrieved and/or a peak serum E2 level 500 pg/mL. In most of the studies, poor responders were identified as patients with one or more of the following characteristics: high basal cycle day 3 FSH (10 mIU/mL) or E2 levels (90 pg/mL), advanced age (37 years), low ovarian volume, reduced number of antral follicles, and/or previous cancelation due to inadequate folliculogenesis (fewer than four dominant follicles after 6 days of gonadotropin stimulation) [30].

There is no one pituitary downregulation protocol that is best suited for all poor responders. Traditional GnRH-agonist flare and long luteal phase protocols do not appear to be advantageous. In poor responders we can try either a stop GnRH-agonist protocol or a micro-flare GnRH-agonist or a GnRH-antagonist protocol. Reduction of GnRH-agonist doses, "stop" protocols, and microdose GnRH-agonist flare regimes all appear to improve outcomes, although the proportional benefit of one approach over another has not been convincingly established. GnRH antagonists improve outcome in poor responders, although, in general, pregnancy rates appear to be lower in comparison with microdose GnRH-agonist flare regimens [31].

7.5 Protocol for Hyper Responders

The management of women who are at risk of developing an exaggerated response to COH represents a formidable challenge. An important consideration is the prevention of OHSS. Women with polycystic ovaries on ultrasound, even in the absence of other clinical features of PCOS, are at greater risk of developing OHSS. The incidence of OHSS has been reported to be as high as 30 % in this patient population. Other known risk factors for OHSS include young age, lean body weight, and a history of OHSS. In women undergoing COH treatment, high gonadotropin doses, high absolute levels (greater than 3,000 pg/ml), and rapidly rising E2 levels also represent risk factors for the development of OHSS [32].

Strategies for the prevention of OHSS include identifying patients at risk, individualization of COH protocols, and judicious use of gonadotropins. In this context, the aim of the COH is to decrease ovarian response, ideally to develop 5–15 follicles, while maintaining an E2 level of less than 3,000 pg/ml. Two effective COH protocols for high responders are the oral contraceptive pill GnRH-agonist dual suppression protocol and the GnRH-antagonist protocol. Antagonist protocol further gives us the option of substituting hCG trigger with a leuprolide acetate trigger which reduces the incidence of OHSS [33].

7.6 Recent Advance: Recombinant FSH-CTP in IVF

The β subunit of hCG is different from gonadotropic hormones as it has a C-terminal peptide extension which is responsible for reduced clearance resulting in major enhancement of in vivo bioavailability. Daily injections of FSH have to be given as it has a short half-life. Genes containing the sequence coding the C-terminal peptide (CTP) of hCG are fused with β subunit of FSH creating an FSH which is long acting with a half-life of 95 h eliminating the need for daily injections. Early follicular phase administration of FSH-CTP avoids the need for daily injections as a single

injection enables follicular growth over a period of 7 days. Maximum serum levels are obtained after 36–48 h. A second injection 7 days later may cause hyperstimulation. Hence, daily doses of recombinant FSH are given thereafter. It is given as a single subcutaneous injection of 180 μg recombinant FSH-CTP on day 3 followed by daily injections of recombinant FSH 150 IU from day 10 onward combined with GnRH antagonist 0.25 mg subcutaneously to prevent premature surge of LH [34]. The pharmacokinetics of corifollitropin alfa and r-FSH are quite different, but their induced pharmacodynamic effects at the dosages used are similar [35]. It is recommended that patients should be treated with the appropriate dose of corifollitropin alfa according to their body weight as a lower dose does not result in milder stimulation and a higher dose does not result in an improved ovarian response. Two strengths of corifollitropin are available (for patients ≤60 kg and >60 kg). Compared with a daily dose of 200 IU of r-FSH, 150 μg of corifollitropin is equivalent in safety and pregnancy outcomes in women using an antagonist protocol.

In normal responder patients undergoing ovarian stimulation with GnRH antagonist cotreatment for IVF, ongoing pregnancy rates of 38.9 % for the corifollitropin alfa group and 38.1 % for r-FSH were achieved showing similar results for the number of embryos transferred. Median duration of stimulation was equal (9 days) and incidence of (moderate/severe) ovarian hyperstimulation syndrome was the same (4.1 and 2.7 %, respectively) [36]. Fertilization rates were high, ranging from 66 to 68 %. Corifollitropin alfa was generally well tolerated, with a tolerability profile similar to that of r-FSH. There were no clinically relevant differences in pregnancy complications and the incidence of infant adverse events between the two drugs [37].

A recent Cochrane review analyzed four RCTs with a total of 2,335 participants [38]. Overall live birth rate (OR 0.92) or OHSS (OR 1.12) was similar between the long-acting FSH and daily dose FSH. Women who received lower doses (60–120 μg) of long-acting FSH compared to daily FSH had lower live birth rates (OR 0.60). However, with medium dose of long-acting FSH, clinical pregnancy rate, ongoing pregnancy rate, multiple pregnancy rate, miscarriage rate, and ectopic pregnancy rate were similar to daily dose of FSH. The review concluded that the use of a medium dose of long-acting FSH is a safe treatment option and equally effective compared to daily FSH. It is a well tolerated and more convenient treatment option to induce multiple follicular growth prior to assisted reproduction. Its use for hyper or poor responders requires further research before a conclusion can be drawn.

Conclusion

The long protocol is considered the 'gold standard' in IVF cycles, in future the use of antagonist for pituitary suppression and agonist for ovulation trigger would eliminate OHSS making ART protocols simpler and patient friendly. GnRh-agonist long downregulation protocols produce more oocytes as well as embryos, but there is always the risk of OHSS. Also this protocol requires longer time for downregulation and large number of gonadotropin injections, which is a cause for concern to the patients, both physically and financially. Even though antagonists are better in this respect, the pregnancy rate is compromised in many studies, particularly in younger age group. With the soft protocols, which utilize antagonist for downregulation, even though oocyte recovery rate is low and does not allow embryo cryopreservation, the pregnancy rate is satisfactory, with least chance of OHSS.

Fine-tuning of COH can be performed presently with the available battery of hormonal preparations and adjuvant therapies. It is now very clear that the "one-size-fits-all" approach may no longer exist. The availability of new markers of ovarian reserve, the improvement in methodology for their measurement, and the huge amount of clinical data have supported the view that individualization in IVF is the way forward. In addition, new developments in the horizon may bring novel alternatives including more bioactive gonadotropin agonists and antagonists with effects of variable duration.

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8 Human Menopausal Gonadotropin, Pure FSH, and Recombinant FSH: A Comparative Analysis

Sudha Prasad and Mona Dahiya

Abstract

Gonadotropins are used very frequently for ovarian stimulation in infertility treatment. Its preparations have evolved gradually over the years from relatively crude urinary extracts to more highly purified urinary extracts to the recombinant preparations. The rationale of developing a pure FSH preparation was to induce ovulation induction using gonadotropins in patients with elevated endogenous LH serum levels. FSH alone could be enough for folliculogenesis, and LH in gonadotropin preparations may increase the incidence of complications like OHSS in patients with elevated serum LH levels. Researchers have tried to extract out the optimal dose requirement with the best effective preparation. Technical improvements have resulted in the introduction of highly purified (hP) hMG, which can be administered subcutaneously. Highly purified hMG contains more hCG and less LH than does traditional hMG. A comparative study has shown r-FSH, uFSH, and hMG among the women going through IVF/ICSI cycle. It was observed that protocol used with r-FSH, uFSH, and hMG showed the 39.9, 36.3, and 34.4 % pregnancy rate. Technology in molecular engineering is undertaking to modify FSH preparations to prolong their half-lives and therapeutic actions. Simultaneously trials are evoking the oral preparation to replace painful injections.

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Follicular-stimulating hormone • Human menopausal gonadotropin • Luteinizing hormone • Human chorionic gonadotropin • COS

8.1 Introduction

Use of gonadotropin therapy is essential to infertility treatment. Gonadotropin preparations have evolved gradually over the years from relatively crude urinary extracts to more highly purified urinary extracts to the recombinant preparations. The history underpinning this development spans close to 100 years and provides a marvelous basic animal experimentation and technological advance for clinical application.

Earlier demonstration proved that pituitary extracts could stimulate follicular development and therefore have potential utility in infertility treatment. Gonadotropin products must be safe and effective. Gonadotropin treatment for induction of ovulation in anovulatory women began in the 1960s and for stimulating multifollicular development in ovulatory women in the 1980s.

8.2 Gonadotropins: Historical Overview

In 1927, Aschheim and Zondek discovered a substance in the urine of pregnant women that have the same action as the gonadotropic factor in the anterior pituitary [1]. They called this substance gonadotropin or "prolan." Furthermore, they believed that there were two distinct hormones, prolan A and prolan B. They subsequently used their findings to develop the pregnancy test that carried their names. In 1930, Zondek reported that gonadotropins were also present in the urine of postmenopausal women [2]. In the same year, Cole and Hart found gonadotropins in the serum of pregnant mares [3]. It was not until 1948, as a result of the work of Stewart, Sano, and Montgomery, that gonadotropins in urine of pregnant women were shown to originate from the chorionic villi of the placenta rather than the pituitary. It was subsequently designated "chorionic gonadotropin" [4].

After years of experimentation, it gradually became apparent that the pituitary factor was needed for the production of mature follicles and that chorionic gonadotropin could induce ovulation only when mature follicles were present [5]. In 1947, Piero Donini, a chemist at the Pharmaceutical Institute, Serono, in Rome, tried to purify hMG from postmenopausal urine. This purification method was based on a method used by Katzman et al., published in 1943 [6]. The first urine extract of gonadotropin contained LH and FSH and was named Pergonal, inspired by the Italian words "per gonadi" (for the gonads) [7]. With Pergonal treatment, the first pregnancy was achieved in a patient with secondary amenorrhea in 1961, who delivered the first normal baby girl in Israel in 1962 [8]. Urinary FSH called Metrodin and highly purified (HP) FSH became available with the development of new technology. The specific monoclonal antibodies were bound with the FSH and LH molecules in the hMG material in such a way that unknown urinary proteins could be removed. Metrodin has a specific activity of 100–200 IU of FSH/mg of protein, whereas Metrodin-HP had an activity of approximately 9,000 IU/mg of protein.

8.3 Structure of Glycoprotein Hormones

The glycoprotein hormone family has four following members:

1. FSH
2. LH
3. hCG
4. TSH

The structure of each hormone consists of:

- α subunit: common to all
- β subunit: hormone specific
- Carbohydrate moieties

The glycoprotein hormones FSH, LH, hCG, and thyroid-stimulating hormone (TSH) are composed of two non-covalently linked protein subunits, the alpha and beta subunits, to which carbohydrate moieties are attached. The alpha subunit is identical among four hormones and is composed of 92 amino acids. In contrast, the beta subunits are distinct and confer the unique biological and immunological properties and the receptor specificity of each of these glycoproteins. The subunits alone have no known biological activity. It is the formation of the heterodimer that provides the hormonal activity through attachment of the carbohydrate moieties and the extent of glycosylation especially sialylation that conveys the spectrum of differences in charge, bioactivities, and elimination half-lives.

8.3.1 Follicle-Stimulating Hormone (FSH)

FSH consists of α and β subunits and carbohydrate moieties. The beta subunit of FSH is composed of 111 amino acids. Each subunit is attached to two carbohydrate moieties with variable compositions of different isoforms. These multiple forms of FSH differ in their plasma half-lives (range: 3–4 h) due to variations in their binding potentials. The distribution of isoform types is under endocrine control and is influenced mainly by estradiol (E2) levels – the higher the E2 levels, the less glycosylated the FSH and the shorter the half-life but the greater the receptor affinity. Therefore, the isoform profile is more acidic during the early follicular to mid-follicular of the menstrual cycle but shifts to become more basic shortly before ovulation. These dynamic changes in sialylation are not mimicked by current controlled ovarian gonadotropin stimulation regimens. It is not clear whether such modification in follicular stimulation protocols would affect oocyte quality.

8.3.2 Luteinizing Hormone (LH) and Human Chorionic Gonadotropin (hCG)

Although the alpha subunits of LH and hCG are identical to that of FSH, the beta subunits are different. Luteinizing hormone has a beta subunit containing 121 amino acids that confers its specific biologic action and is responsible for its interaction with the LH receptor. This beta subunit of LH contains the same amino acids in sequence as the beta subunit of hCG, but the hCG subunit contains an additional 23 amino acids. The two hormones differ in the composition of their carbohydrate moieties, which, in turn, affects bioactivity, and half-life of LH is 20 min and that for hCG is 24 h.

8.3.3 Human Menopausal Gonadotropin

Clinical use of hMG began in 1950, but clinical trials were not started until the early 1960s. Human menopausal gonadotropin (hMG) or menotropin is derived from postmenopausal urine. The early preparations were originally only about 5 % pure and contained varying amount of FSH, LH, and hCG. The LH activity in hMG derives primarily from the hCG component, which preferentially is concentrated during the purification process and sometimes was added to achieve the desired amount of LH-like biological activity.

Human menopausal gonadotropin contains an equivalent amount of 75 IU FSH and 75 IU LH in vivo bioactivity. Cook et al. demonstrated that hMG preparations also contain up to five different FSH isohormones and up to nine LH species [9]. These differences may cause various responses in patients.

Follicle-stimulating hormone, which is the major active agent, accounts for <5 % of the local

protein content in extracted urinary gonadotropin products [10]. The different proteins found in various hMG preparations include tumor necrosis factor-binding protein I, transferrin, urokinase, Tamm-Horsfall glycoprotein, epidermal growth factor, and immunoglobulin-related proteins [11]. Local side effects, such as pain and allergic reactions, have been reported and attributed to immune reactions related to non-gonadotropin proteins [12].

Technical improvements in recent years have resulted in the introduction of highly purified (HP) hMG, which can be administered subcutaneously (SC). Highly purified hMG contains more hCG and less LH than does traditional hMG [13]. Accordingly, hMG and HP-hMG induce different follicular development profiles. The risk of transmission of prion disease by urinary gonadotropins had developed the opportunity of commercially available formulations of human prion proteins. Information is scarce regarding the metabolism of gonadotropin hormones. It was shown that purified preparation of hFSH, hLH, and hCG injected (IV) in humans had serum half-lives of 180–240 min, 38–60 min, and 6–8 h respectively.

8.3.4 Purified Follicle Stimulation Hormone

Further purification of hMG substantially decreased LH-like activity, leading to a commercial purified FSH (pFSH) preparation. Metrodin was introduced in the mid-1980s and is produced from the same source as hMG, but the LH component has been removed by immune-affinity chromatography.

The rationale of developing a pFSH preparation was to induce ovulation induction using gonadotropins in patients with elevated endogenous LH serum levels apart from obtaining a more purified product. It was also suggested that FSH alone could be enough for folliculogenesis and LH in gonadotropin preparations may increase the incidence of complications in patients with elevated serum LH levels [14].

8.3.5 Recombinant Human Gonadotropins (Follicle-Stimulating Hormone, Luteinizing Hormone, and Chorionic Gonadotropin)

Following the development of highly purified urinary FSH, considerable improvements have facilitated both separation of FSH from hLH and its production using recombinant technology which was achieved through genetic engineering. The process involves introduction of the genes encoding the α and β FSH subunits into the genome of a Chinese hamster ovary (CHO) cell line which then synthesizes and secretes a glycosylated bioactive dimeric FSH. This is finally purified by immunochromatography using a specific anti-FSH monoclonal antibody. Recombinant FSH preparations contain less acidic FSH isoforms that have a shorter half-life than those derived from human urine but stimulate estrogen as or even more efficiently.

The advantages of recombinant FSH preparations include:

1. Absence of urinary protein
2. More consistent supply
3. Less batch-to-batch variation in biological activity

The two recombinant FSH preparations currently available are marketed as follitropin alpha and follitropin beta. Both are structurally identical to native FSH and, despite being named follitropin alpha and follitropin beta, each comprising alpha and one beta glycoprotein chain. These dissimilar glycoprotein chains are non-covalently linked, being conjoined by electrostatic and hydrophobic forces, attached to two complex carbohydrate structures. The posttranslational glycosylation process and purification procedures of the two recombinant FSH preparations are not identical, resulting in different sialic acid residue compositions and thus different isoelectric coefficients. The subtle differences in structure have not resulted in any proven differences in clinical preference.

8.3.6 Corifollitropin Alfa

The range of recombinant gonadotropins available for the treatment of subfertility has been expanded through protein engineering. An FSH molecule has been engineered to possess an extended half-life and duration of therapeutic action.

This long-acting protein, designated FSH-C-terminal peptide (FSH-CTP, corifollitropin alfa), was first described by Bouloux and colleagues in 2001 [15]. FSH-CTP consists of the alfa subunit of r-hFSH together with a hybrid beta subunit made up of the beta subunit of hFSH and the C-terminal part of the beta subunit of hCG. FSH-CTP has a longer half-life than standard r-hFSH. FSH-CTP initiates and sustains follicular growth for 1 week, so one dose can replace the first seven daily injections of gonadotropin in controlled ovarian stimulation (COS). A single dose of FSH-CTP induces multifollicular growth accompanied by a dose-dependent rise in serum inhibin-B [16]. The first live birth resulting from a stimulation cycle with FSH-CTP was reported in 2003 [17]. FSH-CTP is now approved for use in Europe in ART cycles in combination with a GnRH antagonist.

Two large studies were conducted to demonstrate the non-inferiority of FSH-CTP to r-hFSH (follitropin beta) [18, 19]. A multicenter randomized, double-blind, double-dummy clinical trial involving 34 centers and 1,506 patients of 60–90 kg was initially performed (ENGAGE study). Patients undergoing ART in a standard GnRH antagonist protocol received a single dose of FSH-CTP 150 mcg or daily doses of r-hFSH 200 IU during the first week of stimulation. Ongoing pregnancy rates per cycle initiated were not significantly different for FSH-CTP or r-hFSH (38.9 % vs. 38.1 %, respectively; estimated difference 0.9; $p=0.71$). The reported incidence of moderate/severe ovarian hyperstimulation syndrome (OHSS) was 4.1 % with corifollitropin alfa versus 2.7 % with follitropin beta.

A further study was conducted to evaluate the efficacy and safety of FSH-CTP in women with low body weight. The ENSURE study was a multicenter, randomized, double-blind, double-dummy clinical trial involving 19 centers and 396 patients weighing <60 kg undergoing ART. Patients undergoing ART in a standard GnRH antagonist protocol received a single dose of FSH-CTP 100 mcg or daily doses of r-hFSH 150 IU during the first week of stimulation. The primary endpoint, number of oocytes retrieved per cycle, was 13.3 (7.3) with FSH-CTP compared with 10.6 (5.9), which was within the predefined equivalence range (−3 to +5 oocytes). The reported incidence of moderate or severe OHSS was 3.4 % for corifollitropin alfa and 1.6 % for follitropin beta.

FSH-CTP was developed with the aim of simplifying ART treatment regimens. However, high incidence of OHSS associated with FSH-CTP in published studies and in clinical practice requires further evaluation. During open-label, Phase III TRUST study, the high rate of severe OHSS was pointed out [20]. In the TRUST study, 25 patients discontinued treatment after the first or second cycle because of an excessive response to COS or signs or symptoms of OHSS. The overall rate of moderate to severe OHSS in the study was 1.0 % in Cycle 1, 1.0 % in Cycle 2, and 0 % in Cycle 3. The effects of FSH-CTP cannot be adjusted to individual patient requirements; therefore, careful assessment of patient suitability is required before treatment is commenced. The availability of recombinant FSH, LH, and hCG has shown much to further our understanding of the specific actions of individual gonadotropins in follicular development and oocyte maturation. Recombinant gonadotropins provide the capability to tailor ovarian hyperstimulation regimens to the needs of the individual woman in an effort to optimize oocyte quality and cycle fecundity.

8.4 Optimizing Outcomes of Ovarian Stimulation

8.4.1 Safety Profile of Gonadotropins

Accumulated data of 1,160 babies born after induction of ovulation with gonadotropins revealed that major and minor malformations

were found in 63 infants, representing on overall incidence of 54.3/1,000 (major malformations 21.6/1,000; minor malformations 32.7/1,000) [21]. This rate of malformation is not significantly different from that of the general population.

8.4.2 Outcomes Achieved With Recombinant FSH (r-hFSH) Versus hMG

r-hFSH and hMG are most frequently used gonadotropins for COS for IVF/ICSI. Outcomes achieved using these gonadotropins have been compared over many years in numerous retrospective studies, RCTs, and meta-analyses. Accumulating data suggest that nil commercially available gonadotropins have similar efficacy and safety profiles [22]. Indeed, there appears to be little overall difference between r-hFSH and hMG in outcomes of fresh ART cycles.

The outcomes have been measured in terms of:

- Days of stimulation
- Gonadotropin dose
- Number of oocytes retrieved
- Final estradiol and progesterone levels
- Cancellation rates
- Pregnancy/live birth rate per woman
- Availability and cost of the gonadotropins

In 2003, Al-Inany et al. published a meta-analysis that compared r-hFSH with urinary FSH products (hMG, pFSH, and FSH-HP) in IVF/ICSI cycles using a long GnRH agonist protocol [23]. Four of the twenty studies compared hMG with r-hFSH and showed no significant difference between hMG ($n=603$ cycles) and r-hFSH ($n=611$ cycles) in terms of clinical pregnancy rate per cycle initiated (OR = 0.01, 95 % CI 0.63–1.05; $p=0.11$) [24–27]. A different meta-analysis from 2003 included six RCTs ($n=2,030$) of women undergoing COS for IVF/ICSI [28]. Pooling of data from five RCTs that used a long GnRH agonist protocol showed that hMG resulted in significantly higher clinical pregnancy rates versus r-hFSH (RR = 1.22, 95 % CI 1.03–1.44). However, there was no difference between groups in ongoing pregnancy rates or live births (RR = 1.20, 95 % CI 0.99–1.45). A related Cochrane systematic review from 2003 also showed no difference in pooled data from four true RCTs in ongoing pregnancy/live birth rate per woman (OR = 1.27, 95 % CI 0.90–1.64) [29].

In 2005, Al-Inany published an updated meta-analysis involving eight RCTs and 2031 participants. It was observed that no significant differences between hMG and r-hFSH in rates of ongoing pregnancy/live birth rate, clinical pregnancy, miscarriage, multiple pregnancy, or moderate/severe OHSS [30]. This group published a third meta-analysis in 2000 including 12 trials involving 1,453 hMG cycles and 1,404 r-hFSH cycles. They showed a significant higher live birth role with hMG versus r-hFSH (OR = 1.2, 95 % CI 1.01–1.42; $p=0.04$) and similar rates of OHSS in each group (OR = 1.21, 95 % CI 0.70–1.06; $p=0.39$) [31]. Also in 2000, Coomarasamy selected seven RCTs that used a long GnRH agonist protocol. A significant increase in live birth per woman randomized was found in favor of hMG versus r-hFSH (RR = 1.10, 95 % CI 1.02–1.30; $p=0.03$) [32]. In 2009, Al-Inany et al. published a meta-analysis of six trials involving 2,371 participants comparing HP-hMG and r-hFSH in women undergoing IVF/ICSI. No significant difference in the overall ongoing pregnancy/live birth rate was found between groups. However, when IVF cycles were analyzed alone, a significantly higher ongoing pregnancy/live birth rate was found in favor of HP-hMG (OR = 1.31. 9,524 CI 1.02–1.60: $p=0.03$).

The largest meta-analysis of r-hFSH and hMG to date was published in 2010 and included data from 16 RCTs involving 4,040 patients undergoing fresh ART cycles [33]. The primary endpoint of this analysis was the number of oocytes retrieved, which was selected to estimate directly the gonadotropin effects during COS. A recent study of more than 400,000 IVF cycles has confirmed that the number of oocytes retrieved is a robust surrogate outcome for clinical success. This large meta-analysis showed that r-hFSH resulted in the retrieval of significantly more oocytes versus hMG ($p<0.001$), and

a significantly lower dose of r-hFSH versus hMG was required ($p = 0.01$). No significant difference was observed in baseline-adjusted pregnancy rates (RR = 1.04; $p = 0.49$) or in OHSS (RR = 1.47: $p = 0.12$).

In another study [34], the authors searched for randomized trials and meta-analyses comparing HP-hMG and r-FSH. Meta-analysis showed no significant difference in live births, but a greater number of oocytes with r-FSH were retrieved. In conclusion a greater number of oocytes with r-FSH allows for more frozen embryo transfer, thereby reducing overall treatment costs.

A prospective randomized and controlled study of 127 consecutive normogonadotropic infertile women ≥35 years old undergoing their first IVF/ICSI cycles receiving ovarian stimulation with HP-hMG ($n = 63$) or with r-FSH in long agonist protocol was carried out [35]. More leading (≥18 mm) follicles and oocytes were obtained in the r-FSH group. The proportion of top-quality embryo from oocyte retrieval and live birth rate per started cycle trended toward improvement with HP-hMG although there were no significant differences between the two groups. Thus superiority of HP-hMG over r-FSH in live birth rate could not be concluded from this study but noninferiority was established.

Similarly in another study, the ongoing pregnancy rate after a fresh cycle was 30 % with HP-HMG versus 27 % with r-FSH. Noninferiority of HP-hMG compared to r-FSH was established [36]. (A comparative study was conducted at Maulana Azad Medical College to assess the effectivity of r-FSH, uFSH, and hMG among the women going through IVF/ICSI cycle. It was observed that protocol used with r-FSH, uFSH, and hMG showed 39.9, 36.3, and 34.4 % pregnancy rates.)

Yet another large study [37] included 42 trials with a total of 9,606 couples. Comparing r-FSH to any of the other gonadotropins irrespective of the downregulation protocol used did not result in any evidence of statistically significant difference in live birth rates. This suggests that for a group with a 25 % live birth rate using urinary gonadotropins, the rate would be between 22.5 and 26.5 % using r-FSH. Thus, choice of gonadotropins should depend on availability, convenience, and cost in patient care.

Effectiveness of highly purified hMG with recombinant FSH in IVF/ICSI was also compared in patients who underwent ovarian hyperstimulation with pituitary suppression. On literature search, it was found that ongoing pregnancy rate per started cycle or per embryo transfer, as well as live birth rate per embryo transfer, was similar between the highly purified hMG and recombinat FSH group [38].

One thousand twenty-eight donors undergoing GnRH agonist protocol were observed randomly to one of the three groups: group I ($n = 346$), only recombinant FSH (r-FSH); group II ($n = 333$), only highly purified menotropin (HP-hMG); and group III ($n = 349$), r-FSH plus HP-hMG [39]. No differences were found among the groups with respect to days of stimulation, gonadotropin dose, final estradiol and progesterone levels, number of oocytes retrieved, and cancellation rate.

Similarly no differences were detected among the groups in terms of embryo development parameters. However, the cost of r-FSH was greater than that of other protocols.

8.5 Necessity of Individualization of Ovarian Stimulation

The objective of fertility treatment is the same for all women – optimization of outcomes with minimization of risks. It has become clear that the "one size fits all" approach to fertility treatment is too simplistic as each woman's ovarian response to stimulation is highly variable. Indeed, the use of flexible gonadotropin dosing during ovarian stimulation is now believed to be essential to optimizing cycle outcomes [40].

Various algorithms have been developed to calculate the optimum FSH starting dose [41, 42]. The CONSORT treatment algorithms were also tried to predict the optimum dose of r-hFSH (follitropin alfa) for individual patient characteristics: age, BMI, basal FSH, and antral follicle count (AFC). This algorithm resulted in an

adequate yield, good pregnancy rate, and low incidence of OHSS. However, cycle cancellation observed due to an inadequate response in frequently used protocol with lowest evaluable dose group (75 IU/day).

Efforts have also been made to identify markers that accurately predict response to the OI regimen to improve the safety, efficiency, and convenience of treatment for women with WHO group II anovulatory infertility [43, 44]. The selection of an appropriate starting dose of r-hFSH would allow physicians to individualize established treatment protocols. This could potentially shorten the time taken to reach the ovulation-triggering threshold and reduce the risk of cycle cancellation because of extreme responses to gonadotropins.

An individualized approach to ovarian stimulation is likely to result in optimal treatment outcomes. Determination of the most appropriate single or combination of drugs for ovarian stimulation, daily dose, and duration of treatment is expected to enhance safety and cost efficacy. Therefore, the identification of groups of patients who are likely to benefit from each available management strategy is essential. Such an approach would incorporate a wide variety of options based on the anticipated ovarian response.

8.6 Future Development in Gonadotropin Preparations

Molecular engineering provides the technology to modify FSH preparations to prolong their half-lives and therapeutic actions, thereby reducing the number of injections required to achieve optimal follicular growth. To this end, novel FSH preparations with modifications in FSH glycosylation or replacements of the carboxy-terminal peptide (CTP) of FSH are undergoing clinical trials.

Additional future developments may utilize the chemical libraries to identify orally active small molecule agonists of human FSH or LH receptors that might obviate the need to inject gonadotropin.

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GnRH Agonists in Controlled Ovarian Stimulation

9

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Abstract

In a rapidly advancing era of science, we are witnessing inventions of new drugs with discovery of newer applications. GnRH agonists are one of the good examples of the same. The short half-life of native GnRH triggered the need for more stable and long-acting molecules with similar actions and effects. That was subsequently achieved by the modification of the original structure. The use of GnRH agonist started in ovarian hyperstimulation for suppression of HPO axis and prevention of premature LH surge. Subsequently with wide use of antagonist protocol, it is found to be applicable as ovulation trigger, preventing OHSS. With this wonderful discovery, the concept of OHSS free clinic appears to be possible. Apart from this, it also appears to be useful for fertility preservation in patients undergoing treatment for cancer. The role in luteal phase support is still controversial and needs further studies.

Keywords

Agonist • Half life • Surge • Trigger • Luteolysis • Depot • POF

9.1 Introduction

Gonadotropin-releasing hormone agonists (GnRHas) are widely used in controlled ovarian hyperstimulation (COH). For over two decades gonadotropin preparations are extensively applied for ovarian stimulation in ovulatory women for empirical treatment of unexplained subfertility.

The GnRH agonists were developed with the goal of use for the treatment of anovulation. But soon their paradoxical ability of inhibition of

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reproductive function was demonstrated in animals. They cause rapid desensitization of pituitary gland as a result of prolonged and non-pulsatile administration [1].

In IVF cycles multi-follicular development is targeted with use of gonadotropins. But incidence of premature LH surge was found to be ranging between 20 and 50 % leading to increased cycle cancellation rates and compromised IVF outcome [1].

The major advantage offered by agonist is efficient prevention of premature LH surge and reduced cycle cancellation rates. It has also shown increase in oocyte yield and number of embryos. So eventually, pregnancy rates are better [1].

9.2 What Is GnRH?

GnRH is one of the four hypothalamic hormones, regulating the function of anterior pituitary. It is a decapeptide. It regulates the production and release of LH and FSH from anterior pituitary.

It was first isolated, characterized and synthesized independently in 1971 by Andrew Shally and Roger Guillman. They were awarded the Nobel Prize for their achievement [1].

GnRH is produced and released from groups of loosely connected neurons in arcuate nucleus of medial basal hypothalamus and preoptic area of ventral hypothalamus [1].

9.2.1 Structure of GnRH

Structure of GnRH is common to all mammals including humans. Action is similar in both males and females [1].

GnRH is a single-chain peptide comprising of ten amino acids with important functions at positions 1, 2, 3, 6 and 10 (Fig. 9.1).

Position 6 is involved in enzymatic cleavage. Positions 2 and 3 are involved in gonadotropin release, and positions 1, 6 and 10 are important for three-dimensional structure [1].

9.2.2 Structure of GnRH Agonist

Native GnRH has a short half-life due to rapid cleavage of bonds between amino acids 5–6, 6–7, 9–10. By altering amino acids at this position, analogues of GnRH can be synthesized with different properties (Fig. 9.2) [2].

Substitution of amino acid glycine at position 10 at 'c' terminus was a first major modification. This was for *increasing the potency*. But 90 % of its biological activity was lost with splitting of glycine at 10. It was restored by attachment of NH_2-ethylamide to proline at position 9 [1].

Replacement of glycine at postion 6 by D amino acids decreases enzymatic degradation. Hence it renders *more stability*. These modifications also have *higher receptor binding affinity*.

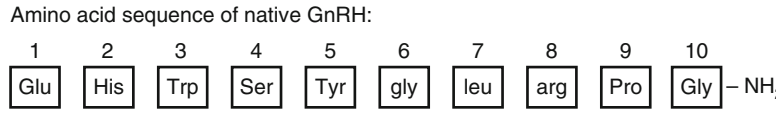

Fig. 9.1 Amino acid sequence of native GnRH

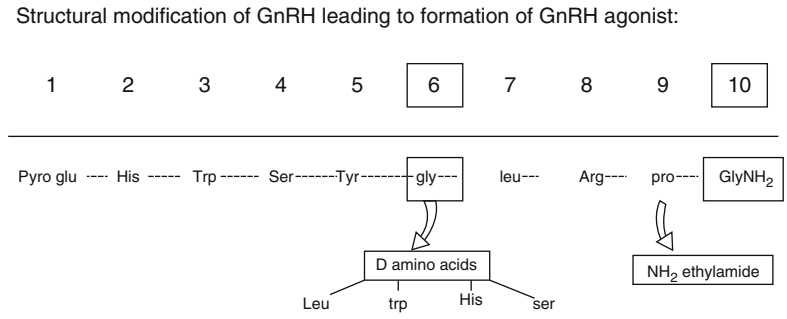

Fig. 9.2 Structural modification of GnRH, leading to formation of GnRH agonist

The introduction of larger, hydrophobic and more lipophilic D amino acids at position 6 can further increase the affinity. Increased lipophilicity is associated with *prolonged half-life* [1].

9.2.3 Structure of Antagonist

Substitution of amino acids at positions 1, 2, 3, 6, 8 and 10 produces antagonist (Figs. 9.3 and 9.4) [3].

9.2.4 Mode of Action of GnRH

Native GnRH has got a half-life of 2–4 min. GnRH neuronal system releases it in pulsatile fashion. It is necessary for rhythmic secretion of FSH and LH. The pulse frequency is approximately 1 per hour during follicular phase and 1 per 3 h in luteal phase.

It results in gonadal stimulation without down-regulation of anterior pituitary.

GnRH can produce its biological effect if it covers receptors episodically. Hence it gives time for replenishment of receptors. Receptors have three important segments which include hormone-specific external binding, transmembranous region and internal site controlling the process of internalization.

Gonadotrophin will be secreted only in response to pulsatile release of GnRH. A change in frequency or amplitude or both is associated with irregular gonadotropin release. Continuous delivery is ineffective and can lead to suppression of gonadotropic pituitary function. Similarly, prolonged stimulation of receptor by GnRH molecule results in down-regulation. (loss of ability of receptor to respond with original sensitivity). The receptor after being internalized does not return to cell surface for further action. So GnRH limits its own activity by down-regulation.

9.3 GnRH Analogues

They are structural modifications of natural GnRH. There are two types of GnRH analogues that are used: GnRH agonist and GnRH antagonist.

9.4 GnRH Agonists

A *gonadotropin-releasing hormone agonist* is a synthetic peptide modelled after the hypothalamic neurohormone GnRH. It interacts with the gonadotropin-releasing hormone receptors resulting in released gonadotropins (FSH and LH) from pitutary.

The agonist was developed with the idea of increasing the stability, potency and receptor affinity.

An increased potency could be achieved by replacing glycine for D amino acids at position 6 and by replacing gly-NH_2 at position 10 by ethyl amide. It has 100–200 times more affinity to the receptors than native GnRH. Such structural modifications render these compounds more hydrophobic and more resistant to enzymatic degradation.

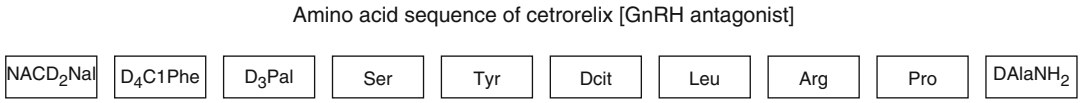

Fig. 9.3 Amino acid sequence of Cetrorelix

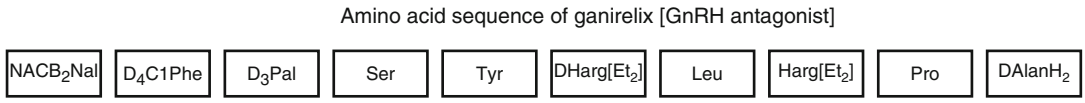

Fig. 9.4 Amino acid sequence of Ganirelix

In 1978, it was discovered that repeated administration of GnRH agonist produced a transient increase in gonadal function. The mechanism of action is 'flare effect' followed by down-regulation. Within 12 h of administration it induces liberation of high amounts of LH and FSH. It also increases the number of receptors (fivefold increase in FSH, tenfold increase in LH and fourfold increase in E2 receptors). This is known as up-regulation. This is rationale for using GnRH agonist as trigger in antagonist cycles.

The continuous occupation of the receptors leads to desensitization due to clustering and internalization of receptors resulting in fall of FSH and LH levels. This is known as down-regulation which results in arrest of follicles and fall in sex steroids. This effect is completely reversible as soon as therapy is stopped. This is a basis for clinical use of agonist in ovulation induction and controlled ovarian hyperstimulation. GnRH agonists, when chronically administered, result in marked reductions in blood levels of testosterone and oestrogen.

9.4.1 Available Preparations

The preparations available include leuprolide acetate, the first GnRH agonist to be approved in the United States, nafarelin acetate, histerelin, triptorelin, buserelin and goserelin acetate.

9.4.2 Routes of Administration

GnRH agonists need to be administered parenterally, as they would be susceptible to gastrointestinal proteolysis. Preparations are available for intramuscular, nasal and subcutaneous administration. The preferred route of administration is the subcutaneous route. As the absorption is rapid, blood concentration remains elevated for many hours without long-term pituitary desensitization.

9.4.2.1 Nasal Spray

Buserelin and naferelin are available as nasal preparations.

By nasal route of administration, the absorption is unpredictable. Considerable losses occur by proteolysis and swallowing, giving a fluctuating desensitization levels. The systemic absorption of nasal buserelin is estimated to be 5 % only. So it needs to be administered two to four times a day to maintain an effective drug concentration. The only advantage is that it is a convenient alternative to parenteral route of administration [4].

In most of the cases it is sufficient to prevent premature LH surge.

9.4.2.2 Subcutaneous Injections (Daily Doses)

This can be given once a day. They are given preference because of more stable effect. After subcutaneous administration agonist is rapidly absorbed and blood concentrations remain elevated for several hours.

Buserelin, histerelin, leuprolide and triptorelin can be effectively used as subcutaneous daily administrations. Histerelin is used in treatment of central precocious puberty.

In controlled ovarian hyperstimalation subcutaneous daily preparations are started in luteal phase of previous cycle or follicular phase of stimulation cycle according to the protocol used (long, short or ultrashort protocol). Commonly used preparation for this is leuprolide.

9.4.2.3 Intramuscular Depot Preparations

Depot preparations are useful where long-term pituitary desensitization is needed. So they are given preference for treatment of endometriosis, adenomyosis or fibroids.

Depot preparations are not first choice of treatment in ART because of long duration of action. Hypogonadotropic hypogonadal state may be sustained for 8 weeks after single depot in regularly cycling women.

It is used in ART practice in cases of frozen embryo transfer cycles, egg donation or embryo donation cycles for suppression of endogenous hormones.

Leuprolide and triptorelin are available as intramuscular depot preparations.

Goserelin acetate is available as 3.6 mg depot preparation for subcutaneous use.

Albuquerque LE found no evidence of a significant difference between depot and daily GnRHa use for pituitary down-regulation in IVF cycles using the long protocol, but substantial differences could not be ruled out [5]. Since depot GnRHa requires more gonadotropins and a longer duration of use, it may increase the overall costs of IVF treatment [5]. Hence, daily administration of GnRH agonist seems to be a more cost-effective option as compared to depot preparation.

9.4.3 Applications of GnRH Agonists in ART

1. In controlled ovarian hyperstimulation
2. An ovulation trigger to prevent OHSS
3. GnRH agonist as luteal phase support
4. For fertility preservation in patients undergoing cancer treatment

9.4.3.1 In Controlled Ovarian Hyperstimulation

Gonadotropin-releasing hormone agonists are used in assisted reproduction technology (ART) cycles to prevent a premature luteinizing hormone surge. After use of GnRH agonist IVF, cycle cancellation rates dropped from 20 to 2 % and the fertilization and implantation rates significantly improved.

The protocols are

1. *Long Protocol*: GnRH agonist is started on day 21 of the previous cycle in a daily dose. Stimulation is started from day 2/3 of cycle after confirming down-regulation. GnRH agonist is continued either in the same dose or reduced dose. The agonist may be stopped on the day of stimulation and subsequently an antagonist started on day 5/6 to prevent premature LH surge.
2. *Ultralong Protocol*: This protocol is followed for patients with endometriosis where GnRH agonist is given for 3 months and then stimulation is started.
3. *Short Protocol*: The GnRH agonist is started on day 1 of the cycle, and after 3 days dose is reduced to half and stimulation is started with gonadotropins. The advantage of flare effect is taken to increase levels of FSH.
4. *Ultrashort Protocol*: The GnRH agonist is started on day 1 of the cycle, and after 3 days it is stopped. Stimulation is started with gonadotropins.

A Cochrane database review in 2011 published analysis of gonadotrophin-releasing hormone agonist protocols for pituitary suppression in assisted reproduction [6]. There was no evidence of a difference in the live birth rate, but this outcome was only reported by three studies. There was evidence of a significant increase of 50 % in clinical pregnancy rate in a long protocol when compared to a short protocol. This difference could range from 16 to 93 % increased chance of pregnancy. There was evidence of about 60 % increased number of oocytes obtained when a long protocol was used as compared to a short protocol. However, gonadotropin requirement was also increased in a long protocol. There was no difference in any of the outcome measures for luteal versus follicular start of GnRHa and stopping versus continuation of GnRHa at the start of stimulation.

Long protocol is an original protocol described and is still considered as the gold standard.

9.4.3.2 An Ovulation Trigger to Prevent OHSS

GnRH agonist trigger instead of human chorionic gonadotropin was introduced in the early 1990s as a means to prevent OHSS.

The GnRH agonist preparations used in practice for triggering ovulation are Triptorelin 0.2 mg SC or Leuprolide 1 mg SC. Buserelin use as trigger is also mentioned in few studies.

GnRH antagonist protocols for pituitary down-regulation in IVF and ICSI allow the use of GnRH agonists for triggering final oocyte maturation. Currently, human chorionic gonadotropin (HCG) is still the standard medication for this purpose. The effectiveness of triggering with a GnRH agonist compared to HCG measured as pregnancy and ovarian hyperstimulation (OHSS) rates are unknown.

Cochrane database review in 2011 showed that, in fresh autologous cycles, GnRH agonist was less effective than HCG in terms of the live birth rate and ongoing pregnancy rate per randomized woman [7]. Incidence of ovarian hyperstimulation syndrome (OHSS) was significantly lower in the GnRH agonist group compared to the HCG group. In donor recipient cycles, there was no evidence of a statistical difference in the live birth rate per randomized woman. In conclusion they did not recommend GnRH agonists to be routinely used as a final oocyte maturation trigger in fresh autologous cycles because of lowered live birth rates and ongoing pregnancy rates. An exception could be made for women with high risk of OHSS, after appropriate counselling [8] stated that this recommendation was too premature and more studies are required before coming to a conclusion on GnRH agonist trigger in ovulation induction [8].

Haas et al. studied GnRH agonist vs. hCG for triggering of ovulation – differential effects on gene expression in human granulosa cells [9]. The fertilization rate was similar in the two groups. The mRNA expression of CYP19A1, CYP11A1 and 3 beta hydroxysteroid-dehydrogenase was significantly lower in the GnRH group. The expression of VEGF and inhibin β B was lower in the GnRH analogue triggered group. Expression of genes related to steroidogenesis is lower at the time of oocyte retrieval in patients triggered with GnRH agonist. The decreased expression of VEGF and inhibin β B in the GnRH agonist group can explain the mechanism of early OHSS prevention.

GnRHa trigger offers important advantages, including virtually complete prevention of ovarian hyperstimulation syndrome (OHSS), the introduction of a surge of FSH in addition to the LH surge and finally the possibility to individualize luteal-phase supplementation based on ovarian response to stimulation. Virtually complete elimination of OHSS is one of the major benefits of GnRHa trigger. The mechanism behind this is the luteolysis. As endogenous LH released after bolus of GnRHa has got short half-life as compared with HCG (Table 9.1).

Table 9.1 Comparison of hCG and GnRH agonist as trigger for ovulation

	HCG	GnRH agonist
Mechanism of action	Surrogate of LH; acts on LH receptors and causes resumption of meiosis in oocytes	Acts on GnRH receptor, and within 12 h induces liberation of high amounts of LH and FSH, which is known as 'flare effect'.
Half life	8–10 days	24–48 h
Effect of luteal phase	No luteal phase insufficiency	Due to shorter duration of action there is early luteolysis. So deficient luteal phase
OHSS	Increased incidence	Decreased incidence

Once the endogenous HCG production from the trophoblast reaches measurable serum concentrations around day 8 after ovulation, it is too late to rescue the corpora luteae, which results in virtual elimination of the late-onset pregnancy-associated OHSS [10, 11]. Taken together, the combination of GnRH antagonist co-treatment and GnRHa trigger is the tool by which the concept of a future OHSS-free clinic could become a reality [11].

9.4.3.3 GnRH Agonist as Luteal Phase Support

Use of GnRH agonist is suggested on day 5/6 after the ICSI procedure for luteal phase support. The effect remains controversial.

A prospective randomized control study in 2009 of 'single-dose GnRH agonist administration in the luteal phase of GnRH antagonist cycles' was designed to evaluate the effect of luteal-phase administration of single-dose GnRH agonist on pregnancy, implantation and live birth rates [12].

The patients in the luteal-phase agonist group had significantly higher rates of implantation and clinical pregnancy rates. There were also statistically significant differences in multiple pregnancy and live birth rates. Administration of single-dose GnRH agonist as a luteal-phase support in

ovarian stimulation-GnRH antagonist cycles in addition to standard luteal support seems to be effective in all cycle outcome parameters.

Olieveira et al. in 2010 published the meta-analysis on administration of 'single-dose GnRH agonist in the luteal phase in ICSI cycles' [13]. The outcomes analyzed were implantation rate, clinical pregnancy rate (CPR) per transfer and ongoing pregnancy rate. In all trials, a single dose of GnRHa was administered at day 5/6 after ICSI procedures. All cycles presented statistically significant higher rates of implantation, CPR per transfer and ongoing pregnancy in the group that received luteal-phase GnRHa administration than in the control group. In trials with long GnRHa protocol, CPR per transfer and ongoing pregnancy rates were not significantly different between the groups, but implantation rate was significantly higher in the group that received luteal-phase-GnRHa administration. On the other hand, the results from trials with GnRH antagonist multi-dose ovarian stimulation protocol showed statistically significant higher implantation, CPR per transfer and ongoing pregnancy rate in the luteal-phase GnRHa administration group.

These findings demonstrate that the luteal-phase single-dose GnRHa administration can increase implantation rate in all cycles. It increases CPR per transfer and ongoing pregnancy rate in cycles with GnRH antagonist ovarian stimulation protocol.

Nevertheless, by considering the heterogeneity between the trials, it seems premature to recommend the use of GnRHa in the luteal phase. Additional randomized controlled trials are necessary before evidence-based recommendations can be provided.

9.4.3.4 For Fertility Preservation in Patients Undergoing Cancer Treatments

In day-to-day practice, we come across multiple patients diagnosed with cancers and hence undergoing radiotherapy and chemotherapy for treatment. In these patients fertility is affected by these therapies to variable extent. But prevention is possible to some extent. GnRH agonists are used to suppress gonadotropins. Rendering the follicular development quiescent does reduce the ovarian damage [14].

A meta-analysis of studies of ovarian preservation by GnRH agonists during chemotherapy was published in 2009. It showed that 93 % women treated with GnRHa during chemotherapy maintained ovarian function as compared to 48 % of women not treated with GnRHa. The use of a GnRHa during chemotherapy was associated with a 68 % increase in the rate of preserved ovarian function compared with women not receiving a GnRHa. Among the GnRHa-treated women, 22 % achieved pregnancy following treatment compared with 14 % of women without GnRHa therapy [15].

The analysis of randomized studies, published in 2014, also shows that the temporary ovarian suppression induced by GnRHa significantly reduces the risk of chemotherapy-induced POF in young cancer patients [16].

9.5 Summary

GnRH agonist is a useful tool to prevent premature LH surge in ovulation induction. Hence, it is increasing the oocyte yield and clinical pregnancy rate. It can also be used along with antagonist protocols as preventive measure for OHSS. It is also coming up as a new hope for fertility preservation in cancer patients undergoing treatment. Its role in luteal phase as support is still controversial and requires further studies.

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GnRH Antagonist in Ovarian Stimulation

10

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Abstract

In this chapter, we discuss the introduction of GnRH antagonists into IVF, their mechanism of action and how they differ from GnRH agonist use in IVF. The chapter discusses the dosing of GnRH antagonists: single versus multiple dose protocols, timing of commencement: fixed versus flexible start protocols and their efficacy. We finally discuss the role of GnRH antagonist in individualized controlled ovarian stimulation (COS) protocols, its reduction of the risk of ovarian hyperstimulation syndrome (OHSS) and it enabling the use of the GnRH agonist trigger with potential elimination of OHSS.

Keywords

GnRH antagonist • Ovarian stimulation • Hyper responder • Fixed protocol • Flexible protocol • Poor responders • Prevention of ovarian hyperstimulation • Mild stimulation

10.1 Introduction

Since the early days, in vitro fertilization (IVF) treatment results have much improved with a 32.2 % live birth rate being reported for women aged under 35 years in the UK in the year 2010 [1]. The paradigm shift from natural unifollicular IVF treatment cycles to multifollicular stimulated IVF treatment cycles has been an important contributing factor to this improvement, largely enabled by the availability of ovulation-inducing drugs such as clomiphene citrate, human menopausal gonadotropin (hMG; menotropins) and subsequent generations of products. It led to the

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evolution of the concept of controlled ovarian stimulation (COS) whereby the ovaries are stimulated to produce high numbers of good-quality oocytes that will compensate in part for the deficiencies in in vitro fertilization and cleavage and facilitate a yield of good numbers of high-quality embryos available for transfer, thereby increasing the probability of pregnancy.

Another aspect of COS is better cycle control and the avoidance of a premature luteinizing hormone (LH) surge which, by either premature ovulation or inappropriate luteinization before oocyte pick-up, leads to high cycle cancellation and poor pregnancy rates. In conjunction with the drugs that cause multifollicular stimulation of the ovaries, pituitary suppression with gonadotropin-releasing hormone (GnRH) analogues which eliminate endogenous gonadotropin interference with exogenous superovulation regimens and timed administration of human chorionic gonadotropin (hCG) which has a similar structure but a prolonged half-life compared to LH, serve as adjuvants for the control of all events in the process of COS.

Porter and colleagues were one of the first to report the use of GnRH agonists to prevent premature LH surge in IVF treatment cycles, thereby improving the outcome [2]. The possibility of desensitizing the pituitary gland with GnRH agonists and thus inhibiting its capacity to respond to rising oestradiol levels with an untimely LH surge has led to their use prior to and during stimulation with gonadotropins in IVF treatment regimens. The introduction of GnRH agonists in assisted reproduction played an important role in the improvement of IVF treatment success by reducing the incidence of a premature LH surge which resulted in fewer cycle cancellations and higher pregnancy rates and allowed cycle programming [3].

10.2 Structure and Mechanism of Action of GnRH Antagonists

At about the same time as the GnRH agonists were being developed, work was being carried out to synthesize the GnRH antagonists. GnRH antagonists result from multiple substitutions of amino acids in the native GnRH molecule. GnRH is a decapeptide (Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) synthesized in the cell bodies of the hypothalamic neurons and secreted by their terminals into the hypophyseal-portal blood supply [4]. (Fig. 10.1) GnRH selectively stimulates the gonadotroph cells in the pituitary to release FSH and LH, which in turn stimulate gonadal production of sex steroids (oestrogen and progesterone) and folliculogenesis [5] (Fig. 10.2).

GnRH antagonists suppress gonadotropin secretion by competing with GnRH for the receptors on the pituitary gonadotroph cell membranes. Administration of the GnRH antagonists produces immediate and transient suppression of the secretion of follicle-stimulating hormone (FSH) and LH. As a result of their mechanism of action,

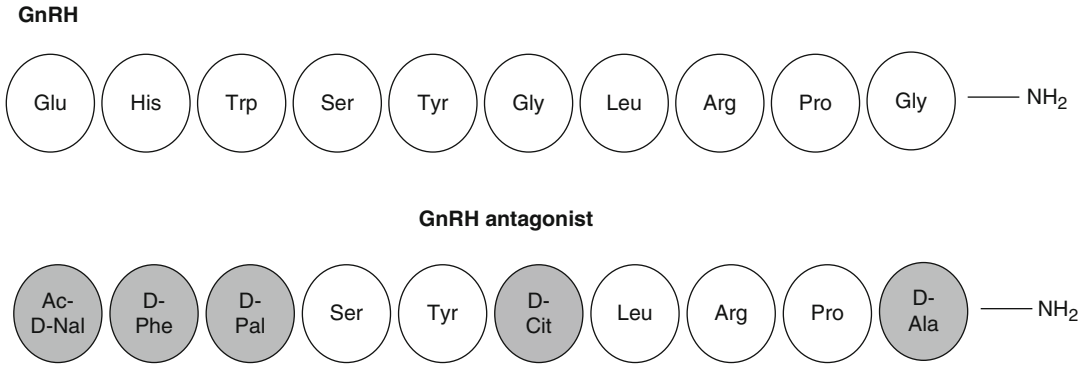

Fig. 10.1 Structure of the native GnRH and GnRH antagonist

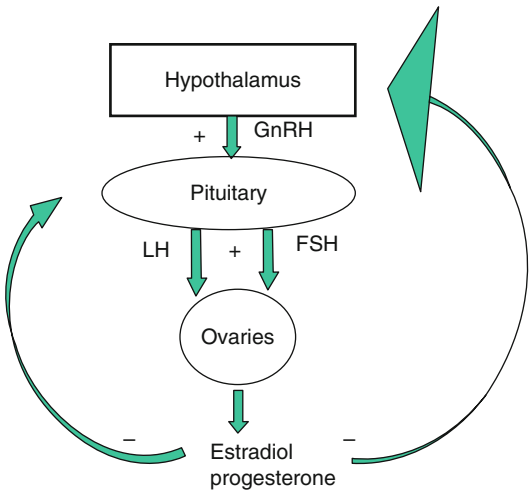

Fig. 10.2 Neuroendocrine axis. *LH* luteinizing hormone, *FSH* follicle stimulating hormone

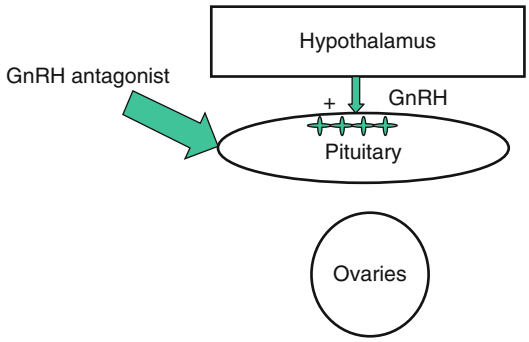

Fig. 10.3 Mechanism of action of GnRH antagonist—blocking of the pituitary receptors

they lack the initial stimulatory 'flare effect' of the GnRH agonists (Fig. 10.3).

The first GnRH antagonist was developed in 1972 by the replacement of the histidine residue at position number 2 of the native GnRH. The initial preparations of the GnRH antagonists had side effects such as an oedematous reaction caused by the release of histamine from the mast cells and depot formation after injection due to 'gelling' resulting in unreliable and unpredictable release [6]. The first-generation product was followed by subsequent generations, and the present (fourth) generation of GnRH antagonists, such as cetrorelix and ganirelix used in assisted reproduction, have been developed to overcome these side effects.

10.3 GnRH Antagonist in Ovarian Stimulation

10.3.1 Dose-Finding Studies

The fourth-generation GnRH antagonists (cetrorelix and ganirelix) were studied in Phase II studies to determine the most effective dose to prevent a premature LH surge but also avoiding oversuppression of the pituitary at the same time. Initial dose-finding studies of cetrorelix and ganirelix demonstrated that administration of 0.25 mg/day in the follicular phase was the minimal effective dose for suppression of serum LH levels and at the same time not compromising the implantation and pregnancy rates [7–10].

Initial studies to determine the minimal effective dose of GnRH antagonist administered as a single dose compared 5 mg versus 3 mg single dose of cetrorelix and 3 mg versus 2 mg single dose of cetrorelix administered on day 8 of the stimulation cycle where the gonadotropin stimulation was commenced on day 2 [11–13]. The results from these studies demonstrated that a single dose of 3 mg of cetrorelix was the minimal effective dose for successfully preventing LH surge in gonadotropin-stimulated cycles. The dose-finding studies therefore established that the GnRH antagonist could be administered either as 0.25 mg/daily in a multiple-dose protocol or as 3 mg in a single-dose protocol to effectively suppress the LH surge and maintain IVF results [14].

10.3.2 Fixed Versus Flexible Protocols

GnRH antagonists due to their mechanism of action by blockade of the pituitary gonadotrophs cause a rapid decrease in LH and FSH levels following administration. This property allows their use in the mid/late follicular phase as they do not require a prolonged desensitization period as with the GnRH agonists. GnRH antagonists can be administered either as a fixed or a flexibe protocol. In a fixed protocol, they are administered on day 6 of stimulation whereas in a flexible protocol the GnRH antagonist is administered

when a lead follicle is present at least 5 days after stimulation. A randomized controlled trial (RCT) comparing the fixed versus flexible start of GnRH antagonist commencing ganirelix 0.25 mg/day either on day 6 of stimulation or when a lead follicle of ≥15 mm found the ongoing implantation rate to be significantly higher with the fixed compared to the flexible start (23.9 % versus 8.8 % respectively) [15]. A subsequent meta-analysis involving four RCTs showed no significant difference in the incidence of premature LH surge and pregnancy rates with the fixed and flexible protocols [16]. Another RCT comparing the fixed start on day 6 of stimulation versus an earlier flexible GnRH antagonist start when the LH levels were >10 IU/l, and/or follicle diameter >12 mm, and/or serum oestradiol (E2) levels >150 pmol/l showed no significant difference in the incidence of LH rise nor ongoing implantation or pregnancy rates [17]. Therefore, based on current evidence, the fixed and flexible protocols seem to be equally effective with most protocols employing GnRH antagonist start on day 6 of stimulation with the fixed and with the lead follicle at ≥14 mm with the flexible start.

10.4 GnRH Antagonist Use in Unselected Patients

Reassuring data from the initial phase II and III studies resulted in a gradual increase in GnRH antagonist use in IVF programmes. There then followed several studies and RCTs comparing the efficacy of GnRH agonists versus GnRH antagonists for pituitary down-regulation in IVF. Early studies and meta-analyses, however, were in favour of GnRH agonists over antagonists in terms of live birth rates [16, 18]. These studies, however, favoured the use GnRH antagonists over agonists in terms of a shorter duration of treatment and lower incidence of ovarian hyperstimulation syndrome (OHSS). Given the earlier reservation of the GnRH anatgonists on the pregnancy rates, the GnRH agonists remained the predominantly used GnRH analogue for pituitary down-regulation in IVF [19]. However, with more confidence in the use of GnRH antagonists, recent studies and updated evidence have shown comparable pregnancy and live birth rates leading to their widespread use with the added benefit of patient compliance and a reduced risk of OHSS [20].

10.5 GnRH Antagonists in Selected Patient Groups

10.5.1 GnRH Antagonists for Hyper-Responders and Women with PCOS

With the embracing of individualization of COS in IVF, GnRH antagonist use has seen a high uptake in the recent past. A worldwide survey in 2010 involving 179,300 IVF cycles from 262 centres in 68 countries showed the use of GnRH antagonist-based protocols in 50 % of IVF cycles among women with polycystic ovarian syndrome (PCOS) [21]. The use of GnRH antagonists in this group of women is substantiated by a significantly lower risk of OHSS compared with GnRH agonist-based protocols [20]. A recent meta-analysis of studies comparing GnRH antagonist versus GnRH agonist protocols in women with PCOS involving nine RCTs from 2002 to 2013 showed comparable clinical pregnancy rates (CPR) between the two groups and a significantly lower incidence in severe OHSS in the GnRH antagonist group [22]. An added advantage with the use of GnRH antagonist-based protocols is the use of GnRH agonist trigger as a substitute for hCG in triggering of final oocyte maturation and potentially eliminating the risk of OHSS.

10.5.2 GnRH Antagonists for Poor Responders

A worldwide survey of GnRH analogue use in poor responders involving 124,700 IVF cycles from 196 centres in 45 countries showed that the GnRH antagonist protocol was used in 53 % of cycles [21]. Numerous studies and reviews on the ideal regimen for poor responders had suggested insufficient evidence in recommending a

particular regimen for poor responders [23–25]. One of the recurring criticisms was the lack of a uniform evidence-based definition of poor ovarian response. A recent RCT comparing the GnRH agonist long regimen versus the GnRH agonist short regimen versus the GnRH antagonist regimen in previous poor responders demonstrated the GnRH agonist and the GnRH antagonist regimens as being most effective in terms of the number of oocytes retrieved [26]. This study was conducted to overcome the previously identified deficiencies in studies of poor ovarian response and defined a poor responder woman as someone who had a previous IVF cycle with ≤3 oocytes retrieved following gonadotropin stimulation with at least 300 IU of gonadotropin daily.

10.6 GnRH Agonist Trigger with GnRH Antagonist Protocols

The GnRH agonist trigger has been proposed as an alternative to the hCG trigger by virtue of inducing an endogenous rise in LH and FSH due to its initial flare effect [27, 28]. The GnRH agonist trigger can only be used with COS regimens where prior pituitary suppression has not been achieved with the GnRH agonist as the mechanism of action of the GnRH agonist in causing down-regulation and desensitization of the pituitary receptors precludes the use of the agonist trigger. On the other hand, the advent of the GnRH antagonist into COS regimens and the recent widespread uptake has enabled the use of the GnRH agonist trigger [7, 12, 21]. Due to the specific mode of action of the antagonist by competitive blockade of the pituitary receptors and a shorter half-life, the pituitary remains responsive to the GnRH agonist, thus enabling its use for triggering ovulation.

The Cochrane review comparing the GnRH agonist versus the hCG trigger in IVF demonstrated a significantly lower incidence of OHSS and a lower live birth rate with the GnRH agonist trigger [29]. It demonstrated significantly reduced live birth rates in fresh autologous cycles with the use of the GnRH agonist trigger, but there was no reduction in live birth rates in oocyte donor/recipient cycles. Following initial use of the GnRH agonist trigger, it was soon recognized of the need to modify the standard luteal support to obtain reliable reproductive outcomes [30]. Study groups have since endeavoured to fine tune the luteal phase support in IVF cycles using the GnRH agonist trigger to optimise clinical outcomes [31, 32]. Recent suggestions and developments in overcoming the luteal insufficiency with the GnRH agonist trigger are use of

1. A ‘dual trigger’ [33]
2. Low-dose hCG supplementation [30, 32]
3. Intensive luteal oestradiol and progesterone supplementation [31]
4. Rec-LH supplementation [34]
5. Luteal GnRH agonist administration [35]

A recent RCT demonstrated that an individualized luteal support based on the number of follicles following the GnRH agonist trigger optimized the pregnancy rates [36]. This study proposed ovulation triggering with 0.5 mg buserelin S.C followed by a bolus of 1,500 IU of hCG after oocyte retrieval when the total number of follicles ≥11 mm was between 15 and 25 on the day of trigger and an additional 1,500 IU hCG bolus when the total number of follicles was ≤14 mm. All women received micronized progesterone vaginally, 90 mg twice daily, and 4 mg of oestradiol orally commencing on the day of oocyte retrieval and continuing until 7 weeks of gestation.

10.7 GnRH Antagonist Use in Mild Stimulation and Modified Natural Cycle Protocols

Finally, GnRH antagonists are the mainstay for suppression of LH surge in the context of minimal, mild stimulation protocols and modified natural cycle IVF due to the property that GnRH antagonist-based protocols are associated with a reduction in the stimulation dose of gonadotropins used compared with the GnRH agonist-based protocols. This property resulting from

their mechanism of action of competitive pituitary blockade enables their use in mild stimulation protocols and in modified natural cycles.

Conclusion

GnRH antagonist-based COS regimens are increasingly becoming the mainstay in IVF programmes given their advantage of a shorter duration of treatment, a reduction in the risk of OHSS and at the same time associated with pregnancy rates comparable with GnRH agonist-based protocols. GnRH antagonist-based protocols also enable the use of GnRH agonist trigger over hCG with potential elimination of OHSS. Although the last decade has seen a significant increase in the use of GnRH antagonist protocols, it remains to be seen if their use is likely to surpass GnRH agonist-based protocols in IVF programmes.

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GnRH Agonist Versus Antagonist in ART

11

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Abstract

Most protocols for ovarian stimulation using gonadotrophins incorporate GnRH-agonist and GnRH-antagonist co-treatment, to prevent a premature rise in LH in in vitro fertilization (IVF) cycles. Its use in IUI cycles is controversial, though the pregnancy rates may be slightly higher with the use of analogues. But one must remember that the use of GnRH agonist in IUI cycles is associated with a higher incidence of ovarian hyperstimulation syndrome (OHSS) and multiple pregnancies. On the other hand the GnRH antagonist may not be cost effective as one knows that to achieve one extra pregnancy, the number needed to treat (NNT) is 20. Protocols using GnRH antagonists are effective in preventing a premature rise of LH and induce a shorter and more cost-effective ovarian stimulation compared to the long agonist protocol.

We know that for more than 20 years, GnRH agonists have been the "gold standard" protocol in ovarian stimulation but today with more and more clinics utilizing the GnRH antagonist has had several advantages like lower total dosages of gonadotrophins, less incidence of hyperstimulation syndrome, lower cost, lack of side effects, shorter duration of treatment, and more individualized and less aggressive protocol.

With the long protocol of GnRH agonist started either in the midluteal phase or in the early follicular phase of the preceding cycle, pituitary desensitization in 2 or 3 weeks of treatment can be achieved. The initial stimulatory effect ("flare up") may lead to ovarian cyst formation. On the

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other hand, GnRH antagonists cause an immediate suppression of gonadotrophin secretion, without the initial stimulatory effect; hence, they can be given after starting gonadotrophin administration.

Thus, the GnRH-antagonist protocol is a handy protocol with good clinical outcome and a definite reduction in incidence of severe OHSS. It is the protocol of choice as the Cochrane review also has demonstrated no evidence of statistically significant differences in the rates of live births or ongoing pregnancies when comparing GnRH-agonist long protocols with GnRH-antagonist protocols.

Keywords

GnRH agonist • GnRH antagonist • Ovarian stimulation • Premature LH surge • Live-birth rates

11.1 Introduction

The role of endogenous luteinizing hormone (LH) levels during ovarian stimulation is very important. Both low and high baseline LH will affect the outcome of assisted reproductive techniques (ART) and should be taken into consideration when controlled ovarian stimulation (COS) is planned. It is important to prevent a premature LH surge during COS to be able to collect oocytes at oocyte retrieval and also prevent premature luteinization, which can affect the endometrial receptivity. For years GnRH agonists have been used in the long and short protocol to prevent premature LH surge. With the introduction of GnRH antagonist early in the twenty-first century to prevent premature LH surge, it has made the clinicians think about its efficacy compared to GnRH agonist. GnRH antagonist had several advantages like avoidance of an acute stimulation of endogenous gonadotrophins (GT), a dramatic reduction in the length of analogue treatment because of their ability to inhibit directly the premature LH surge, and a reduction in the gonadotrophin 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 analogue used. The initial use showed that the probability of clinical pregnancy was shown to be significantly lower compared with GnRH agonists [1]. But today the GnRH antagonist in ART is the first-choice analogue instead of GnRH agonist after the meta-analysis published by Cochrane in 2002 [1] showed that there is no significant difference in the probability of clinical pregnancy and live births. Moreover, the lower incidence of ovarian hyperstimulation syndrome (OHSS) in a GnRH-antagonist cycle, where there is an option of using GnRH agonist for trigger, has made them the analogue of choice.

Apart from clinical pregnancy and live-birth rate, we also need to take into account certain secondary outcomes like duration of analogue treatment, FSH requirement, duration of FSH stimulation, number of cumulus-oocyte complexes (COCs) retrieved, incidence of premature LH rise, incidence of OHSS, and probably cost.

The other thing one needs to consider is the gonadotrophin used, as the use of GnRH analogues can suppress the LH levels to an extent that may affect the folliculogenesis and in turn the outcome of ART. At present, available evidence suggests that among women with normal ovulation or World Health Organization (WHO) II oligo-anovulation, low endogenous LH levels during ovarian stimulation for IVF using gonadotrophin-releasing hormone (GnRH) analogues are not associated with a decreased probability of ongoing pregnancy beyond 12 weeks [2] However, it cannot be excluded that LH supplementation during the follicular phase might be beneficial for pregnancy achievement, independently of any effect of endogenous LH levels. The type of

downregulation (agonist or antagonist) did not seem to modify the effect of LH addition to FSH.

11.2 Differences Between GnRH Agonist and Antagonist

11.2.1 Endocrinological Profile Differences Between the Two GnRH Analogues

A progesterone rise during the late follicular phase has a negative predictive value for clinical outcome in both GnRH-agonist [3–5] and GnRH-antagonist protocols [6, 7]. This is because high serum progesterone levels on the day of hCG administration induce both advanced endometrial histological maturation [8] and differential endometrial gene expression [9, 10] which may have a negative effect on the implantation failure.

Though a previous meta-analysis failed to demonstrate any relationship between progesterone levels and clinical pregnancy rates [11], data from large prospective randomized studies like the Merit study [12] and a retrospective study of 4,000 cycles [6] consistently support that pregnancy rates are inversely related to progesterone levels on the day of hCG administration, when a threshold of 1.5 ng/ml is adopted.

Papanikolaou et al. [13] published that there was no difference in the incidence of progesterone rise, but in both protocols elevated progesterone results in a significant decrease in pregnancy rates. There was no difference observed in the live-birth rates between the two GnRH analogues (28.1 % with GnRH antagonist versus 24.5 % with GnRH agonist) with or without premature progesterone rise.

11.2.2 Advantages of GnRh Antagonists over GnRH Agonists

1. Prevention of premature LH increase is easier and takes less time. GnRH antagonists' action is within a few hours after their administration [14] and thus they can be administered only when there is a risk for an LH surge. This is in contrast to GnRH agonists where pituitary downregulation occurs only after 7–10 days.
2. GnRH antagonists are not associated with the initial period of stimulation, which increases both FSH and LH, which occurs with GnRH-agonist administration.
3. The initial stimulation by GnRH agonists can induce cyst formation, which is avoided with GnRH antagonists.
4. No hot flushes are observed with GnRH antagonists, as their use does not result in profound hypoestrogenemia observed with GnRH agonists [15].
5. Inadvertent administration of the GnRH analogue in early pregnancy can be avoided as GnRH antagonist is administered in the mid-follicular phase.
6. Requirements for exogenous gonadotrophins are reduced, rendering ovarian stimulation less costly.
7. Duration of ovarian stimulation protocols is therefore shortened, improving patient discomfort.
8. GnRH agonist can be used to trigger final oocyte maturation in GnRH-antagonist cycles [16]. Replacing hCG with GnRH agonist in a high-risk patient has led to a decreased risk of developing ovarian hyperstimulation syndrome (OHSS) [17].

11.2.3 Advantages of GnRH Agonists over GnRH Antagonist

1. GnRH antagonists offer less flexibility regarding cycle programming as compared with the GnRH-agonist long protocol, but not with the short GnRH-agonist protocol.
2. The GnRH-agonist long protocol provides a more synchronous growth of follicles as the cycle is downregulated. Use of GnRH antagonist has been found with asynchronous development of follicles as compared to agonist. This is due to decrease in FSH levels, first after the inter-cycle rise of FSH and second after initiation of antagonist.
3. Most comparative studies report a minor reduction in pregnancy rates per cycle with GnRH antagonists as compared with GnRH agonists.

It was also observed that the number of COCs retrieved was also fewer in a GnRH-antagonist cycle. Though for both these disadvantages, statistical significance was not reached.

4. Use of GnRH-agonist administration instead of hCG for triggering final oocyte maturation induces an LH surge which is not identical to that occurring in the natural cycle and is supposed to be shorter in duration [18, 19]. This results in a deficient luteal phase, despite support with progesterone. This in turn will result in a lower pregnancy rate [20–22]. The use of alternative luteal support schemes would improve the pregnancy outcome. At this point one must remember that if pregnancy occurs, OHSS can still result, and therefore, it is best to cryopreserve all embryos and transfer in a subsequent hormone replacement (HRT) cycle.

11.2.4 Duration of Analogue Treatment

The duration of analogue administration was significantly longer in the agonist group [23].

11.2.5 Use of Exogenous FSH in GnRH-Antagonist and GnRH-Agonist Co-treatment Cycles

The required starting dose of FSH in GnRH-antagonist cycles is lower compared to GnRH agonist, due to the presence of higher endogenous FSH levels during the inter-cycle phase [24]. However, a lower number of cumulus-oocyte complexes (COCs) were retrieved with the use of GnRH antagonists compared with GnRH agonists [1]. A higher starting dose of FSH in an agonist cycle may result in an increased number of COCs retrieved but it does not appear to be associated with higher pregnancy rates [25, 26]. In addition, the increase of gonadotrophin doses at GnRH-antagonist initiation did not appear to result in higher probability of pregnancy [27].

In a GnRH-antagonist cycle, it is possible to start FSH stimulation later in the follicular phase by extending the FSH window for multi-follicular development [28, 29] resulting in milder stimulation. This flexibility of starting FSH later in the follicular phase can also be used in modified natural cycle for IVF, in which the development of a single follicle is supported by addition of exogenous FSH latter in the cycle in combination with GnRH antagonist to control the endogenous LH production [30]. Addition of LH for ovarian stimulation does not increase the probability of pregnancy in either group.

11.2.6 Duration of FSH Stimulation

The duration of stimulation was significantly longer in the GnRH-agonist group.

11.2.7 LH Supplementation

The increased pregnancy loss observed with low LH levels in GnRH-agonist cycles [31] and decreased probability of pregnancy associated with low LH levels, observed using high GnRH-antagonist doses [32], as a result of abrupt suppression of endogenous LH by GnRH antagonist occur in the mid-follicular phase, at a critical stage for follicular development. It was thus assumed that LH supplementation might improve pregnancy outcome in both groups, where one could add LH or increase the dose of LH in the form of rec-LH or rec-hMG.

Kolibianakis et al. and Merviel et al. published that there was no indication that low endogenous LH levels after GnRH-antagonist initiation are associated with a decreased probability of pregnancy in IVF cycles [33, 34].

On the basis of the currently available data, it appears that LH supplementation in ovarian stimulation for IVF using GnRH-antagonist cycles is not necessary but can be used in GnRH-agonist cycles associated with low LH levels on day 2 of the cycle in a GnRH-agonist long protocol.

11.2.8 LH Surge/Rise

The likelihood of LH surges and LH rises was significantly higher with GnRH-antagonist than with GnRH-agonist treatment especially in the flexible GnRH-antagonist protocol.

11.2.9 Criteria for hCG Administration

There is a marked variation in the criteria used for triggering final oocyte maturation in IVF both in GnRH-agonist and GnRH-antagonist cycles [35]. Recent data indicate that the timing of hCG administration might be important for the probability of pregnancy. Prolongation of the follicular phase was shown to be associated with decreased pregnancy rates [35] in GnRH-antagonist cycle.

11.2.10 Cumulus-Oocyte Complex (COCs) Retrieved

Significantly more oocytes were retrieved in the agonist group compared with the GnRH-antagonist group.

11.2.11 Luteal Phase Supplementation

The existing evidence in GnRH-antagonist cycles suggests that luteal supplementation remains mandatory as is the case with GnRH agonists.

11.2.12 OHSS

The incidence of OHSS associated with hospital admission was significantly lower in the antagonist than in the agonist group. The incidence of grade I and II OHSS did not differ significantly between the two GnRH analogues but was in favor of GnRH-antagonist group, in which the incidence of OHSS was lower.

11.3 Comparative Efficacy of Various GnRH-Analogue Protocols in IVF

11.3.1 The Evidence

In the meta-analysis of randomized comparative trials between GnRH analogues, the absolute treatment effect of clinical pregnancy rate on an intention-to-treat basis was 5 % in favor of the GnRH agonists [36]. Later meta-analysis by Al-Inany et al. in 2011 [36] and another meta-analysis [37] did not show any significant difference in the live-birth rates, suggesting that both GnRH analogues result in comparable pregnancy rates.

The differences in reported outcome measurements could be the consequence of the large variation in the regimens of GnRH antagonist. It depends on whether we use the fixed or flexible protocol and when using the fixed protocol on what day we start the GnRH antagonist. Several studies today show a better outcome when antagonist is started as early as day 5 of stimulation.

The two phenomena that play an important role to facilitate optimal IVF results when GnRH analogues are used are:

1. Stable and low LH and progesterone levels throughout the stimulation phase to achieve optimal conditions for implantation.
2. Sustained low levels of endogenous FSH before stimulation are started to allow optimal synchronization of the follicular cohort.

11.3.2 Long Versus Short or Ultrashort GnRH-Agonist Regimen

The long protocol (starting in the midluteal phase of the preceding cycle) gave the best IVF results with regard to oocyte yield and pregnancy rates [38]. It results in profound suppression of endogenous release of gonadotrophins during the early follicular phase, allowing the early antral follicles to grow coordinately in response to exogenous gonadotrophins to accomplish simultaneous maturation. Basically it results in extended widening of the FSH window, increased FSH requirement, and in the end more mature follicles and retrieved oocytes [38].

11.3.3 Fixed Versus Flexible GnRH-Antagonist Regimens

Fixed GnRH-antagonist regimens started the antagonist relatively late in the follicular phase, mostly stimulation day 6. Normally, the luteo-follicular transitory rise of endogenous FSH

starts the stimulation of a cohort of follicles that vary in stage of development as there is a decrease in FSH concentration just before exogenous FSH is started. The start of exogenous FSH allows further development of a few leading large follicles and several smaller follicles [39–44]. Further there is again a small fall in the level of FSH, when the antagonist is started. As the criteria for administration of hCG are based on the size of the leading largest follicles, there are several immature follicles at that time. Though the stimulation period will be shorter with less FSH required, the number of mature oocytes obtained is definitely less compared to GnRH-agonist long protocol [39–42, 45].

Thus, GnRH-antagonist regimens result in less synchronization of the follicular cohort as compared to a long GnRH-agonist cycle with lesser mature and more immature follicles.

Significantly lower ongoing pregnancy rates are seen in patients with elevated progesterone at initiation of stimulation in GnRH-antagonist cycle, which is more common in a flexible protocol when the antagonists are initiated only after the dominant follicle is 14 mm. The high estradiol levels when the antagonist is initiated late may result in premature LH rise with early rise in progesterone levels with luteinization. This may result in early closure of the implantation window [46] through earlier expression of progesterone receptors in the follicular phase and downregulation of estrogen receptors by the exposure to supraphysiological steroid hormone levels [47, 48].

We know that once the endometrium is primed by estradiol, the duration of progesterone exposure is the crucial point leading to a receptive endometrium. Thus, the fixed protocol has better pregnancy rates as compared to the flexible protocol due to better control of hormonal levels (estradiol, LH, and progesterone).

One study indicated that the stability of LH levels rather than absolute LH values is associated with clinical pregnancy, as no pregnancies occurred if the LH and progesterone levels changed too markedly (either increase or decrease) during GnRH-antagonist administration [44].

Thus, fixed protocols where the antagonist is started on day 6 are better than flexible protocols, which allow higher LH, estradiol, and progesterone levels and are associated with lower pregnancy rates [49]. An earlier start (cycle day 4 or 5) of GnRH antagonists is associated with improved pregnancy rates [50]. So if we start GnRH antagonist on day 1 compared with day 6, there will be even further decrease in the exposure to LH and estradiol during the early follicular phase [51] and it would be beneficial in PCOS women. However, the pregnancy rates (52 % per embryo transfer) were not different in this small study. Additionally, this regimen will increase the cost due to the extended period of GnRH-antagonist administration.

11.3.4 Long GnRH-Agonist Versus Flexible GnRH-Antagonist Regimens

The GnRH-agonist long protocol is more favorable compared to flexible start antagonist protocols with respect to the number of dominant follicles on the day of hCG and number of oocytes retrieved [29, 52, 53]. The incidence of asynchronous follicle development through absent suppression of early endogenous FSH secretion is seen only in the antagonist protocol. The low gonadotrophin levels prior to stimulation created by the long agonist protocol are of particularly favorable to IVF/ICSI yield and outcome.

11.3.5 Comparisons of GnRH-Antagonist Versus GnRH-Agonist Protocol in Poor Ovarian Responders Undergoing IVF

Poor ovarian response is defined as reduced follicle/oocyte production (<4) after controlled ovarian hyperstimulation (COH) for IVF

[54, 55]. It may be associated with high cancelation rates, impaired fertilization rates, and lower embryo quality [56]. Therefore, the management of poor responders has been one of the most difficult challenges in ART with overall poor IVF success rates.

Various treatment regimens and interventions have been investigated in an effort to improve ovarian response and IVF outcome. These include the use of high doses of gonadotrophins [57], the change to a "flare-up" protocol with OC pretreatment [58], and the use of growth hormone or growth hormone-releasing factor [59] or aspirin [60] as adjunct therapies. However, most of these interventions have only limited success in poor responders. The availability of GnRH antagonists has offered an alternative protocol for poor responders [61, 62] as GnRH-agonist long protocol may cause over-suppression of endogenous gonadotrophin secretion at the stage of follicular recruitment [61, 63].

Although the results of the GnRH antagonist in COS protocols offer a number of potential advantages [64] compared with the conventional GnRH-agonist long protocol, the efficacy of GnRH antagonist and GnRH agonist in poor-responder IVF patients is still controversial.

As early as 2009 Nelson et al. [65] published that treatment with a GnRH-antagonist protocol reduced the burden of treatment in poor responders compared with a GnRH-agonist protocol [66] but did not influence either the proportion of cases achieving egg collection or pregnancy rates [65]. Later a Cochrane review [67] published in 2010 and a meta-analysis published in 2011 showed that the duration of stimulation was significantly lower in GnRH-antagonist protocols than GnRH-agonist long protocols in poor responder, and no improvements were found in the number of oocytes and mature oocytes retrieved, the cycle cancelation rate (CCR), and the clinical pregnancy rate (CPR) with the use of GnRH antagonist. GnRH antagonist resulted in an LH surge in 9 % of poor responders, which was a cause for concern [65].

In a recent retrospective study [68], patients with extremely low AMH concentrations had a moderate but reasonable chance of pregnancy (7.9 % per cycle started) when treated with a microdose agonist protocol, a daily gonadotrophin dose of 600 IU, and dehydroepiandrosterone supplementation.

11.3.6 Comparisons of GnRH-Antagonist Versus GnRH-Agonist Protocol in Hyperresponders Undergoing IVF

AMH and AFC are a good predictor of excessive ovarian response [69]. Thus, in women with a high AMH concentration and high AFC, an individualized (reduced) dose of FSH and use of GnRH antagonist with GnRH-agonist trigger can improve both safety and pregnancy outcomes.

The antagonist protocol eliminated the need for complete cryopreservation of embryos due to excessive response ($P<0.001$), coupled with significant reductions in the incidence of hospitalizations owing to the development of OHSS (13.9 % in the agonist group versus 0.0 % in the antagonist group; $P=0.02$) [37, 65, 70].

The antagonist protocol, in high responders, was also associated with higher fresh-cycle clinical pregnancy rates (odds ratio 4.40, 95 % confidence interval 1.95–9.93; $P<0.001$), required fewer days of FSH stimulation, and was associated with lower egg yields compared with the agonist protocol [65]; these patients with low egg yields achieved pregnancy rates comparable with those with normal or high egg yields [65, 71]. Patients with AMH serum concentrations >40 pmol/l still remain at risk of developing an excessive response and OHSS despite the use of a "mild" antagonist protocol with hCG trigger.

The use of GnRH-antagonist protocols as part of the AMH- and AFC-tailored treatment strategy may result in improvement of efficacy and safety in high responders.

Individualized COS protocols using the AMH also helped in reducing the cost of treatment as well as the cost involved in the clinical management of OHSS in high responders.

11.3.7 Early Initiation of GnRH-Antagonist (Day 1) Versus GnRH-Agonist in GnRH-agonist long protocol

Initiation of GnRH antagonist on day 1 of stimulation for IVF when compared with the long agonist protocol is associated with a more rapid follicular development [72], an earlier rise in E2 levels, and significantly higher levels of progesterone. This is accompanied by significantly lower LH levels in the early follicular phase and significantly higher LH levels in the late follicular phase in the antagonist group. The exposure to LH, E2, and progesterone in the early follicular phase was higher in the antagonist when compared with the GnRH agonist group but did not reach statistical significance.

11.3.8 GnRH Analogues in Oocyte Donation (OD) Cycles

OD cycles, both the short GnRH agonist and antagonist, appear to be similar in ovarian response and embryo quality and comparable in terms of recipients' pregnancy and implantation rates. The GnRH-antagonist protocol could be the protocol of choice for ovarian stimulation in OD cycles, as the risk of OHSS could be reduced by the triggering of ovulation with a GnRH agonist [73].

11.3.9 Oral Contraceptive Pill Pretreatment in Ovarian Stimulation with GnRH Antagonists and Agonist

The use of OCP has been advocated for programming IVF cycles using GnRH antagonists [74, 75] and improved synchronization of the recruitable cohort of ovarian follicles as against a GnRH-agonist cycle, where it is used to prevent ovulation, which in turn will reduce the cyst formation after initiation of the agonist in a long protocol.

Estrogen or OC pretreatment offers a simple alternative to achieve gonadotrophin suppression during the early follicular phase [76, 77]. Gonadotrophin can be started 2 or 3 days [75, 78–81] after OC withdrawal in either flexible or fixed GnRH-antagonist protocols. OC pretreatment using GnRH antagonists with subsequent starting of FSH 2 or 3 days after the last OC intake is associated with deep suppression of LH and FSH levels and improved synchronization of the follicular cohort development compared with GnRH-antagonist-only protocols [80, 81]. Similarly, improvement of the synchronization of the follicular cohort was observed only if stimulation was started 3 days after estradiol pretreatment in GnRH-antagonist protocols in a general population [82] and in poor responders with optimal pregnancy rates [83]. This effect is not seen when FSH stimulation was started on day 5 after the last OCP [84, 85]. Apparently, timing the start of exogenous gonadotrophin administration after OCP pretreatment affects follicular development [86].

But it was also observed that pretreatment with OCP has been associated with a longer duration of treatment [87] and increased gonadotrophin requirement [88]. No significant effect of OCP pretreatment was noted on the probability of pregnancy in GnRH-antagonist cycles which was shown in a large RCT [85], suggesting that programming of IVF cycles with the use of OCP is feasible.

Some studies have shown a lower implantation rates after OC pretreatment [80, 81] or increased pregnancy loss compared with GnRH-antagonist-only regimens [85]. Similar luteal endometrial development was found in OC-pretreated flexible GnRH-antagonist protocol [8] or fixed day 6 antagonist protocol [89] in comparison to a GnRH-agonist long protocol or a short GnRH-agonist protocol [90].

11.4 GnRH Analogues in Ovarian Stimulation for IUI

LH surge is an absolute requirement for luteinization, final maturation of the oocyte, and follicle rupture. Premature LH surge occurs in 25–30 % of stimulated IUI cycles and may interfere with timing of IUI or result in cancelation of IUI cycle and more treatment failures with IUI [91, 92].

So we need to see whether use of GnRH agonist or antagonist in IUI cycles is cost effective and helps in improving the outcome.

Moreover when IUI is done with gonadotrophins, the response may vary, ranging from no response to hyperresponse (more than four follicles of >12 mm developed). Among hyperresponders, where follicular recruitment is excessive, a decision must be made to either cancel the cycle or allow the multiple follicles to mature and thus risk the incidence of multiple pregnancy and OHSS or convert it into an IVF cycle.

Here is then the role of GnRH analogues, and GnRH antagonists have the advantage over GnRH agonist as they could be added later in the cycle.

11.4.1 GnRH Agonists in Ovarian Stimulation for IUI

There seems to be no role for GnRH agonists in IUI programs as they increase cost as the dose of gonadotrophins is increased tremendously. Its use also increases the incidence of multiple pregnancies without increasing the probability of conception. Thus, the use of GnRH agonists with gonadotrophins should be carefully considered in an intrauterine insemination program [93].

11.4.2 GnRH Antagonists in Ovarian Stimulation for IUI

When GnRH antagonists are used for ovarian stimulation in combination with IUI [94–96] (Ragni et al. 2001, 2004; Gomez-Palomares et al. 2005), there may be a small increase in probability of pregnancy and the number needed to treat is 20. In addition, they may be helpful in cycle programming and avoidance of inseminations during weekends.

Conversion of high-response gonadotrophin-IUI cycles to "rescue" IVF using a GnRH antagonist is a cost-effective strategy that produces better results than regular IVF with relatively minimal morbidity and shorter duration to achieve pregnancy. Implantation and ongoing clinical pregnancy rates tend to be higher than those from hyperresponder regular IVF patients.

Whether or not GnRH antagonists are going to play a role in mild ovarian hyperstimulation/IUI programs needs to be determined in future trials [93].

The GnRH antagonist resulted in more monofollicular development, less premature luteinization, and less cycle cancelation in IUI cycles of patients with PCOS; however, the cost of stimulation increased without an improvement in pregnancy rates [97].

Patients with a previous canceled cycle because of premature luteinization are candidates for this treatment.

11.5 Discussion

Ovarian stimulation is applied to restore monoovulatory cycles in anovulatory women (ovulation induction) or to induce the development of multiple dominant follicles for ART. Ovarian response is the endocrine and follicular reaction of the ovaries to stimulation. Achieving an appropriate ovarian response to exogenous gonadotrophins without much variation in hormonal milieu and preventing complications is most important during COS. To achieve adequate response without cycle cancelation and adverse effects, it is important to predict the patient's ovarian response to medication and to individualize the starting dose and type of exogenous gonadotrophin and select the correct GnRH analogue.

Antimüllerian hormone (AMH) and antral follicle count (AFC) can predict response to COS and identify women who are at risk either for

OHSS or poor response. Thus, AMH and AFC have the potential to determine the optimal treatment protocol for an individual undergoing ART. This knowledge could be used to address safety and efficacy issues associated with COS by varying the type of GnRH analogue used or the type and daily dose of gonadotrophin.

In women who are at risk of developing OHSS, one can adjust the stimulation strategy to incorporate GnRH antagonists [98] and can also completely eliminate the possibility of OHSS by adopting a GnRH-agonist trigger before oocyte retrieval [99]. This unique approach has tremendous benefits in women undergoing altruistic oocyte donation, eliminating completely the risk of IVF [73, 100]. For anticipated normal responders, one could continue to use GnRH-agonist protocols, due to higher ongoing pregnancy (28 randomized, controlled trials: odds ratio [OR] 0.87; 95 % confidence interval [CI], 0.77–1.00) and live births (9 randomized, controlled trials: OR 0.86; 95 % CI, 0.69–1.08) favoring agonist-based rather than antagonist-based strategies [98]. In potential poor responders, currently the use of flare strategy, because of its reduced treatment burden and ability to capitalize on endogenous luteinizing hormone (LH) activity, makes GnRH-agonist short protocol the treatment of choice. This is in accordance with recent studies supporting a beneficial role of LH in older women [101]. Today it is a great challenge to determine the optimal protocol in poor responders and to improve clinical outcomes, while minimizing treatment burden would be the ultimate goal of future prospective research.

The probability of live birth is not dependent on the type of GnRH analogue (GnRH agonists and GnRH antagonists) used for suppression of premature LH rise/surge. A significantly higher incidence of premature LH rise/surge in GnRH-antagonist cycles may be seen in a GnRH-antagonist flexible protocol, where the analogue is started only after the dominant follicle reaches 14 mm. Here if there are many developing follicles, there could be high estradiol levels resulting in rise of LH levels. Moreover, the timing of LH assessment in relation to antagonist administration is also very important. Ideally, antagonist administration should occur immediately after blood is collected for hormonal analysis [102].

It is important for us to remember that the endocrine environment in cycles which are down-regulated with GnRH agonist is more controlled than cycles controlled by GnRH antagonists, and all follicular growth is dictated only by the exogenous gonadotrophins.

The application of GnRH antagonist in ovarian stimulation for IVF was associated with a significantly lower probability of OHSS associated with hospital admission.

Conclusion

The achievement of a simple, safe, and cost-effective treatment protocol in controlled ovarian hyperstimulation (COH) is of paramount importance to improve the quality of care in assisted reproduction. Both GnRH-agonist and GnRH-antagonist co-treatment during ovarian hyperstimulation for IVF are effective in preventing an undesirable premature rise in serum LH. When using GnRH antagonist, the daily low-dose protocol should be preferred over a single high-dose regimen. GnRH antagonist could produce a more physiological follicular selection than the long luteal GnRH-agonist protocol, recruiting a smaller number of follicles and thus reducing OHSS risk.

Initial publications suggested that OCP pretreatment in GnRH-antagonist cycles reduced the pregnancy rates, but the clinical evidence generated recently suggests that OCP pretreatment can be used for planning IVF cycles.

In patients treated with FSH and GnRH analogues for IVF, the addition of rLH does not increase live-birth rate or have any beneficial effect on secondary outcome variables. So addition of LH from initiation of stimulation or from antagonist administration does not appear to be necessary. There is also no need to increase the starting dose of gonadotrophins or to increase gonadotrophin dose at antagonist initiation.

Progesterone elevation (PE) on the day of hCG administration is associated with a decreased probability of pregnancy in fresh IVF cycles in women undergoing ovarian stimulation using GnRH analogues and gonadotrophins. On the other hand, a negative association between PE on the day of hCG administration in the fresh cycle and the probability of preg-

nancy after transfer of frozen-thawed embryos originating from that cycle does not seem to be present. GnRH-antagonist initiation on day 6 of stimulation appears to be superior to flexible initiation by a follicle of 14–16 mm, and probably initiation of GnRH antagonist earlier in the cycle if the estradiol levels are more than 200 pg/ml on day 4 of COS may prevent early rise of progesterone and therefore improve the pregnancy rates.

Today the evidence suggests that the choice of GnRH analogue for inhibiting the premature LH surge does not alter significantly the probability of live birth. But the OHSS rate in women receiving the antagonist is significantly lower compared with the agonist protocols as hCG can be replaced by GnRH agonist for triggering final oocyte maturation. This may be associated with lower probability of pregnancy if a fresh transfer is done not using the modified luteal phase support protocol where hCG is given in the dose of 1,500 IU on the day of oocyte retrieval. The pregnancy rates remain the same if all embryos are frozen and transferred in the subsequent cycle. GnRH-antagonist protocol may be used for patients at high risk of developing OHSS to make the clinic an OHSS-free one.

Luteal phase supplementation is required following both GnRH-agonist and GnRH-antagonist co-treatment protocols with gonadotrophins.

GnRH antagonists may have a role in ovarian stimulation for IUI as well as their application in mild stimulation protocols for IVF. Use of GnRH agonist does not improve the outcome in IUI cycles.

Today with the availability of new markers of ovarian reserve, the improvement in methodology for their measurement allows a scientific estimate of the pool of follicles that potentially respond to ovarian stimulation. This then has

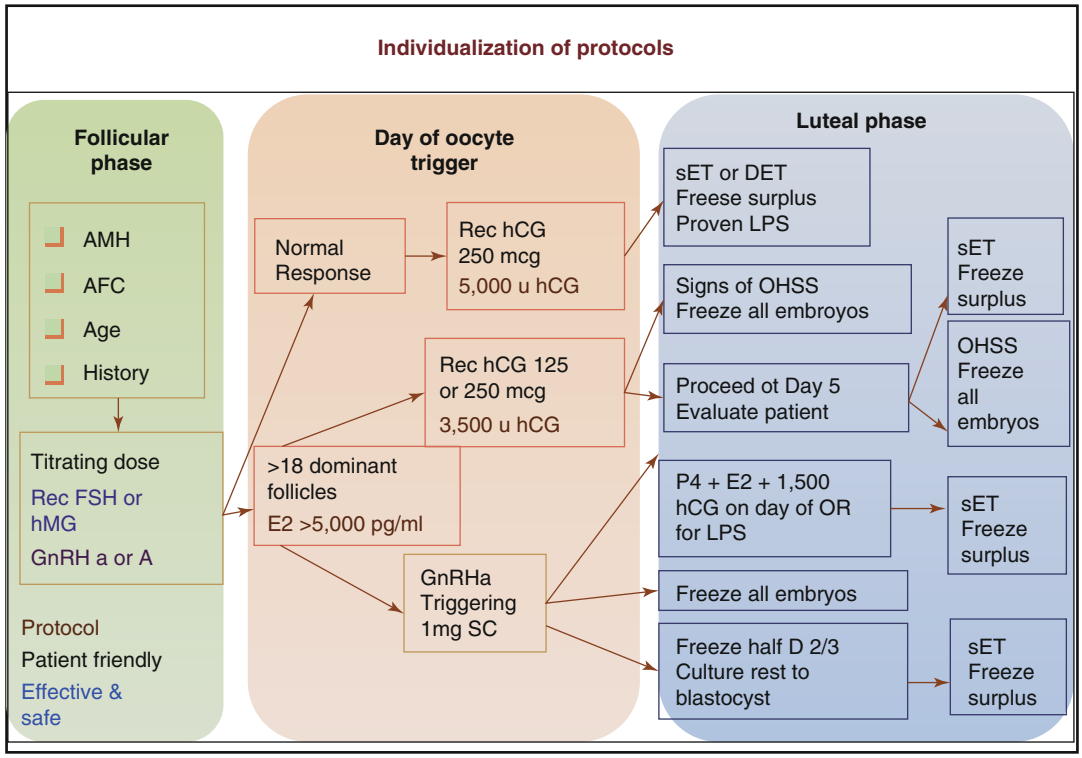

Fig. 11.1 Key points in selection of stimulation protocols to improve results in IVF. *AFC* antral follicle count, *FSH* follicle stimulating hormone, *hMG* human menopausal gonadotropin, *hCG* human chorionic gonadotropin, *SC* subcutaneous, *LPS* luteal phase support, *sET* single embryo transfer, *OHSS* ovarian hyperstimulation syndrome, *DET* double embryo transfer, *AMH* anti mullerian hormone

supported the use of individualization of COS in ART cycles (Fig. 11.1). Today most protocols are selected on values of AMH and AFC. This protocol enables the correct selection of the different GnRH analogues and the gonadotrophin dose. The benefits of a personalized therapy are reduction in the risk of poor response or hyperresponse thus reducing the incidence of cancelation of the cycle and at the same time optimizing the outcome of ART.

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Mild Stimulation Protocol

12

Nalini Mahajan and Shivani Singh

Abstract

More than three decades of COH for in vitro fertilization have exposed us to its various shortcomings like higher incidence of chromosomal abnormalities, high cost, low compliance and limited results in POR, and side effects like OHSS and higher-order multiple pregnancies. In order to overcome these problems associated with conventional stimulation protocols, ISMAAR has proposed *mild stimulation* whose aim is to limit the number of oocytes obtained to less than eight by the administration of low doses or fewer days of exogenous gonadotrophins in GnRH antagonist co-treated cycles or use of oral compounds (like antiestrogens or aromatase inhibitors) with or without gonadotrophins with antagonist co-treatment for ovarian stimulation. Many have proposed natural and modified natural cycle IVF, especially for women with poor ovarian reserves.

Mild stimulation entails decreased dose of gonadotrophins, decreased days of injections, reduced chances of OHSS, equivalent CPR, decreased aneuploidy rate, better endometrial receptivity, lower rate of HOMP, and overall lower per cycle cost because of lower drug cost and delivery of singletons. On the other hand, there are distinct disadvantages: a decreased number of oocytes are recovered, no or few embryos are available for cryopreservation, and ultimately cost, physical, and psychological burden may go up because of repeat fresh cycles.

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Mild IVF • Conventional IVF • Natural cycle IVF • Modified natural cycle IVF • ISMAAR

12.1 Introduction

The advent of IVF saw oocyte retrieval from a single follicle in a natural cycle. The disadvantages of having only one oocyte to work with lead to the introduction of ovarian stimulation (OS) for IVF. More oocytes meant more embryos which offered the possibility of embryo selection; this in turn helped to improve pregnancy rates – ART had finally taken a step forward.

Three seminal events changed the course of IVF: introduction of gonadotrophins which increased oocyte yield, GnRH agonist to prevent the premature LH surge, and availability of cryopreservation to freeze supernumerary embryos. Availability of cryopreservation initiated a trend to maximize the number of oocytes through hyperstimulation of the ovaries. Unfortunately this leads to the ovarian hyperstimulation syndrome (OHSS) which increased patient morbidity and mortality. Cycle programming to ease out the work schedule of physicians and embryologists added to the physical burden of treatment. Contraceptive pills given in the previous cycle and agonist injections continued till timing are convenient for the clinic, lead to increased requirement of gonadotrophins, and probably compromise the reproductive performance [1].

Today the pendulum has started swinging back. Problems associated with OHSS, complex and expensive protocols, weeks of daily injections, and the resultant high drop-out rate forced physicians to rethink their stand on OS protocols. Additionally improved laboratory conditions and culture media have reduced the need for a large number of oocytes. Edwards et al. in 1996 were the first to advocate milder stimulation for IVF [2]. Advent of GnRH antagonist paved the way for development of more patient-friendly protocols which involved mild stimulation. The aim of mild protocols is to reduced treatment burden without compromising the pregnancy rate.

Introduction of a new concept does not gain immediate acceptance – there always are proponents and opponents. This article aims to examine the pros and cons of the process. The decision to adopt either rests with the treating physician based on the evidence presented.

12.2 Definition

Over the years there have been no well-defined criteria to define mild stimulation, in fact even the terminology has been varied with terms such as soft, gentle, minimal, mild stimulation being used when a deviation was made from the standard stimulation protocol. This leads to the formation of "ISMAAR" (International Society for Mild Approaches in Assisted Reproduction), which aimed to clearly define the various non-conventional stimulation protocols [3] (Table 12.1).

Mild IVF stimulation is defined by ISMAAR as the administration of:

1. Low doses or fewer days of exogenous gonadotrophins in GnRH antagonist co-treated cycles
2. The use of oral compounds (like antiestrogens or aromatase inhibitors) with or without gonadotrophins with antagonist co-treatment for ovarian stimulation

The aim of mild stimulation is to limit the number of oocytes obtained to less than eight.

12.3 Mild Stimulation Protocol

The concept of "FSH Threshold" and "FSH Window" forms the basis of all our stimulation protocols. FSH threshold being the level of FSH required to initiate follicular growth and FSH window is the time frame for which this FSH level

Table 12.1 ISMAAR terminology with protocols

Recommended	To replace	Aim	Protocol
Natural cycle IVF	Unstimulated IVF Spontaneous cycle IVF	Single oocyte	No medication
Modified natural cycle IVF	Seminatural IVF Controlled Natural IVF	Single oocyte	hCG only GnRH antagonist and FSH/HMG add back
Mild IVF	Soft stimulation IVF Minimal stimulation IVF Friendly IVF	2–7 oocytes	Low dose FSH/HMG, oral compounds and GnRH antagonist
Conventional IVF	Standard IVF Routine IVF Controlled ovarian stimulation IVF	≥8 oocytes	GnRH agonist or antagonist along with conventional FSH/HMG dose

From Nargunde et al. [3]

plateau's to obtain cohort recruitment. Drop in FSH levels following rise in estradiol level is responsible for selection of the dominant follicle. It follows then that the wider the FSH window, the more the follicles recruited [4]. Thus, in conventional IVF, multifollicular recruitment is achieved by administering a high dose of gonadotrophins for a longer duration; thus keeping the FSH window open for a longer time. In mild IVF a moderate elevation is sought since the aim is to have a small cohort of follicles; hence, the gonadotrophin dose is reduced. In the conventional protocol, follicular recruitment is totally dependent on exogenous FSH since the pituitary is downregulated. In the mild stimulation protocol, on the other hand, initial recruitment is by the endogenous FSH rise in the late luteal phase. Exogenous FSH added subsequently (cycle days (CD) 2–5) prevents decrease of FSH levels inducing multifollicular development by preventing follicular dominance [5].

12.3.1 Drugs Used for Ovarian Stimulation (OS) in "Mild Stimulation Protocol"

Both oral and injectable ovarian-stimulating agents can be used since the pituitary is not downregulated. The drugs used are:

1. Gonadotrophins – rFSH, uFSH, and uHMG
2. Antiestrogens – clomiphene citrate and tamoxifen
3. Aromataze inhibitor – letrozole

12.3.1.1 Gonadotrophins

Gonadotrophins with or without oral ovulogens still remain the mainstay of OS. The choice between recombinant and urinary drugs is a question of availability and economics since it has been shown unequivocally that there is no difference in outcome [6].

Dose of Gonadotrophin

The dose of gonadotrophin is kept at 75–150 IU starting from days 2–5 of the cycle. A fixed daily dose of 150 IU rFSH compared with 100 IU/day was found to be more effective in inducing multifollicular growth when ovarian stimulation was started on CD5 [7]. The starting dose of gonadotrophin in the conventional protocol varies between 225 and 300 IU though it may be lower in PCOS patients.

Day of Starting Stimulation

Gonadotrophin administration can be initiated from cycle days (CD) 2–5 in the mild protocol, while it starts from CD2 in the conventional protocol. A cancellation rate of almost 15–20 % is observed when starting from CD5 because of mono- or bifollicular response. Starting on CD2 allows for more follicles to be recruited. De Jong et al. suggested that ovarian stimulation could be initiated as late as CD7 [7]; however, the number of women showing multifollicular development with this protocol was lower than with those starting stimulation on CD2–5 [8], and it never became popular.

12.3.1.2 Clomiphene Citrate (CC)

CC is an antiestrogen and has been used very successfully in ovulation induction for many decades. Trounson et al. were the first to use CC for OS in IVF in 1981 [9]. Once gonadotrophins were introduced they replaced CC as they were far more effective in getting a multifollicular response. Introduction of GnRH agonist for pituitary downregulation in IVF protocols spelt a death knell for CC since CC needs an intact hypothalamo-pituitary-ovarian axis for its action. The reintroduction of this antiestrogen came about with the use of antagonist in IVF. Addition of CC reduces the dose of gonadotrophin required for stimulation [10], thereby reducing the cost of the IVF cycle. The dose used is 50–100 mg for 5 days from cycle day 2 along with 150 IU of gonadotrophin. The dose of CC has not been standardized. CC can be used by itself as well; however, the number of follicles recruited is lower, and its antiestrogenic effects can be detrimental to implantation.

12.3.1.3 Aromatase Inhibitors (AI)

AIs inhibit the aromatization of androgens to estrogens thereby providing a negative feedback to the pituitary. In addition, the increased intraovarian androgens increase the sensitivity of the antral follicles to FSH [11] and may increase the number of pre-antral and antral follicles [12]. Advantage over clomiphene is thus twofold – no antiestrogenic effect on the endometrium and no depletion of E2 receptors [13] and an improvement of antral follicle sensitivity to FSH thus improving recruitment. The dose varies from 2.5 to 5 mg daily for 5 days starting from cycle day 2 and is administered orally.

AIs have been used with gonadotrophins extensively in poor responders and patients requiring fertility preservation as it keeps the E2 levels low. At present letrozole is an off-label drug, and its use as an ovulation induction agent is banned in India due to concerns about teratogenicity.

12.3.2 Preventing the Premature LH Surge

GnRH analogs are used in IVF to prevent the premature LH surge. The agonist has been in use for more than 20 years, and after many years of experience, it has been established that the long downregulation regime gives the best results in IVF. The antagonist was introduced in 2000 and after some initial hiccups is slowly gaining ground.

12.3.2.1 GnRH Antagonist

GnRH antagonists prevent the premature LH rise by competitive binding to the pituitary GnRH receptor. This leads to an immediate suppression of gonadotrophin secretion. Unlike the GnRH agonists, they do not cause an initial flare of FSH and LH, and there is rapid recovery of pituitary action once the effect wears off in 24 h.

GnRH antagonist is typically started as a daily injection of 0.25 mg administered s/c, in a fixed protocol from CD5/6 or a flexible protocol when the follicle size is between 12 and 14 mm and E2 >200 pgm/ml/. This allows the use of endogenous FSH action for initial follicular growth and helps to reduce the dose of gonadotrophins. It is also given as a single dose of 3 mg s/c, but this is not available in India.

The advent of GnRH antagonists with its rapid and reversible action brought to fore a surge of protocols using oral and a combination of oral and injectable ovarian-stimulating agents. These protocols helped to reduce the physical and financial burden of ART treatment. The introduction of antagonist protocols was met with a lot of skepticism since they were reported to give lower pregnancy rates [14]. The ease of administration and reduced medication used in patient especially one's with poor ovarian reserve overrode these concerns, and as experience grew with the drug, claims of lower pregnancy rates were nullified [15]. Antagonist protocols using gonadotrophin only and a combination of clomiphene citrate/letrozole with gonadotrophins are popular for mild stimulation IVF.

12.3.2.2 GnRH Agonist

Long downregulation protocol involves starting GnRH agonist in the luteal phase of the previous cycle. This protocol is the most favored IVF protocols. Deep suppression of the pituitary necessitates the use of heavy doses of gonadotrophins for ovarian stimulation. So mild stimulation protocols cannot be used effectively with agonist suppression. GnRH acts by receptor depletion,

and hence there is an initial gonadotrophin flare from the pituitary. Protocols using this action of the agonist are called "agonist flare protocols."

Two major problems associated with agonist suppression are the need for higher doses of gonadotrophin with a consequent increase in the chances of hyperstimulation and almost 21 days of agonist injection.

12.3.3 Implications of Mild Stimulation IVF

Acceptance of any IVF protocol is intimately connected to the pregnancy rate and live birth rate achieved. This, in turn, would depend on the oocyte and embryo quality and alterations in endometrial receptivity. Physical and emotional burdens of a regime also play an important role.

Van der Gaast et al. have shown that the ideal number of oocytes after a conventional long protocol is 13 [16]. When the number is lower or higher, the pregnancy rate is compromised. In this context, aiming for a lower number of oocytes would seem both contradictory and counterproductive. The reduction in complications reduced physical and emotional burden, and the reasonable pregnancy rates achieved with mild stimulation have obligated physicians to consider this approach to improve patient experience. Reduced oocyte numbers obtained through mild stimulation appear to differ from reduced numbers obtained in the conventional regime. It appears that poor oocyte yield after classical ovarian stimulation probably reflects a poor ovarian response to FSH and that is associated with poor IVF outcome. However, low number of oocytes after mild stimulation probably represents a "quality selection," i.e. stimulation of only the most mature follicles which result in high-quality embryos and in a pregnancy.

12.3.4 Comparison of Pregnancy Rates

Studies have compared the success rate of mild vs. standard ovarian stimulation in women with normal and poor ovarian reserve. In fact the advantage of mild stimulation was first recognized in poor responders.

12.3.4.1 PR in Women with Normal Ovarian Reserve

Three RCTs compared mild with the classical stimulation regimen. Pooled data showed an ongoing pregnancy rate per started cycle of 15 % in the mild group and 29 % in the conventional group showing that mild stimulation is not as effective as the conventional strategy [17]. Freeze-thaw cycles were not included in these studies. Inclusion of freeze-thaw cycles would improve the CPR (cumulative pregnancy rate) in the conventional group, as cycles with mild stimulation may not generate supernumerary embryos.

Of the three RCTs, the first by Hohmann et al. [18] included 142 normal responders who were divided into three groups: group A, long downregulation protocol, and groups B and C, antagonist protocol. In group B stimulation was started on CD2, and in group C it was started on CD5. Gonadotrophin dose was 150 IU. There were no differences in PR between the three groups though women in group C had a higher cancellation rate because of insufficient response.

Baart et al. [19] compared mild protocol with the conventional long downregulation protocol in 111patients. A dose of 150 IU of rFSH was started from CD5 in the mild group and 225 IU in the long protocol. The ongoing pregnancy rate per started cycle was 21 % in the "mild" group and 18 % in the control group, which was not statistically significant. PGS was performed on these embryos, and there were fewer numbers of aneuploid embryos in the mild stimulation group.

The largest RCT by Heijnen et al. [20] included 404 women who had approximately 800 cycles. In this study, the group with mild stimulation had a selected single embryo transfer, while the conventional group had two embryos transferred. The number of oocytes retrieved was lower, and the pregnancy rate per cycle was significantly lower in the "mild" stimulation group (17.6 % vs. 28.6 %, $p<0.0001$). The patients however tolerated this protocol better, and the rate of discontinuation of treatment was lower. The cumulative live birth rate after 1 year of IVF

treatments was comparable in the two groups (43.4 % with mild protocol, 44.7 % with the conventional regimen); the twinning rate was also significantly lower in the "mild" stimulation-SSET transfer group (0.5 % vs. 13.1 %, $p<0.0001$). According to the authors, a reduced chance of birth per cycle in the "mild" regimen might be compensated by the increased number of IVF attempts in a fixed time.

Ovarian aging, ovarian reserve, and high BMI predict the risk of insufficient response to "mild" stimulation, and a predictive model has been developed in order to minimize the need of cancelling [21].

12.3.4.2 Women with Poor Ovarian Reserve

OS of women with poor ovarian reserve is beset with problems and frustration. Despite high doses of gonadotrophins, oocyte yield remains poor, and cancellations are high. It has been the trend to use doses as high as 600 IU to achieve good follicular recruitment. Unfortunately such strategies have not proven very useful [22] primarily because you cannot force out of a bank what it does not have. The poor pregnancy rates cannot justify the greatly increased cost of medicine; hence, there has been a shift toward mild stimulation.

Land et al. [23] observed that the IVF outcome of patients given a starting dose of 225 FSH UI/day vs. those receiving 450 UI/day was similar, even though more oocytes were obtained with the higher dose. High gonadotrophin dosage may prevent cycle cancellation but provides no advantage in terms of pregnancy rate, live birth rate, or miscarriage rate. It is believed that high doses of FSH recruit "resistant" follicles rescuing them from atresia, but the oocytes that they host are of poor quality and usually do not result in the generation of good quality embryos [24].

CC/Gonadotrophin/Antagonist Regimes

Reduce the cost and physical burden of treatment. In most studies, gonadotrophins 150/225 IU are combined with CC in a dose of 50–100 mg/day for 5 days from cycle day 2, during the early follicular phase. Unfortunately there is a high rate of heterogeneity in studies.

Two randomized trials that compared CC/HMG antagonist protocol to conventional agonist protocol came up with contradictory results. In the study by Dhont et al. [25], there was a significantly higher cycle cancellation rates and lower pregnancy rates per cycle ($p=0.002$). The study by Lin et al. [26] concluded that PRs were similar in the two protocols, gonadotrophin used and number of stimulation days, and a number of oocytes retrieved were lower in the CC group. A similar outcome was achieved by other authors in retrospective studies.

Aromatase Inhibitors

Aromatase inhibitors are administered orally and help to reduce the cost of treatment by reducing the requirement of gonadotrophins, especially in patients with poor ovarian reserve. Grabia et al. [27] observed a PR of 27 % in good prognosis patients. Most studies have used letrozole with the standard dose of gonadotrophins in antagonist protocols. Verpoest et al. [28] randomized 20 good prognosis patients for the use of 150 IU rFSH from CD2 with or without the addition of 2.5 mg letrozole. GnRH antagonist co-treatment was started from CD6. The use of aromatase inhibitors resulted in higher numbers of oocytes and a tendency toward higher clinical pregnancy rates per started cycle in the letrozole group.

In conclusion oral ovulogens in combination with gonadotrophins have a place in cost-effective mild ovarian stimulation treatments especially in poor responders. More RCTs however are needed to assess the true benefit of these protocols.

12.3.5 Comparison of Embryo Quality

High estradiol levels have a negative impact on the developmental and implantation potential of embryos [29]. An increase in aneuploid embryos has also been reported [30]. It has been hypothesized that ovarian stimulation might disrupt mechanisms involved in maintaining accurate chromosome segregation [31]. Baart et al. [19] found a higher number of aneuploid embryos in the conventional protocol

suggesting that more oocytes do not necessarily mean more good quality or more chromosomally normal oocytes. These findings imply that mild stimulation selects less oocytes but with a better quality that lead to the production of euploid embryos.

12.3.6 Comparison of Endometrial Receptivity

Supraphysiological levels of estradiol negatively impact endometrial receptivity [32] and are responsible for implantation failure. This point has been amply proved by the higher pregnancy rates in oocyte donation cycles where the endometrium is not subject to high steroids. Global gene profiling of the endometrium has revealed that there are alterations in the endometrial gene profiles during the phase of receptivity, in patients who have undergone stimulation [33]. The comparisons of gene expression from the same patients between natural and stimulated cycles revealed that endometrial profiles showed moderately altered receptivity in most cases (86 %) and a strongly altered receptivity in14 % during COS [34]. Mild stimulation protocols aim at a more physiological response and hence would improve implantation rates [35]. Between agonist and antagonist, the endometrial gene expression pattern is closer to the natural cycle in the GnRH antagonists protocols [36].

12.3.7 Psychological Aspects

Couples faced with infertility are under immense emotional stress, which is compounded by the stress-related to treatment. Patients are on an emotional roller coaster oscillating between hope, anxiety, and bitter disappointment cycle after cycle. With respect to treatment failure, patients have symptoms of depression, anger, and guilt; psychological stress is the most important reason for patients to discontinue treatment [37].

Mild stimulation protocols have fewer symptoms of depression after IVF failure, the drop-out rate is lower, and patients go for repeat cycles earlier, thus improving their CPR [38–40]. However, lower per cycle pregnancy rates and repeated IVF attempts by themselves would increase stress. Devroy et al. [41] failed to observe a difference in anxiety levels or depression between patients in the mild and conventional protocol. So far there is inconclusive evidence to confirm a psychological benefit with mild protocols.

12.3.8 Cost Comparison

Cost per cycle is lower in the mild stimulation protocol, but since more fresh attempts are required to achieve pregnancy, the cost evens out. Cost of antagonist is still much higher than agonist. There is however an overall reduced cost till delivery because of the reduction in multiple pregnancies [42]. Low cycle cost may provide accessibility to patients in the lower socioeconomic strata giving them an opportunity to have at least one cycle.

12.3.9 Physical Burden

Reduction in the days and number of injections, reduced visits for monitoring, reduced blood tests, and finally a reduction in OHSS [20] dramatically alleviate the physical burden of treatment in mild protocols. Long-term health risks related to excessive ovarian stimulation need to be kept in mind though so far studies on this front have been reassuring.

Listed below are the advantages and disadvantages of "mild stimulation IVF."

12.3.10 Advantages

1. Decreased dose of gonadotrophins
2. Decreased days of injections.
3. Decreased chances of OHSS
4. No difference in CPR
5. Decreased aneuploidy rate
6. Decreased alteration of endometrial receptivity

7. Lower rate of twins
8. Lower per cycle cost because of lower drug cost and delivery of singletons

12.3.11 Disadvantages

1. Decreased number of oocytes recovered – 35 % reduction.
2. No or few embryos available for cryopreservation. An overall increase in PR of 10–15 % with availability of frozen embryos for transfer.
3. Ultimately cost and physical burden may go up because of repeat fresh cycles.
4. No decrease in emotional burden.
5. Optimization of OS protocols still awaited.

12.4 Natural Cycle and Modified Natural Cycle IVF

12.4.1 Natural Cycle IVF

Natural cycle IVF consists of simply monitoring the spontaneous cycle and retrieving a single oocyte post the spontaneous LH surge. Natural cycle IVF is more patient friendly in terms of requirement of no or far less hormonal medication, but needs more intense cycle monitoring and LH surge monitoring on the part of treating physician and round the clock working embryology laboratory. The per cycle costs of natural cycle IVF have been calculated to be 20–25 % of those of stimulated IVF [43]. Ongoing pregnancy rates per started natural cycle IVF have been reported to be dismal 7.2 % only.

12.4.2 Modified Natural Cycle (MNC-IVF)

To improve outcomes while preserving the advantages of natural cycle IVF, modifications have been made. In the "*modified*" *natural cycle* (*MNC-IVF*), rather than waiting for the spontaneous LH surge, inj. Hcg is given once the diameter of the leading follicle reaches 17–18 mm. To prevent the occurrence of a premature LH rise, which is seen in as many as 20 % of cycles, some protocols in MNC-IVF use GnRH antagonist during the late follicular phase. The ongoing growth of the dominant follicle is supported by the addition of exogenous gonadotrophins (referred to as "add back"). In most studies, GnRH antagonist and gonadotrophins (75–150 IU/day) are initiated at a follicle diameter of 12–17 mm.

Promoters of natural cycle IVF offer it as a series of treatment cycles, for it is safer, less stressful compared with conventional stimulation. It has been postulated that after four cycles of natural cycle IVF, the cumulative pregnancy rates are as high as 46 % with an associated live birth rate of 32 % in selected groups of patients [44]. Even though four cycles of natural cycle IVF were found to be comparable to a single cycle of conventional IVF in terms of pregnancy rates and cost effectiveness, the added investment of time and increased number of oocyte retrieval procedures also should be taken into account.

Most studies regarding modified natural cycle IVF include patients with a previous poor response to conventional ovarian stimulation. In this population, success rates between 0 and 14 % per started cycle have been reported in non-randomized studies [45–47]. One large cohort study analyzed the cumulative pregnancy rate after three modified natural IVF cycles in good prognosis patients [48]. The ongoing pregnancy rate per cycle was 8.3 and 20.8 % after up to three cycles.

Conclusion

IVF is an ever evolving technology. There has been a sea change in technique both in the clinic and the laboratory and an improvement in drug quality and mode of administration. Despite these changes, there is still an immense physical and emotional burden attached to treatment. The treatment involves daily injections, frequent ultrasounds and blood tests, and anesthesia general or local, for oocyte retrieval. Among the complications,

the most terrifying one is ovarian hyperstimulation syndrome which can be life threatening.

Mild stimulation protocols resulted from a desire to make the procedure more safe and simple. The fact that there has been an immense improvement in the IVF laboratory gave courage to the physician to aim for less eggs reducing the dose of gonadotrophins required and consequently the cost and complications. Unfortunately the change was not universally accepted because the per cycle pregnancy rates are lower, and the cumulative pregnancy rate though projected to be similar takes many more cycles of stimulation since there are less embryos available for cryopreservation. The cost too though low per cycle ultimately levels out.

The social scenario is also changing with more and more older women coming for IVF. Studies comparing the two protocols specifically in women over 38 years are not available, and more are required even in the younger age group. The contention that the emotional distress is lower has also been challenged. The decision to use either protocol has to be based on physician discretion and patient acceptance after full information of the pros and cons; the future may well be different.

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Individualizing Ovarian Stimulation Protocols in ART

13

Pratap Kumar

Abstract

The ideal ovarian stimulation regimen for IVF should have a low cancelation rate, minimize drug costs, have low risks and side effects, require limited monitoring for practical convenience, and maximize singleton pregnancy rates. Individualization starts from an assessment before the start of IVF cycle of the ovarian reserve by antral follicle count (AFC), antimullerian hormone (AMH), FSH, and age of the patient. AFC and AMH are the most sensitive markers of ovarian reserve identified to date and should be used to plan individualized treatment. Once the patient is categorized as a hypo-/hyper- or normoresponder the dose of gonadotropin is decided. The selection of dose is of paramount importance for optimal outcome of controlled ovarian stimulation (COS). In case of poor responders and hyper-responders, GnRH antagonist regimes are preferred. This helps in explaining the prognosis and in appropriate counseling and also ensures a safe controlled ovarian stimulation with optimal results which prevent unnecessary psychological and financial burden on the couple. Cycle monitoring is important to decide any alteration in dose or when to add GnRH antagonist. In women at high risk for ovarian stimulation, it is important to start with low doses and intensive monitoring. In case there are indications of hyperstimulation, the regime may be altered by decreasing dose or coasting. Many factors are interdependent, and hence, a careful selection of the type of ovarian stimulation will be the key factor in deciding the success of the same.

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13.1 Introduction

The ideal ovarian stimulation regimen for IVF should have a low cancelation rate, minimize drug costs, have low risks and side effects, require limited monitoring for practical convenience, and maximize singleton pregnancy rates, but this regimen has not yet been defined. It should be tailored according to the patient's characteristics. Numerous regimens have been described, ranging from no stimulation (natural cycles) to minimal stimulation (clomiphene citrate) or mild stimulation to aggressive stimulation (high dose exogenous gonadotropins) alone or in combination with a gonadotropin-releasing hormone (GnRH) agonist or antagonist. Each has its advantages, disadvantages, and applications. The stimulation regimen selected for any one individual should be based on age, response to any previous stimulation, and ovarian reserve. This would predict response and help individualize the drug regime which is to be followed. Individualization would be needed to decide the gonadotropin type and dose as well as whether an agonist or antagonist is to be used for suppression of LH. It is important to individualize to prevent hyper-response or poor response, improve results, and prevent unnecessary psychological and financial burden on the couple.

13.2 Evaluation Before IVF to Individualize Ovarian Stimulation

The following are recommended to decide on the dosage and the type of stimulation.

13.2.1 Ovarian Reserve

Ovarian reserve testing has prognostic value and is recommended for all women planning assisted reproductive technology (ART). Because ovarian response is inversely related to the day 3 serum FSH level, results of FSH may help to guide the choice among treatment regimens and the dose of gonadotropins to be used for stimulation. However, estimating the antimullerian hormone (AMH) levels will also give an idea about the response.

La Marca reported the AMH level of 0.7 ng/ml had a good sensitivity and specificity of identifying 75 % of poor responders [1]. AMH of >3.5 ng/ml predicted hyper-response and OHSS. AMH levels are higher in PCOS [2]. Eldar-Geva et al. reported an additional increase in AMH levels were observed in PCOS patients due to abnormal activity of granulosa cells, hyperandrogenism, and obesity [3]. Hence, the dosage should be monitored for ovulation induction depending upon the AMH values. The dosage is more for a lesser value and less for a higher value. In PCOS the dosage may start with 112.5/150 IU, whereas in a woman with lower reserve, it can be as high as 600 IU.

Antral follicle count on days 2–3 of the cycle gives a good estimate of the number of eggs to be obtained by ovarian stimulation. Each ovary having about six to eight antral follicles seems to be a good responder.

AFC and AMH are the most sensitive markers of ovarian reserve identified to date and should be used to plan individualized treatment. It has been suggested by a recent review that either of the two markers can be used as they have equal accuracy in prediction of response [4]. So once the patient is categorized as a hypo-/hyper- or normoresponder, the dose of gonadotropin is decided. The selection of dose is of paramount importance for optimal outcome of COS. Also in case of poor responders and hyper-responders, GnRH antagonist regimes are preferred. This helps in explaining the prognosis and in appropriate counseling and also ensures a safe controlled ovarian stimulation with optimal results.

13.2.2 Age of the Woman

Age is an important factor in fertility, and chances of conception decrease with advancing years, usually after the 30s. The reason why fertility decreases with increasing age is the diminished number of eggs left in the ovary. Unlike men, who produce new sperm daily throughout most of their lifetime, women are born with all their eggs in two ovaries. To be more precise, a woman is born with about one to two million immature eggs, or follicles, in her ovaries.

Throughout her life, the vast majority of follicles will die through a process known as atresia. Atresia begins at birth and continues throughout the course of the woman's reproductive life. When a woman reaches puberty and starts to menstruate, only about 400,000 follicles remain. With each menstrual cycle, 1,000 follicles are lost and only 1 follicle will actually mature into an ovum (egg), which is released into the fallopian tube, kicking off ovulation. That means that of the one to two million follicles, only about 400 will ever mature.

Relatively little or no follicles remain at menopause, which usually begins when a woman is between 48 and 55 years of age. The remaining follicles are unlikely to mature and become viable eggs because of the hormonal changes that come along with menopause.

Most infertility specialists define an older woman as one who is more than 35 years, but this is an arbitrary number. A woman's fertility does not fall at a particular age, but starts declining gradually after the age of 30. After 35, the drop is fairly dramatic; and after 38, it's even more so. However, there is no magic number at which fertility disappears, and this decline is a progressive irreversible process.

In the past, it was assumed that as the woman got older, her entire reproductive system started failing. However, today we know that the uterus and the fallopian tubes remain relatively unaffected by age and that the reason for the decline in fertility is the diminished number of eggs left in the ovary. The infertility specialist is really not interested in the woman's calendar (or chronological age), but rather her biological age – or how many eggs are left in her ovaries.

13.2.3 Weight and BMI

Women having a normal BMI have maximum oocyte retrieval, fertilization, implantation, and clinical pregnancy rates in comparison to obese females [5].

In a recent study it was found that increased doses of gonadotropins were required with increase in patient's BMI. No difference was seen in the number of oocyte retrieved, but a decreased fertilization and cleavage rate was seen with decreased number of cryopreserved embryos with increasing BMI. This study shows that poorer oocyte quality is seen with increasing BMI which results in reduced clinical pregnancy rate [6]. Hence, these women should be started at higher doses.

The CONSORT study developed a dosing algorithm which individualizes recombinant human FSH (r-hFSH) doses for assisted reproductive technologies, assigning 37.5 IU increments according to patient characteristics: basal FSH, body mass index, age, and antral follicle count [7].

13.3 Individualizing Protocols for Prevention of Premature LH Surge

13.3.1 Exogenous Gonadotropin Stimulation After Downregulation with a Long-Acting GnRH Agonist: Long Protocols

The introduction of long-acting GnRH agonists in the late 1980s revolutionized the approach to ovarian stimulation in ART by providing the means to downregulate endogenous pituitary gonadotropin secretion and thereby prevent a premature LH surge during exogenous gonadotropin stimulation. Adjuvant treatment with a GnRH agonist eliminated the need for frequent serum LH measurements and assuaged fears of premature luteinization which previously had necessitated cancelation of approximately 20 % of all IVF cycles before oocyte retrieval. Because fewer than 2 % of cycles are complicated by a

premature LH surge after downregulation with a GnRH agonist, stimulation could continue until follicles were larger and more mature. Clinical trials subsequently demonstrated that oocyte yields and pregnancy rates were significantly higher than in cycles stimulated with exogenous gonadotropins alone [8]. Moreover, GnRH agonist treatment offered the welcome additional advantage of scheduling flexibility, allowing programs to coordinate cycle starts for groups of women simply by varying the duration of GnRH agonist suppression. Not surprisingly, the long protocol quickly became the preferred ovarian stimulation regimen for all forms of ART.

13.3.1.1 Disadvantage

GnRH agonist treatment sometimes blunts the response to subsequent gonadotropin stimulation and increases the dose and duration of gonadotropin therapy required to stimulate follicular development. However, with poor ovarian reserve this is not the preferred protocol. The combined costs of the additional gonadotropins and the agonist itself increase the total cost of treatment.

Gonadotropin stimulation also yields more follicles and oocytes when agonist treatment begins during the luteal phase, possibly because LH-stimulated androgen production and circulating androgen levels are more effectively suppressed throughout folliculogenesis. Because the egg yield is greater, the number of embryos available is also increased. Consequently, the probability of having an optimal number of embryos for transfer and excess embryos for cryopreservation is greater. GnRH agonist treatment may be scheduled to begin on cycle day 21 (assuming a normal cycle of approximately 28 days of duration). Hence, in a woman with polycystic ovary syndrome (PCOS), a long protocol may be useful to reduce the LH levels and have a better oocyte recovery and ongoing pregnancy rates.

Overall, the weight of available evidence suggests that women who require longer durations of GnRH agonist treatment to achieve suppression or who develop cysts are more likely to respond poorly to gonadotropin stimulation; those who do are understandably less likely to succeed. The initial dose of exogenous gonadotropins used to stimulate ovarian follicular development after GnRH agonist-induced downregulation should be tailored to the needs of the individual woman. Typical starting doses range between 225 and 300 IU of urinary FSH (uFSH), recombinant FSH (rFSH), or urinary menotropins (hMG) daily, depending on the age, the results of ovarian reserve testing, and the response observed in any previous superovulation or IVF cycles. Either a "step-up" (beginning with a low dose, increasing as necessary based on response) or "step-down" (beginning with a higher dose, decreasing as necessary based on response) may be used, but the latter approach is generally preferred.

Concerns persist that GnRH agonist treatment may suppress endogenous LH levels below those necessary for normal follicular development in at least some women. Because only about 1 % of LH receptors must be occupied to support normal follicular steroidogenesis, the low levels of LH secretion after downregulation with a GnRH agonist are sufficient to meet the need in most women stimulated with uFSH or rFSH alone. However, LH concentrations also may be inadequate in those who are more profoundly suppressed. Indeed, LH levels are markedly suppressed (less than 1 IU/L) in many who are treated only with FSH, and in such cycles, the dose and duration of gonadotropins required are higher and peak estradiol levels are lower; the number of oocytes and embryos may also be reduced. Another evidence suggests that fertilization, implantation, and pregnancy rates may be adversely affected when LH levels are extremely low [9]. The evidence indicates that there may be a subgroup of eugonadotropic women that could benefit from supplemental hMG or rLH during ovarian stimulation.

13.3.2 Stimulation with Exogenous Gonadotropins with Addition of a GnRH Antagonist

The relatively recent introduction of GnRH antagonists into clinical practice has provided another option for ovarian stimulation in ART. GnRH antagonists, being more complex than agonists, act through a completely different mechanism for inhibiting gonadotropin secretion.

GnRH antagonists bind competitively to GnRH receptors preventing the action of endogenous GnRH pulses on the pituitary. Not only the secretion of gonadotropins is decreased within hours of antagonist administration, but in addition no flare-up effect occurs. Moreover discontinuation of GnRH antagonist treatment results in rapid and predictable recovery of the pituitary-gonadal axis as the pituitary receptor system remains intact.

In contrast to the agonists, antagonist treatment is highly dose dependant, relying on the balance between endogenous GnRH present and antagonist administered. Most importantly, within 6–8 h of administration, any imminent LH surge is blocked. The first generation of GnRH antagonists, besides binding to pituitary receptors, could in addition bind to GnRH receptors in mast cells resulting in degranulation and histamine release, consequences that impeded their use in assisted reproductive technologies for almost a decade. Thus, until some time back, downregulation in IVF was accomplished almost exclusively with the use of GnRH agonists.

13.3.2.1 Advantages of GnRH Antagonists

- The duration of treatment for an antagonist is substantially shorter than for an agonist.
- Since its only purpose is to prevent a premature endogenous LH surge and its effects are immediate, antagonist treatment can be postponed until later in follicular development (after 5–7 days of gonadotropin stimulation), after estradiol levels are already elevated, thereby eliminating the estrogen deficiency symptoms that can emerge in women treated with an agonist.
- Because any suppressive effects that agonists may exert on the ovarian response to gonadotropin stimulation are also eliminated, the total dose and duration of gonadotropin stimulation required may be decreased.
- By eliminating the flare effect of agonists, GnRH antagonists avoid the risk of stimulating development of a follicular cyst.
- The risk of severe ovarian hyperstimulation associated with the use of antagonists appears lower than with agonists.

13.3.2.2 Disadvantages of GnRH Antagonists

- When administered in small daily doses, strict compliance with the prescribed treatment regimen is essential.
- Low levels of LH observed during agonist treatment are usually sufficient to support normal follicular steroidogenesis when uFSH or rFSH is used for stimulation; the even lower concentrations in women treated with an antagonist may not be sufficient however.
- There is evidence to suggest that pregnancy rates in antagonist treatment cycles may be modestly lower than in cycles using agonists in the long protocol, possibly because GnRH antagonists may influence the mitotic programming of cells involved in folliculogenesis, blastomere formation, and endometrial development.

The two GnRH antagonists available for clinical use are equally potent and effective. For both, the minimum effective dose to prevent premature LH surge is 0.25 mg daily, administered subcutaneously [10]. Either of the antagonists can be administered in a series of small daily doses (0.25 mg). The treatment protocol can be fixed to begin after 5–6 days of gonadotropin stimulation or tailored to the response of the individual, starting treatment when the lead follicle reaches approximately 13–14 mm in diameter. Alternatively, a single larger dose of cetrorelix (3.0 mg) will effectively prevent an LH surge for 96 h. If given on days 6–7 of stimulation, the interval of effective suppression will encompass the day of hCG administration in most women (75–90 %); the remainder may receive additional daily doses (0.25 mg) as needed, ending on the day of hCG treatment. The single-dose antagonist treatment regimen also may be withheld until the lead follicle reaches 13–14 mm in diameter [10].

ART stimulation regimens are still uncertain, but antagonists have been viewed as holding particular promise for women with polycystic ovary syndrome and those who respond poorly to stimulation after treatment with an agonist. The antagonist treatment regimens currently in use

also have potential disadvantages for women with polycystic ovary syndrome. Their tonically elevated LH levels will remain high until antagonist treatment begins. Consequently, LH levels can rise prematurely, particularly if antagonist treatment is withheld until the lead follicle reaches 14 mm or more. Moreover, evidence indicates that increased LH exposure during early follicular development may be detrimental and predispose to lower pregnancy rates.

In theory, pretreatment with an oral contraceptive might prove quite useful by suppressing LH and androgen levels before stimulation begins, decreasing exposure during early follicular development and the risk of rising LH levels before antagonist treatment begins. Preliminary oral contraceptive suppression and later antagonist treatment may also help to limit the follicular response to gonadotropin stimulation while preserving the option to use an agonist to trigger final oocyte maturation. An earlier start to antagonist treatment may offer similar advantages. These and other considerations simply serve to illustrate that GnRH antagonists are not a panacea and may not even be the best choice for women with polycystic ovary syndrome.

- Poor responders are another group for which GnRH antagonists may have particular value because antagonist treatment eliminates any suppressive effects that the long-acting agonists may have on follicular response and can prevent the premature LH surges commonly observed in women stimulated with gonadotropins alone.

13.4 Cycle Monitoring to Individualize Dose Adjustments in Ovarian Stimulation

The response to stimulation is monitored with serial measurements of serum estradiol and transvaginal ultrasound imaging of ovarian follicles. The first serum estradiol level usually is obtained after 3–5 days of stimulation to determine whether the chosen dose of gonadotropins requires adjustment. Thereafter, serum estradiol concentrations and ovarian scans are obtained every 1–3 days, based on the quality of the response and the need to evaluate the impact of any further adjustments in the dose of gonadotropin treatment. Most women require a total of 9–10 days of stimulation. In general, the goal is to have at least two follicles measuring 17–18 mm in mean diameter, ideally accompanied by a few others in the 14–16 mm range, and a serum estradiol concentration that is consistent with the overall size and maturity of the cohort (approximately 200 pg/ml per follicle measuring 14 mm or greater). Typically, endometrial development is also monitored during stimulation by measuring the endometrial thickness. Although multiple studies have examined the prognostic value of endometrial thickness and echotexture in ART cycles, the issue remains controversial. The results are best when endometrial thickness measures 8–9 mm or greater and poor when the endometrium is less than 6–7 mm in thickness or appears homogeneous on the day of hCG administration. Once the targeted thresholds of response are met, hCG (10,000 IU) is administered to induce final follicular maturation.

13.5 Individualization on the Basis of Patient Response

13.5.1 High Responders

Occasionally, stimulation generates an exaggerated follicular response, characterized by massive ovarian enlargement, extremely large numbers of follicles of all sizes, and markedly elevated serum estradiol concentrations (>3,000 pg/ml). Under such circumstances, the risk for ovarian hyperstimulation syndrome is substantially increased and may lead to cycle cancelation.

Canceling the cycle and starting another using a more conservative stimulation regimen may ultimately decrease overall costs and

maximize the chances for success. The prognosis for high responders in subsequent cycles is generally very good. Hence, in a woman with PCOS, the stimulation is always at a lower dose.

13.5.2 Poor Responders

The challenges presented by "poor responders" are far greater. Poor responders include women in whom a previous cycle yielded three or fewer oocytes or was canceled because of observations of three or fewer follicles 16 mm or greater, a single dominant follicle, or a peak serum estradiol less than 500 pg/ml. In such women, a more aggressive or alternative stimulation regimen is warranted, and there are several options from which to choose:

- The long protocol, beginning with higher doses of gonadotropin stimulation.
- Decreasing the doses of GnRH agonist or discontinuing agonist treatment immediately before or soon after gonadotropin stimulation begins. However, in agonist cycle, the response in a poor responder is not good.
- A short follicular phase GnRH agonist treatment regimen using a standard or microdose "flare" protocol.
- Using a GnRH antagonist instead of a long-acting agonist.

13.6 Individualizing Controlled Ovarian Stimulation for Prevention of Ovarian Hyperstimulation

Ovarian hyperstimulation syndrome (OHSS) is an exaggerated response to ovulation induction therapy. The OHSS is typically associated with exogenous gonadotropin stimulation and is only rarely observed with the use of other agents. Clinicians who prescribe ovulation-inducing agents must be prepared to recognize and manage OHSS [11].

The syndrome has a broad spectrum of clinical manifestations, from mild illness needing only careful observation to severe disease requiring hospitalization and intensive care [12, 13].

13.6.1 Risk Factors

The following factors increase the risk independently for developing OHSS [14]:

- Young age
- Low body weight
- Polycystic ovary syndrome (PCOS)
- Higher doses of exogenous gonadotropins
- High absolute or rapidly rising serum E2 levels
- Previous episodes of OHSS

13.6.2 Prevention

Ovulation induction regimens need to be individualized, carefully monitored, and used with the minimum dose and duration of gonadotropin therapy necessary to achieve the therapeutic goal. Caution is indicated when any of the following indicators for increasing risk of OHSS are present:

- Rapidly rising serum E2 levels
- An E2 concentration in excess of 2,500 pg/ml
- The emergence of a large number of intermediate-sized follicles (10–14 mm)

Withholding further gonadotropin stimulation and delaying hCG administration until E2 levels plateau or decrease significantly can reduce risks of OHSS [15]. A lower dose of hCG may be prudent for patients judged to be at a high risk for OHSS. The use of exogenous P (e.g., 50 mg P in oil IM, 200 mg P vaginal suppositories, or 8 % P vaginal gel, daily) for luteal phase support rather than supplemental doses of hCG may further reduce the risks of OHSS.

Another method is "coasting" or "controlled drift" – here further gonadotropin stimulation is stopped and administration of the ovulatory trigger is deffered till the estradiol concentration has returned to "safe" levels. The general consensus is that coasting should be initiated when the serum estradiol levels exceed 3,000 pg/ml but not unless the leading follicle is 15–18 mm in diameter; the duration should not exceed 4 days as reduced implantation and pregnancy rates are seen when the coasting is done for longer periods of time. A GnRH analog can be used in antagonist cycle instead of hCG.

Serum progesterone must be controlled during the ovarian stimulation cycle in order to avoid premature luteinization. Regarding the effect of GnRH antagonists on human endometrium, Simon et al. studied the gene expression profile on the endometrium of women undergoing ovarian stimulation for oocyte donation and observed that the endometrial development after GnRH antagonist mimics the natural endometrium more closely than after GnRH agonist [16].

One of the significant advantages that antagonists have over agonists is the duration of the analog used. The initial flare effect of the agonist necessitates the longer duration of treatment to achieve adequate downregulation. GnRH antagonists competitively block pituitary receptor sites and induce a rapid and reversible suppression of gonadotropins. The reduction in both duration of therapy and gonadotropin requirement make its use attractive.

Conclusion

Each woman is different in her capacity to produce mature oocytes with the controlled ovarian stimulation. In clinical practice the dosage is decided by the age of the woman, antimullerian hormone levels, basal antral follicle counts, and presence or absence of polycystic ovary syndrome. Many factors are interdependent, and hence, a careful selection of the type of ovarian stimulation will be the key factor in deciding the success of the same.

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14 Impact of Mutations and Polymorphisms of Gonadotrophins and Their Receptors on the Outcome of Controlled Ovarian Stimulation

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Abstract

Controlled ovarian stimulation is a mainstay of assisted reproductive technologies and leads to optimal follicular growth and steroidogenesis in the majority of cases. Nonetheless, some women defined as "hyporesponders" require higher amount of exogenous gonadotrophin to achieve an adequate number of oocytes retrieved despite an apparently good prognosis. Clinical observational trials suggest that hyporesponse to exogenous gonadotrophins, including initial poor response, could be a genetically determined trait with specific genotype profile associated with this condition. Specifically, mutation and polymorphisms involving luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and their receptors LH-R and FSH-R have been thoroughly investigated. Among all the mutations discovered, it seems that that carriers of common LH variant and FSH receptor Ser/680 variants require higher doses of exogenous FSH to achieve a normal ovarian response.

In conclusion, the idea of a tailored gonadotrophin administration based on a pharmacogenomic approach may be considered in specific situations and could represent the future research target for a better understanding of the underlying mechanisms that regulate human fertility.

Keywords

Polymorphism • Controlled ovarian stimulation • Pharmacogenomics • IVF Follicle-stimulating hormone • Luteinizing hormone • Follicle-stimulating hormone receptors (FSH-R) • Luteinizing hormone receptors (LH-R)

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14.1 Introduction

Controlled ovarian stimulation (COS) is an essential step in most "in vitro" fertilization (IVF) programs. In this context, GnRH agonist (GnRH-a) long protocol in association with recombinant FSH (r-hFSH) still constitutes the most utilized strategy for normogonadotrophic patients. The use of GnRH antagonists (GnRH-ant) plus r-hFSH does represent a valid alternative. These approaches lead to optimal follicular growth and steroidogenesis in about 85–90 % of women. Conversely, COS results in a very different clinical outcome, from poor ovarian response to the risk of hyperstimulation syndrome (OHSS) in a relevant number of cases. In addition, some women, defined as "hyporesponders," require higher amounts of r-hFSH to obtain an adequate number (i.e., >4) of oocytes retrieved, despite an apparently good prognosis.

To sort this problem out, several markers have been proposed to predict ovarian response such as age, basal FSH, inhibin-B, anti-Müllerian hormone (AMH), and the count of antral follicles by ultrasonography (AFC). Yet, there is an increasing interest on the possible effect of specific genotype patterns on ovarian response.

In the present chapter, the potential effect of specific mutations/polymorphisms of the gonadotrophins and their receptors on the outcome of COS is explored. Confirmation of these observations would reinforce the idea of a tailored gonadotrophins administration based on a pharmacogenomic approach.

14.2 The Physiology of Gonadotrophins and Their Receptors

The classical "two cells-two gonadotrophins" model is based on the idea that follicle-stimulating hormone (FSH) and luteinizing hormone (LH) exert their roles on two different compartments, *granulosa* and *theca*, respectively. According to this model, LH exerts its activity in *theca* cells, which express enzymatic pathways of androgen synthesis [1, 2]. *Theca* involucres surround the *granulosa* cells, whose activities and proliferation are directly regulated by FSH. This hormone induces the expression of the aromatase enzyme, which in turn converts *theca*-deriving androgens into estradiol (E_2).

This theory, reinforcing the notion that *granulosa* and *theca* cells are distinct compartments regulated by FSH and LH, respectively, has been revised. More specifically, it has been found that LH receptors are also detected on the *granulosa* compartment at the intermediate follicular phase [2–5]. Therefore, it appears that LH regulates both *granulosa* and *theca* cells.

FSH and LH cooperate in inducing the *granulosa* cell-specific production of inhibin-B and other TGB-β growth factors. In addition, insulin growth factors (IGF) I and II, which are expressed by both *granulosa* and *theca* cells throughout folliculogenesis, are important in promoting follicular maturation [6, 7]. Locally produced peptides, rather than estrogens, are known to be the key factor regulating primate follicle growth and development [8–11]. In light of these finding, we can conclude that (1) both gonadotrophins contribute (*via granulosa*) to maintain the autocrine-paracrine system governing dominant follicle growth and (2) LH is crucial in sustaining FSH activity in the *granulosa* during intermediate-late stages of folliculogenesis. On this basis it is possible to argue that high levels of one gonadotrophin can counteract the lack of the other. This hypothesis is consistent with the observation that FSH activity can be totally substituted by LH once *granulosa* cells express adequate amounts of LH receptors [5, 12]. Conversely, higher exogenous FSH doses during COS are able to compensate GnRH-a-related reduction of LH. It could be argued that if LH concentration and/or activity falls below a hypothetical threshold, an impairment in *granulosa* paracrine activities will occur, which in turn can lead to higher requirement of FSH.

On the basis of the above information, it could be hypothesized that during COS, different "adaptative" mechanisms may occur. For instance, lack of LH activity in *granulosa* cells may be counteracted by higher exogenous FSH. Conversely, administration of exogenous

Table 14.1 Mutations and polymorphisms of LH

Location	Type	Amino acid involved	Effect	Reference
Exon 3	Missense	Gln^{54} to Arg	Absence of spontaneous puberty in male	Weiss et al. (1992) [17]
Exon 2	Missense	Trp^{8} Arg Ile^{15} Thr	Delayed pubertal progression in male and infertility in female	Petterson et al. (1991) [18] Furui et al. (1994) [19] Haavisto et al. (1995) [20]
Exon 3	Missense	Ala $^{-3}$ Thr	Normal bioactivity	Jiang et al. (2002) [21]
Exon 3	Missense	Gly^{102} Ser	Infertility in male, menstrual disorders in female	Liao et al. (1998) [22] Ramanujam et al. (1999) [23]

Adapted from Lamminen and Huhtaniemi [24]

LH may optimize FSH activity on the same compartment, which in turn can increase steroidosynthesis and reduce FSH requirement. In the clinical practice, COS protocols are often chosen empirically. As consequence, same protocols are administered in most patients, despite potential biological differences. Adaptation capability of follicles leads ovarian response to an "adequate" profile in almost all women. Nevertheless, this adaptation requires "integrity" of *granulosa-theca* system. Aging and some genetic characteristics may reduce this capability, leading to "suboptimal" ovarian response. In the following paragraphs, the potential role of some polymorphisms of gonadotrophins and their receptors in conditioning ovarian response to gonadotrophins will be discussed.

14.3 The LH System: A Crucial Variable During COS

14.3.1 LH Polymorphism

Recently, it has been reported that hyporesponders who benefited from LH activity had endogenous levels of LH in the normal range. In addition, endogenous LH concentrations of these patients during early phases of COS was always comparable with those observed in women who had optimal response to FSH and who did not require any change of FSH dose during stimulation. This observation led to the hypothesis that hyporesponse to r-hFSH is associated with a less bioactive LH [13–16].

Among the most valuable β-LH polymorphisms identified (Table 14.1) [17–24], we have recently performed an observational trial [25] aimed to evaluate whether the presence of the most common of them, v-LH, is associated with different profiles of ovarian response to r-hFSH.

Pettersson and Söderholm [18] were the first to describe this common variant of LH (v-LH) as an immunologically anomalous form of LH. The occurrence of the v-LH varies according to geographic areas (Fig. 14.1) [24]. v-LH is due to two point mutations in the β subunit gene, both altering the amino acid sequence (Trp^{8}Arg and Ile^{15}Thr). v-LH has elevated bioactivity in vitro but significantly shorter (5–9 min) half-life in circulation when compared with the wild type LH (wt-LH) (12–22 min). As the pulse frequency of the v-LH is normal, this results in an overall LH action that is more potent at the receptor site but shorter in duration in vivo.

The v-LH is common worldwide, with carrier frequency varying from 0 to 52 % in various ethnic groups. Its incidence in Italy ranges between 12 and 13 %. The v-LH differs functionally from wt-LH, and it seems to predispose its carrier to mild aberrations of reproductive function menstrual irregularities causing infertility [19] and recurrent pregnancy loss [26].

In our observational trial, 60 normogonadotrophic patients undergoing a GnRH-a long downregulation plus r-hFSH for IVF/ICSI, and in

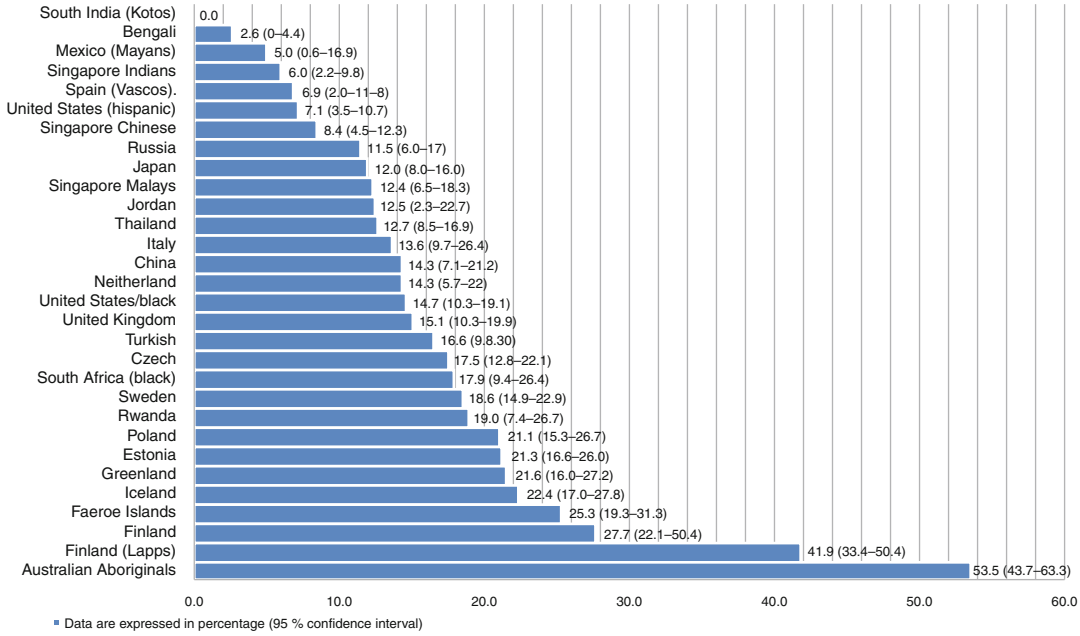

Fig. 14.1 Worldwide occurrence of the common v-LH (From Lamminen and Huhtaniemi [24])

whom at least five oocytes were retrieved, were divided into three groups: 22 women requiring a cumulative dose of r-hFSH >3,500 IU constituted group A, 15 patients requiring 2,000–3,500 IU were included in group B, and 23 women requiring <2,000 IU served as control group (group C). The presence of the v-LH was evaluated using immunoassays able to detect both wt-LH and polymorphism. Group A showed a significantly lower ($p<0.05$) number of oocytes retrieved when compared with group B and C (7.3 ± 1.5, 11.7 ± 2.4, and 14.7 ± 4.1 in the three groups, respectively). Seven carriers (32 %) of v-LH were found in group A, whereas only one variant (7 %) was observed in group B; no variant was detected in group C. This study suggested, for the first time, an association between a less bioactive LH and a higher FSH requirement. In addition, it supports the idea that hyporesponders represent a specific subgroup of patients. In fact, all women requiring >3,500 IU of FSH had at least five oocytes retrieved and showed peak estradiol >500 pg/ml, which in turn would have lead physicians to classify them as normal responders. Nevertheless, they had a statistically significant reduction of the number of oocytes retrieved and estradiol levels when compared with woman requiring lower FSH doses.

On the basis of these finding we further investigated the relationship between v-LH and ovarian response to FSH [27, 28] in a Danish population. v-LH was present in 11 % of patients, whereas the allelic frequency was 12 %. Patients were divided into two groups according to their LH genotype. Group A included 196 wt/wt women, and group B was constituted by 24 individuals with v-LH (21 heterozygous and 3 homozygous). The mean number of oocytes retrieved, fertilization rate, and pregnancy rate per cycle were similar in the two groups. Group B received a significantly higher cumulative dose of r-hFSH than group A (2,435.86 ± 932.8 IU versus 1,959.8 ± 736.45; $P=0.048$). LH genotype had a statistically significant effect ($P<0.01$) on the cumulative dose of r-hFSH, showing a progressive increase from wt/wt (1,959.8 ± 736.45 IU) to v-LH heterozygotic (2,267.5 ± 824.3) and homozygotic women (3,558.3 ± 970.9). These results confirmed that carriers of v-LH have hyposensitivity to exogenous FSH during COS.

14.3.2 LH Receptors (Mutations and Polymorphisms)

The luteinizing hormone/choriogonadotrophin receptor (LHCGR) is a member of the superfamily of guanine nucleotide-binding protein-coupled receptors (GPCRs) and belongs to the glycoprotein hormone receptors [29]. LHCGR is expressed in Leydig cells and in ovarian *theca*, *granulosa*, as well as luteal cells. These receptors exert a fundamental role in reproductive process since puberty [30]. Several mutations have been identified in LHCGRs, and some of them have been related to reproductive disorders such as male-limited gonadotrophin-dependent precocious puberty, Leydig cell hypoplasia, and anovulation/amenorrhea [30]. In addition, some authors have observed an increased risk for endometrioid adenocarcinoma when rs13405728 mutation in gene LHCGR is associated with SNPs rs2479106 in gene DENND1A [31].

LHCGR mutation can be didactically divided into two categories:

1. Activating mutations (such as missense Leu368Pro, missense Asp578His), which were associated with precocious puberty and Leydig cell neoplasia
2. Inactivating mutations, characterized by pseudohermaphroditism and in some cases (such as deletion of exon 10) by normal sexual development with no sign of puberty [32]

In addition to the LHCGR mutations, more than 200 single nucleotide polymorphisms have been discovered. One of the most widespread polymorphisms is due to the presence of a two-amino acid insertion at position 18 in exon 1 (insLQ) and has been detected in breast cancer patient with lower survival rate [33]. Subsequently, another group have analyzed the same polymorphism in PCOS patients, but found no significant association [34].

A detailed phenotype of novel homozygous inactivating nonsense and missense mutations of the LH-receptor gene (Arg 554 stop codon 554 [TGA] and Ser 616 → Tyr 616, respectively) has been described in a woman with compromised ovulation and luteinization processes but apparent normal pubertal feminization [35]. This aforementioned patient presented with high LH and FSH levels and normal estradiol end progesterone values [36].

Evidence about the relationship between LHCGR and reproductive outcome during COS is scarce. In addition to the previously mentioned Kerkala et al.'s observations [34], some authors have recently observed that a higher expression of LH receptors by human cumulus granulosa cells is associated with lower fertilization rate [37].

14.4 The FSH System: From Physiology to COS

14.4.1 FSH Receptor (Mutations and Polymorphisms)

The FSH receptor (FSH-R), likewise its homologue LH, is a glycoprotein hormone receptor that belongs to subfamily of G protein-coupled receptors (GPCRs). FSH mutations have been extensively studied with more than 1,000 polymorphic variants identified to date [38]. Like LHCGR, FSH-R mutations are categorized in "activating" or "inactivating" mutations.

The first "activating" FSH-R mutation was discovered in a hypophysectomized man who surprisingly showed normal spermatogenesis despite undetectable FSH levels [39].

Other two peculiar cases of constitutively activated FSH-R were characterized by heterozygous Thr449Ile and Asp567Asn mutations. Both affected women had a history of spontaneous OHSS syndrome during pregnancy. The probable explanation for this phenotype is linked to the altered ligand site, which becomes activated in the presence of high hCG levels as normally seen during pregnancy [40, 41].

Carriers of "inactivating" mutations are usually affected by hypergonadotrophic hypogonadism, primary or early-onset secondary amenorrhea, variable sexual development, arrest of follicular maturation between primordial and

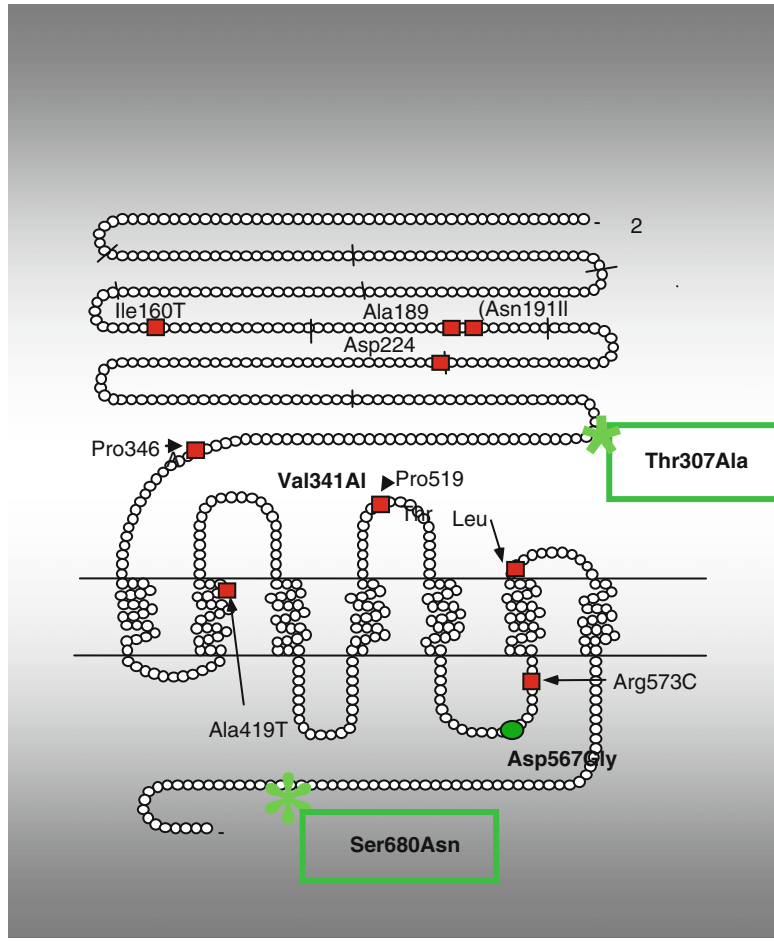

Fig. 14.2 Human FSH receptor mutations. The Ser680Asn is in linkage disequilibrium with Thr307Ala (*green*) (From Huhtaniemi and Themmen [32])

preantral stage, and poor semen quality. While severe phenotypes have been described in carriers of Ala189Val and Pro348Arg mutations, mild forms have been detected in patients with a compound heterozygous mutation of Ala189Val and Ala419Thr [42–44].

The most investigated variant of the FSH-R consists in the replacement at position 680 of the amino acid asparagine by serine (Fig. 14.2) [32]. This polymorphism has been associated with higher basal FSH levels and an increased number of antral follicles during the early follicular phase [45]. In an observational trial, Perez Mayorga et al. [46] evaluated the relationship between the presence of the Ser/680 FSH-R variant and ovarian response to COS in 161 normo-ovulatory women undergoing IVF. All women were below 40 years. The distribution of genotypes in the study population was 29 % for the Asn/Asn, 45 % for the Asn/Ser, and 26 % for the Ser/Ser FSH-R variant. Both estradiol levels at the day of human chorionic gonadotrophin (hCG) and number of retrieved oocytes were similar in the three groups. Conversely, basal FSH levels were significantly different among the three groups (6.4 ± 0.4 IU/l, 7.9 ± 0.3 IU/l, and 8.3 ± 0.6 IU/l for the Asn/Asn, Asn/Ser, and Ser/Ser groups, respectively, $P < 0.05$). In addition, the mean number of FSH ampoules required for successful stimulation was significantly different among groups (31.8 ± 2.4, 40.7 ± 2.3, and 46.8 ± 5.0 for the Asn/Asn, Asn/Ser, and Ser/Ser groups, respectively, $P < 0.05$). These clinical findings demonstrated that ovarian response to FSH stimulation depends on the FSH-R genotype. Following these observations, Behre et al. [47]

tested whether the same daily dose of FSH resulted in lower levels of estradiol in women homozygous for the Ser/Ser and whether the difference could be overcome by higher FSH doses. Fifty-nine women undergoing COS for IVF or ICSI and homozygous for the FSH-R polymorphism Ser/680 were randomly allocated in three groups. Group I (Ser/Ser, $n=24$) received a daily FSH dose of 150 IU/day, and group II (Ser/Ser, $n=25$) received a FSH dose of 225 IU/day. In group III (Asn/Asn, $n=44$), FSH dose was 150 IU/day. Age and basal FSH levels were not different between groups. Total FSH doses were comparable in group I (1,631 ± 96 IU) and group III (1,640 ± 57 IU) but significantly higher in group II (2,421 ± 112 IU) ($P<0.001$). Peak estradiol levels were significantly lower in group I (5,680 ± 675 pmol/l) compared to group III (8,679 ± 804 pmol/l) ($P<0.05$). Increasing the FSH dose from 150 to 225 IU/day overcame the lower estradiol response in women with Ser/Ser (group II, 7,804 ± 983 pmol/l). The authors concluded that patients with the Ser/Ser FSH-R variant have lower FSH receptor sensitivity, which can be overcome by higher FSH doses. This study represented the first case of a pharmacogenomic approach to COS.

Recently, we have evaluated the occurrence of the Ser/680 FSH-R variant among women classified as "hyporesponders" (Alviggi et al. 2013). Forty-two normogonadotrophic patients in whom at least five oocytes were retrieved after GnRH-a long downregulation protocol followed by stimulation with r-hFSH for IVF/ICSI were retrospectively studied. On the basis of the total r-hFSH consumption, patients were divided into two groups: 17 women requiring a cumulative dose of r-hFSH >2,500 IU constituted group A, whereas 25 patients requiring <2,500 IU served as controls (group B). DNA was analyzed to determine the FSH receptor genotype. Estradiol peak levels were significantly lower in group A (997 ± 385 pg/ml) when compared with group B (1,749 ± 644; $P<0.001$). The number of oocytes retrieved was also significantly lower in group A compared with group B (7.1 ± 1.5 versus 9.6 ± 2.4; $P<0.001$). Homozygous Ser/Ser receptor variant at codon 680 was observed in 47.0 % of women of group A and in 28.0 % of women of the control group. The homozygous Asn/Asn receptor variant was found in 23.6 and 20.0 % of patients in the two groups, respectively. Heterozygosis Ser/Asn was detected in 29.4 % of patients of group A and in 52.0 % of patients of group B. These results indicated that FSH-R Ser 680/variant is more frequent in women with hyporesponse to r-hFSH.

Although some investigators found a positive association between pregnancy rate and presence of Ser680 genotype [48, 49], a recent meta-analysis confirmed that Ser/Ser genotype carriers have significantly higher basal FSH levels and require higher exogenous FSH doses for COS [50].

Nakayama et al. in 2006 identified another polymorphic variant of FSH-R with possible implication in COS [51]. It consisted of a polymorphism in the 5′-UTR of the FSH-R gene (position 29 A/G; rs1394205), which seems to be associated with a lower luciferase activity compared with G/G 29 allele. Subsequently, Desai and colleagues observed a reduced FSH-R expression in *granulosa* cells of AA genotype carriers [52].

In women undergoing assisted reproduction, variants A/A have been associated with poor ovarian response with respect to number of oocyte retrieved and doses of exogenous FSH for COS [53].

Lastly, the impact of a new FSH-R polymorphism has been investigated in a female Indian population. Specifically, 50 patients undergoing ART and 100 fertile patients have been recruited. The authors observed that Ala307Ala carriers required lower amount of exogenous FSH for ovulation induction in comparison with Thr307Thr and Thr307Ala subjects. Estradiol levels and incidence of OHSS were higher in the former [54].

FSH-R polymorphisms and the ovarian outcome in women undergoing ovarian stimulation have been widely studied [46, 52–58].

14.4.2 FSH Mutations and Polymorphisms

Several β subunit mutations of FSH have been identified in the literature. Most of them inactivate

the FSH effects. In females, primary amenorrhea, impaired fertility, and compromised pubertal development are the most frequent clinical manifestations. In contrast, FSH mutations in males do not affect sexual maturation although they result in azoospermia. Most of FSH mutations interfere with a specific cysteine knot region that is crucial for dimerization with α subunit and biological activity [32].

Unlike LH β, most of FSH polymorphic variants have been found in noncoding regions confirming that FSH β is strongly conserved in the human species [32, 59].

To date, only a single nucleotide polymorphism located into FSH β promoter-211G/T seemed to influence the FSH concentration in males [60, 61]. In addition, it seems that Ser 680 Asn polymorphism may influence serum FSH levels in the male population [62]. The same effects were also reported in the female population. Higher FSH serum levels were observed in women with the FSHB-211 GT + TT/associated with FSHR2039 AA genotype [59]. The impact of FSH polymorphisms and their combination with different FSH-R genotypes is yet to be evaluated.

Conclusion

The unraveling of the mechanisms that regulate the interaction between the gonadotrophins and their receptors is a step forward to a better understanding of why an impaired ovarian response to stimulation occurs in apparent good prognosis patients. There are clinical observational trials suggesting that hyporesponse to exogenous gonadotrophins, including initial poor response, could be a genetically determined trait. This phenomenon has been associated with the presence of at least two common polymorphisms involving LH and FSH-R, respectively. Carriers of v-LH and FSH-R Ser/680 variants, despite normal levels of endogenous gonadotrophins and regular AMH/AFC, require higher doses of exogenous FSH to achieve a normal ovarian response. Thus, the idea of a tailored gonadotrophin administration based on a pharmacogenomic approach may be considered in specific situations. As examples, LH supplementation may be considered in the presence of v-LH, whereas a timely identification of Ser/680 FSH-R variant may represent an indication to administer higher doses of FSH.

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The Role of Androgens in Controlled Ovarian Stimulation

15

Ariel Revel and Jordana Hadassah Hyman

Abstract

Endogenous androgens, which are synthesized in the adrenal glands and the ovary, play a crucial role in folliculogenesis. Androgens undergo aromatization into estrone and estradiol in the granulosa cells. Androgens also exert an influence on the follicular cycle, acting via granulosa cell receptor to FSH.

In states of androgen excess, there is usually a high number of antral follicles and enhanced response to gonadotropin therapy. This has led to the hypothesis that increasing intraovarian androgen, either via exogenous testosterone administration or by increasing local ovarian testosterone concentration, can potentially increase the quantity and quality of oocytes retrieved during controlled ovarian (hyper)stimulation (COS) and improve pregnancy and live birth rates.

Women who are poor responders to COS classically have shortened follicular phase, with a smaller window for recruitment of follicles, as well as reduced sensitivity to FSH. Poor responders may exhibit improved response to treatment with the addition of exogenous testosterone, as well as adjuvant LH administration, which can increase endogenous androgens. Treatment options include transdermal testosterone, DHEA, aromatase inhibitors, and adjuvant LH.

Aromatase inhibitors, which block aromatization of androstenedione and testosterone to estrone and estradiol, are also used in COS in women undergoing treatment for fertility preservation. In these cases, it is desirable to preserve low estradiol levels. The addition of aromatase inhibitor also reduces the required gonadotropin dosage.

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Current research and meta-analyses regarding androgen therapy in COS are presented in this chapter, as well as clinical application and recommendations.

Keywords

Androgen therapy • Poor responders • Controlled ovarian stimulation • Testosterone • DHEA • Aromatase inhibitor

15.1 Rationale for Androgen Therapy

Folliculogenesis is the process of development of primordial follicles into primary, pre-antral, and then antral follicles, which are then recruited in the menstrual cycle. A dominant or preovulatory follicle is selected and undergoes follicular rupture with ovulation of the oocyte, while the rest become atretic. The follicular cycle is influenced by a complex interplay between FSH, LH, inhibin, activin, and follistatin. FSH and LH synthesis and secretion are regulated by GnRH, with negative feedback from estrogen and progesterone. These hormones control the recruitment of follicles, selection of the dominant follicle, timing of ovulation, and luteal phase. In most spontaneous cycles, a single oocyte is ovulated per cycle [1–5].

Controlled ovarian stimulation (COS) involves exogenous hormonal stimulation of the ovaries in order to recruit and develop multiple dominant follicles, enabling retrieval of several oocytes with potential for fertilization [6, 7]. Traditional protocols for COS include stimulation with gonadotropins, combined with prevention of premature ovulation by either downregulation with GnRH agonist (long protocol) or concurrent treatment with GnRH antagonist (short protocol) and control of the timing of ovulation by either mimicking (hCG) or inducing (GnRH agonist) the endogenous LH surge [8]. Endogenous androgens, which are produced in both the ovary and the adrenal glands, also have an important role in follicular development. According to the two-cell two-gonadotropin theory, follicular steroid hormones synthesized in the theca cells – androstenedione and testosterone – undergo aromatization into estrone and estradiol in the granulosa cells [9]. Androstenedione and testosterone are synthesized from cholesterol, with dehydroepiandrosterone (DHEA) as a steroid precursor [10, 11]. DHEA is mainly synthesized in the adrenals. Androgen production in the ovary is regulated by FSH, LH, and inhibin.

In addition to acting as a substrate for estrogen production, androgens also influence the follicular cycle [12–16]. They appear to act via granulosa cell androgen receptors (ARs) to promote FSH-induced granulosa cell differentiation and may enhance the follicular response to FSH, particularly in early antral stages of folliculogenesis [17]. Androgen receptor mRNA and androgen concentrations in follicular fluid are correlated with FSH receptor mRNA expression in granulosa cells from small antral follicles [18, 19]. Androgens may also increase the number of FSH receptors expressed in granulosa cells. Immunohistological staining of androgen receptors during different times of the menstrual cycle showed varying intensities during the cycle, with strongest expression during the antral to preovulatory phase, suggesting that androgens, mediated by androgen receptors, may play an essential role in follicular growth and maturation, atresia, and luteinization [20]. FSH receptor expression can be modulated by testosterone added to granulosa cells [21].

Women with excessive androgens, either in cases of ovarian hyperandrogenism such as polycystic ovarian syndrome (PCOS); adrenal hyperandrogenism, such as congenital adrenal hyperplasia; or exogenous androgen treatment, such as testosterone-treated transsexuals, have a high number of antral follicles [13, 22, 23]. While the hyperandrogenism often is associated with dysovulation or anovulation, such women are

usually extremely sensitive to low doses of exogenous FSH stimulation. In ovarian stimulation cycles, the addition of exogenous androgens may affect the hormonal milieu of the ovary, by increasing the number of small antral follicles and improving the follicular sensitivity to FSH [24]. This could potentially enhance the ovarian response to stimulation and lead to better reproductive outcome, especially in women who are poor responders. Androgens may also stimulate follicular steroidogenesis via IGF-1 and IGF-2 stimulation and IGFBP1 inhibition [25–28].

Increased circulating insulin and IGF-I, exogenous testosterone, and increased local ovarian testosterone concentrations due to aromatase inhibition or exogenous luteinizing hormone/human chorionic gonadotropin are all associated with an increased ovarian response to gonadotropins.

The hypothesis underlying androgen use in ovarian stimulation is that increasing intraovarian androgens, either via exogenous testosterone administration or increased local ovarian testosterone concentrations due to aromatase inhibition or exogenous LH/hCG, would result in an increase in the number, maturity, and reproductive potential of the oocytes.

15.2 Patients with Potential Benefit from Androgen Therapy

15.2.1 Poor Responders

During physiological reproductive aging, the primordial follicle count declines rapidly [29]. This is associated with decreased oocyte quality, increased aneuploidy, and reduced fertility and fecundity [30, 31]. The associated diminished ovarian reserve and reproductive potential is usually age related, but can be accelerated in certain women. Poor responders to ovarian stimulation include women with decreased ovarian response due to physiological age, as well as those with accelerated reproductive aging, due to a variety of etiologies, such as genetic or immunologic premature ovarian failure, endometriosis, malignancy, or exposure to gonadotoxic agents.

There is no universal agreement regarding the definition of poor responder. The Bologna criteria, described in 2011 [32], however, represent the ESHRE consensus. This defines poor responders as women with at least two out of the three following criteria: (i) advanced maternal (age ≥40 years) or any other risk factor for poor ovarian response, (ii) a poor ovarian response (≤3 oocytes with a conventional stimulation protocol), and (iii) an abnormal ovarian reserve test result (i.e., AFC < 7 follicles or AMH <1.1 ng/ ml).

15.2.1.1 Poor Responders: Treatment Options

Many treatment options for poor responders have been proposed and evaluated. The most recent Cochrane review of interventions for poor responders [33] concluded that there was insufficient evidence to support any particular treatment protocol or adjuvant treatment. However, a review of randomized trials of interventions for poor responders performed in 2011 [34] showed that in 47 trials there were 41 different definitions of poor ovarian response. It is challenging to interpret and compare data from trials performed before the Bologna criteria, which offers a standardized definition of poor responders, and difficult to exclude potential benefit of any options for treatment. It is also difficult to perform randomized controlled trials on this group of women.

Treatment for poor responders includes both specific ovarian stimulation protocols and adjuvant therapy [35–37]. Ovarian stimulation protocols that have been proposed include natural cycle IVF, minimal stimulation IVF, minimal stimulation with aromatase inhibitor (letrozole), short or antagonist protocol, and microdose flare protocol. There is also much debate regarding the dose and type of gonadotropin. High or very high dose? Single dose or multiple doses? FSH plus LH? Purified gonadotropins? No single treatment protocol has been proven.

Many adjuvant therapies have been proposed by both medical and alternative practitioners. These include growth hormone [38–41], androgens including DHEA and testosterone, arginine [42], glucocorticoids, pyridostigmine, and aspirin [43]. More recently, COQ10 has been advocated for poor responders [44]. Acupuncture has also been

promoted for poor responders. Intracytoplasmic sperm injection (ICSI) and assisted hatching are laboratory techniques which may increase pregnancy rates in older women or poor responders.

15.2.1.2 Poor Responders: Rationale for Androgens

Poor responders typically have shorter or irregular cycles, with a shortened follicular phase. This shortens the potential window for recruitment of follicles. An additional challenge is reduced sensitivity of FSH receptors, which leads to the use of very high gonadotropin doses in this patient group. Women with poor response to gonadotropins exhibit lower expression of FSH receptor granulosa cells [45, 46].

Exogenous androgen therapy causes an increase in follicular levels of testosterone and androstenedione, as does treatment with aromatase inhibitor [27, 47], which acts as a mediator by blocking aromatization to estrogens and thus effecting an endogenous increase. LH administration in addition to FSH also causes increased endogenous androgen production and is also recommended for poor responders [48, 49]. Increased intraovarian androgens have been reported to increase follicle numbers, increase antral follicle count [50, 51], improve follicular survival, reduce apoptosis/atresia, and enhance IGF-1 concentration [52, 53]. The outcome of this improved follicular microenvironment includes increased quantity and superior quality of oocytes available for fertilization, with improved fertilization, reduced cancellations, improved pregnancy and live birth rates, and decreased aneuploidy and miscarriage rates. It has been postulated that low pregnancy rates and embryo aneuploidy may result from low intrafollicular androgens; adjuvant androgen therapy may serve to rectify this deficiency [54–57].

15.2.1.3 Hypoandrogenism in Poor Responders

Adrenal and ovarian androgen levels undergo changes during reproductive aging and menopause, with a natural decline commencing as early as age 30 [58, 59]. Follicular levels of testosterone have been shown to be reduced in poor responders who underwent COS, as compared with normal responders [60, 61]. The number of zona reticularis cells, the site of DHEA synthesis in the adrenal gland, also undergoes decline with reproductive aging and in poor responders [57]. Conversely, De Los Santos [62] reported similar intrafollicular androgen levels in poor responders compared with fertile women. The authors suggested that follicular androgen secretion does not seem to be reduced; rather, other mechanisms such as lower FSH receptor expression or aromatase activity insufficiency may be the main challenges in poor responders.

Basal testosterone has been proposed as a potential marker for poor ovarian reserve. Low basal testosterone and DHEAS are associated with decreased ovarian sensitivity to FSH, reduced response to stimulation [63], and low pregnancy rates after IVF. In women with high FSH, low basal testosterone may be predictive of expected number of large follicles and oocytes, as well as pregnancy outcome [64]. Gleicher [65] suggested that older women with poor ovarian reserve, who have been treated with DHEA supplementation, also show low conversion rates of DHEA to testosterone. The authors present an association between the potential benefit of DHEA supplementation in improving pregnancy rates and the conversion rate of serum DHEA to testosterone, which may serve as a marker in poor responders.

Recently Guo et al. [66] analyzed basal testosterone as a predictor of poor ovarian response and found that it was a predictor but, used alone, had limited use. This study proposed a multivariate model for prediction of poor ovarian response, which included age, AFC, FSH, FSH/LH, and testosterone. Ratios of androgen to AMH and androgen to FSH may also be useful markers for poor response [61]. The use of basal testosterone as a marker for poor ovarian reserve, combined with the declining ovarian and adrenal androgens observed in reproductive aging, implies a need for androgen supplementation in these women.

15.2.2 Fertility Preservation

Women of reproductive age who must embark on potentially gonadotoxic chemotherapy and radiotherapy for treatment of malignancy are another group that may benefit from androgen therapy as

part of ovarian stimulation. Chemotherapy can damage or destroy the primordial ovarian follicles, including oocytes and granulosa cells, which can render the woman with premature ovarian failure [67]. Radiotherapy can cause various insult and damage to the reproductive organs, depending on the exact location and dosage of radiation [68]. As more clinicians are aware of fertility preservation options, and as cancer detection and treatment continue to improve, there continue to be more women seeking fertility preservation before cancer treatment.

Depending on the woman's age and status, the desired fertility preservation technique may be oocyte or embryo cryopreservation or ovarian tissue cryopreservation. Oocyte harvesting involves ovarian stimulation and retrieval. The ovarian stimulation protocol will depend on the actual malignancy, as well as the window of time available prior to commencement of chemotherapy or radiotherapy. Women with breast cancer, particularly estrogen receptor-positive disease, should not be exposed to high levels of estrogen, for fear of cancer recurrence. Therefore, natural cycle IVF or stimulation in combination with aromatase inhibitor may offer the only options for these women.

15.2.2.1 Rationale for Aromatase Inhibitor in Fertility Preservation

Aromatase inhibitors block the aromatization of androstenedione and testosterone to estrone and estradiol, causing higher androgen levels, but suppressed plasma estradiol, estrone, and estrone sulfate levels. The lower levels of estrogen are clearly desirable in women with breast cancer, and these drugs, specifically letrozole and anastrozole, are used as treatment for breast cancer itself, not only for fertility preservation.

Aromatase inhibitors, most commonly letrozole, may be used itself as a form of ovulation induction or in combination with gonadotropins as part of COS [69, 70]. The suppression of estradiol caused by aromatase inhibitors leads to negative feedback, with resultant increased gonadotropin stimulation. Both letrozole only and letrozole-gonadotropin cycles result in very low serum estradiol. However, the addition of letrozole appears to reduce the number of oocytes retrieved for preservation [71]. Aromatase inhibitors used in combination with gonadotropin for ovarian stimulation for fertility preservation have been demonstrated to have significantly lower estradiol levels [72]. They also decrease the amount of gonadotropin required for stimulation [69]. Surveillance after letrozole-gonadotropin ovarian stimulation protocols revealed no negative effects on survival, with no difference in recurrence rate or relapse-free survival [73, 74]. Letrozole has been compared with alternative aromatase inhibitors (anastrozole) [72, 75] as well as to selective estrogen receptor modulators (tamoxifen) [70] and found to be superior for fertility preservation cycles in terms of maintaining low estradiol levels and achieving higher oocyte and embryo yields.

15.3 Androgen Therapy: Options

There are several options including exogenous androgens such as transdermal testosterone and oral DHEA, as well as aromatase inhibitors, which modulate androgen levels.

15.3.1 Transdermal Testosterone

Transdermal testosterone as an adjunct to conventional IVF for poor responders was reported by both the French [76] and Spanish [77] groups in 2006. These studies were based on the hypothesis that androgens act synergistically with FSH receptors and may improve small follicle recruitment and sensitivity to FSH, in poor responders. Balasch [77] enrolled women with two previous failed cycles, who had normal basal FSH levels. All women [25] underwent long GnRH agonist cycle and received 5 days of transdermal testosterone before gonadotropin stimulation was commenced. In comparison with the previous cycles, there was significant improvement in peak E2 levels, number of follicles (>10 mm), number of oocytes retrieved, and clinical pregnancy rate.

Massin et al. [76] reported a randomized controlled trial of poor responders, who either had a

previous IVF cycle with poor response (defined as peak E2 of <1,200 pg/ml or <5 oocytes retrieved) or who had basal hormonal profile suggestive of decreased ovarian reserve. Women were randomized to transdermal testosterone gel or placebo. Women were treated with the gel for 15 days prior to gonadotropin stimulation. Although serum testosterone increased significantly in the treated group, there were no significant differences in ovarian response or treatment outcome.

Balasch and Fabregues [78] also performed a randomized controlled study of transdermal testosterone, published in 2009. These were women who had undergone cancellation of their first IVF cycle due to poor response. Patients were randomized to pretreatment with 5 days of transdermal testosterone, in addition to standard long protocol (starting dose 150 U) or mini-dose agonist protocol with high-dose gonadotropin (300 U). More women in the testosterone group achieved oocyte retrieval, and significantly fewer women in the testosterone group were considered low responders in the study treatment cycle. The authors suggest that transdermal testosterone may improve ovarian sensitivity to FSH and follicular response to gonadotropin; however the study had low power to determine significant differences between the study groups. Kim et al. [79] randomized 110 poor responders (<3 oocytes retrieved in the previous cycle) to treatment with transdermal testosterone or control, in conjunction with multidose GnRH antagonist treatment. They reported significant improvement in number of oocytes retrieved, mature oocytes, fertilized oocytes, and good-quality embryos, as well as implantation rate and clinical pregnancy rate per number of women treated with testosterone.

15.3.2 DHEA

Dehydroepiandrosterone (DHEA) supplementation is the other form of adjuvant androgen therapy promoted for poor responders. DHEA is recommended by over 25 % of IVF practitioners, according to the 2010 survey [80]. DHEA treatment was initially reported by Casson in 2000, with a small case series of five poor responders, following their experience with DHEA for postmenopausal women [81]. Later, Gleicher reported the case of a 43-year-old patient, who self-treated with DHEA, improving her oocyte yield from a single oocyte in her first cycle to a maximum of 17 oocytes [82]. This case inspired the interest in investigating DHEA use for poor responders. Barad and Gleicher [83] then reported a case-control study of 25 women, showing increased quantity and quality of embryos in IVF cycles following DHEA treatment. They further reported higher pregnancy rates [84], decreased miscarriage rates [54], and reduced aneuploidy [55] following DHEA administration. Increased number of oocytes, number and quality of embryos, and pregnancy rates, in IVF cycles after DHEA, have been reported in several studies [85–91]. Increased spontaneous pregnancies [84, 92] as well as pregnancies following IUI treatment have been reported in poor responders after DHEA treatment, while waiting for further IVF treatment. They reported 10 spontaneous pregnancies in 39 young (<39 years) women treated with DHEA.

Several studies have also reported improvements in ovarian reserve including reduced FSH [85, 89, 93] and increased AMH [89, 93, 94], as well as increased AFC [88, 93, 95] and inhibin B [93] after DHEA. There have been three randomized controlled trials (RCTs) published to date regarding DHEA supplementation. Wiser [86] reported an RCT of 33 poor responders undergoing long protocol. They noted significantly improved embryo quality and live birth rate in women treated with DHEA. Clinical pregnancy rate was improved, but this was not statistically significant. Kara performed an RCT with 208 women, but concluded that there was no clear evidence of benefit with DHEA [96]. The number of oocytes retrieved and the fertilization rate were slightly higher in the study group, but the pregnancy rate was higher in the control group. None of the differences were statistically significant. The most recently published RCT by Yeung [95], of 32 women randomized to DHEA or placebo, reported no difference in markers of ovarian reserve, response to treatment, or treatment outcome.

Reviews of DHEA use for poor responders have yielded conflicting conclusions. Gleicher and Barad [56] summarized the benefits of

DHEA supplementation – improved ovarian function, increased pregnancy rates, and reduced aneuploid and miscarriage rates. They hypothesized that DHEA may be able to revert the ovarian aging process specifically in younger women with premature ovarian failure. However, Urman [97] concluded that there was a lack of evidence to support DHEA. They cited regression to the mean and variability of gonadotropin responsiveness as potential explanations for improvements in treatment cycle and dismissed the only published RCT at that stage as insufficiently designed or powered to allow valid scientific conclusions.

15.3.3 Aromatase Inhibitors

Aromatase inhibitors act by inhibiting the activity of aromatase, which is responsible for conversion of androstenedione and testosterone to estrone and estradiol. This results in higher androgen levels and lowered estradiol, estrone, and estrone sulfate levels. The resultant reduced estrogen feedback causes increased gonadotropin secretion. Aromatase inhibitors, specifically letrozole and anastrozole, were introduced for ovulation induction in women with PCOS who were clomiphene resistant and for women with unexplained infertility [98, 99]. In poor responders, the resultant increase in intraovarian androgens may also be of benefit in a similar mechanism to increased exogenous androgens, with an impact on folliculogenesis, small follicle recruitment, and FSH receptor expression.

Letrozole for poor responders was initially reported in 2002 [100]; treatment with letrozole prior to commencement of gonadotropins led to increased oocyte yield, with lower total dose of FSH. Garcia-Velasco [27] performed an observational pilot study of 147 women with previously cancelled cycles. Women underwent high-dose gonadotropin antagonist protocols, with or without addition of letrozole. The letrozole group showed significantly higher levels of follicular fluid testosterone and androstenedione as well as significantly higher oocyte yield and implantation rate. Pregnancy rate was increased, but not significantly. A large prospective study of 534 women [101] compared microdose flare protocol with a letrozole – an antagonist protocol in poor responders. There were no significant differences in treatment parameters, except that peak estradiol was lower in the letrozole group. Pregnancy rates were found to be significantly higher, with a trend toward improvement of implantation rate in the microdose flare group. Yarali [102] reported a case-control study of 885 poor responders treated with microdose flare or letrozole-antagonist protocols. Total gonadotropin consumption and peak E2 were significantly lower in the letrozole-antagonist group. Oocyte yield was also significantly lower with letrozole treatment, but clinical pregnancy rates were comparable, and fertilization and implantation rates were higher, with more number of top-quality embryos. Lee [103] more recently reported a retrospective study of poor responders treated with multidose antagonist protocol, with or without adjuvant letrozole. Oocyte yield was significantly higher in the letrozole group. Clinical pregnancy, implantation, and miscarriage rates were similar between the groups; however dosage and duration of both gonadotropin and antagonist were significantly lower in the letrozole group. Rate and miscarriage rate were similar in the two groups. Total dose and days of recombinant human follicle-stimulating hormone (rhFSH) administered were significantly fewer in the letrozole group than in the control group.

There have been three randomized controlled trials for letrozole in poor responders. Goswami [104] sought to examine letrozole as a low-cost protocol. Poor responders, with 1–3 previous failed IVF cycles, were randomized to GnRH agonist long protocol or letrozole plus low-dose rFSH protocol (75 IU on days 3 and 8) responders. The letrozole-FSH had comparable pregnancy outcomes, as well as other treatment parameters, and clearly the most cost-effective protocol with considerably less gonadotropin exposure. Omzen [105] randomized 70 poor responders (previous cancellation or low E2 or <4 oocytes retrieved) into fixed-dose (450 IU) rFSH antagonist cycle with and without letrozole. Gonadotropin dose and peak E2 were significantly lower in the letrozole group, as was

cancellation rate. Higher clinical pregnancy rate was reported in the letrozole treatment group. Mohsen [106] randomized 60 women with at least one failed IVF cycle (four or less oocytes retrieved) to mild stimulation letrozole-antagonist or microdose flare agonist treatment. Dose and duration of gonadotropin were significantly lower, as was peak E2, in the letrozole group, and clinical pregnancy rate was comparable. Other parameters were comparable between the groups.

15.4 Results of Meta-Analysis Reviews

There has been one meta-analysis specifically examining transdermal testosterone, one meta-analysis regarding DHEA, and two which examine androgen therapy for poor responders. In the review and meta-analysis of transdermal testosterone, Gonzalez-Comadran [107] analyzes the three RCTs previously presented [76, 78, 79], a total of 225 women. It was noted that the inclusion criteria and definition of poor responder was not consistent between the three trials. There was also a variation in the actual treatment protocol and in the regime for application of transdermal testosterone, with different doses and length of treatment ranging between 5 and 21 days. Pooled analysis of the data showed significantly increased clinical pregnancy and live birth rate in the treated group. Clinical pregnancy per transferred embryo, however, proved not to be significantly different between the two groups. Women in the testosterone group required significantly less FSH stimulation than the controls. There was no significant difference detected in other parameters including cycle cancellation rate, peak estradiol, number of oocytes retrieved, or miscarriage rate.

Narkwichean et al. performed a meta-analysis of DHEA for poor responders [108]. Of the 22 studies, only 3 were eligible for meta-analysis – Wiser [86], Gleicher [55], and Barad [84]. Clinical pregnancy rate was assessed by analyzing two studies; no significant difference was demonstrated. There was no difference in miscarriage rate; however oocyte yield was lower in the DHEA-treated group compared with controls. Given the small sample size, any conclusions of this meta-analysis should be cautiously interpreted. Two meta-analyses examined the use of androgens and androgen-modulating agents on IVF outcome for poor responders. These came to discordant conclusions. Sunkara et al. [109] included nine studies, of which five were randomized controlled studies. Two studies were of transdermal testosterone [76, 77], two of DHEA [84, 86], and the remaining of aromatase inhibitor [27, 101, 102, 104, 105]. Clinical pregnancy rate was not significantly increased in the treated women in the meta-analysis of RCT and of non-RCT studies. There was also no significant increase in clinical pregnancy in women treated with aromatase inhibitor. Analysis of the four studies using testosterone and DHEA showed significantly increased clinical pregnancy rate. Total gonadotropin dosage was significantly lower in androgen-treated women compared with controls, but length of treatment was not different. There was no difference detected in cycle cancellation rates, oocytes retrieved, or ongoing pregnancy rate. Sunkara concluded that there was insufficient evidence to support androgen use for poor responders.

Bosdou [110] published a meta-analysis in 2012. This analysis included 13 RCTs reporting use of aromatase inhibitors [105, 111], DHEA [86], transdermal testosterone [76, 79], recombinant LH [112–118], and recombinant hCG [115]. Analysis of the studies reporting transdermal testosterone revealed significantly increased oocytes retrieved, clinical pregnancy and live birth rates, lower GT dose, and shorter duration of GT (gonadotropin) stimulation. In contrast, the single DHEA study analyzed showed no difference in number of oocytes retrieved, clinical pregnancy, or live birth rates. Clinical pregnancy was not shown to be significantly different in the meta-analysis of studies reporting treatment with aromatase inhibitor; live birth rates were not reported. Total gonadotropin dose was significantly reduced with aromatase inhibitor, while oocyte yield showed no difference. All forms of androgens and androgen-modulating agents presented in the meta-analysis demonstrated a trend toward increased clinical pregnancy rate; however

there was lack of scientific evidence to confirm these findings.

The results of the meta-analyses do not provide consistent conclusions; however, all analyses showed higher clinical pregnancy rates with transdermal testosterone. Analysis of DHEA supplementation, aromatase inhibitor, rLH, and RhCG were not able to show clear advantage to enable clinical recommendation of androgens or androgen-modulating agents for poor responders.

15.5 Potential Adverse Effects of Androgens

Androgens have the potential for various adverse effects including oily skin, acne vulgaris, deep voice, hair loss, and other masculinizing features. There is one report of a patient who presented with posttraumatic seizure after 1 month of DHEA supplementation [119]. Gleicher et al. reported no adverse effects in over 1,000 women treated with DHEA [56]. Wiser also showed no adverse effects of androgens [86]. Massin and Kim concluded that there are no adverse effects in women treated with transdermal testosterone and no evidence of congenital malformations [76, 79]. Aromatase inhibitors were thought to be associated with congenital malformations [120], but a subsequent study of five Canadian centers demonstrated that there was very low risk of congenital malformations or chromosomal abnormalities [121]. In women treated with long-term aromatase inhibitors for breast cancer, carpal tunnel syndrome and musculoskeletal events have been reported [122, 123].

It is prudent to note that testosterone is used for continuing replacement therapy in both men and women, as well as in transsexuals. In female-male transsexuals treated with testosterone, incidence of cardiovascular disease and myocardial infarction was comparable to rates in women [124]. DHEA is also used in postmenopausal women long term. It has been shown to be beneficial in terms of bone density [125, 126] with possible benefits for memory, cognitive function, and libido. A study of women treated with 1 year of DHEA showed no adverse effects in terms of either lipid or insulin profiles [127].

15.6 Clinical Applications and Recommendations

Recent evidence suggests that the problem in poor responders is not low intraovarian androgens in the actual phase of oocyte retrieval, but rather long-term deficiency, which impacts on the ovarian microenvironment [56, 62]. It appears logical that androgen therapy should be used as priming rather than adjuvant therapy during the treatment cycle. The process of folliculogenesis, reaching the antral stage where the follicle would express androgen receptor, takes weeks, and it is unclear how long treatment with exogenous androgens would be required in order to improve the functional ovarian reserve [128]. DHEA synthesis in the theca cells occurs approximately 70 days prior to ovulation, so supplementation for at least 3–4 months seems logical [57]. Long-term androgen priming seems to be necessary to increase recruitment of small antral follicles [84, 88]. Long-term aromatase inhibitor therapy, as suggested by Feigenberg [24], may also potentially improve the cohort of early follicles.

DHEA can be offered to patients waiting for their next IVF cycle, which can often be motivating as women feel that they are doing something practical to improve their chances of success. DHEA appears to be dispensed widely among poor responders, while transdermal testosterone seems to be less popular, despite the stronger scientific evidence for transdermal testosterone in terms of clinical pregnancy and live birth outcomes. Like the initial patient reported by Barad who self-medicated with DHEA [82], many poor responders are purchasing DHEA of their own volition, not necessarily in conjunction with their treatment plan. Retrospective and case control studies seem to point toward a benefit for androgen therapy in poor responders, even if level 1 evidence is still lacking.

Aromatase inhibitors, while not strictly androgens, work by increasing endogenous androgens. Although these also have not been proven for poor responders, they are being increasingly used in minimal stimulation protocols for poor responders. The benefit of aromatase inhibitors for ovulation induction and as an adjunct to gonadotropin stimulation for IVF in fertility preservation therapy is obvious.

15.7 Future Research

Much of the research to date regarding androgen use in controlled ovarian stimulation, including the meta-analyses presented in this chapter, appears to be confounding and vague. This is in part due to lack of uniform definition of poor responders, as well as wide variation in treatment protocols. Further RCTs with clear definition of patient cohorts, as well as standardized adjuvant androgen therapy protocol, will hopefully provide more distinct evidence to either support or invalidate use of androgens for poor responders.

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The Role of LH in Controlled Ovarian Stimulation

16

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Abstract

Although exogenous FSH is the main regulator of follicular growth in stimulated cycles, LH plays a key role in promoting steroidogenesis and follicle development. Stimulation protocols with LH supplementation are mandatory in patients with hypogonadotropic hypogonadism who do not achieve adequate steroidogenesis by stimulation with FSH alone, but resume adequate estrogen production by LH supplementation. In normogonadotropic women undergoing controlled ovarian stimulation (COS), the hypogonadotropic state after GnRH analogues is short in duration, and the resting levels of LH are usually sufficient for promoting optimal follicular development. An increased body of evidence otherwise indicates that at least three subgroups of normogonadotropic patients indeed seem to benefit from the addition of LH activity to the stimulation protocol: (1) patients >35 years, (2) patients with a decreased ovarian reserve/poor response to COS (poor responders), and (3) patients with an initial poor response to rec-hFSH (hyporesponders). Possible reasons for a beneficial effect of LH activity supplementation include the biological aging of the ovary and pharmacogenetics involving the LH molecule and its receptor. The three gonadotropins containing LH activity are human menopausal gonadotropin (hMG), with 1:1 ratio of FSH/LH in which LH activity is driven by hCG; recombinant

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human LH (rec-LH), with only LH activity driven by pure LH; and a combination of recombinant FSH and recombinant LH, with 2:1 ratio of pure FSH/LH activity. In addition to the higher purity and specific activity of rec-hLH compared with hMG, LH activity is markedly different at the molecular and functional levels between these gonadotropins. The choice of the type of gonadotropin preparations containing LH activity should be considered when tailoring COS with LH supplementation because they may influence cycle outcome.

Keywords

Gonadotropins • Luteinizing hormone • Human chorionic gonadotropin • Controlled ovarian stimulation • Assisted reproductive technology

Abbreviations

3β-HSD	3β-Hydroxysteroid dehydrogenase
AA	Amino acids
ART	Assisted reproductive techniques
Asn	Asparagine
cAMP	Cyclic adenosine monophosphate
CHO	Chinese hamster ovary
CI	Confidence interval
COS	Controlled ovarian stimulation
DHEA	Dehydroepiandrosterone
FbM	Filled by mass
FSH	Follicle-stimulation hormone
GalNAc	N-acetylgalactosamine
GC	Granulosa cells
GlcNAc	N-acetyl glucosamine
GnRH	Gonadotropin-releasing hormone
hCG	Human chorionic gonadotropin
hMG	Human menopausal gonadotropin
HP-hMG	Highly purified human menopausal gonadotropin
ICSI	Intracytoplasmic sperm injection
IGF	Insulin-like growth factor
IVF	In vitro fertilization
LH	Luteinizing hormone
LMW	Low molecular weight
OR	Odds ratio
P4	Progesterone
P450arom	P450 aromatase
P450scc	Cholesterol side-chain cleavage enzyme
RCT	Randomized controlled trial
RD	Risk difference
rec-hCG	Recombinant human chorionic gonadotropin
rec-hFSH	Recombinant human follicle-stimulating hormone
rec-hLH	Recombinant human luteinizing hormone
RR	Relative risk
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SE-HPLC	Size-exclusion high-performance liquid chromatography
SO3-4GalNAc	Sulfonated β1–4-linked N-acetylgalactosamine
StAR	Steroidogenic acute regulatory protein
WMD	Weighted mean difference

16.1 Introduction

Gonadotropin therapy has a central role in ovarian stimulation. Its introduction in medical practice dates from almost one century ago and represents a major upgrade in infertility treatments. Treatment of anovulatory women with exogenous gonadotropin administration started in the 1960s and expanded to ovulatory women to promote multifollicular development in the 1980s [1–3]. Gonadotropins were first extracted from urine in the 1940s, and a decade later the first urinary forms of human chorionic gonadotropin (hCG) and human menopausal gonadotropin

(hMG) became commercially available [2, 3]. Improvements in the purification methods led to the production of follicle-stimulating hormone (FSH) – only products in the 1980s and advances in DNA technology enabled the development of recombinant human gonadotropins, which became commercially available approximately two decades later [2–4]. In 2000, recombinant human luteinizing hormone (rec-hLH) became commercially available, and recently, in 2007, a fixed combination of recombinant FSH (rec-hFSH) and rec-hLH was launched [3].

Although exogenous FSH is the main regulator of follicular growth in stimulated cycles, the question whether the LH hormonal environment achieved after administration of gonadotropin-releasing hormone (GnRH) analogues is really optimal for all categories of patients undergoing controlled ovarian stimulation (COS) or whether subgroups of patients exist that might actually benefit from exogenous LH supplementation has received increased attention. While FSH is the main antral follicular growth regulator, LH plays a key role in promoting steroidogenesis and development of the leading follicle and has different functions in different stages of both natural and stimulated cycles. During the early follicular phase, LH stimulates the production of androgens by theca cells. Androgens are then transferred to the GC and transformed into estrogens via aromatization [4]. From the mid-follicular phase onwards, LH upregulates FSH receptor expression and sustains FSH-dependent granulosa cell activities, including aromatase production and growth factors' release. In addition, LH sustains follicular growth and final follicular maturation via its direct effects on the GC in the late follicular phase [4]. Therefore, during recent years an increasing body of evidence has emerged examining the possible beneficial role of exogenous LH activity supplementation in stimulated ART cycles.

The purposes of this chapter are (1) to review the glycoprotein structure and action of luteinizing hormone (LH), (2) to examine the rationale of using luteinizing hormone (LH) supplementation during controlled ovarian stimulation, (3) to present the clinical evidence supporting LH supplementation during COS in different subset of infertility patients, (4) to describe the commercially available preparations containing LH activity, and lastly (5) to analyze the differences in LH activity provided by rec-hLH and hMG preparations.

16.2 Structure and Function of LH

LH is a protein covalently linked to a carbohydrate (glycoprotein). It is synthesized and secreted by gonadotrophs of the anterior pituitary gland under stimulation of the pulsatile secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus [5]. The LH molecule comprises two non-covalently linked protein subunits, alpha and beta. The three-dimensional structure and the active conformation of the subunits are maintained by internal disulfide bonds [6]. The alpha subunit contains 92 amino acids (AA) and is identical in all gonadotropins (i.e., LH, FSH, and hCG). The beta subunit differs in the aforementioned gonadotropins and confers unique receptor specificity as well as differential biological and immunological properties (Fig. 16.1) [7]. Protein subunits alone have no biologic activity; the latter is provided by glycosylation, which is achieved by the attachment of carbohydrate moieties forming heterodimers [3]. The extent and pattern of glycosylation convey the differential spectrum of charges, bioactivities, and half-lives of each gonadotropin [7, 8]. The LH molecule is further modified in vivo by the addition of a sialic acid (sialylation) or sulfonic group (sulfonation) to the carbohydrate moieties. Both sialylation and sulfonation are physiological processes with major roles in gonadotropin biological activity modulation [3, 7–9]. Elimination of LH from circulation is modulated by the number of glycosylation sites and sialic acid residues attached to the carbohydrate moieties [10]. LH beta subunits contain a single site of N-linked glycosylation (Asn 30) and few sialic acid residues (only 1 or 2); as such, native LH has a short half-life of only 20–30 min (Fig. 16.2) [8, 10]. LH shows physiologic fluctuations in isoform profile during the menstrual cycle. More

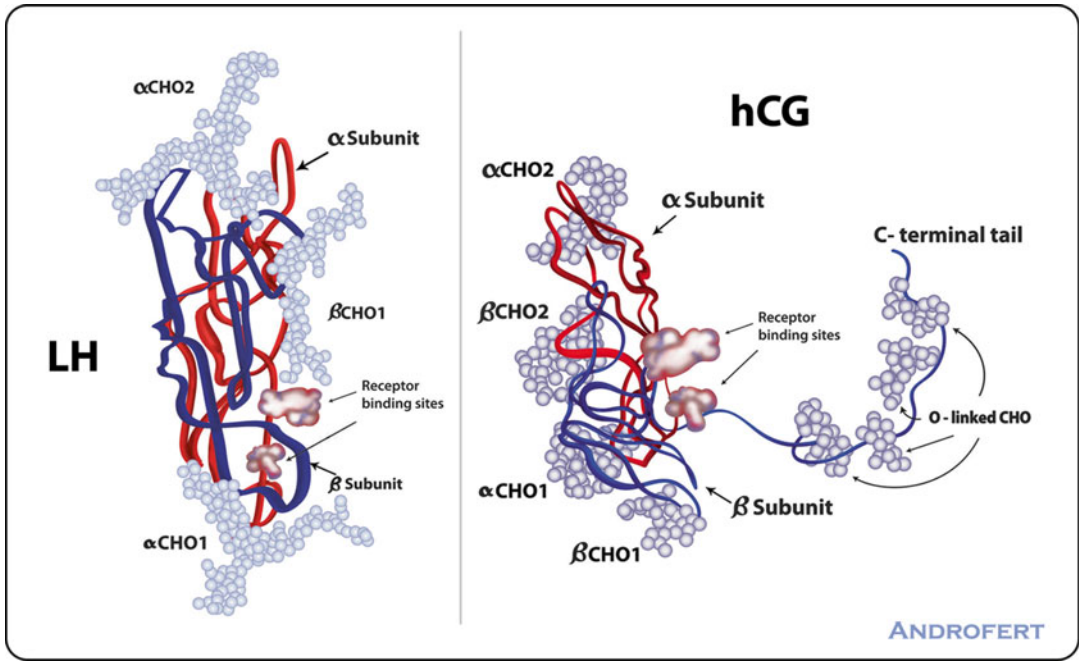

Fig. 16.1 Luteinizing hormone and human chorionic gonadotropin molecules. (**a**) LH is a glycoprotein with two subunits, the alpha subunit (*red*), similar to that of FSH and hCG with two carbohydrate attachment sites, and the beta subunit (*blue*), with only one carbohydrate attachment site. The light blue balls represent the carbohydrate chains. (**b**) hCG is similar in its structural attributes to LH. A notable exception is the presence of a long carboxyl terminal segment that is O-glycosylated (O-linked CHO), conferring longer half-life to hCG. The alpha and beta subunits are represented in *red* and *blue* strands, respectively, whereas the *light blue balls* represent the carbohydrate chains (Adapted from Leão and Esteves [3])

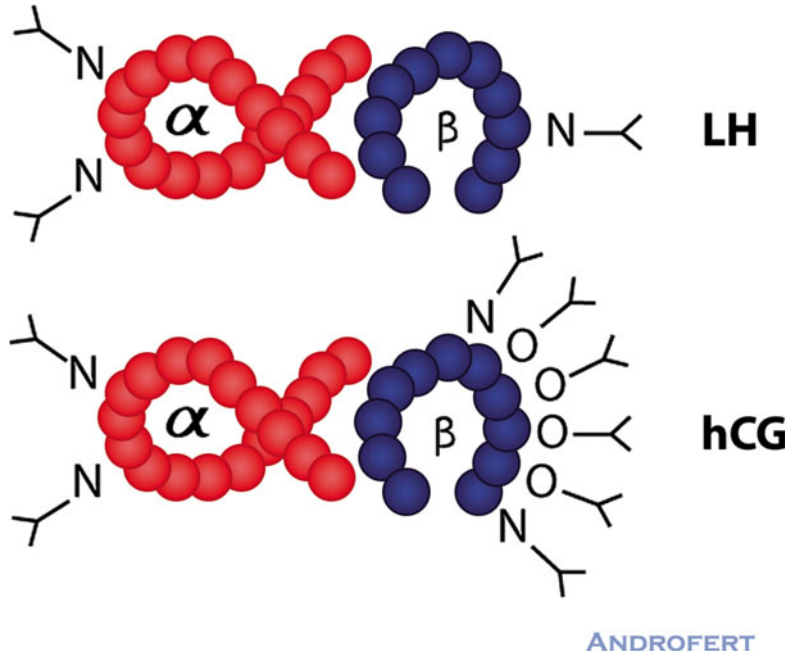

Fig. 16.2 Glycosylation patterns of LH and hCG. The alpha subunits of each hormone are identical in amino acid sequence and contain two sites of N-linked glycosylation. The beta subunit confers hormone specificity and contains variable amounts of N-linked glycosylation. LH beta subunit contains a single site of N-linked glycosylation, while hCG beta subunit contains two sites of N-linked glycosylation. In addition, hCG has an extended C-terminal that contains four sites of O-linked glycosylation

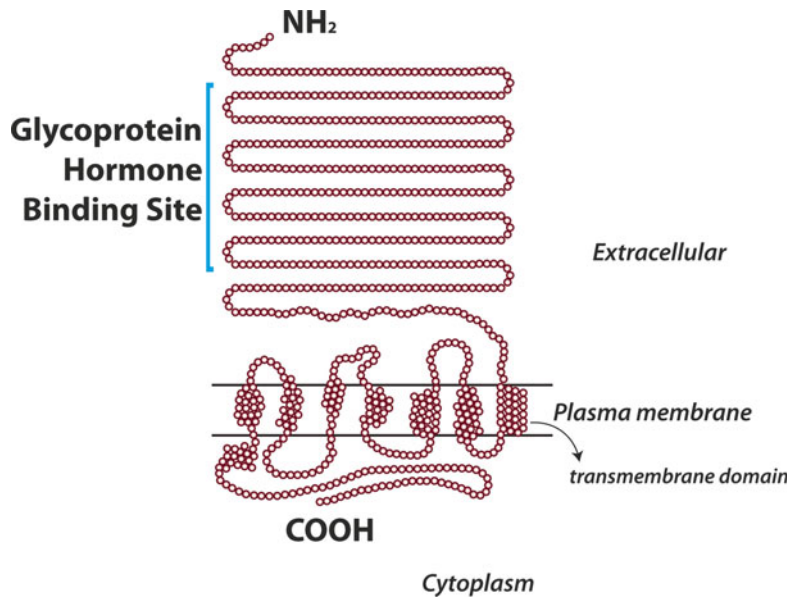

Fig. 16.3 LH/hCG receptor. LH receptors are included in the subgroup of G protein-coupled receptors. Such receptors are characterized by having 7-transmembrane domains and a large N-terminal extracellular region, which is the predominant site of hormone binding

basic LH isoforms are seen at midcycle due to considerably decreased sulfonation concomitant with slightly increased sialylation. Both changes increase LH half-life in the circulation, thus explaining the increased levels of serum LH at this period. This change in isoform profile seems to be physiologically important for ovulation triggering [11].

LH binds to a subgroup of G protein-coupled receptors with 7 transmembrane domains and a large N-terminal extracellular region (Fig. 16.3) [12, 13]. Receptor activation requires that hormones bind to the N-terminal region, thus leading to intramolecular signal transduction from the ligand–receptor complex to the transmembrane domains. Although the mechanism that underlies this intramolecular signaling pathway is not fully understood, it involves stimulation of adenyl cyclase via coupling to Gs proteins [12, 13]. Unlike FSH receptors that are expressed exclusively in the granulosa cells (GC), LH receptors are expressed in both GC and theca cells. The LH receptor expression is at its maximum in the GC of preovulatory follicles, but antral follicles with 3–10 mm in diameter have already expressed these receptors at approximately 10 % of the maximum [14].

16.2.1 The Role of LH on Ovarian Steroidogenesis

The two-cell system, first proposed by Falck in 1959, is based on the assumption that while FSH receptors are present only in the GC, LH receptors are present in the theca cells and absent in the GC during the early follicular stages [15–18]. Theca cells are characterized by exhibiting steroidogenic activity in response to LH stimulation. Specifically, cholesterol is converted into androgens (i.e., testosterone and androstenedione) by transcription activities of cholesterol side-chain cleavage enzyme (P450scc), P450c17, and 3β-hydroxysteroid dehydrogenase (3β-HSD) genes. The starting point of steroid biosynthesis is cholesterol, a carbon 27 (C27) steroid. Cholesterol is converted to pregnenolone (C21) by P450scc (CYP11A – cytochrome P450, family 11, subfamily A, polypeptide 1), whose regulation is mediated by steroidogenic acute regulatory protein (StAR). StAR facilitates the influx of cholesterol into the mitochondria where P450scc is located. StAR expression is enhanced by cAMP and by stimulation of GC with FSH and LH or hCG [3, 4, 18, 19].

The primary route of pregnenolone metabolism is via the delta 5 pathway, the first two steps of which

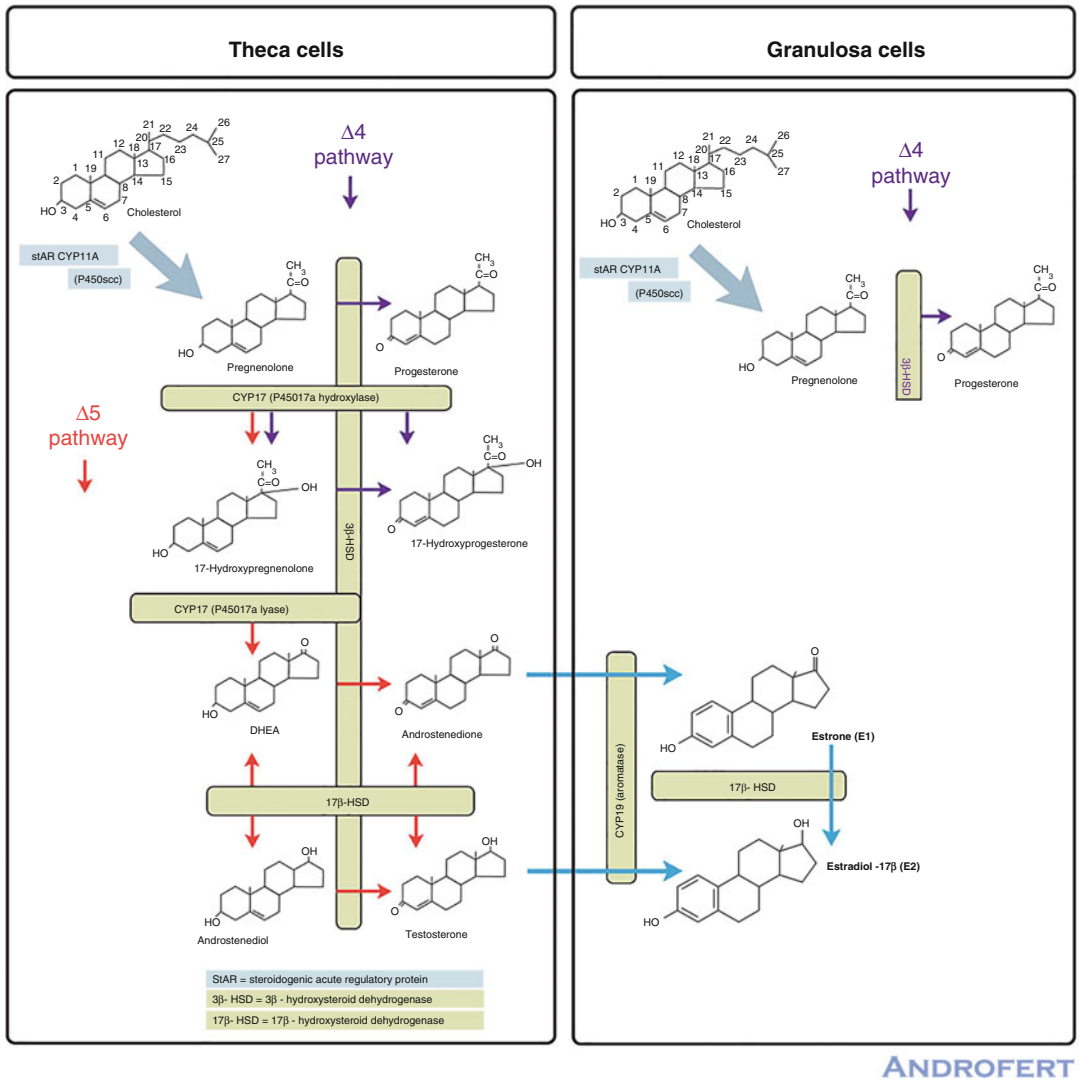

Fig. 16.4 Human ovarian steroidogenesis. The starting point for steroid biosynthesis is the conversion of cholesterol in pregnenolone by P450scc. One route of pregnenolone metabolism is the delta-5 pathway (*red arrows*) by the action of CYP17 (P450c17). Hydroxylation of pregnenolone at the C17a position forms 17-hydroxypregnenolone, and subsequent removal of the acetyl group forms the androgen precursor dehydroepiandrosterone (DHEA). Another route of pregnenolone metabolism is the delta-4 pathway (*purple arrows*) in which pregnenolone is converted to progesterone by the action of 3b-HSD (an irreversible conversion). Progesterone is then converted to 17-hydroxyprogesterone by CYP17. In humans, 17-hydroxyprogesterone cannot be further metabolized. Aromatization of androgens to estrogens is a distinct activity within the granulosa layer induced by FSH via activation of the P450 aromatase (P450arom) gene (From Leão and Esteves [3])

are driven by the same enzyme, CYP17 (P450c17). The hydroxylation of pregnenolone at the C17a position forms 17-hydroxypregnenolone, and the subsequent removal of the acetyl group forms the androgen precursor dehydroepiandrosterone (DHEA). Accordingly, CYP17 has both hydroxylase and lyase activity. Lastly, DHEA is converted to androstenedione by 3β-HSD [15, 19, 20]. A secondary route of metabolism involves the conversion of pregnenolone to progesterone by the action of 3β-HSD via the delta 4 pathway. Progesterone is then converted to 17-hydroxyprogesterone by CYP17 (Fig. 16.4) [20]. Importantly, CYP17 is located exclusively in thecal and interstitial cells, the

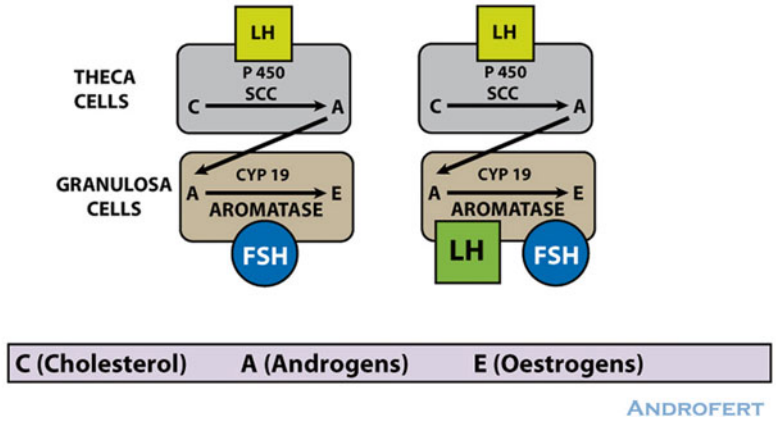

Fig. 16.5 The "two-cell" system. FSH receptors are present exclusively in the granulosa cells. LH receptors are present in the theca cells and initially absent in the granulosa cells. In response to LH, theca cells convert cholesterol to androgens (testosterone and androstenedione). CYP17 is located exclusively in thecal cells, whereas CYP19 (aromatase) is expressed only in the granulosa. Thus, androgens must diffuse into the granulosa layer to be converted to estrogen via aromatization induced by FSH. Both FSH and LH act via AMPc production. In the late follicular phase, FSH induces LH receptor formation in the granulosa cells, which acquire LH responsiveness. In the granulosa, LH enhances FSH action (increasing estrogen production)

extrafollicular compartment of the ovary, whereas CYP19 (aromatase), that converts androgens to estrogens, is expressed exclusively in GC, the intrafollicular compartment [20–22]. Thus, aromatization of androgens to estrogens is a distinct activity within the granulosa layer induced by FSH via activation of the P450 aromatase (P450arom) gene. Androgens produced in the theca layer must therefore diffuse into the granulosa layer to be converted to estrogens (Fig. 16.5). Hence, increasing levels of estradiol in the peripheral circulation during the follicular phase reflect the release of estrogen from granulosa cells into blood vessels [15, 22]. Theca and granulosa cells also secrete peptides that act as both autocrine and paracrine factors. Insulin-like growth factor (IGF) is secreted by theca cells and enhances LH-mediated androgen production within the thecal compartment as well as FSH-mediated aromatization in granulosa cells [15]. Inhibin and activin are produced in the granulosa cells in response to FSH and modulate the expression of steroidogenic enzymes, especially P450c17 in theca cells. While inhibin enhances androgen synthesis, activin has an opposite effect. Activin also has the important autocrine role of enhancing FSH action mainly by increasing the expression of FSH receptors [15] (Fig. 16.6).

LH also acts in GC to stimulate progesterone production. Most circulating progesterone (~95 %) is produced in the intrafollicular compartment by the granulosa cells via the action of 3b-HSD that catalyzes conversion of pregnenolone (delta-4 pathway) under the LH influence (see Fig. 16.4) [18, 22]. Despite a marked increase in progesterone levels measured at the veins of the active ovary in the mid-follicular phase, peripheral concentrations increase only slightly probably due to active liver metabolism [23]. Progesterone can be further converted to 17-hydroxyprogesterone by CYP17 (via delta-4 pathway). However, very little 17-hydroxyprogesterone is converted to androstenedione, since human CYP17 catalyzes this reaction at only 3 % of the rate for the conversion of 17-hydroxypregnenolone to DHEA [18, 22, 24]. Therefore, 17-hydroxyprogesterone is basically the final product of the delta-4 pathway in humans. Moreover, progesterone itself cannot be metabolized in the GCs because CYP17 is not expressed within this cell compartment; as such, progesterone is the final product of the delta-4 pathway in the intrafollicular compartment and cannot be converted to estradiol in the GC under the effect of LH [20]. The preovulatory rise in progesterone facilitates the positive feedback action of

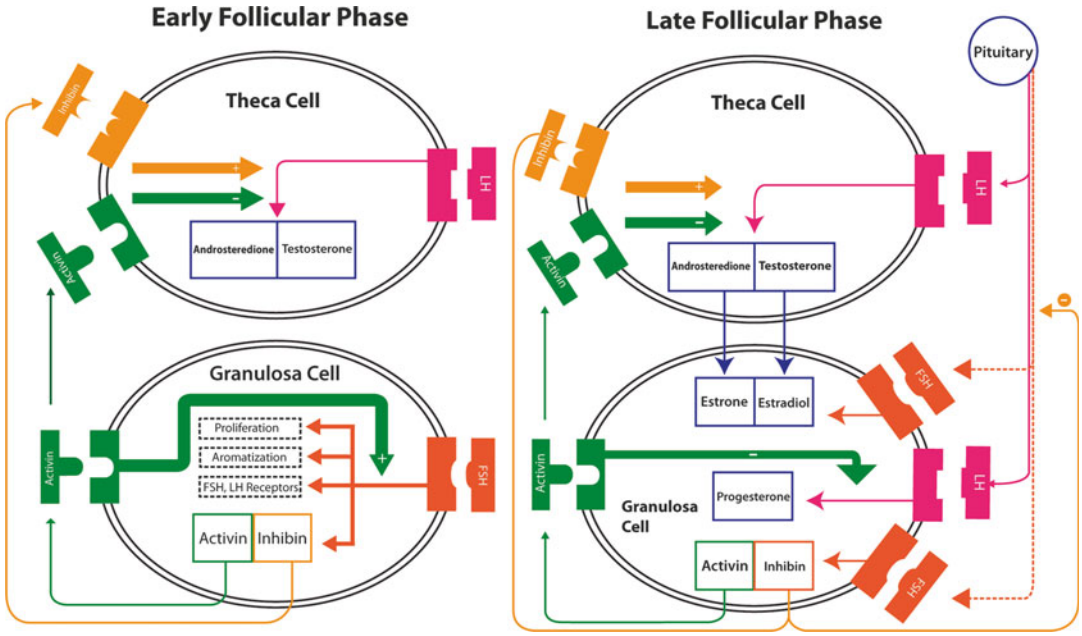

Fig. 16.6 Modulation of steroidogenic enzymes. In the early follicular phase, inhibin and activin are produced in the granulosa cells in response to FSH. They have important paracrine functions to modulate the expression of steroidogenic enzymes, especially P450c17 in theca cells. Inhibin enhances LH function, thus stimulating androgen synthesis to latter aromatization to estrogen in the granulosa, whereas activin suppresses androgen synthesis. Activin has also an important autocrine role of enhancing FSH action, especially by increasing the production of FSH receptors. Production of inhibin by the granulosa cells is increased in the late follicular phase while activin is decreased, with a positive effect on androgen production by theca cells. FSH induces LH receptor formation in the granulosa cells, which acquire LH responsiveness and therefore less FSH dependence. In granulosa, LH enhances FSH action that in turn increases estrogen production, initiates progesterone production (negatively modulated by activin), and control granulosa production of inhibin. The increase in inhibin, in turn, suppresses FSH secretion by the pituitary, important to ensure the dominance of the follicle

estrogen on the pituitary; the latter is the key factor to induce the midcycle LH peak in the natural cycle. Progesterone also stimulates a midcycle FSH surge, important to support the full expression of LH receptors at the granulosa layer [22, 25].

In summary, ovarian steroidogenesis is the result of combined LH and FSH stimulation of the two cell types, theca and granulosa, influenced by autocrine and paracrine factors.

16.2.2 The Role of LH on Follicular Maturation and Luteal Phase Support

In the mid-follicular phase, FSH induces LH receptor expression in the granulosa cells of developing follicles [26]. The action of LH on its receptors activates cyclic AMP-protein kinase A (cAMP/PKA) pathway, which represents an additional stimulus to follicular growth [27]. Thereby, the maturing follicle also reduces its dependency on FSH by acquiring LH receptors and LH responsiveness [26–30]. FSH and LH cooperate in inducing the local production of the soluble molecule inhibin B and growth factors. Among these, insulin growth factors (IGF) I and II, which are expressed by both granulosa and theca cells throughout folliculogenesis, are important in promoting follicular maturation [31, 32]. Furthermore, LH exerts an antiapoptotic effect on the GCs, mediated by the production of fibroblast growth factors that maintain calcium homeostasis and granulosa cell viability by stimulating calcium efflux via a protein kinase C (PKC) delta-dependent pathway [33]. Additional signaling

pathways (e.g., AKT and ERK1/2 pathways) involve the expression of EGF-like growth factors that influence GC proliferation, differentiation, and survival (apoptosis blockage) [34, 35]. Lastly, aromatase expression and steroidogenic function via LH receptor activation are likely to involve cAMP/PKA, extracellular signal-regulated (ERK) 1 and 2, and AKT pathways, all playing a crucial role in the final stages of maturation of human oocytes and follicles [36, 37].

LH activity during the luteal phase is totally responsible for the maintenance and the steroidogenic activity of the corpus luteum [38]. LH is responsible for the upregulation of growth factors like vascular endothelial growth factor A [39, 40], which plays a dynamic role in luteal angiogenesis, and epidermal growth factor-like ligands, amphiregulin and epiregulin, which regulate apoptosis in luteinized human granulosa cells [34, 41–44]. Furthermore, LH stimulates expression of extragonadal LH receptors in the endometrium [45, 46] and production of cytokines involved in implantation [47].

Therefore, LH regulates both granulosa and theca cells and has a pivotal role in follicular development and maturation. In light of the aforementioned findings, we can conclude that (1) both gonadotropins contribute (via granulosa) to maintain the autocrine–paracrine system governing dominant follicle's growth; (2) LH is crucial in sustaining FSH activity in the granulosa during intermediate–late stages of folliculogenesis; and (3) LH is critical for maintaining corpus luteum function during the luteal phase.

16.3 Rationale of LH Supplementation in Stimulated Cycles

The "LH window" concept, as outlined by Shoham in 2002, proposes that in the absence of a threshold level of serum LH, estradiol production will be insufficient for follicular development, endometrial proliferation, and corpus luteum formation [48]. This concept can be clearly observed in patients with hypogonadotrophic hypogonadism who do not achieve adequate steroidogenesis by stimulation with FSH alone, but resume sufficient estradiol production by LH supplementation [49]. Evidence therefore suggests that in reproductive cycles optimal follicular development occurs within a "LH window," that is, above an LH threshold of 1.1 and below an LH ceiling of 5.1 IU/L [48, 49].

After pituitary suppression, still widely used in association with COS, residual circulating levels of endogenous LH are usually adequate to support multiple follicular growth and oocyte development in COS with gonadotropins devoid of LH activity [50, 51]. In fact, only 1 % of LH receptors need to be occupied to drive adequate ovarian steroidogenesis. Fair evidence indicates that most normogonadotropic women have sufficient levels of endogenous LH and do not require exogenous LH supplementation [52–54]. Despite of that, a recent large meta-analysis including a total of 40 RCTs and 6443 women aged 18–45 years found a small relative increase (estimate of 9 %) in clinical pregnancy rate in patients treated with of rec-hFSH plus rec-hLH versus rec-hFSH alone (RR 1.09; 95 % CI 1.01–1.18) [55]. More importantly, ovarian response to COS with FSH-only-containing gonadotropins has shown to be suboptimal in subsets of normogonadotropic women, including those with advanced reproductive age (≥35 years old) [56, 57], diminished ovarian reserve [54, 58], and highly suppressed levels of endogenous LH, in whom LH activity falls below the LH threshold [59–63]. In addition, a subgroup of normogonadotropic patients who had normal estimated ovarian reserve but suboptimal responses to FSH-alone stimulation has also been identified and termed "hyporesponders" [64–67].

Clinical evidence indicates that the aforementioned subgroups have less responsive ovaries in stimulated cycles with FSH, which could be explained by a wide range of factors, including reduced paracrine ovarian activity [68], genetically determined reduced LH bioactivity [69], reduced androgen secretory capacity [70], and decreased number of functional LH receptors [71]. Serum androgen levels, especially total testosterone (T), calculated free T, dehydroepiandrosterone sulfate, and androstenedione, decline

steeply with age, with the decline of each being greater in the early reproductive years than the later decades [72, 73]. Hence, it has been hypothesized that such women would benefit from LH-containing gonadotropin preparations. Action of LH at the follicular level could promote an increase in ovarian steroidogenesis and androgen production for its later aromatization into estrogens, with a positive impact on the follicular milieu. Furthermore, LH has also a direct effect on follicular growth and maturation via different signaling pathways that positively impact oocyte quality [74].

16.4 Clinical Evidence Supporting LH Supplementation During COS in Selected Patients

16.4.1 Hypogonadotropic Hypogonadism

The European Recombinant Human LH Study Group investigated the efficacy of rec-hLH for supporting FSH-induced follicular development in hypogonadotropic hypogonadal women (LH levels of <1.2 IU/l; WHO group I anovulation) [49]. Thirty-eight patients were randomized to receive 0, 25, 75, or 225 IU/day of rec-hLH in addition to a fixed dose of rec-hFSH (150 IU/day). The authors found that rec-hLH was able to promote a dose-related increase in estradiol and androstenedione secretion by rec-hFSH-induced follicles. Serum concentrations on the last day of FSH administration were 65 ± 4, 195 ± 94, 1392 ± 585, and 2441 ± 904 pmol/L for E2 and 3.6 ± 0.9, 5.1 ± 1.3, 6.4 ± 1.3, and 6.7 ± 1.3 nmol/L for androstenedione in the patients treated with 0, 25, 75, and 225 IU rec-hLH, respectively. LH supplementation also increased ovarian sensitivity to FSH, as shown by the proportion of patients who developed follicles after the administration of a defined dosage of FSH. While only 12.5 % of the patients treated with FSH alone developed follicles, the proportion substantially increased, according to the varying doses of rec-hLH (42.8 % in 25 IU and 77.8 % and 80 % in 75 IU and 225 IU, respectively). Furthermore, follicles that had been exposed to rec-hLH showed an increased ability to luteinize after hCG exposure.

In the aforementioned study, a daily dose of 75 IU rec-hLH was effective in the majority of women in promoting optimal follicular development (defined as >or = 1 follicle >or = 17 mm; E2, >or = 400 pmol/L; midluteal phase progesterone, >or = 25 nmol/L) and maximal endometrial growth. Lastly, rec-hLH was shown not to be immunogenic and was well tolerated by the patients.

In conclusion, exogenous LH activity supplementation is mandatory in stimulation protocols applied to women with hypogonadotropic hypogonadism.

16.4.2 Older Women (>35 Years Old)

The impact of luteinizing hormone administration in ovarian stimulation with gonadotropin-releasing hormone (GnRH) antagonist cycles was examined by Bosch and colleagues in a randomized controlled trial (RCT) involving 720 women undergoing their first or second IVF [75]. The authors compared cycle outcome, according to the use of rec-hFSH or rec-hFSH + rec-hLH in an age-adjusted analysis. For the patients <36 years old, the total starting dose of gonadotropins was 225 IU/d for both stimulation protocols. In the rec-hFSH-alone group, 225 IU/d SC of rec-hFSH was administered, and the starting dose for the rec-hFSH + rec-hLH group was 150 IU/d of rec-hFSH and 75 IU/day of rec-hLH. As noted, LH supplementation was started on stimulation day 1. For those patients aged 36–39 years, the total starting dose of gonadotropins was 300 IU/d for both study groups: rec-hFSH-alone, 300 IU/d SC of rec-hFSH was administered, and for the rec-hFSH + rec-hLH group, 225 IU/d of rec-hFSH + 75 IU/d rec-hLH. The rec-hLH dose remained fixed across the cycle. In the younger population (up to 35 years old), implantation rates were similar, 27.8 % versus 28.6 %, odds ratio (OR) 1.03 (95 % confidence interval [CI] 0.73–1.47), as was the ongoing pregnancy rate per started cycle, 37.4 % versus 37.4 %, OR 1.0 (95 % CI 0.66–1.52). In older patients

(36–39 yrs.), the implantation rate was significantly higher in the rec-hFSH + rec-LH group: 26.7 % versus 18.6 %, OR 1.56 (95 % CI 1.04–2.33). Ongoing pregnancy rates per started cycle were not statistically different: 33.5 % versus 25.3 %, OR 1.49 (95 % CI 0.93–2.38).

Contrary results have been reported by Konig et al. in an RCT involving 253 couples undergoing IVF/ICSI [76]. In their study, women were 35 years or older and received ovarian stimulation in a GnRH antagonist protocol with either rec-hFSH 225 IU/day or rec-hFSH + rec-hLH 150 IU/d starting on stimulation day 6. The intention-to-treat analysis revealed implantation rates (18.8 % vs. 20.7 %; mean difference −1.9 %, 95 % confidence interval [CI] −8.0 to 11.7) and clinical pregnancy rates (28.0 % vs. 29.7 %; mean difference −1.5 %, 95 % CI −9.4 to 12.7).

A systematic review and meta-analysis of the studies examining the age-related effects of LH supplementation in COS were conducted by Hill and colleagues [57]. The authors demonstrated that LH supplementation in women aged >34 years old undergoing COS with rec-hFSH was beneficial. Their study included 7 RCTs (902 women) and compared COS using rec-hFSH alone or in combination with rec-hLH. GnRH-agonist downregulation was used in five trials, while GnRH antagonist and GnRH-agonist micro-flare were used in the remaining trials. The dose and day of starting rec-hLH supplementation varied among trials. In five of them a fixed dose of 150 IU rec-hLH, which started either on the sixth or seventh stimulation day, was used. One trial used a fixed 2:1 ratio of rec-hFSH and rec-hLH, while another used a fixed dose of 75 IU rec-hLH regardless of the FSH dose; in both of them LH supplementation was given from the first day of stimulation on. Implantation (OR = 1.36; 95 % CI: 1.05–1.78, $I^2 = 12$ %) and clinical pregnancy rates (OR = 1.37; 95 % CI: 1.03–1.83, $I^2 = 28$ %) were significantly higher for women who received rec-hLH in addition to rec-hFSH compared with those in whom rec-hFSH was administered alone.

The meta-analysis by Hill et al. was subsequently reexamined by Konig and colleagues, who replaced the study of Bosch and cols. by their own and concluded that no effect whatsoever could be observed by adding LH to older patients [77]. Nevertheless, a methodological bias could have been produced by replacing the Bosch and colleagues' study, which represented 36 % of the weight of all studies pooled in the aforementioned meta-analysis, by the one of Konig and cols. because the protocols of COS differed with regard to the day LH supplementation has started; LH supplementation was started in the mid-follicular phase in the latter in contrast to the former, in which rec-LH was administered since the first day of stimulation. It has been suggested that LH supplementation should be initiated on the beginning day of stimulation to get total advantage of the LH effects on both theca and granulosa cells [78].

In conclusion, evidence suggests that rec-hLH supplementation has a positive effect on cycle outcome of older women (>35 years old), particularly when used from the start of COS. Nevertheless, given the heterogeneity of the published data, additional large RCTs examining the impact of rec-hLH supplementation from the early phases of COS are needed to draw a conclusive recommendation about the routine incorporation of rec-hLH in older women undergoing IVF/ICSI.

16.4.3 Poor Responders

Mochtar et al., evaluating poor responders, have demonstrated the usefulness of adding rec-hLH to COS. These authors pooled three RCTs including 310 participants and showed that higher ongoing pregnancy rates (OR = 1.85; 95 % CI: 1.1–3.11) were obtained in patients treated with the combination of rec-hFSH and rec-hLH compared with rec-hFSH alone [54]. In another meta-analysis of Bosdou et al., which included 7 RCTs and 603 patients classified as poor responders, differences in clinical pregnancy were not detected in the group of patients receiving LH supplementation [58]. Nevertheless, the definition criteria for poor responders were not uniform among the included studies, and two of the RCTs evaluated slow/hyporesponders rather than poor responders.

The protocols of stimulation also varied as GnRH antagonists and agonists were applied in two trials each, and GnRH-agonist short protocol was used in three subjects. The way rec-hLH supplementation was given also varied as daily doses of either 75 IU or 150 IU were used, and the starting day differed or was not traced. Rec-hLH was added to rec-hFSH from the first stimulation day in one trial, at stimulation day 7 in three trials, at day 8 in one trial, and on the day of the first GnRH antagonist injection in another trial. Although statistical significance was not reached, the magnitude of the effect size and the width of the 95 % CI regarding the clinical pregnancy rates (RD = +6 %; 95 % CI: −0.3 to +13 %; $p = 0.06$) suggested a potential clinical benefit of LH supplementation. Nevertheless, the authors of the aforesaid meta-analyses did find that rec-hLH supplementation was beneficial in terms of live birth rates after IVF (RD = +19 %; CI: +1 to +36 %), but their results were derived from a single RCT.

Lately, a large meta-analysis assessed the outcomes of rec-hFSH plus rec-hLH or rec-hFSH alone for ovarian stimulation in association with GnRH analogues during ART [55]. A total of 40 RCTs involving 6443 women aged 18–45 years were included, of which 14 studies (1129 patients) specifically investigated poor responders. Poor response (POR) was defined according to study authors' criteria and although the studies were published prior to the European Society of Human Reproduction and Embryology (ESHRE) consensus definition of POR [79], in 10 of the 14 studies reporting POR data, the definition of POR employed was aligned with the subsequently reported ESHRE definition. According to the ESHRE consensus, POR is defined by the presence of at least two of the following three features: (1) advanced maternal age (≥40 years) or any other risk factor for POR, (2) a previous POR (≤3 oocytes with conventional stimulation), and (3) an abnormal ovarian reserve test (antral follicle count [AFC] <5–7; anti-Mullerian hormone [AMH] <0.5–1.1 ng/mL), but two episodes of POR after maximal COS per se are sufficient to define a patient as poor responder. Patients of advanced age with an abnormal ORT may be classified as POR since these features indicate reduced ovarian reserve and act as a surrogate of ovarian stimulation cycle outcome. In this case, the patients should be defined as "expected poor responder" [79]. In the aforementioned study by Lehert and colleagues, significantly more oocytes were retrieved with rec-hFSH plus rec-hLH versus rec-hFSH alone in poor responders (12 studies, $n = 1077$; weighted mean difference +0.75 oocytes; 95 % CI 0.14–1.36). Also, significantly higher clinical pregnancy rates were observed in this patient category with rec-hFSH plus rec-hLH versus rec-hFSH alone (14 studies, $n = 1179$; RR 1.30; 95 % CI 1.01–1.67; ITT population).

In conclusion, current evidence suggests that there is an increase in both the number of oocytes retrieved and clinical pregnancy rates in poor responders treated with rec-hLH in addition to rec-hFSH.

16.4.4 Hyporesponders

The concept of "hypo-response" to COS has been proposed to identify those at first hand good prognosis normogonadotropic young women with normal ovarian reserve who turn out to require high amounts of rec-hFSH (e.g., >2500 IU total dose) to obtain an adequate number (i.e., >4) of oocytes retrieved [64–67]. Such normoovulatory and normogonadotropic women differ from classical poor responders because they are usually young (<39 years) and ovarian biomarkers (AMH/AFC) are within normal ranges. Although the pathogenesis of hyporesponsiveness to FSH is unknown, it has been speculated that hypo-response is a genetically determined condition (*see Chapter 14: Pharmacogenomics Approach to Controlled Ovarian Stimulation*). More specifically, ovarian resistance to exogenous FSH has been associated with the presence of at least two genetic variations, including a polymorphic allele of the LH beta-subunit gene (v-betaLH), which has been shown to have altered in vitro and in vivo activities, and FSH receptor (FSH-R) Ser/680 variant [69, 80–83]. Given a possible association between ovarian resistance to FSH stimulation (hypo-response)

and a genetically determined less bioactive LH molecule or FSH-R dysfunction, several investigators have examined the roles of exogenous LH activity supplementation and increased FSH doses on ART cycle outcome.

The role of LH supplementation and increased FSH doses in hyporesponders were evaluated by Ferraretti and colleagues, who conducted an RCT involving 184 patients (age <38 years) undergoing COS for IVF after pituitary desensitization [67]. Hyporesponsiveness to rec-hFSH was defined by the observation of a steady follicular growth (>10 antral follicles ≥8 mm in diameter) and estradiol levels (≥100 pg/mL) between stimulation days 7–10 despite continuous rec-hFSH administration. Upon reaching this stage, patients were randomized to receive (1) an increased rec-hFSH dose alone (max 450 IU/daily; $n=54$), (2) LH activity supplementation with rec-hLH (75 IU/day or 150 IU/day) in addition to an increased FSH dose ($n=54$), and (3) LH activity supplementation with hMG in addition to an increased rec-hFSH dose ($n=26$). Fifty-four age-matched women with normal responses to COS were included as a control group. The average number of oocytes retrieved was significantly lower in hyporesponders treated with rec-hFSH step-up (8.2) versus the other three groups (11.1, 10.9, 9.8), respectively. Pregnancy rates were significantly higher in the group treated with rec-hLH plus increased rec-hFSH dose (54.4 %) compared with both the patients receiving rec-hFSH alone (24.4 %) and hMG (11 %; $p<0.05$). Pregnancy rates in the group of women receiving rec-hLH supplementation and an increased rec-hFSH dose were not different from controls (41 %). Although live birth rates in both rec-hLH (40.7 %) and control (37 %) groups were twofold higher than the other two groups (22 % and 18 %, respectively), the difference did not reach statistical significance.

The role of rec-hLH supplementation per se in hyporesponders was evaluated by De Placido and colleagues, who conducted a multicenter RCT involving a total of 117 IVF/ICSI cycles [66]. Hyporesponders (age <37 years, basal FSH ≤10 IU/l) were defined by the presence of low serum estradiol levels (below 180 pg/mL) and at least 6 follicles ranging between 6 and 10 mm, but no follicles over 10 mm on stimulation day 8 with rec-hFSH. After pituitary desensitization and stimulation with a fixed dose (225 IU/day) of rec-hFSH for the first 8 days, the patients were randomized to receive either an additional 150 IU/day of rec-hLH supplementation ($n=65$) or an increase in the daily dose of rec-hFSH by 150 IU/day ($n=65$; rec-hFSH "step-up" protocol). An age/BMI-matched population of "normal responders" (i.e., tripling E_2 levels between stimulation days 5 and 8 and more than 4 follicles >10 mm on stimulation day 8) was selected as a control group ($n=130$). The number of oocytes retrieved was significantly higher in the patients who received rec-LH supplementation (9.0 ± 4.3) compared with those in whom an increased dose of rec-hFSH was administered (6.1 ± 2.6; $p<0.01$), and the results of both aforementioned groups were lower than those obtained in the control group (10.49 ± 3.7; $p<0.05$). Implantation and ongoing pregnancy rates were similar in "hyporesponders" treated with rec-hLH and "normal responders" (14.2 % and 32.5 % vs. 18.1 % and 40.2 %, respectively). Conversely, both parameters were significantly lower ($p<0.05$) in "hyporesponders" treated with step-up rec-hFSH (10.0 and 22.0 %).

In conclusion, these studies reinforced the idea that hyporesponders benefit from LH supplementation and that hyporesponsiveness to rec-hFSH could be related to the presence of less bioactive LH due to specific genetic-determined variations in the beta subunit of the LH molecule.

16.4.5 Deeply Suppressed LH Levels

Profound suppression of LH concentrations as a consequence of GnRH-agonist downregulation has been found in 7–48 % of normogonadotropic women undergoing controlled ovarian stimulation [60, 61, 84, 85]. This wide variation might be justified by the type and mode of GnRH-agonist action. Intranasal administration of buserelin resulted in significantly less depressed levels of mid-follicular LH levels compared with the subcutaneous route. Moreover, the inhibitory

effect on ovarian steroidogenesis and follicular development is more evident with the more potent buserelin than with leuprolide acetate [61, 85].

Early studies have suggested that ovarian response and IVF cycle outcome are negatively impacted when mid-follicular serum LH levels are below a certain threshold (between 0.5 and 0.7 UI/L) after downregulation with GnRH agonists and ovarian stimulation with FSH monotherapy [59–63, 84]. Westergaard and colleagues, retrospectively analyzing 200 normogonadotropic women, reported that as many as 49 % of those stimulated with rec-hFSH under pituitary suppression with buserelin acetate (0.5 mg SC daily) for 14 days achieved very low concentrations of LH (<0.5 IU/L) in the mid-follicular phase, albeit GnRH-a dose was reduced to 0.2 mg SC per day during ovarian stimulation [60]. In comparison with the normal LH group, these women had serum estradiol concentrations significantly lower on Sd8 (1349 ± 101 vs. 2908 ± 225 pmol/L; $p < 0.001$). Although the proportion of patients with a positive pregnancy test was similar in the two groups (30 % vs. 34 % per started cycle in the low and normal LH groups, respectively), a fivefold higher risk of early pregnancy loss was observed in the low LH group (45 % vs. 9 %; $p < 0.005$). In another study, Fleming and colleagues found that 26 % of women treated with highly purified or recombinant FSH and GnRH agonist (type and dose not reported) had suppressed LH concentration (≤0.7 IU/l) on Sd7 [84]. Patients with suppressed LH had lower estradiol concentrations ($p = 0.001$) irrespective of whether the FSH is derived from purified urinary or recombinant sources. However, the negative impact on cycle outcome (longer treatment duration combined with a reduced oocyte yield) was observed only in women treated with urinary FSH, thus indicating that the more potent recombinant FSH treatment could overcome the impact of LH suppression upon gross ovarian response. Furthermore, no effect upon pregnancy outcome was observed irrespective of the level of LH suppression and type of FSH preparation. Humaidan and colleagues also studied the effect of LH levels on stimulation day 8 on ovarian response and pregnancy outcome [61]. The authors retrospectively analyzed 207 normogonadotropic women receiving pituitary downregulation with buserelin acetate (0.8 mg SC daily until pituitary downregulation and 0.4 mg/day during ovarian stimulation) and ovarian stimulation with rec-hFSH. LH levels on Sd8 were directly related to estradiol levels and inversely related to the total consumption of exogenous FSH and duration of gonadotropin stimulation ($p < 0.002$). In their study, however, only 12 % of the patients showed LH levels <0.5 IU/L. While the number of retrieved oocytes was not affected by LH suppression, the frequency of fertilized oocytes was significantly lower in the group with profound LH suppression ($p < 0.05$). Likewise in the study of Fleming and cols., pregnancy and implantation rates were not significantly affected by profound mid-follicular LH suppression. Taken together, these aforementioned studies indicate that deeply suppressed LH levels in GnRH agonist-treated women have a significant impact on the ovarian response during ovarian stimulation, but its impact on pregnancy outcome is controversial.

Contrary results have been reported by Balasch and colleagues studying 144 infertile women undergoing IVF/intracytoplasmic sperm injection (ICSI) treatment, in whom pituitary desensitization was carried out by the administration of leuprolide acetate (1 mg SC daily, then reduced to 0.5 mg after downregulation was confirmed) [85]. Using a receiver-operating characteristic (ROC) analysis, the authors showed that the serum LH concentration on Sd7 was unable to discriminate between conception and non-conception cycles (AUC = 0.52; 95 % CI: 0.44–0.61). In this study, only 7 % of the patients had mid-follicular LH serum concentration <0.5 IL/L, and no significant differences were found with respect to ovarian response, number of oocytes retrieved, IVF/ICSI outcome, implantation, and the outcome of pregnancy between these patients and those with normal LH on Sd7.

In conclusion, conflicting evidence exists regarding the impact of deeply suppressed levels of mid-follicular serum LH levels on ovarian response to stimulation with rec-FSH. Current data indicate that the choice of GnRH-a plays a role in the frequency of patients exhibiting profound mid-follicular LH suppression. Whether these women would benefit from supplementation with LH activity during ovarian stimulation remains to be proven.

16.5 Gonadotropin Preparations Containing LH Activity

Currently, there are three commercially available gonadotropin preparations containing LH activity: (1) urinary hMG, in which LH activity is dependent on hCG rather than LH, (2) pure LH glycoprotein produced by recombinant technology (lutropin alfa), and (3) a combination of FSH (follitropin alfa) and LH (lutropin alfa) in a fixed ratio of 2:1 also manufactured by recombinant technology (Sect. 16.1) [3].

16.5.1 Menotropin

Menotropin, or human menopausal gonadotropin (hMG), was first extracted from the urine of postmenopausal women in 1949 [2]. Early preparations contained varying amounts of FSH, LH, and hCG in only 5 % pure forms [1–3]. Improvements in the purification techniques standardized FSH and LH activities to 75 IU for each type of gonadotropin in 1963, as measured by standard in vivo bioassays (Steelman–Pohley assay). Human menopausal gonadotropin preparations have both FSH and LH activity, but the latter is primarily derived from the hCG component present in postmenopausal urine and concentrated during purification [2, 86, 87, 88]. Sometimes hCG is added to achieve the desired amount of LH-like biological activity [2]. In 1999, purified hMG gonadotropins were introduced, allowing its subcutaneous (SC) administration [2, 3]. At present, both conventional hMG and highly purified hMG (HP-hMG) are commercially available in vials containing lyophilized powder of FSH and LH at 1:1 ratio [3]. The enhanced purity of HP-hMG enabled subcutaneous delivery [3].

16.5.2 Lutroprin Alfa

Lutroprin alfa was introduced in the market in the year 2000 intended for promoting ovarian stimulation in women with WHO type I anovulation. The manufacturing process of lutropin alfa involves recombinant technology in which the genes coding for human LH alpha and beta subunits are incorporated into the nuclear DNA of Chinese hamster ovary (CHO) cells via a plasmid vector [2, 3, 88]. As a result, a master LH-producing cell bank is built [88, 89]. A working cell bank is then made by growing cells in culture flasks, which are afterwards combined with a suspension of microcarrier beads and transferred to a bioreactor vessel with continuous culture media infusion. The cell culture supernatant medium, containing the proteins secreted by the cells, is collected from the bioreactor. The harvested "crude LH" is then purified by chromatography, followed by ultrafiltration. Each purification step is rigorously controlled in order to ensure batch-to-batch consistency of the final purified product that is the recombinant human LH (rec-hLH) [3].

Lutropin alfa is highly pure and has high biological activity (9000 IU/mg protein) [3, 90]. It is presented in vials of 82.5 IU lyophilized pure glycoprotein powder to be reconstituted with diluent before administration using a conventional syringe and needle (75 IU of lutropin alfa is delivered per vial). Lutropin alfa is intended for subcutaneous daily injections, which represents an important gain for patients as better tolerability (lower pain at injection site) has been reported with SC injections compared with the intramuscular route. Importantly, SC injections allow self-administration that is more convenient and less time consuming as patients need fewer visits to the clinic or hospital for injections [91, 92]. Due to the relatively short half-life of LH, daily injections of lutropin alfa are needed during the stimulation period [3, 90]. After each injection, terminal half-life is reached within 10–12 h, and then LH levels decline until the next injection.

16.5.3 Follitropin Alfa in Combination with Lutropin Alfa

A preparation containing both rec-hFSH (follitropin alfa) and rec-hLH (lutropin alfa) at 2:1 ratio was launched in 2007 [93]. The 2:1 ratio of FSH and LH in a fixed dose combination was obtained by recombinant technology and vial

Table 16.1 Differences in LH activity among gonadotropin preparations

	Purity (LH content)	FSH activity (IU/vial)	LH activity (IU/vial)	hCG content (IU/vial)	Specific activity (LH/mg protein)
Lutropin alfa	>99 %	0	75[a]	–	9000
Follitropin alfa + lutropin alfa 2:1 ratio	>99 %	150	75	–	9000
HP-hMG	Unknown[b]	75	75[b]	~8	–

[a]1 μg of lutropin alfa = 22 IU
[b]Derives primarily from the hCG component, which preferentially is concentrated during the purification process and sometimes was added to achieve the desired amount of LH-like biological activity
HP-hMG highly purified human menopausal gonadotropin

filling using protein mass (FbM). The use of FbM as opposed of filled-by-bioassay was possible due to the specific activity; isoform distribution and sialylation profile of both gonadotropins are highly consistent among manufactured batches [88]. It is intended for subcutaneous daily injections and is presented in vials of lyophilized pure glycoprotein powder to be reconstituted with diluent before administration using a conventional syringe and needle (150 IU of follitropin alfa and 75 IU of lutropin alfa is delivered per vial). The results of two phase I, randomized, crossover studies demonstrated bioequivalence between rec-hFSH and rec-hLH administered alone or in fixed 2:1 combination, thus allowing administration of both recombinant gonadotropins in a single injection [94].

16.6 Differences in LH Activity Between rec-hLH and hMG Preparations

Recombinant LH has three major differences compared with hMG preparations. First, rec-hLH has a better quality and safety profile compared with hMG [3, 90]. High purity and specific activity are common features of gonadotropin preparations manufactured using recombinant technology. Each product batch of recombinant LH is routinely characterized and controlled using physicochemical techniques, including size-exclusion high-performance liquid chromatography (SE-HPLC), which allows assessment of both the integrity and the amount of glycoproteins, and isoelectric focusing (IEF) and glycan mapping, which characterize protein glycoforms present in each preparation [95, 96]. Conversely, the manufacturing process of urine-derived gonadotropins is less stringent, as urine is pooled and the donor source cannot be fully traced. As the pool is constantly changing, standardization is difficult to ascertain [97–99]. Although sophisticated purification techniques are currently available, which allow the safe clinical use of urinary formulations, extraneous urinary proteins may account for more than 30 % of the protein content in high-purified hMG products (Table 16.1) [3, 100].

Second, rec-hLH is associated with better dose precision due to fill-by-mass (FbM) technology that virtually eliminates batch-to-batch variation [2, 3, 100, 101]. The conventional method used to quantify the glycoprotein activity in gonadotropin products is the Steelman–Pohley assay, which is an in vivo rat bioassay. As well as being costly and subject to ethical concerns related to the use of animals, this technique has an inherent variability of up to 20 % [101, 102]. In 2003, Driebergen and Baer demonstrated the batch-to-batch consistency of follitropin alfa in terms of specific activity, isoform pattern, and sialylation profile [101]. The authors showed that there was a constant relationship between FSH mass and its biological activity. Following these observations, a new method was developed to calibrate each batch of follitropin alfa, and also lutropin alfa, using SE-HPLC, which measures glycoprotein content by protein mass. This technique has enabled lutropin alfa to be filled and released on the basis of mass (75 IU of

Table 16.2 Structural characteristics, half-life in serum, and downstream effects of LH and hCG following receptor binding

	LH	hCG
Amino acid number		
Alpha subunit	92	92
Beta subunit	121	145
N-linked glycosylation sites		
Alpha subunit	2	2
Beta subunit	1	2
O-linked glycosylation sites	–	4
Carboxyl terminal segment	Nonexistent	Present
Half-life (hours)		
Initial, range of mean	0.6–1.3	3.9–5.5
Terminal, range of mean	9–12	23–31
Response		
ED50 (pM)[a]	530.0 ± 51.2	107.1 ± 14.3
Time to maximal cAMP accumulation[a]	10 min	1 h
ERK 1/2 activation[b]	Strong	Weak
AKT activation[b]	Strong	Minimal
CYP19A1 expression in presence of ERK1/2 pathway blockade[b]	Increased	Unaffected

Initial half-life (distribution): time for the plasma concentration to decrease steeply because of the distribution into tissues

Terminal (elimination) half-life = time that it takes for the concentration in blood plasma of a substance to reach one-half of its steady-state value

ERK extracellular signal-regulated kinases, *AKT* protein kinase B, *CYP19A1*0 cytochrome P450, family 19, subfamily A1

[a]Median effective dose to produce a response 50 % of the COS-7/LHCGR cells, which constitutively express LH receptors

[b]Effect on human granulosa cells

LH assessed by the Steelman–Pohley assay corresponds to 3.4 μg of lutropin alfa), with dose variability of only 2 % [90, 101].

Third, LH activity is derived from pure LH glycoprotein unlike hMG, in which hCG is concentrated during purification or added to achieve the desired amount of LH-like biological activity [2, 3, 90]. LH and hCG differ in the composition of their carbohydrate moieties which, in turn, affect bioactivity and half-life. Although hCG amino acid sequence is similar to that of LH, a notable difference is the presence of a long carboxyl terminal segment with 24 AA containing four sites of O-linked oligosaccharides [3, 103] (see Fig. 16.1). Furthermore, hCG beta subunits contain two sites of N-linked glycosylation compared with a single site in LH. Due to the higher number of both glycosylation sites and sialic acid residues (approximately 20) than LH, hCG exhibit a markedly longer terminal half-life. After administration, recombinant human LH is eliminated with a terminal half-life of 10–12 h in contrast to 23–31 h of hCG [10, 104] (Table 16.2).

16.6.1 Differences Between LH and hCG: Evidence from In Vitro Studies

Although both human LH and hCG act on the same LH/hCG receptor (LH-R), evidence from in vitro models indicates that LH receptors differentiate between LH and hCG coupling. While LH exclusively stimulate the targeted LH-R by cis-activation, hCG is also capable of inducing transactivation, thus affecting the kinetics of cAMP production and downstream ERK1/2- and AKT-pathway activation [105, 106]. Using equimolar concentrations of LH and hCG in human granulosa cells obtained from women undergoing oocyte retrieval for ART, Casarini and colleagues have shown that hCG is fivefold more potent in vitro than LH at the receptor

level based on the measurement of intracellular cAMP [105]. Using equipotent doses of LH and hCG, however, the aforementioned authors showed that accumulation of intracellular cAMP by LH was significantly faster, with maximal activation achieved in 10 minutes, while by hCG the same levels of the maximal stimulation were attained only after 60 min of stimulation (see Table 16.2). Interestingly, LH and hCG were equipotent in terms of progesterone production in spite of overall lower cAMP levels after LH stimulation. Despite being mainly dependent on the cAMP/PKA-pathway [107], progesterone production in preovulatory GCs may involve other signaling pathways modulated by ERK1/2 and AKT [108], including [109] molecules of the EGF family such as neuregulin 1 and amphiregulin [34, 110].

In additional experiments using the same in vitro model, Casarini and cols. also evaluated the effects of LH and hCG at activating the ERK1/2 and AKT pathways. ERK and AKT are cell cycle regulators; while AKT is involved in cell signaling leading to cell survival (by blocking apoptosis), ERK represents a range of cell proteins that communicate a signal from a receptor on the cell surface to the DNA in the nucleus. Stimulation with equimolar concentrations of LH resulted in a strong, rapid (10 min), and sustained (45 min) activation of ERK1/2, while hCG induced a much weaker and short-lived stimulation, reaching significance only at 10 min. As far as AKT is concerned, LH provoked a substantial increase in AKT between 10 and 30 min, while hCG stimulation at same doses was virtually nonexistent. Given the different intracellular signaling of hLH and hCG on acute ERK and AKT activation, these authors assessed whether the expression of genes known to be under LH and/or hCG control, epiregulin (AREG) and neuregulin 1 (NRG1) and of CYP19A1 (aromatase), would be differentially affected by the type of gonadotropin. While both LH or hCG stimulation resulted in a marked stimulation of the expression of such genes, LH was significantly more potent than hCG on AREG. Epiregulin may play a role in the ovulatory process and oocyte maturation [108, 111] exerted via both ERK- and AKT-pathway activation [34, 112]. In conclusion, LH and hCG action on the same receptor results in quantitatively and qualitatively different intracellular signaling. While equimolar concentrations of LH and hCG possess different in vitro potency (in terms of cAMP), equipotent concentrations of LH and hCG stimulate intracellular cAMP accumulation with significantly different kinetics. Moreover, LH is more potent than hCG on the ERK and AKT pathways and elicits different kinetic response.

The investigation of the functional role of the cAMP, ERK, and AKT signaling pathways in human fertility has revealed that LH/hCG stimulation of the same receptor results in activation of different, complex signal transduction pathways and molecules [111–113]. In vitro activation of cAMP-pathway by gonadotropins is traditionally associated with structural changes, consisting in cell-rounding [114, 115], apoptotic events [115–117] and in the prevention of meiosis resumption of the oocyte [118]. In contrast, gonadotropin-dependent activation of antiapoptotic pathways [34, 119] and proliferative effects [120] seems to be mediated by ERK1/2 and AKT, and reduction of ERK1/2 signaling activates apoptotic signals in the GCs [121]. Taken together, these results indicate that hCG and hLH action on the regulation of cell cycle and apoptosis in granulosa cell might be divergent and/or dependent on which signal transduction pathway is activated. This is especially relevant in influencing the cell fate during folliculogenesis, when the activation of different signal transduction pathways mediates a delicate balance between pro- and antiapoptotic signals [122]. While the in vivo effects of the differential activation of the various pathways remain to be investigated, the nonequivalence of LH and hCG deserves consideration in the application of therapeutic strategies involving LH activity supplementation in COS protocols.

16.6.2 Differences Between LH and hCG: Evidence from Clinical Studies

It has been shown that the expression of the LH receptor gene, as well as genes involved in the biosynthesis of cholesterol and steroids in granulosa

cells (CYP11A activity decreased by 2.4-fold), is lower in patients treated with hMG preparations compared with those treated with FSH preparations [123]. Such effects are caused by a constant ligand exposure during the follicular phase due to long half-life and high receptor binding affinity of hCG. In animal models, downregulation of LH receptors is maintained for up to 48 h after hMG administration [124]. These findings indicate that the GCs have lower LH-induced cholesterol uptake, a reduction in the de novo cholesterol synthesis, and a reduction in steroid synthesis and thus could explain the observed lower serum progesterone levels achieved in patients treated with hMG compared with FSH [123, 125].

In a study prospectively evaluating 60 normogonadotropic women who, when undergoing induction of multiple follicular growth, showed an insufficient ovarian response in terms of follicular growth (defined as "low responders"), Ruvolo and colleagues examined the impact of LH supplementation on cumulus cell apoptosis [126]. On stimulation day 8, one group was treated with rec-hFSH combined with rec-hLH, while the other was stimulated with rec-hFSH alone. Terminal deoxynucleotidyl transferase-mediated digoxigenin-deoxyuridine-triphosphate (dUTP) nick-end labeling (TUNEL) assay and anti-caspase-3 cleaved immunoassay were used to measure apoptosis in the cumulus cells. A statistically significant increase in the number of immature oocytes was observed in the group treated with rec-hFSH alone (2.33 in the rec-hFSH group vs. 0.58 in the rec-hLH group; $p<0.01$). In contrast, apoptosis markers were lower in the group who received LH supplementation by rec-hLH. A lower rate of cells with chromatin fragmentation (TdT, 18.2 % vs. 12.1 %) and lower presence of caspase-3 cleaved (17.0 % vs. 11.0 %) were observed in the rec-hLH group compared with the rec-hFSH group. Implantation rates were significantly higher in the rec-hLH group (15.6 %) compared with the rec-hFSH group (12.5 %, $p<0.01$). The authors concluded that supplementation with rec-hLH reduced the number of immature oocytes collected after pickup. Furthermore, they speculated that the increase in implantation rate might be correlated with the reduction of apoptosis seen in the cumulus cells of patients treated with rec-hLH, due to a direct action of rec-hLH on the cumulus and granulosa cells, or because of the paracrine effect mediated by secreting factors in the theca and oocyte cells. Thus, maintaining their physiological function for a longer time, cumulus cells are better able to support nuclear and cytoplasmic maturation of the oocyte until ovulation, thus allowing the collection of oocytes with better "intrinsic" qualities that are necessary for sustaining fertilization and the early phases of embryogenesis. Hence, if cumulus cells are preserved from apoptotic processes, the oocyte receives no molecular signal able to activate apoptotic pathways [126].

The clinical implications of the aforementioned observations have been investigated by "tail to tail" comparison between hMG and rec-LH preparations. An open-label RCT in 2012 compared HP-hMG and a fixed combination of rec-FSH and rec-hLH in 35 women with hypogonadotropic hypogonadism. Eighteen patients received 150 IU hMG-HP (150 IU FSH + 150 IU LH-like activity) and seventeen received 150 IU rec-hFSH/75 IU rec-hLH daily for a maximum of 16 days. The proportion of patients reaching ovulation did not differ between the groups (70 % vs. 88 %, respectively), but the pregnancy rate was significantly higher in those treated with the combination of recombinant gonadotropins (55.6 % vs. 23.3 %; $p=0.01$) [127]. In another RCT, Pacchiarotti and colleagues enrolled 122 women with low baseline endogenous LH levels (<1.2 IU/L) in the presence of normal FSH levels undergoing IVF. The patients were treated with a downregulation protocol consisting of triptorelin 0.1 mg at day 21 of the cycle and were randomized to receive an ovarian stimulation with 225 IU/day of either HP-hMG or rec-FSH plus rec-hLH in a 2:1 ratio. Fewer days of stimulation (10.9 ± 1.1 vs. 14.1 ± 1.6; $p=0.013$) and a higher number of retrieved oocytes (7.8 ± 1.1 vs. 4.1 ± 12; $p=0.002$) were noted in the group that received follitropin alfa + lutropin alfa 2:1 compared with the group who received HMG. However, differences were not observed in estradiol levels on hCG day (1987 ± 699 pg/mL vs. 2056 ± 560 pg/mL),

pregnancy rates per cycle (28.3 % vs. 29.3 %), and implantation rates (12.1 % vs. 12.2 %), despite higher cancelation rates due to excessive response in women receiving follitropin + lutropin alfa (11.1 % vs. 1.7 %; $p = 0.042$), which therefore indicates that the latter is a more potent preparation for COS [128].

Furthermore, the German experience with the use of rec-hLH compared with hMG in daily ART practice was recently reported by analyzing data from the National IVF Registry (DIR), in which patients undergoing IVF from approximately 85 % of the German IVF centers are prospectively enrolled [129]. A total of 4719 women, 1573 per group, matched by age, body mass index, indication, and number of previous ART cycles, treated with either rec-hFSH and rec-hLH in a fixed 2:1 ratio or hMG, either alone or in combination with rec-hFSH, after down-regulation in a long GnRH-agonist protocol, was analyzed. The mean gonadotropin consumption (in ampoules of 75 IU) was significantly lower in the group treated with the fixed combination of rec-hFSH and rec-hLH (34.3) compared with the two hMG groups (hMG alone: 36.4, $p < 0.001$; hMG in combination with rec-hFSH: 46.3, $p < 0.001$). Pregnancy rates per cycle (25.5 % vs. 21.5 %, $p = 0.006$; 25.5 % vs. 21.7 %, $p = 0.02$) and per embryo transfer (31.3 % vs. 26.0 %, $p = 0.02$; 31.3 % vs. 25.6 %, $p = 0.008$) and implantation rate per embryo transferred (19.0 % vs. 14 % in both pairwise comparisons, $p < 0.001$) were higher in the group treated with the fixed combination of rec-hFSH and rec-hLH compared with the aforesaid hMG groups, respectively. Lastly, in 2013, a crossover study evaluated 33 patients using HP-hMG in their first IVF cycle and 2:1 rec-hFSH plus rec-hLH in their second IVF attempt [130]. Estradiol levels on the day of hCG (2633 ± 871 vs. 2101 ± 816; $p < 0.05$) and the number of oocytes retrieved (9.8 ± 3.3 vs. 7.3 ± 3.1; $p < 0.01$) were higher in the group that received the 2:1 rec-hFSH plus rec-hLH formulation. Despite implantation and clinical pregnancy rates per started cycle were not different between the groups (29.6 % and 48.4 %, respectively, for hMG and 28.4 % and 48.4 % for rec-hFSH plus rec-hLH), 2/3 of the patients in rec-FSH + rec-LH group (vs. 1/3 hMG group) would have frozen embryos to transfer if a fresh transfer failed.

Despite being developed for stimulation of follicular growth in women with severe LH and FSH deficiency, the 2:1 formulation of follitropin alfa and lutropin alfa has expanded to normogonadotropic women undergoing ART. In a 3-year, multicenter, open-label, observational, post-marketing surveillance study involving 2200 German women (21–45 years) undergoing ART, the most common reasons for physicians to prescribe the 2:1 formulation of follitropin alfa and lutropin alfa were poor ovarian response (39.4 %), low baseline LH level (17.8 %), age (13.8 %), and low baseline E_2 level (7.3 %) [131]. Recently, a cost-effectiveness model compared rec-FSH + rec-hLH and HP-hMG for ovulation induction in hypogonadotropic hypogonadal women, according to the Italian Health Service perspective, in which only direct costs (drugs, specialist visits, patient examinations, and hospitalizations) are included [132]. A Markov model was developed, considering the probability of pregnancy and miscarriage in three cycles of therapy. In that model, the patients started the therapy with recombinant or urinary gonadotropins following pregnancy evaluation. If a woman became pregnant, the possibility of miscarriage was considered. Women who did not become pregnant during the first series of treatment or had a miscarriage underwent a second cycle of therapy, maintaining the same treatment of the previous cycle. The same process was applied to the third cycle. Consumption of gonadotropins and outcome of HP-hMG and rec-hFSH + rec-hLH cycles were based on the study by Carone and cols [127]. Rec-hFSH + rec-hLH was associated with a higher acquisition cost (€3453.50) and higher efficacy (0.87) compared with HP-hMG (€2719.70 and 0.50). The average cost per pregnancy was estimated to be €3990.00 for recombinant strategy and €5439.80 for urinary strategy, thus indicating that the combination therapy with rec-hFSH + rec-hLH is associated with a better cost-effectiveness compared to HP-hMG in the treatment of infertility in hypogonadotropic hypogonadal women.

In conclusion, evidence from experimental and clinical studies indicates that LH activity driven by hCG and rec-hLH is not equivalent neither at the molecular level nor at the functional level. COS protocols involving LH supplementation with rec-hFSH appear to be more effective and efficacious than those with hMG (hCG activity). Nevertheless, large RCTs are needed to confirm these observations.

Conclusions

- Ovarian steroidogenesis is the result of combined LH and FSH stimulation of the two cell types, theca and granulosa, influenced by autocrine and paracrine factors.
- LH has a pivotal role in follicular development and maturation. LH is crucial in sustaining FSH activity in the granulosa during intermediate–late stages of folliculogenesis and is critical for maintaining corpus luteum function during the luteal phase.
- Exogenous LH activity supplementation is mandatory in stimulation protocols applied to women with hypogonadotropic hypogonadism.
- Review of the studies exploring the supplementation of rec-hLH to COS regimens indicates that, at present, the addition of rec-hLH to the general population of infertile women undergoing IVF/ICSI cycles remains controversial.
- Fair evidence indicates that rec-hLH supplementation has a positive effect on cycle outcome of older women (>35 years old), particularly when used from the start of COS.
- Fair evidence indicates that both the number of oocytes retrieved as well as the clinical pregnancy rates are increased in poor responders undergoing COS with a combination of rec-hLH and rec-hFSH.
- Hyporesponsiveness to rec-hFSH could be related to the presence of less bioactive LH due to specific genetic-determined variations in the beta subunit of the LH molecule. Limited data indicate that hyporesponders benefit from LH supplementation during COS.
- Conflicting evidence exists regarding the impact of deeply suppressed levels of mid-follicular serum LH levels on ovarian response to stimulation with rec-FSH. Current data indicates that the choice of GnRH-a plays a role on the frequency of patients exhibiting profound mid-follicular LH suppression. Whether these women would benefit from supplementation with LH activity during ovarian stimulation remains to be proven.
- Evidence from experimental and clinical studies indicate that LH activity driven by hCG and rec-hLH is not equivalent neither at the molecular level nor at the functional level. COS protocols involving LH supplementation with rec-hLH, particularly using a fixed 2:1 combination of rec-hFSH and rec-hLH, appear to be more effective and efficacious than those with hMG (hCG activity). Nevertheless, large RCTs are needed to confirm these observations.

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Ovulation Trigger: HCG vs. GnRH Agonist

17

Neeta Singh and Malti Madhu

Abstract

HCG triggering is vital for the final oocyte maturation in the in vitro fertilization cycle. In recent years, with the increase in the prevalence of polycystic ovarian disease and use of antagonist protocol for ovarian stimulation, it is possible to minimize the risk of ovarian hyperstimulation syndrome with the use of GnRH agonist in place of HCG for triggering ovulation. Some of the initial studies reported lower pregnancy rates due to defective luteal phase after GnRH trigger. But the results are encouraging with addition of rescue dose of HCG (15,000 IU) and after modified luteal phase support. It is a boon for primary prevention of ovarian hyperstimulation (OHSS) in PCOS patients.

Keywords

Ovulation triggering • GnRH agonist • Ovarian hyperstimulation syndrome (OHSS)

17.1 Introduction

Ovulation is one of the most crucial steps in female reproduction. In natural cycles a single dominant follicle ruptures and releases a viable oocyte from the ovary; only after this process will the oocyte be made available to the sperm for fertilization. The oocyte is arrested at the prophase of first meiotic division, which resume only after preovulatory LH surge. LH receptors are present on the granulosa cells and in response to LH surge; there is expression of epidermal growth factors which act on cumulus cells, thereby triggering oocyte maturation.

It is mainly the LH surge that initiates a cascade of events including resumption of meiosis in the oocyte, expansion of cumulus, synthesis of prostaglandins responsible for follicular rupture, and luteinization of granulosa cells that produce

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progesterone following ovulation. Increasing concentration of progesterone causes a rise in activity of proteolytic enzymes that result in rupture of the follicular wall.

Since the endogenous LH surge is blocked in IVF cycle with the help of GnRH agonist or antagonist, some trigger agent is required for artificially inducing LH surge for scheduling oocyte retrieval.

17.2 Agents Used for Ovulation Trigger

Ever since the discovery of human-assisted reproductive techniques, there has been lots of research regarding the ideal trigger agent. Though recombinant LH has also been used, choice revolves around HCG and GnRH agonist.

17.2.1 Recombinant LH as a Trigger Agent

Study done by Aboulghar et al. to evaluate LH as ovulation trigger agent concluded significant increase in cost of treatment, as well as lower implantation rate and a higher OHSS rate (upto 12 %) with the use of LH [1].

17.2.2 HCG as a Trigger Agent

HCG has been used for decades for the final maturation of the follicle. The logic behind the use of HCG is its homology to LH. So both molecules bind to the same receptor, the LH/HCG receptor. It can be used by i.m. or s.c. route.

17.2.2.1 Dose

In an early randomized trial [2], 2,000, 5,000, and 10,000 IU HCG were administered intramuscularly. The proportion of patient with oocyte recovery was significantly lower in the 2,000 IU-dose group (77.3 %) as compared to 5,000 and 10,000 IU groups, which were comparable (99.5 % and 98.1 %). Therefore the recommended dose schedule is 5,000–10,000 IU.

17.2.2.2 Pharmacokinetics

There is difference in pharmacokinetics of LH and HCG. The half-life of HCG is more than 24 h, as compared to LH which is 60 min only [3, 4]. The longer half-life is responsible for prolonged luteotropic effect, resulting in supraphysiological level of steroid [5], increasing the risk of OHSS [6]. Its prolonged luteotropic effect is sometimes responsible for false-positive pregnancy test when HCG is given in the luteal phase.

17.2.2.3 Timing of Oocyte Retrieval After HCG Injection

Timing of oocyte retrieval after HCG administration is very important as if it is given too early, this will lead to greater number of metaphase II oocyte, whereas a delayed retrieval might result in premature ovulation, resulting in decreased number of oocyte retrieved. Based on the available evidences [7, 8], oocyte retrieval should be timed between 34 and 38 h.

17.2.2.4 Disadvantage of HCG

Increased risk of ovarian hyperstimulation syndrome (OHSS) is the main disadvantage when using HCG as the trigger. This is the most feared complication of ART with incidence of 0.7 % in all the stimulated cycles [9]. Moreover, in some cases, it can even be life threatening. According to the UK-based Confidential Enquiry into Maternal and Child Health 7th report, 2007, there have been three deaths due to OHSS in the year 2003–2005 [10]. Ovarian hyperstimulation syndrome is still underreported in many parts of world. It is often the prime cause for cycle cancellation.

Another problem is requirement of large quantities of urine for their extraction. This leads to its unreliable pharmaceutical activity and possibility of allergic reaction [11]. This issue has been sorted out with the use of recombinant HCG, 250 μg s.c., which is considered equally effective, and the incidence of local reaction at injection site is significantly lower [12, 13].

17.2.2.5 Advantages of hCG

hCG can be used in both GnRH agonist as well as antagonist cycle. The biggest advantage of hCG

is the vast experience of its use which is obviously lacking with GnRH agonists.

17.2.3 GnRH Agonist as a Trigger Agent

In the recent years, there has been a shift of interest toward the use of GnRH agonist as an ovulation trigger agent to avoid OHSS in stimulated cycles, particularly in patients with polycystic ovarian syndrome (PCOS).

17.2.3.1 Indication for Use

In the long agonist protocol in which GnRH agonist are used for downregulation, GnRH agonist cannot be used as a trigger agent. But in the short protocol involving GnRH antagonist, GnRH agonist has got its role. As demonstrated by several studies, a single bolus of GnRH agonist causes LH surge and triggers ovulation.

17.2.3.2 Pharmacokinetics and Dose

Chillik et al. [14] in their studies on monkeys has stated that tonic gonadotropins remain suppressed under the effect of GnRH antagonist treatment, but acute LH release can be elicited in a GnRH bolus. In humans, Felberbaum et al. [15] has demonstrated that the pituitary retains its responsiveness to GnRH agonist under GnRH antagonist treatment. Similar reports were demonstrated by Olivennes et al. [16]. The extent of pituitary suppression by use of GnRH antagonist is somewhat dose dependent [17].

With the minimal effective dose of GnRh antagonist (0.25 mg daily; Gainerelix Dose Finding group, 1998), it is suggested that ovulation can be safely and effectively triggered by a single dose of GnRH agonist (triptorelin 0.2 mg s.c., buserelin 0.5 mg s.c., or leuprolide acetate 1 mg) [15, 17, 18].

There exists a difference between the natural LH surge and that which is GnRH agonist induced. The LH surge of natural cycle is characterized by three phases, with a total duration of 48 h [19], whereas that induced by GnRH agonist is of two phases only, with duration of 24–36 h [6]. Thus the response elicited by GnRH agonist is not that pronounced in contrast to that produced by HCG which is exaggerated due to its prolonged luteotropic effect [20]. Because of this, the risk of OHSS is almost abolished by use of GnRH as reported in various studies [5, 20–26].

17.2.3.3 Advantage of GnRH Agonist as Ovulation Trigger

OHSS is prevented to a great extent when GnRH agonist is used for ovulation trigger. It becomes especially important in those patients who are more prone to develop OHSS. This has been recently confirmed in a Cochrane meta-analysis [26]. The same concept was used by Sismanoglu et al. [27] in their study, which they designed to evaluate GnRH agonist in the donor population. Egg donors are usually selected from the young, normally fertile women and thus more prone to develop ovarian hyperstimulation.

The retrieval of more number of mature oocyte [metaphase 2] has been reported with GnRH agonist triggering [28].

17.2.3.4 Disadvantage of GnRH Agonist as a Trigger Agent

Use of GnRH agonist as a trigger agent is possible in the GnRH antagonist ovarian stimulation protocol only. The difference in LH surge of natural cycle and that of GnRH-induced cycle is responsible for the reduction in the total amount of gonadotropins being released from the pituitary when GnRH agonist is used for final oocyte maturation. This may be the reason behind low reproductive outcome due to higher early pregnancy loss [29], as concluded by Youssef et al., in their Cochrane meta-analysis. GnRH agonists as a final oocyte maturation trigger in fresh autologous cycle should not be used routinely due to associated significantly lower birth rate [26].

17.2.3.5 Future of GnRH Agonist as a Trigger Agent

Griesinger et al. [18] performed a meta-analysis and systemic review in 2006, showing comparison of GnRH agonist and HCG as trigger agents for final oocyte maturation. The outcome assessed were clinical pregnancy rate per randomized patient, no. of oocyte retrieved, proportion of

metaphase 2 oocyte, fertilization rate, embryo quality score, first trimester abortion rate, and incidence of OHSS. The number of oocyte retrieved was not significantly different (–0.94, –0.33–0.14), as well as the proportion of metaphase 2 oocyte (–0.03, –0.58–0.52), fertilization rates (0.15, –0.09–0.38), or embryo quality score (0.05, –0.18–0.29). No case of OHSS was reported in some of the studies; hence no conclusion could be drawn as regarding effect of GnRH agonist on incidence of OHSS. But as compared to HCG, GnRH agonist was associated with a significantly reduced likelihood of clinical pregnancy rate (0.21, 0.05–0.84; $P=0.03$). The odds of first trimester pregnancy loss are increased after GnRH agonist triggering [18]. They concluded that the use of GnRH agonist as a final triggering agent in IVF yielded a number of oocyte capable to undergo fertilization and cleavage which is comparable to that by HCG. However, the likelihood of clinical pregnancy was lower with GnRH agonist triggering than achieved with HCG.

The combined effect of GnRH agonist trigger and ovarian stimulation leads to defective luteal phase responsible for higher early pregnancy loss [30]. Therefore, the need for modification in luteal phase support was felt [28]. Various studies have been done using progesterone only, progesterone with estradiol, or dual trigger in which GnRH agonist is used along with small bolus of hCG to normalize the luteal phase not only in terms of mid-luteal serum progesterone concentration, but also in terms of better clinical pregnancy rates if bolus of 1,500 IU HCG was administered 35 h after GnRH triggering [31].

Shapiro et al. [32] have done retrospective analysis in OHSS patients and concluded that there is a higher pregnancy rate and no OHSS by using dual trigger. In their study, patients received leuprolide acetate [4 mg] along with HCG [1,000–2,500 IU], followed by estradiol and progesterone for luteal support.

There is another alternative for patient with high risk of OHSS. GnRH agonist triggering can be combined with cryopreservation of oocytes or embryos to be used later on in subsequent cycles [33]. Clinical pregnancy rates and cumulative ongoing pregnancy rates in cryopreserved cycles after GnRH agonist triggering were 33 % and 37 %, respectively, while OHSS was completely avoided [33, 34].

Conclusion

It is suggested that while GnRH agonist triggering can be highly effective in terms of OHSS prevention and thereby holds promise in the establishment of "friendly IVF," further studies are needed to establish its role.

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The Pharmacoeconomics of Ovarian Stimulation

18

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Abstract

It is important to consider the economics of ovarian stimulation in order to limit the cost of each ART cycle, which translates into reduced dropout rates and maximum cumulative pregnancy rates for the couple. Major cost of IVF cycle is attributable to the drugs for ovarian stimulation.

Various options to optimize the cost include intensive weight loss prior to stimulation, use of GnRH antagonist protocols, natural IVF and mild stimulation regimes. Use of urinary gonadotropins or human menopausal gonadotropins instead of recombinant products, lower starting dose of gonadotropins and correct choice of ovulation trigger will also cut down the cost of ovarian stimulation. A good understanding of the physiology of ovarian stimulation and finer aspects of the drugs used is imperative to make IVF more cost effective.

Keywords

Direct and indirect costs of IVF cycle • Individualizing stimulation • Optimizing costs • Right protocol • Mild regimes • Gonadotropin preparation • Starting dose gonadotropins • Ovulation trigger

18.1 Introduction

Approximately 15–20 % of married couples in the fertile age-group suffer from infertility, which is on the rise because of various reasons like urbanization, pollution, chemical exposure, stress, competitive work environment, fast-paced lifestyle, more women opting to work and increased incidence of diabetes and pelvic inflammatory disease (PID), etc. Today, an array of treatment options are readily available to treat infertility,

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and these include medications for ovulation induction, endoscopic surgery to correct anatomical problems and the assisted reproductive technologies (ARTs) including IUI, IVF and ICSI.

Despite the increasing demand for ART treatment, many patients withdraw from IVF treatment mainly for two reasons: poor prognosis and the inability to afford further treatment [1]. Many patients withdraw from treatment or choose not to pursue treatment because of cost, especially in developing countries, where there is no insurance covering infertility treatment and they have to pay for their own treatment. It is therefore of growing importance to limit the cost of each treatment cycle and to maximize the chances of pregnancy for patients, as it is well known that the cumulative pregnancy rates in ART are much better.

The maximum cost in an ART cycle is attributable to the drugs for ovarian stimulation, which contribute approximately 60 % to overall cost. The conventional protocols aim at quantitative and qualitative factors in oocyte production and have a positive influence on the IVF outcome. Today when we are looking at the economics of ART, we aim at not only making ART affordable but at the same time not compromising with the quantity or quality of oocytes. As we do understand that the conventional ovarian stimulation protocols are expensive and also have been shown to have detrimental effects on the luteal phase, so there lies merit in looking at protocols that are easy on the pocket without affecting the outcome of pregnancy.

18.2 Costs

Costs associated with ART treatment can be characterized as indirect cost, those occurring as a consequence of ART treatment, and as direct cost, attributed to providing ART treatment itself, related to the costs involved, especially in countries like India where there is no medical coverage for these expenses.

18.2.1 Indirect Costs

Multiple-birth infants and the possibility of ovarian hyperstimulation syndrome (OHSS) resulting from ART need to be considered, as the patient's and the family's happiness or stress is directly

18.2.2 Direct Costs

The direct cost of ART mostly includes the cost of pre-investigations, the pre-preparation (down-regulation) followed by the cost of drugs for ovarian stimulation (gonadotropins) or luteal support (progesterone), etc. all of which can lead to a high degree of expenditure to get the desired one live birth. Additional costs include that of medical consultation, laboratory and embryology services, ultrasound scanning, medical procedure such as oocyte retrieval and embryo transfer, hospital charges, nursing and counselling services and administrative and overhead charges.

Along with this, there could be additional cost of cryopreservation, laser-assisted hatching, IMSI, etc. According to the available evidence, there is a difference between cost and cost-effectiveness. And what one should be looking at is cost-effectiveness, which can depend on the following factors:

1. Experienced and estimated treatment success rate
2. Age of the woman
3. Multiple pregnancy
4. Cost of the treatment

So calculating the cost-effectiveness may not be as simple as it appears because of the variation of the different components. However, to keep the discussion simple in this chapter, we will consider only the various ways of optimizing the cost of ovarian stimulation in ART.

A detailed analysis of different cost components per treatment cycle demonstrates that the hormonal stimulation stage is the most expensive part. This percentage could be higher if we consider older women who have increased cost per cycle than younger women, because of higher mean dosage of FSH needed during hormonal stimulation. Aim is to make IVF affordable by changing the stimulation protocol without affecting the pregnancy rates or affecting the luteal phase. Nowadays, individualizing the ovarian

stimulation protocol is a more feasible approach, and individualizing in each group of women will help make it more affordable while reducing the complications and not compromising with the cryopreservation programme. Though such a situation is a win-win for all, it is difficult to achieve and will need a thorough understanding of the patient profile, type of treatment required, understanding the drugs being used for ovarian stimulation and adequate monitoring of the response of stimulation.

So the question is: How and what can be done to modulate the ovarian stimulation? To economize it when we know that in ART the ultimate justification is by live birth rate. We can consider using one protocol over the other, keeping in mind that a larger number of mature oocytes retrieved and high fertilization rates translate into more embryos for cryopreservation with ultimately increased cumulative pregnancy rate.

The options available too are many, but the trick lies in fitting the glove to the therapy. Let's look at what can be done to optimize the costs.

18.3 Optimizing the Costs

18.3.1 Weight Loss

High BMI is associated with a higher FSH requirement during ovarian stimulation, fewer normally fertilized oocytes, lower oestradiol levels, frequent cycle cancellation and lower pregnancy and live birth rates. Infertile women requiring IVF should be encouraged to maintain a normal weight during treatment [2]. Maintaining a certain amount of physical activity irrespective of weight loss should be highly recommended and it helps not only in improving the pregnancy outcome by threefold [3] but also cuts down the cost of stimulation.

18.3.2 Choosing the Right Protocol

18.3.2.1 Antagonist Protocol

Antagonist regime provides a more patient-friendly alternative with shorter and more cost-effective ovarian stimulation protocol compared to agonist [4]. It has also been seen that the dropout rates have been much lesser with antagonist cycles than agonist.

GnRH antagonist protocol when compared with long agonist protocol is shorter, rapidly reversible and requires fewer injections and lesser amount of gonadotropins, which definitely makes it more patient friendly and that too at a lower cost. Despite initial studies about the pregnancy outcome being inferior to agonist protocol, more recent studies have indicated that there is not much difference as far as pregnancy rates are concerned and GnRH-antagonist regimen is as effective in preventing a premature rise of LH. Now the flexible dosing regimen is definitely showing promise amongst PCOS [5].

Also GnRH-antagonist protocol becomes a preferred protocol in cases at high risk of developing OHSS and is the protocol of choice for oocyte donation programme because it allows use of agonist trigger for the final maturation, further enabling reduction in OHSS without compromising on the oocyte quality.

18.3.2.2 Natural Cycle IVF

Natural cycle IVF is carried out without use of any drugs for ovarian stimulation. The first test tube baby Louise Brown was conceived with natural cycle. But the success rates of IVF dramatically increased only with the use of gonadotropins. Though it provides a cheaper alternative to patients who are ovulating spontaneously and may be indicated in patients with hormone-dependent tumours [6], they need to be counselled regarding the possibility of cancelled oocyte retrieval, fertilization failure and failure to reach embryo transfer [7]. According to HFEA data, only 1/26 cycle in women less than 35 years opting for natural cycle IVF resulted in a live birth in the year 2008.

18.3.2.3 Minimal or Mild Stimulation Regimes

Minimal or mild stimulation regimes are designed to recruit a fewer number of eggs, thus avoiding the risks of hyperstimulation and reducing the number of injections also dramatically reduce the cost of medications [8]. Frequently low-dose gonadotropins 75–100 IU are used or combined with oral agents like clomiphene with or without use of

GnRH antagonist [9]. Sample regime: low dose of clomiphene 50 mg is given without discontinuing the clomiphene after 5 days as is usually the custom but to continue the clomiphene until ultrasound monitoring shows the follicle size ready for ovulation and gonadotropins (150 IU of uFSH) are added on days 8, 10 and 12. Clomiphene not only stimulates the pituitary to release FSH but also blocks the oestrogen-stimulated release of LH so prolonged downregulation with lupride is not required. With this 'mini- IVF', though lesser number of oocytes are obtained, less oocyte and embryo aneuploidy is reported and the pregnancy rates are acceptable and similar to conventional protocols.

Mild treatment strategy for in vitro fertilization was shown in a randomized non-inferiority trial by Heijnen et al. [10]to have much lower dropout rates: Mild cycle 5–11 % vs standard cycle 9–19 % and similar cumulative pregnancy with live birth at 1 year: mild: 43 % vs standard: 44 %. In this study, mean total cost of mild IVF was €8,333 while that of a standard IVF protocol was €10,745. Also these regimes drastically lower the multiple pregnancy rates and the associated indirect costs.

Milder stimulation protocols can be used for patients who are presumed high responders, normal responders as well as those with poor ovarian reserve [9] as an option to reduce the cost of stimulation.

In both natural or minimal stimulation protocol, cost is reduced because of

- Fewer office visits
- Less need for monitoring
- Decreased risk of multiple birth
- Possible avoidance of anaesthesia during oocyte retrieval

18.3.3 Type of Gonadotropin Preparation

18.3.3.1 Urinary Gonadotropins

Recombinant FSHs have a higher cost compared with urinary gonadotropins. Initially, a lot of studies were carried out to also prove the superiority in safety, purity and effectiveness of recombinant products when compared with urinary gonadotropins [11]. None of these studies focussed on the cost which is an equally important issue especially in the developing countries. Currently there is no clear evidence of the superiority of r-FSH over urinary gonadotropins in effectiveness [12]. There are number of meta-analyses which suggest that recombinant products may be more costly without much difference in the pregnancy rates, live birth rates and complication rates. Baker et al. compared the efficacy of highly purified hFSH (HP-hFSH) versus rFSH in volunteers undergoing controlled ovarian stimulation for IVF [13], and in this report the authors concluded that there were no statistically significant differences in live birth rate between HP-hFSH and rFSH treatment groups (38.2 % in each group) but there is a lot of difference in the cost.

18.3.3.2 Human Menopausal Gonadotropins

Use of human menopausal gonadotropins (HMGs) instead of recombinant gonadotropins has never gone out of the prescription of ART practitioners for the simple reason that there are certain groups of patients who would actually do well with the HMG due to the addition of LH. Even for other patients also currently, there is no clear evidence of the superiority of rFSH over HMG in effectiveness. In terms of clinical efficacy, there are a number of meta-analyses demonstrating no significant difference in clinical/ongoing pregnancy/live birth rate, miscarriage rate, multiple pregnancy rate and incidence of ovarian hyperstimulation syndrome between rFSH and hMG [14] and between rFSH and hFSH [11]. A study by Wes-Wechowski et al. published in 2010 [15] analyzing the economic implications of choice of gonadotropin on IVF cycles including fresh and up to two fresh or frozen cycles, conditional on the availability of cryopreserved embryos, demonstrated not only a superior cumulative live birth rate for HP-HMG compared with rFSH but also showed that the mean costs per IVF treatment were significantly less for HP-HMG. When maternal and neonatal costs were applied, the median cost per IVF baby delivered was still significantly less with HP-HMG. This cost

saving from using HP-HMG depicted in this model would allow an additional treatment cycle for every seven patients treated [15].

18.3.4 Starting Dose of Gonadotropins

The optimal starting dose of FSH for controlled ovarian stimulation is an important issue in IVF cycles since drugs contribute a major part to the cost involved. Already having discussed the type of gonadotropins, the dosage used can also have major implications for the economics.

For a conventional IVF cycle, the starting dose can vary from 100 IU to 450 IU depending on the assessment of expected response. The decision of what dose to start with is also influenced by whether minimal or large number of oocytes are considered a success. With the legal restriction in some European countries on number of oocytes that can be inseminated, a balance has to be drawn between the number of oocytes and the cost of drugs with minimal wastage of both, hereas Oliviennes et al. in the CONSORT study [16] have suggested an FSH dosage normogram based on age, basal FSH levels, BMI and antral follicle count where dose adaptations, ranged from 75 up to 225 IU/day, revealed that in 30 % of patients, a dose of 100 IU/day or less is sufficient to obtain moderate oocyte numbers with high pregnancy rates.

There is still no universal consensus regarding the optimal starting dose of FSH in presumed normal responders. A recent meta-analysis of ten studies by Sterrenburg et al. [17] has suggested that the optimal starting dose of rFSH for IVF/ICSI is 150 IU daily in young normal responders. This dose was associated with a more modest oocyte yield but an equal pregnancy rate compared with higher doses.

This study demonstrates that though the average number of oocytes retrieved per pickup is increased when FSH doses over 100 IU/day are given, there is no difference in the pregnancy rates. Moreover, the number of frozen embryos and cumulative pregnancy rates does not improve with dosages exceeding 150 IU/day. The use of a standard lower dose of 150 IU/day, instead of 225 IU/day, would reduce per-cycle costs of gonadotropin medication by 30 % with added decline in the risk for OHSS and reducing the need for intense monitoring of ovarian response. At the other end of the spectrum, no difference was seen in the number of oocytes obtained or pregnancy rate in poor responders on doubling the starting dose of rec FSH from 150 to 300 IU [18].

18.3.5 Trigger for Ovulation: hCG or GnRH Agonist?

Controversy regarding trigger for final oocyte maturation has been going on; however, one must realize that the trigger for final oocyte maturation can vary from urinary HCG to recombinant HCG and to agonist trigger. This individualization should come after analyzing the stimulation. Urinary HCG has a longer half-life and may not be suitable for hyperstimulated ovaries. Reducing the dose can affect the number of oocytes retrieved and still may not reduce the incidence of delayed ovarian hyperstimulation. Triggering with recombinant HCG as compared to urinary HCG is costly.

Triggering of final oocyte maturation with a single bolus of low-cost GnRH agonist (GnRHa) Lupride 1 mg or Decapeptyl 0.1 mg as an alternative to hCG is a viable option with the introduction of GnRH antagonist protocols. However, GnRH agonist-induced LH surge lasts for shorter duration of 24–36 h. And first reports from prospective RCTs subsequently showed a very poor clinical outcome when GnRH agonist was used to trigger final oocyte maturation, due to a deficient luteal phase [19–21].

But in oocyte donation programmes, GnRHa triggering has been successfully applied with pregnancy rates similar to hCG triggering in recipients and additional advantage of no OHSS in the donors [22]. The largest study of 2,077 stimulated donor cycles in 1,171 egg donors reported an incidence of 1.26 % of moderate or severe OHSS in the rhCG group compared with no cases in the GnRHa group [23].

In case of high reponders, hCG triggering had a 3.79 times greater risk of developing any form

of OHSS and a 1.35 times greater risk of developing moderate to severe OHSS when compared to GnRH agonist [24]. Still, controversy exists in literature regarding the pregnancy outcome in GnRH agonist-triggered cycles and the best luteal phase support. Good live birth rates are reported in frozen thawed embryo replacement cycles in which embryos were derived from GnRHa-triggered cycles [25]. Also more mature oocytes (4 %) in the GnRHa-triggered group supported previous clinical findings of a possible beneficial effect of the midcycle FSH surge [19].

Triggering with urinary hCG 5,000 IU as compared to standard dose of 10,000 IU reduces the risk of OHSS without impairing the clinical outcome in terms of oocyte retrieval rate, egg quality and fertilization rate [26, 27]. Thus the indirect cost because of OHSS and related hospitalization and procedures is reduced. Cornell low-dose protocol has been suggested which determines hCG dosage according to serum E2 levels on the day of hCG administration using sliding scale between 5,000 and 3,300 IU of hCG administered to women with E2 levels of 2,000–3,000 pg/mL [28]. Significant reductions in early OHSS (occurring before ET; $P < .001$) and severe OHSS (post-ET, requiring hospitalization; $P < .05$) have been reported with this low-dose hCG protocol [29].

In patients undergoing IVF, urinary hCG still remains a feasible and cost-effective outcome compared to recombinant hCG as supported by the Cochrane database. Cochrane review by Mohamed AFM Youssef et al. concludes that there is no evidence of difference between rhCG or rhLH and uhCG in achieving final follicular maturation in IVF, with equivalent pregnancy rates and OHSS incidence [30]. According to these findings, uhCG is still the best choice for final oocyte maturation triggering in IVF and treatment cycles.

Conclusion

To conclude, considering the pharmacoeconomics of ovarian stimulation protocol in ART cycles can have long-term economic implications and also can have impact on the number of cycles the patient tries to achieve a live birth. However, due understanding of the patient profile and the drugs available along with type of infertility treatment required will play a major role in this decision-making, and we strongly feel that this should be encouraged.

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Part III

Monitoring During Controlled Ovarian Stimulation

Endocrine Monitoring of ART Cycles

19

Neena Malhotra

Abstract

Controlled ovarian stimulation is a determining factor in the success of ART cycles. Clinics the world over use ultrasound and endocrine hormone assessment to monitor these cycles to optimize success by getting the ideal number of oocytes and therefore good embryos. Further monitoring helps avert complications of hyperstimulation besides a poor response. The commonly indicated hormone assays for monitoring cycles include oestradiol, luteinizing hormone and lately progesterone. Endocrine monitoring is combined with ultrasound tracking; even though the role of intensive monitoring combining the two is controversial, it is recommended as ultrasound with serum oestradiol is utilized as a precautionary good practice.

Keywords

Hormone • Assays • Luteinizing hormone • Estradiol • Progesterone • Monitoring • ART cycles

19.1 Introduction

Assisted reproductive techniques (ARTs) have been declared a major medical breakthrough ever since the birth of the first in vitro fertilization (IVF) baby in 1978. By the end of 2013, approximately five million babies have been born worldwide from ART techniques including IVF and intra-cytoplasmic sperm injection (ICSI) [1]. While the first IVF baby was the outcome of a natural cycle, a remarkable increase in IVF success has been attributed to effective controlled ovarian hyperstimulation (COH). COH is apparently a key factor in the success of in vitro fertilization-embryo transfer (IVF-ET) and essentially involves programming ovarian stimulation and monitoring of cycles. The aim of COH in ART cycles is to procure as many mature oocytes, with better chance of good embryos that can be transferred and the spare ones frozen for future use, increasing the cumulative pregnancy rate. While monitoring ovulation induction gives

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us an idea of how long to go, essentially in ART cycles where the number of embryos transferred can be restricted reducing the risk of multiples, the risk of ovarian hyperstimulation syndrome (OHSS) or poor response is a prime concern of clinicians such that the cycle culminates in a successful embryo transfer.

What is the need to monitor cycles? Essentially monitoring during ART cycles is for

1. Predicting ovarian response to gonadotropins in advance
2. Monitoring the effect of pituitary down-regulation
3. In the course of stimulation to evaluate whether the doses of gonadotropins have been adequate
4. To avoid the major complication of ovarian hyperstimulation syndrome (OHSS)
5. To optimize the timing of administration of human chorionic gonadotropin for ovulation trigger

Monitoring is therefore essential before the start of COH to identify poor responders as well as those likely to hyperstimulate [2]. Of the methods described to monitor ART cycles, ultrasound imaging of the utero-ovarian response to gonadotropins is clinically most relevant. However used alone, it was realized that the mean size and the volume of the developing follicles seemed to vary greatly [3]. This combined with hormone analysis mainly oestradiol levels is most commonly utilized in clinics the world over. Hormonal control of ovarian activity by gonadotropins plays an important role in folliculogenesis. Besides gonadotropins, steroid hormones are also key players and are usefully measured in monitoring ART cycles. While gonadotropin assays do not adequately reflect the biological activity of gonadotropins, the endocrine characteristics of COH cycles can be assessed by steroids, including oestradiol and progesterone, as they reflect the biological activity of the gonadotropins on the ovary. Also steroids have an effect on implantation process and may play a paracrine and autocrine role on the cumulus complex.

In this chapter we see how the currently used pharmacologic agents (GnRH analogues, gonadotropins) modify the endocrine milieu and how useful are their measurements in cycle monitoring in terms of control and prediction of outcome.

19.2 Gonadotropin Analysis During Controlled Ovarian Hyperstimulation

Over the years with availability of assisted reproductive techniques, using GnRH analogues has given a better understanding of the role of follicular stimulating hormone (FSH) and leutinizing hormone (LH) in folliculogenesis. According to the two cell-two gonadotropin theory [4], both FSH and LH are important for follicular growth and development. The role of either of them in cycle control is discussed.

19.2.1 Follicle Stimulating Hormone (FSH)

During the follicular phase of cycle, FSH is involved in recruitment, selection and dominance of follicle. It is involved in the recruitment of the cohort at an early follicular phase and stimulates the transcription of genes within the granulosa cells, initiating the synthesis of aromatase enzyme, inhibins and LH receptors that are involved in follicle differentiation and growth [5].

A certain amount of FSH secretion - 'FSH threshold ' is required to induce follicle growth, and as this threshold is not identical for follicles even of the same cohort, the FSH dose for inducing multi-follicular development should cross the threshold of the least sensitive follicles. Thus the endogenous FSH is crucial to the cycle in inducing follicular recruitment. The other aspect of 'FSH window' is also crucial as this means that follicular growth is maintained as long as the FSH levels are above the threshold for the follicle. While in natural cycle the progressive decline in FSH secretion due to the feedback effect of ovarian hormones on the pituitary leads to dominance of a selected follicle and atresia of others, during COH the FSH levels are above the threshold and the window that remains open until the

final stage of follicular development resulting in multiple follicles at the time of trigger. Thus FSH is the main therapeutic drug that controls the folliculogenesis in all cases except hypogonadotropic hypogonadism where LH supplementation is necessary for production of steroid hormones.

During COH both gonadotropins and GnRH analogue are used to attain multi-follicular development, but the effects of each on the levels of FSH are variable. With gonadotropins there is a plateau of plasma FSH levels due to the long elimination half-life of FSH molecules (30–35 h). This FSH accumulation lasts for 5 days, and despite cessation of exogenous FSH administration follicles continue to mature. Also plasma levels of FSH after an intramuscular or subcutaneous dose cause a transient (4–8 h) and modest rise in plasma FSH levels, which are further not reflective of the actual bioactivity of the molecule. For these reasons there appears little justification in measuring FSH levels to adjust FSH doses or to establish the threshold above which ovarian response can be observed. This was further supported by a study by Van Weissanbrunch et al., who measured serial FSH levels to determine the adequate threshold FSH dose [6]. FSH was administered in a pulsatile manner to adjust the daily requirement according to the plasma FSH levels. The study found poor correlation between plasma FSH levels and the FSH threshold, as there was an overlap of plasma FSH levels between the groups of patients who demonstrated follicular recruitment as against those who did not [6].

The effects of agonist on FSH levels depend on the preparation and duration of use. There is an initial rise or flare effect of agonist at the pituitary resulting in a significant rise in plasma FSH levels with resultant recruitment that is exploited in a short protocol. The amplitude of FSH response to GnRH-agonist is lower than LH [7], and the desensitizing effect of prolonged GnRH-a administration on FSH secretion is much less than LH [8, 9]. Further FSH bioactivity may not decrease during GnRH-a administration [10]. Therefore, measuring plasma FSH is unlikely to be of benefit in the course GnRH-a therapy. Even in antagonist cycle, the gonadotroph suppression was less marked for FSH than for LH as suggested in studies available [11]. Summing up from available evidence on FSH variations during ART treatment, there seems to be no contribution of FSH estimation in deciding gonadotropin doses or regimen in clinical practice.

19.2.2 Luteinizing Hormone (LH)

Luteinizing hormone acts on the theca cells while producing androgens throughout the follicular phase. Androgens are the substrate for the production of oestradiol (E2) by granulosa cells. LH induces a dose-dependent production of E2, and this is foremost in endometrial preparation for embryo implantation [12]. There is a minimal amount of LH, described as the 'LH threshold', required for pregnancy; however, higher levels are detrimental with a negative impact on endometrium rather than on oocytes/embryos [13, 14]. Using high doses of LH has a negative influence on follicular development, a concept of 'LH ceiling' [15], wherein LH beyond a certain level suppresses granulosa cell proliferation and results in atresia of less mature follicles (11–15 mm) [15]. Substitution of LH in the later follicular phase with recombinant LH alone is known to cause reduction in size and number of large follicles as seen in both type I and type II WHO anovulation category of women [16]. LH therefore synergizes with FSH during the whole follicular phase of folliculogenesis.

As regards LH in ART cycles, urinary human menopausal gonadotropin (hMG) commonly used in earlier years contains LH, and this is cleared rapidly from circulation owing to a short half-life [17] approximately 12 h as compared to 30 h for FSH. Therefore there is little evidence of accumulation following hMG injection. The use of agonist in ART cycles is known for the initial flare effect, as used in ultra-short and short protocols where rise promotes early follicular recruitment. The endogenous FSH and LH rise within 24 h of GnRH agonist administration, the flare effect being more marked for LH than FSH [18]. This subsequently stimulates secretion of E2, which is considered a predictor of ovarian

response to gonadotropins. Thus estimation of LH does not reflect adequacy of flare-up response.

Measurement of plasma LH is routinely done to confirm pituitary desensitization and confirm adequate down-regulation after long-term administration of GnRH agonist. Adequate down-regulation is suggested by LH levels <3 IU/L. The rate and extent of LH suppression is dependent on the type of agonist, its route and dose used [18]. As long as the GnRH-a is given, the hypophysis is refractory to endogenous GnRH and there are no pulsatile LH secretions. The extent of LH suppression is variable after desensitization as when there are profound falls in LH levels higher doses of gonadotropins are needed to achieve ovarian stimulation. Thus in GnRH agonist-based cycles, levels of LH may have a bearing on ART outcome. The endogenous LH levels are within the normal limits (1–4 IU/L) or are within the limits defined by LH ceiling and LH threshold as sufficient for steroidogenesis and folliculogenesis.

Though low LH levels are associated with little difference in birth rate, women with LH levels less than 1.2 IU/L require higher doses of gonadotropins during ovarian stimulation [19]. Thus in poor responders lower doses of GnRH-a are suggested for adequate response to gonadotropins. In keeping with the two-cell–two-gonadotropin theory, administration of pure or recombinant FSH should not be sufficient to stimulate E2 production. However, results from large studies suggest that FSH administration is sufficient to obtain adequate number of good-quality oocytes and embryos with high implantation rate, as there is still endogenous LH, despite profound down-regulation when LH levels are measured to be low [20]. Later studies evaluated outcome in ART cycles according to the plasma levels of LH at the time of desensitization or later in mid-follicular phase [21, 22]. Those patients with LH levels <0.5 IU/L had reduced E2 concentrations at the time of hCG trigger and lower number of oocytes and embryos when stimulated with FSH alone. However, the rate of blastocyst development was unaffected. Thus measurement of LH during the cycle fails to define a sub-group of women who would need additional LH to achieve ovarian stimulation. Therefore, the threshold of LH below which folliculogenesis may be impaired cannot be assessed by measuring LH after down-regulation. There is however no relation between endogenous LH levels and ovarian response, implantation rate and pregnancy rate when normogonadotropic women undergo IVF cycles as suggested by a later meta-analysis [23].

19.3 Steroid Hormone Profile During COH

Steroid hormone estimations including oestradiol and progesterone are routine parts of hormone monitoring of ART cycles. Plasma E2 levels are a good indicator of granulosa cell differentiation and are useful in evaluating follicular maturity. Plasma progesterone (P) is useful in assessing premature luteinization, though with the use of GnRH analogues this should be uncommon. Plasma androgen are rarely performed clinically in monitoring ART cycles.

19.3.1 Oestradiol (E2)

Plasma E2 levels are useful in assessing follicular maturity as the synthesis of E2 associates with dominant follicles in natural cycles. During ART cycles plasma E2 levels are performed combined with ultrasound to adjust doses of gonadotropins. E2 synthesis is related to follicle size, and a mature follicle has an output of approximately 200 pg/ml. In days before the use of GnRH analogues to prevent endogenous LH surge, serial E2 levels were estimated as they correlated well with cycle outcome [24]. With the availability of GnRH-a protocols the problem of premature LH surges is taken care of, but the estimations of E2 are still recommended to confirm pituitary desensitization. After effective desensitization plasma E2 levels must be lower than 50 pg/ml, that is, after 2 weeks of GnRH-a, when the initiation of gonadotropins for ovarian stimulation can be given. While plasma LH levels are also estimated to confirm desensitization, they cannot adequately reflect the same for reasons discussed in previous sections. Mid-luteal start of GnRH-a and use of

long-acting preparations of agonist are associated with profound and immediate desensitization [25, 26]. Whether the prompt desensitization is associated with ovarian refractoriness and the need for higher doses of gonadotropins is debatable [25]; ovarian stimulation with FSH alone should be initiated only once ovarian activity is suppressed. During the ovarian stimulation, levels of E2 guide in determining the optimal response. Plasma E2 levels closely follow stages of development of growing follicles. After 6 days of gonadotropins, an increase in plasma E2 levels is defined as optimal response; however, due to extreme diversity of protocols, the ideal levels of E2 are not defined. A plateau in plasma E2 for more than 3 days suggests poor response to gonadotropins. Conversely excessive response can be gauged by an exponential rise in E2, which helps decide coasting or cancellation. An E2 window of 1,000–1,500 pg/ml is optimal once follicles reach 15 mm [27]. The risk of hyperstimulation is significant with levels more than 3,000 pg/ml [28]. Therefore, E2 monitoring is relevant and should be a part to define optimal response, even though some studies suggest that ultrasound monitoring is sufficient to make decisions during stimulation [29].

Further in antagonist protocols the pattern of E2 during stimulation differs from that in agonist protocols. Plasma E2 levels are higher before the addition of GnRH antagonist, and after the addition of antagonist to control the LH surge, the E2 levels may rise moderately, remain the same or even decline [30]. But these variations in E2 levels do not compromise the cycle outcome. Unlike agonist cycle E2 levels are of little help in adjusting gonadotropin doses after the antagonist has been added to cycle.

The levels of E2 have an important bearing on day of hCG trigger. A value more than 200 pg/ml per dominant follicle suggests adequate response and should be correlated with follicle monitoring on ultrasound. Despite a debatable role of E2 in pathogenesis of OHSS, E2 assessment is an important marker to predict women at risk of OHSS. The relationship between E2 levels and OHSS is controversial [28]. Absolute levels and rate of rise in E2 levels have been described to predict OHSS; however, no value is shown to be an independent predictor [31]. In general, the risk of OHSS is felt to increase variably with E2 levels >3,000–4,000 pg/mL [27, 28]. Papanikolaou and colleagues have shown that if a threshold of 3,000 pg/ml had been used, only a third of the total OHSS cases would have been predicted. Because severe cases are the more clinically significant, only 37.0 % of them would have been predicted (specificity 87 %) [32]. Using ROC curves, a cut-off value of 2,560 ng/L could not predict more than half of the severe cases (49 % sensitivity; 77 % specificity) [33]. There is no clear cut-off limit of E2 levels that predicts the risk and severity of the syndrome. Although Asch and colleagues showed that values >6,000 pg/mL in IVF cycles were associated with a severe OHSS rate of 38 %, others reported an 8.8 % rate with the same cut-off [34, 35]. These observations actually suggest that considering only high E2 levels as a risk factor is unreliable for the prediction of OHSS. While absolute values have poor predictive value for OHSS, the combination of E2 and follicle measurement produces a criterion as given in a study by Paanikolaou et al. [32]. More than 18 dominant follicles and/or E2 of 5,000 pg/L had a significant positive likelihood ratio (LR = 5.19) that can predict 83 % of the severe OHSS cases, including both early and late cases, with an acceptable specificity of 84 % [32]. A level less than 3,000 pg/ml is safe for hCG trigger.

Coasting may be a method to avert OHSS wherein levels of E2 are lowered to safe levels by withholding gonadotropins and reducing the risk of severe OHSS [28]. At what levels of E2 should coasting be initiated is debatable. Some investigators consider that an E2 level >3,000 pg/mL is enough to start coasting [35, 36], while another group only initiate coasting if the E2 level is >6,000 pg/mL [37]. Garcia-Velasco recommend initiating coasting when >15–20 follicles >16 mm are detected by trans-vaginal ultrasound, and serum E2 levels are >4,500 pg/mL on the day that hCG triggers [38]. The role of serial oestradiol estimation once coasting is initiated is paramount. The serum E2 level is evaluated on a daily basis because serum E2 behaviour is erratic and sudden unexpected drops might occur, which

usually are associated with a marked decrease in oocyte quality and a lower pregnancy rate [39]. The decline in E2 is estimated to begin on an average 1.7 days of coasting [38] and as soon as the levels drop to <3,500 pg/mL, either 5,000 IU of urinary hCG or 6,500 IU of recombinant hCG are given, egg retrieval is scheduled and the cycle continues as planned.

Besides the risk of OHSS, and role of E2 during coasting, high levels of E2 have a negative impact on endometrial receptivity [40]. This deleterious effect on endometrial receptivity is seen in high responders with E2 levels above the 75th percentile (>2,446 pg/ml), an improved embryo quality without a concomitant rise in pregnancy rate [40].

19.3.2 Progesterone

Prior to the availability of GnRH agonist, detection of premature LH surges was mandatory as these LH surges were associated with high rates of fertilization failure. Measurement of progesterone was used as a surrogate test to detect partial luteinization of granulosa cells as short surges could not be detected by daily LH monitoring. With the use of GnRH agonist and antagonist in varying protocols to prevent LH surges the need to monitor with progesterone is limited. However there are situations when progesterone is a useful hormonal tool to monitor COH.

Measurements of progesterone at the time of down-regulation are of value as they indicate that corpus luteum is inactive and not inadvertently rescued by GnRH agonist flare-up or uncommonly by a spontaneous pregnancy. After mid-luteal start of GnRH-a, the formation of cysts is associated with rise in progesterone levels and justifies the puncture of such cysts before starting FSH. Since the rise in progesterone could have deleterious effects on ovary and endometrium, it is to be ensured that ovarian stimulation should not be started in a hormonal environment that is hostile to the ovary and endometrium. Extending the administration of GnRH agonist or postponing ovarian stimulation is the best strategy. It is recommended that plasma P levels be measured even before an antagonist cycle and ovarian stimulation should be postponed with levels >1.4 ngm/ml [41].

During the latter part of ovarian stimulation measurements of progesterone are considered to indicate premature luteinization. However it is not uncommon to find this progesterone rise, as in 5–35 % of stimulated cycles it may be associated without a concomitant rise in LH levels. In such situations a rise in P cannot be considered a premature luteinization. There are several questions as to what is the mechanism of this rise in P levels. Indeed rising progesterone could be the consequence of higher production from granulosa cells in response to high doses of FSH or may be considered as an early expression of occult ovarian failure [42]. This may perhaps be one of the reasons why lower doses of FSH improve pregnancy rate, perhaps by lowering levels of progesterone during follicular growth in ART cycle. The high levels of progesterone on the day of hCG may have a negative effect on the pregnancy rates.

The Menotropin Versus Recombinant FSH In Vitro Fertilization trial (merit) study compared stimulation with highly purified human menopausal gonadotropin or recombinant FSH following a long GnRH-agonist protocol [43, 44]. The critical value for defining elevated progesterone in the study was 4 nmol/L on the day of hCG trigger. The serum progesterone was higher in r-FHS-treated patients with the resultant lower implantation rate as compared to patients treated with HP-hMG. Bosch et al. in a subsequent study reported that high serum progesterone concentration on the day of hCG (>1.5 ng/per ml or 4.77 nmol/L) was associated with a decreased pregnancy rate [45]. These findings suggest that a pre-hCG rise in progesterone may be responsible in advancement of endometrial maturation, leading to asynchrony with embryo development and negative impact on implantation [45].

Elgindy in a prospective study correlated progesterone/oestradiol (P: E) ratio on the hCG to pregnancy rates [46]. Using ROC curves a cut of 1.5 ng/ml and 0.55 were defined for P and P/E ratio respectively. Patients with P less than 1.5 ng/ml and P/E less than 0.55 undergoing cleavage stage embryo transfers had higher clinical pregnancy rates. Theses cut-offs did not correlate with pregnancy rates after blastocyst transfer. This

study highlighted that the detrimental effects of progesterone on pregnancy outcome are attributed to temporarily defected endometrial receptivity that recovers a few days later. There was a distinct difference in endometrial gene expression with a progesterone concentration above or below the threshold of 1.5 ng/ml on the day of hCG administration [47]. It seems that progesterone rise (>1.5 ng/ml or 4.77 nmol/l) affects endometrial receptivity by accelerating the endometrial maturation process that narrows the implantation window thereby decreasing pregnancy rates.

However, a meta-analysis by Venetis et al. found no difference in pregnancy rates with raised follicular phase progesterone [48]. However, the flaw in this meta-analysis was that it did not take into account the different threshold values of progesterone used in these studies. A retrospective study assessed the impact of P and P/E on the day of hCG in agonist cycle and concluded that there was no difference in pregnancy rate between patients with elevated P and P/E ratio as compared to those with normal range indicating these hormone assays are of limited value in decision to cryopreserve embryo or go ahead with fresh transfer [49].

19.4 Ultrasound Versus Endocrine Monitoring of ART Cycles

The gold standard for IVF monitoring includes both trans-vaginal ultrasound and E2 monitoring. Though ovarian stimulation is monitored in ART cycles with serial measurements of estradiol and ultrasound monitoring, results comparing cycles monitored with or without hormonal estimations as adjunct to ultrasound do not support superiority of combined monitoring. Murad in a study concluded that ultrasound only monitoring was cheaper, less time consuming, and more convenient for both patients and the team when compared to hormonal and ultrasound monitoring of IVF cycles [50].

Lass in a multicenter, prospective randomized trial from UK did not show any benefit of additional E2 estimation over ultrasound only monitoring in terms of pregnancy rate, decision to time hCG trigger or risk of OHSS [51]. Further, although a systematic review did not show that E2 monitoring prevented OHSS, it did conclude that E2 monitoring should continue to be routinely performed as a 'precautionary good practice point' [29]. While E2 alone was used in the earlier years of IVF, additional hormone assays have a disadvantage of frequent blood sampling, the need for a reliable laboratory setup and costs involved. While units worldwide are involved in developing stimulation protocols that minimize monitoring and therefore costs to the patient, use of minimal hormone analysis as in minimal oestradiol estimations with ultrasound monitoring of cycle is likely to stay.

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Ultrasonography in Controlled Ovarian Stimulation

20

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Abstract

The field of infertility and reproductive endocrinology relies heavily on imaging. Of all imaging modalities, ultrasonography has emerged as the key modality in artificial reproductive technologies. Its role begins with assisting in the diagnosis of the cause of infertility, through deciding on the optimal treatment strategy, execution of personalized fertility treatments and diagnosis and treatment of potential complications arising during treatment. This chapter provides an evidence-based review of the role of ultrasound in COS. Prior to the initiation of treatment, ultrasound assists in pelvic evaluation (predicting treatment success, assuring a dominant follicle has not emerged and diagnosing ovarian abnormalities that should be addressed). After designing the personalized treatment protocol, ultrasound is fundamental in monitoring the response to treatment (endometrial and ovarian follicular response). In in vitro fertilizations it has a key role in guiding embryo transfer. Lastly, its role does not end with achieving pregnancy but is also essential in the diagnosis and treatment of COS complications (abdominal haemorrhage, pelvic infections, adnexal torsion,

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ectopic pregnancy and ovarian hyperstimulation syndrome). In fact, so indispensible is its use that it would be almost impossible to imagine COS achieved successfully and safely without pelvic ultrasonography.

Keywords
Ultrasound • US • Controlled • Ovary • Stimulation • COS • Complication

20.1 Introduction

Ultrasound, having evolved from a simple two-dimensional B mode picture to a comprehensive three-dimensional real-time image, enables its application in various fields in infertility and reproductive endocrinology. In COS ultrasonography is useful not only during execution of the fertility treatment but also assists in choosing the suitable treatment protocol, performing embryo transfer and diagnosing and treating associated potential complications. This chapter reviews the role of ultrasound in COS, from diagnosis of infertility through monitoring the treatment regimen to treating possible complications.

20.2 Pelvic Evaluation Prior to Treatment

Every patient who is a candidate for COS due to infertility has had a pelvic ultrasound performed during the evaluation of infertility. Moreover, many women have also undergone ovulation induction and intra-uterine insemination and failed to achieve pregnancy, before attempting COS. Nonetheless, a pelvic ultrasound prior to treatment selection and initiation should be performed. The purposes of this are the following.

20.2.1 Predicting Treatment Success

20.2.1.1 Evaluation of Ovarian Reserve by Antral Follicle Count (AFC)

Ovarian reserve can be assessed by various means, including inhibin B, FSH, AMH and AFC. AFC is calculated by adding up the number of antral follicles in both ovaries, optimally performed between 1 and 7 days after the beginning of menstruation. A positive correlation has been shown between AFC and the number of oocytes and the percentage of MII oocytes (metaphase 2) retrieved in ART [1]. The predictive performance of AFC towards poor response in ART is significantly better than that of basal FSH [2]. When comparing different parameters (sonographic and hormonal) of ovarian response, it has been previously shown that AMH and AFC are the two most significant predictors of ovarian response in COS and ART and their predictive accuracy is similar. As a combined test their predictive accuracy is not further improved [3]. While evaluation of AFC and AMH prior to ART is not yet considered routine practice, it is becoming a more popular tool, and it might be considered in women with previous ART failure when advising them regarding further treatment.

20.2.1.2 Ovarian Vascular Indices

Ovarian vascularity indices (vascularization index [VI], flow index [FI] and vascularization-flow index [VFI]) – Various studies performed have not found a correlation between these Doppler values and ovarian response and pregnancy in IVF treatments [4–6].

20.2.1.3 Ovarian Volume

Compared with AFC, ovarian volume is a weak predictor of pregnancy and response to ART. It is not suitable as a routine test for ovarian reserve or response to ART [6].

20.2.2 Assuring a Dominant Follicle Has Not Already Emerged

When the GnRH agonist short protocol is applied an ultrasound prior to the initiation of hormonal

treatment can determine if folliculogenesis has not already chosen a dominant follicle.

20.2.3 Diagnosis of Abnormal Ovarian Findings That Should Be Addressed

Ovarian cysts or masses may require treatment before initiation of ART. A controversy exists in the literature regarding aspiration of ovarian cysts versus expectant management before beginning hormonal treatment. Adnexal cysts may interfere in treatment is several ways:

- Disrupted folliculogenesis due to compression of the ovarian parenchyma
- Monitoring of follicular growth
- Complicating ovum pickup

Despite these potential limitations trials have not shown that simple cyst aspiration prior to ART increases the number and quality of retrieved oocytes [7, 8]. As for endometriomas, a Cochrane review in 2010 showed that while surgery (aspiration or cystectomy) versus expectant management does not improve clinical pregnancy rates, aspiration versus expectant management is associated with greater retrieval of MII oocytes and increased ovarian response in ART. Nonetheless, surgical treatment may sometimes be more harmful than the cyst itself to the ovarian reserve. This is especially true in women with endometriosis, in whom oocyte quality is reduced to begin with. Therefore, due to the negative impact ovarian surgery has on the decrease in ovarian reserve, when performing surgery for endometriomas, the conservative laparoscopic approach is more suitable.

20.2.4 Baseline Ultrasound in Between Cycles of Treatment

Baseline ultrasound in between cycles of treatment may be considered between consecutive cycles of stimulation with exogenous gonadotropins. Higher fecundity rates have been observed with consecutive cycles than with alternating cycles [9]. However, when one or more residual ovarian cysts are diagnosed it might be prudent to postpone the treatment briefly, as success rates are lower with the presence of cysts [10].

20.3 Ultrasound in Monitoring Fertility Cycles

The use of ultrasound in fertility treatments, both ovulation induction and IVF, is indispensible and extends from monitoring and timing the interventions to diagnosing and treating complications and predicting success rates.

20.3.1 The Endometrium

20.3.1.1 Endometrial Thickness

Successful implantation depends upon endometrial receptivity. Endometrial thickness is measured as the sum thickness of the two opposing endometrial layers in the mid-sagittal plane (Fig. 20.1). A controversy exists whether endometrial thickness has a positive predictive value on pregnancy rates, or only a negative predictive value. In ovulation induction cycle fecundity increases with endometrial thickness, correlating with serum oestradiol levels. Few pregnancies have been noted in gonadotropin-induced

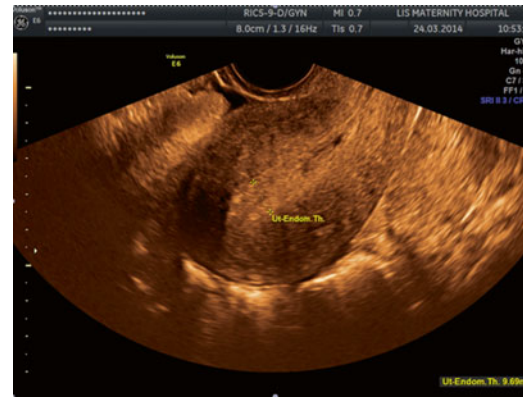

Fig. 20.1 Calculating endometrial thickness – the sum thickness of the two opposing endometrial layers in the mid-sagittal plain

IUI cycles when the endometrium measured <7 mm on the day of hCG (human chorionic gonadotropin)-induced ovulation [11]. Too thick of an endometrium does not seem to negatively impact success [12, 13].

Use of clomiphene citrate for ovulation induction in anovulatory women is common, since it will induce ovulation in approximately 70–80 % of properly selected women [14]. However, in a minority of these women treatment with clomiphene may have anti-oestrogenic effects and inhibit endometrial growth [15]. Contrary to the belief, several studies in women treated with clomiphene and IUI exhibiting a thin endometrial stripe did not find a reduction in pregnancy rates [16, 17].

In ART cycles numerous trials have failed to observe a clear correlation between endometrial thickness or appearance (trilaminar vs. homogenous) on the day of hCG administration and cycle outcomes. Since these endometrial characteristics are not predictive of ongoing pregnancy outcomes, its routine assessment may not be justified.

20.3.1.2 Doppler Studies

Doppler velocimetry is a useful tool for evaluating uterine receptiveness in fertility treatments. Uterine artery Doppler flow indices (vascularization index (VI), flow index (FI) and vascularization flow index (VFI)) of endometrial and sub-endometrial regions have been linked to pregnancy outcome in ART. It has been shown that greater vascularization on the day of oocyte retrieval correlates with higher live birth rates [18]. In COS and IUI treatment a lower pulsatility index (PI) in uterine and intra-ovarian arteries around the time of ovulation was exhibited by women who conceived, as opposed to those who did not [19]. A uterine PI >3 is a poor predictor of pregnancy outcome in ART [20].

20.3.2 The Follicles

20.3.2.1 Measuring Follicular Size

During fertility cycles routine assessment of follicles is mandatory in order to predict ovulation (in IUI cycles or timed coitus) or time hCG administration. In the normal ovulatory cycle the recruited cohort of antral follicles can be identified by cycle day 5–7 and the dominant follicle by day 8–12. The growth rate thereafter is approximately 1–3 mm per day. When the LH surge occurs the dominant follicle measures about 20–24 mm in diameter. Follicles which arrive at maturity 'naturally' (without ovulation induction) may not be quite the same as those that reach maturity via ovulation induction. Moreover, different induction protocols achieve oocyte maturity at different time frames, according to the gonadotropin supplied. In gonadotropin-stimulated cycles, dominant follicles reach maturity at a lesser diameter and over a wider range of sizes. While 80 % of the follicles will ovulate when measuring 19–20 mm in diameter, those measuring less than 14 mm ovulate in less than 40 % of the cases [21]. Generally speaking, most agree that follicular maturity is reached at a follicle size ranging between 16 and 22 mm [22]. Follicular growth is monitored differently according to stimulation protocol. We monitor growth as follows:

- In natural cycles (in women with regular menstruation) or IUI cycles without stimulation – ultrasound is performed typically from day 10 of menstrual cycle.
- In gonadotropin-stimulated cycles and IUI – ultrasound is performed typically from day 10, after 5 days of stimulation, except for special cases (poor responders in whom the first ultrasound is performed later than day 10 and hyper-responders, with risk factors for OHSS, in whom the ultrasound is performed earlier than day 10 of stimulation).
- In IVF cycles – ultrasound is performed after 5 days of stimulation.

Follow-up intervals are scheduled according to follicular size, growth rate and blood hormone concentrations of oestradiol. Normally for IUI and natural cycles we perform follow-up every 2–4 days, according to the response rate. For IVF cycles we usually perform follow-up every other day.

20.3.2.2 Timing of Ovulation

Timing ovulation induction by ultrasound only is limited, as it may miss premature LH surges, if

given too late, or result in triggering an immature follicle, when given too early. Therefore, while some clinicians monitor ovulation induction by ultrasound only, most coordinate IUI cycles with LH surge detection in urine or blood. A Cochrane review concludes that either way is possible, since both result in similar rates of pregnancy and live birth rates [23].

20.3.2.3 hCG Administration

hCG should be administered according to the size of the dominant follicle and the number of follicles. For ovulation induction a single follicle, and no more than 3–4 dominant follicles, is the goal. The physician may opt to cancel the cycle if more than two follicles are likely to ovulate. For super-ovulation multiple follicular development is the goal. We administer hCG as follows:

- In natural cycles and clomiphene citrate-stimulated cycles – when the dominant follicle is 20–24 mm
- In gonadotropin-stimulated cycles and IUI – when the dominant follicle is larger than 18–20 mm
- In IVF cycles – when three follicles above 17–18 mm are present

Measurement of ovarian follicles can be achieved by two- and three-dimensional ultrasound, as well as by automatic ultrasound counting software. Two-dimensional measuring is performed by measuring the greatest follicular diameter and averaging the two greatest diameters measured (Fig. 20.2). In super-ovulation, when the goal is obtaining multiple large follicles, such a task can be tedious and inaccurate. An emerging technology, Sonographic Automated Follicular Volume Calculation (Sono AVC) using 3D US software, may be preferable in such cases; however, it is still under investigation. A recent review comparing this method to other validated measurement methods found that automated volume measurements are in very good agreement with actual volumes of the assessed structures. This technique seems to provide fast, reliable and highly reproducible results under a variety of conditions, including COS and IVF. It can replace or be used interchangeably with conventional 2D measurements as a method of quality control and may also create opportunities for developing hCG criteria based on follicular volume [24]. Its main disadvantage is in the evaluation of follicles smaller than 10 mm (Fig. 20.3).

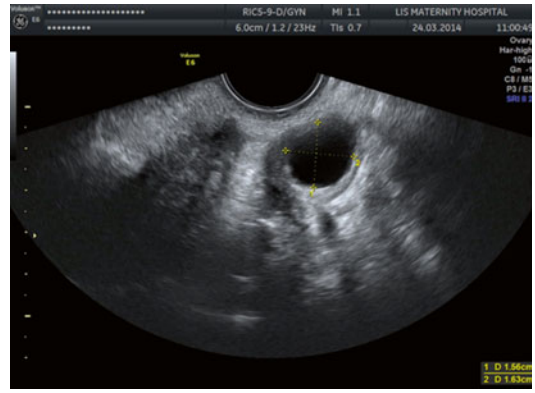

Fig. 20.2 Calculating follicular size. Two dimensional measuring is performed by measuring the greatest follicular diameter and averaging the two greatest diameters measured

20.4 Monitoring COS with Ultrasonography Only

Fertility cycles are monitored traditionally by both ultrasonography and measurements of oestradiol (E2) serum levels. Current evidence from meta-analysis of RCTs concludes that monitoring COS cycles with ultrasonography only is unlikely to substantially alter the chances of achieving a clinical pregnancy or affect the number of oocytes retrieved per cycle [25]. Moreover, the addition of E2 measurements to ultrasound monitoring of IVF cycles in normal responders seldom changes the timing of hCG administration or the risk of OHSS [26]. According to a Cochrane review, when monitoring IVF and ICSI (intra-cytoplasmic sperm injection) cycles similar pregnancy and live birth rates were achieved with combined ultrasound and E2 as with ultrasound alone. However, until a sufficient RCT to test different ways of monitoring OHSS (a rare but life-threatening complication) is done, cycle monitoring by ultrasound and serum oestradiol is recommended as a precautionary good practice point [27].

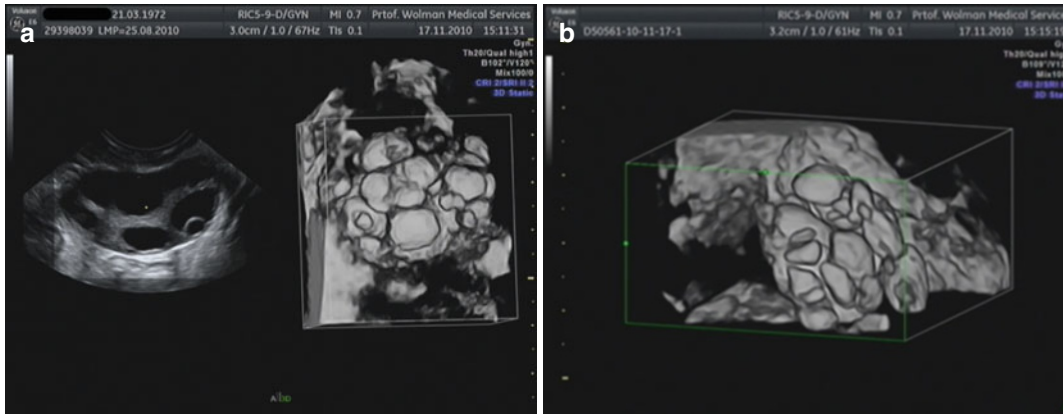

Fig. 20.3 (**a**, **b**) Follicle count and volume in a stimulated ovary with SonoAVC

20.5 Embryo Transfer

Embryo transfer can be performed under ultrasound guidance or in a tactile manner. With the ultrasound guided transfer technique an abdominal ultrasound transducer is placed suprapubically to allow clear visualization of the uterus. The transfer catheter loaded with the embryo(s) is gently guided through the cervical opening into the uterine cavity under observation. Once the ultrasound image confirms that the catheter is properly placed, the embryos are injected into the uterus, and the catheter is slowly withdrawn (Fig. 20.4). Several studies have examined the effect of the distance of blastocyst dislodgement, as determined by air bubble position, from the uterine fundus on pregnancy rates. Air bubbles within 10 mm from the fundus are associated with greater pregnancy rates as opposed to placement further away from the fundus [28]. While some believe that the optimal placement of the inner catheter tip is 1.5–2 cm from the fundal endometrial surface [29], others state that pregnancy rates do not differ as long as the catheter tip is placed in the middle to upper third of the uterus [30].

Ultrasound-guided transfer has many potential advantages, such as facilitating the placement of soft catheters, avoiding stimulation of the fundus and endometrial lining and blocking of the catheter tip with blood or mucus and placement

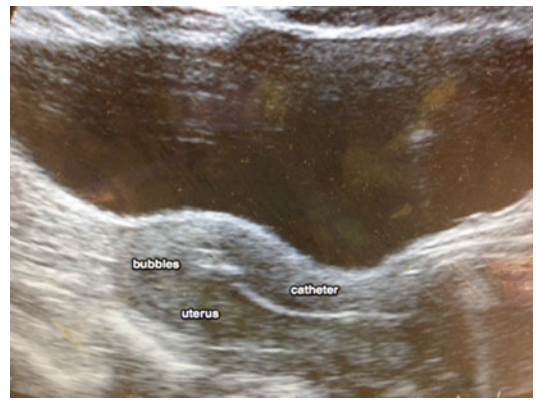

Fig. 20.4 Ultrasound guided embryo transfer

of the catheter in cases of an elongated cervical canal. Also, when performed under ultrasound guidance a full urinary bladder is required, which straightens the cervical canal and facilitates access to the uterus [31].

A Cochrane meta-analysis of four trials concluded that the clinical pregnancy rate and ongoing pregnancy rates were significantly greater with ultrasound-guided embryo transfer as opposed to the 'clinical feel' approach. The rate of ectopic pregnancies was not affected by the method of transfer [32]. Other trials have found that the advantage of ultrasound-guided transfer is culture day dependent – exists if transfer is performed after 3 or 4 days of culture but not when performed for 5-day-old embryos [33].

20.6 Complications During COS: Sonographic Diagnosis and Treatment

Although generally safe, COS treatments are associated with complications that, albeit rare, can have significant morbidity. The rate of major complications is 0.7 % [34]. All physicians, and not only those performing COS treatments, should be aware of the increased frequency of potential complications and should keep them in mind when treating women undergoing fertility treatments. The role of ultrasonography is not only in the diagnosis of these conditions but may also aid in resolving them.

20.6.1 Follicle Rupture and Bleeding

Bleeding during oocyte aspiration is divided into minor and major episodes. Minor vaginal bleeding is reported to occur in 0.5–8.6 % of oocyte retrievals [35]. In most cases it subsides after brief local compression, and only rarely suturing is required.

According to a recent retrospective study of 973 cycles of COS, cyst rupture and intra-abdominal bleeding following oocyte retrieval are very rare complications, occurring in 0.1 % of the cases [34]. Similar rates were also reported previously [36]. It is caused by puncture of vessels, such as the ovarian capsule vessels, the sacral plexus or the iliac vessels. This life-threatening complication can be avoided by careful ultrasound visualization of the peripheral follicles in a cross-sectional view before puncture and by the use of colour Doppler if available [37] (Fig. 20.5).

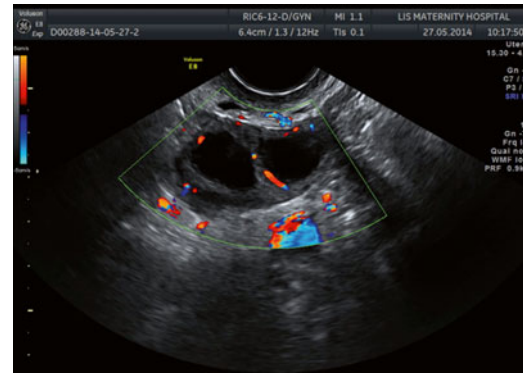

Fig. 20.5 Color Doppler aids in visualization of blood vessels during ovum pick up

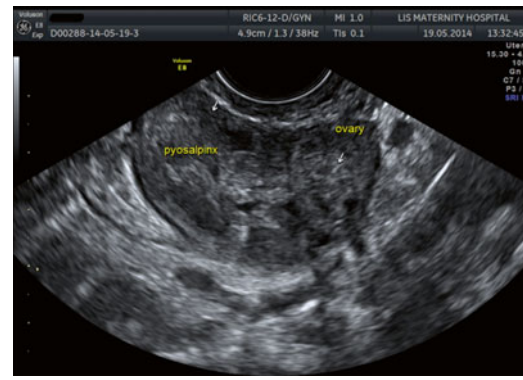

Fig. 20.6 Tubo-ovarian abscess with pyosalpinx

20.6.2 Pelvic Infections

The risk of infectious complications following oocyte retrieval is low (0.3–0.6 %), even without antibiotic prophylaxis. Therefore, the use of prophylactic antibiotics 30–60 min before retrieval or immediately after the procedure is controversial. Alternatively, some reserve it for women at increased risk of infection (those with a diagnosed endometrioma or a history of pelvic inflammatory disease) [35, 38]. Nearly half of the cases of infection present as tubo-ovarian abscesses, 1–6 weeks after oocyte retrieval [35] (Fig. 20.6). An ultrasound is key to the diagnosis of this important complication. A complex adnexal cystic lesion following trans-vaginal oocyte retrieval that is accompanied by persistent fever and leukocytosis should lead to an early presumptive diagnosis of tubo-ovarian abscess.

20.6.3 Adnexal Torsion

Torsion of the ovary is a serious complication of ovarian stimulation and should be considered in any patient with complaints of sudden abdominal

pain accompanied by nausea, appearing during or after ovarian stimulation. Since ovarian cysts are the main risk factor for torsion, the rate is higher among women exhibiting OHSS. It is 11 times more common in ART pregnancies than in non-ART pregnancies [39]. While the absolute incidence of ovarian torsion is 0.8 % in all IVF cycles [36], it can reach 7.5 % in patients with OHSS [40]. The most consistent imaging finding is asymmetric enlargement of the twisted ovary [41], frequently due to an underlying mass. Obstruction of venous outflow in torsion also causes enlargement, as well as stromal oedema and stromal heterogeneity due to haemorrhage and oedema. Peripheral displacement of follicles due to oedema may also be noted, as well as free pelvic fluid. Doppler sonography can aid in the diagnosis and decrease the morbidity associated with this condition. However, studies have found normal Doppler findings in 45–61 % of torsion cases, so they should not be relied on too heavily. Lastly, a sensitive sign is the 'whirlpool sign', seen at both grayscale and colour Doppler US as coiled vessels representing a twisted vascular pedicle [42, 43]. In rare cases the fallopian tube can undergo torsion without involving the ipsilateral ovary. In these cases colour Doppler may not facilitate the diagnosis. Due to the limited diagnostic value of sonography in ovarian torsion, management decisions should still be based on clinical grounds.

Distinguishing ovarian torsion from mild OHSS can be challenging, both clinically and radiologically. Clinically, both entities may manifest with abdominal pain, nausea and vomiting. Radiologically, there is overlap in the grayscale US appearance of these conditions, since both may demonstrate ovarian enlargement and heterogeneous ovarian stroma [41]. Peripheral migration of follicles, a finding associated with torsion, may not be evident in the hyperstimulated ovary, due to the increase in follicles. Moreover, free pelvic fluid, a common finding in torsion, is also present in nearly all cases of OHSS. A possible diagnostic hint to differentiate between the two entities is the finding of cortical cysts separated by thickened parenchyma in the twisted ovary, as compared with ovarian follicles separated by thin walls in OHSS [44] (Fig. 20.7).

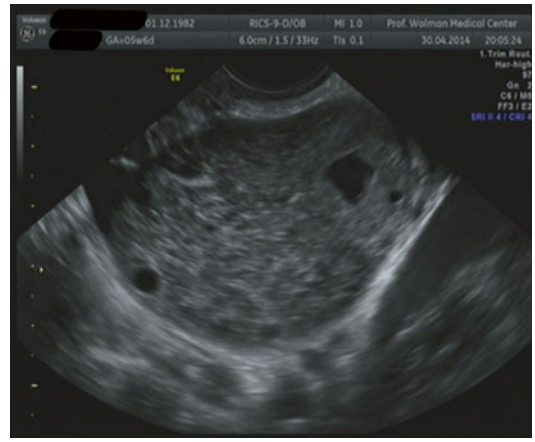

Fig. 20.7 Torsion of an enlarged (hyperstimulated) ovary. Thickened echogenic parenchyma separates the cortical cysts, distinguishing torsion from OHSS

20.6.4 Ectopic or Heterotopic Pregnancy

While the incidence of ectopic pregnancy in spontaneous gestations is approximately 2 % [45], its incidence following ART ranges from 2.1 to 8.6 % [46]. Many of the risk factors associated with ectopic pregnancies, such as increasing age, prior tubal pregnancy and history of salpingitis, are also characteristic of the ART population. In contrast to the discriminatory threshold of an intra-uterine pregnancy of a beta-hCG level of 1,500, there is no such threshold for the diagnosis of an extra-uterine pregnancy. Moreover, it is important to note that up to 35 % of ectopic pregnancies may not display any extra-uterine mass [47]. The sensitivity of trans-vaginal sonography can be improved by performing 3D imaging in asymptomatic patients [48]. In cases of high clinical suspicion, a normal ultrasound does not exclude the diagnosis. Most ectopic pregnancies resulting from ART are tubal, as in spontaneous pregnancies; however, the frequencies of cervical, interstitial and abdominal pregnancies, though remaining rare, are increased in ART treatments [49, 50]. Interstitial pregnancy is worth noting, due to its potential catastrophic outcome. This type of pregnancy occurs when an embryo implants in the intra-myometrial portion of the

fallopian tube. Its sonographic appearance is of an eccentrically located gestational sac surrounded by a thin layer of myometrium measuring less than 5 mm [51] (Fig. 20.8). A specific additional finding is the 'interstitial line sign', an echogenic line that likely represents the interstitial portion of the fallopian tube [52]. These ectopic pregnancies tend to manifest later, and rupture may result in the rapid extravasation of blood to the abdominal cavity due to proximity to the uterine artery.

Heterotopic pregnancy refers to simultaneous intra-uterine and ectopic pregnancies and has an incidence of 1–3 % in ART patients [41]. In order to avoid a late diagnosis due to rupture, as is the case in 50 % of heterotopic pregnancies [53], it is critical to visualize both adnexa when performing an US in women undergoing ART, even when an intra-uterine pregnancy is diagnosed. The most common location of a heterotopic pregnancy is tubal, in 88 % of the cases [52] (Fig. 20.9). Since these pregnancies cannot be followed reliably by the beta-hCG level, the recommended treatment is surgical.

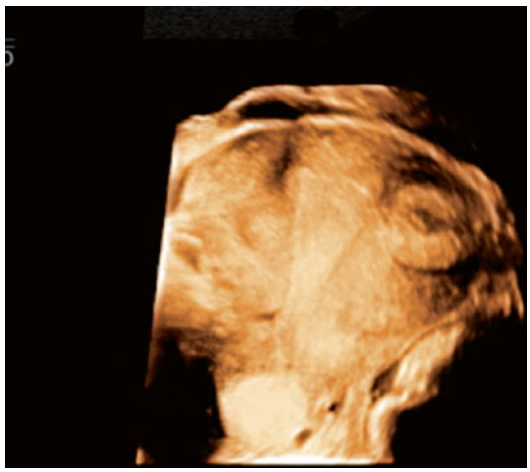

Fig. 20.8 Interstitial pregnancy. A yolk sac within a gestational sac, located in the interstitial portion of the fallopian tube

20.6.5 OHSS

Ovarian hyperstimulation syndrome is a potentially life-threatening iatrogenic complication of infertility treatments, which can occur in the setting of ovulation induction with exogenous gonadotropin therapy of IVF. Rarely it can also occur in its mild form after treatment with clomiphene citrate. Mild ovarian hyperstimulation probably occurs in 8–23 % of stimulated cycles, moderate forms in <1–7 % and severe forms in ~0.5 % of stimulated cycles [54, 55].

The most frequently used classification for the severity of OHSS, proposed by Golan, is based on the use of ultrasonography to quantify ovarian enlargement and ascites [54, 56] (Table 20.1). Here we will focus on the sonographic appearance of hyperstimulated ovaries. Bilateral

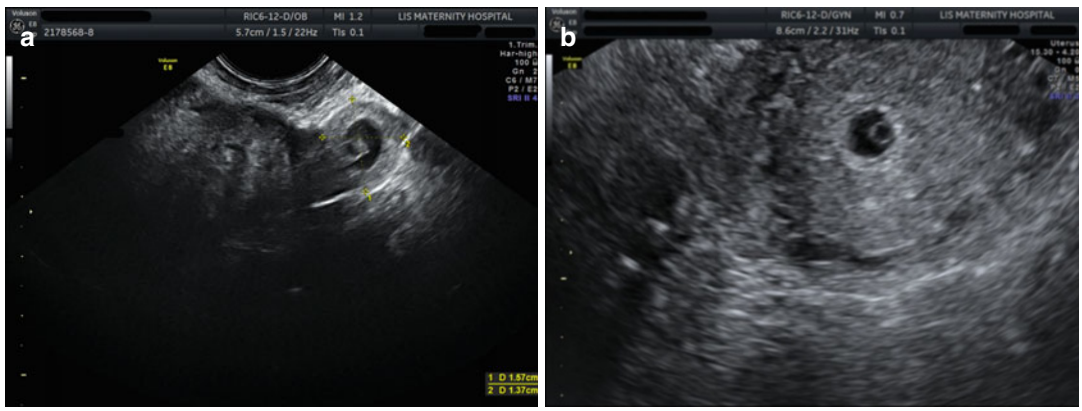

Fig. 20.9 (**a**, **b**) Heterotopic gestation (**a**) An ectopic gestational sac, yolk sac and embryo in the right fallopian tube along with (**b**) An intra-uterine pregnancy with a gestational sac and yolk sac

Table 20.1 Modified Golan Classification scheme for OHSS

Category	Ovarian size (cm)	Grade	Signs and symptoms
Mild	<6	I	Abdominal distention
		II	Abdominal distention, nausea, vomiting, diarrhea
Moderate	6–12	III	Abdominal distention, nausea, vomiting, diarrhea; ascites at US; weight gain
Severe	>12	IV	Ascites/hydrothorax
		V	Ascites/hydrothorax, hypovolemia, hemo-concentration, coagulation disorder, oliguria, shock

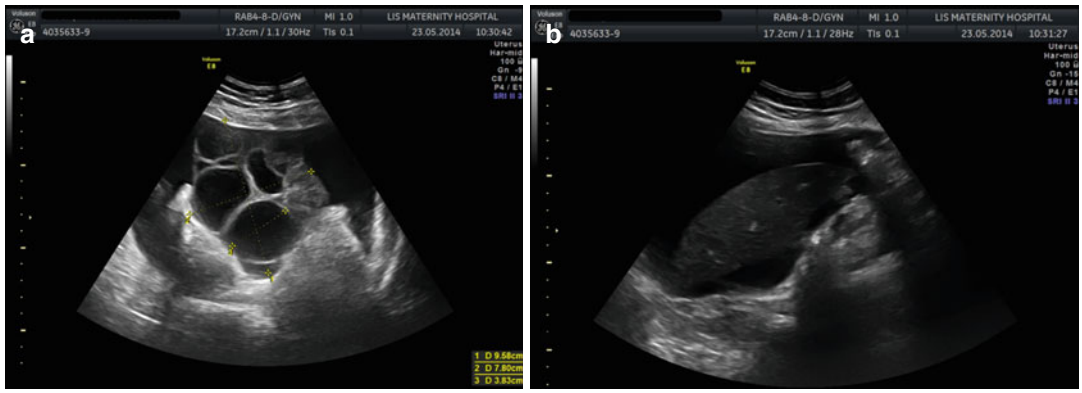

Fig. 20.10 (**a**, **b**) OHSS. (**a**) enlarged ovary with multiple variable-sized cystic lesions and ascites; (**b**) ascitic fluid around liver associated with severe OHSS

symmetrically enlarged ovaries containing multiple variable-sized cystic lesions representing enlarged follicles or corpus luteum cysts, in the presence of ascites, is the hallmark image [41] (Fig. 20.10). The characteristic peripheral location of the follicles surrounding a central core of ovarian stroma has been described as creating a 'wheel spoke' appearance, with thin septa separating the cysts [57]. The cysts are usually anechoic but may be complicated by haemorrhage. Associated complications such as deep vein thrombosis or pleural effusion can also be diagnosed with ultrasonography. Follow-up imaging should demonstrate resolution of the ascites/effusion. Ascites drainage should be considered when there is dyspnoea or tense ascites and abdominal pain. It can be performed both vaginally and intra-abdominally, always under ultrasound guidance. Quick symptom relief is usually achieved along with improvement in venous return, cardiac output, diuresis, creatinine clearance and lung ventilation [58].

Conclusions

The role of ultrasound in COS begins with the initial evaluation of the infertile patient through planning and executing the fertility treatment and culminates with treating potential complications. Ultrasound is commonly used to assess the process of folliculogenesis during ovulation induction and to follow the emergence and growth of a dominant follicle in natural cycles. During the early follicular phase ultrasound can assure adequate follicle recruitment, while during the late follicular phase it can assist in timing hCG injection and ovulation according to follicular size and endometrial thickness and velocimentry. In IVF cycles oocyte aspiration is performed under trans-vaginal ultrasound guidance, and in embryo transfer it is used to map the endometrial cavity and determine the optimal location for embryo placement. Complications, including extra-uterine pregnancy, adnexal torsion, OHSS and intra-abdominal bleeding

or infection, can also be diagnosed with ultrasonography, while OHSS can sometimes be prevented with careful adjustments of gonadotropin regimens when ultrasound reveals excessive follicular recruitment. In conclusion, in ART trans-vaginal ultrasonography is indispensible, as it has revolutionized both the evaluation and treatment of infertility.

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Part IV

The Endometrium and Luteal Phase

Effect of Ovarian Stimulation on the Endometrium

21

Manish Banker and Arati Gupte-Shah

Abstract

The endometrium is a dynamic endocrine organ. Its role in implantation is the single most vital step in the management of infertility, yet it is least understood.

In this chapter, we offer an insight into the endometrium. We discuss its physiology and functions, its molecular dynamics, the hormonal interplay involved in the menstrual cycle and its role in conception. We also discuss the factors regulating endometrial receptivity, how it is affected by various hormones, how the natural hormonal interplay affects the window of implantation and what effect different stimulation protocols have on its structure and functions.

Keywords

Endometrium • Implantation • Receptivity • Stimulation protocols • GnRH agonists • Antagonists

21.1 Introduction

Implantation is the single most important step in the management of infertility and is also the most poorly understood part of reproduction. The endometrium is an active endocrine organ. It synthesizes and secretes lipids like prostaglandins and thromboxane, cytokines like interleukin and interferon and peptides like prolactin, growth factors, relaxin and renin. The interplay of all these substances is essential to create a receptive environment for the implanting blastocyst. This environment is labile and easily disturbed by exogenous as well as

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endogenous hormonal fluctuations. To understand these factors, we must first review the natural mechanisms of the endometrium and its functions.

21.2 Physiology of the Endometrium and Hormonal Interplay

The growth of the endometrium in every cycle is stimulated by the rising levels of oestrogen. There is increase in stromal thickness as well as increase in ciliated and micro-villous cells of the endometrium. Spiral arteries, the end arterial branches of the uterine arteries, are sensitive to hormonal changes. Glandular formation begins under the influence of oestrogen, in this phase. Glands become enlarged, filled with vacuoles and tortuous. Endometrial thickness increases. Oestrogen also stimulates VEGF synthesis, which helps in angiogenesis.

Epithelial proliferation stops 3 days after ovulation. This inhibition is brought about by the rising levels of progesterone that begin in the secretory phase. Tortuosity of glands increases, and there is intensive coiling of spiral arterioles in response to the progesterone. This heralds the beginning of the secretory phase. At the time of implantation, there is increased oedema of the endometrial stroma. Oestrogen and progesterone at this stage cause increase in production of prostaglandins, which leads to increased capillary permeability and thus increased stromal oedema.

Decidualization of the endometrium takes place around day 21–23 under the influence of progesterone. This decidualization helps to control the invasion of trophoblast after implantation. If there is no implantation, endometrial breakdown begins. Drop in the oestrogen and progesterone levels leads to withdrawal of support leading to vasomotor reactions that cause apoptosis, and subsequently tissue loss, which in turn leads to menstruation. At a cellular level, MMP (matrix metalloproteinase) secretion caused by progesterone withdrawal leads to cell membrane breakdown and dissolution of the cell membranes. MMP expression is suppressed postmenstrually by the rising oestradiol levels [1].

21.3 Role of Endometrium in Conception

The endometrium performs numerous functions to achieve conception [2]:

- Sperm transport from cervix to oviducts
- Nourishment of blastocyst
- Removal of zona pellucida from fertilized ovum
- Attachment and implantation of blastocyst

21.4 Endometrial Receptivity and Window of Implantation [WOI]

Endometrial receptivity is defined as a temporary unique sequence of factors that make the endometrium receptive to implantation of the embryo. The endometrium is normally a non-receptive environment for an embryo. The window of implantation is the window of time when the uterine environment is conducive to blastocyst acceptance and subsequent implantation. Embryo transfer data from assisted-conception cycles suggests a window lasting approximately 4 days, from days 20–24 of a 28-day normal cycle [3].

21.5 Markers of Endometrial Receptivity

21.5.1 Pinopodes

The beginning of the WOI is heralded by the progesterone-induced formation of pinopodes. Pinopodes are bleb-like short irregular surface projections found on the apical surface of the endometrial epithelium [4]. They are usually seen between the 19th and the 21st day of the menstrual cycle and persist for 24–48 h. They are considered transient markers of endometrial receptivity [5]. Their formation is stimulated by rising levels of progesterone seen in the luteal phase. Administration of oestradiol leads to their

rapid loss, usually within 24 h. Thus, their detection during the mid-secretory phase is useful as a marker for endometrial receptivity. Blastocyst attachment has been shown to occur on top of pinopodes.

There are numerous other markers that help to define endometrial receptivity.

21.5.2 Biochemical Markers

Adhesion molecules: Mainly αvβ3 integrin appears in endometrial glands and luminal surface on cycle days 20 to 21 and is among the best-described markers of endometrial receptivity.

Anti-adhesion molecules: MUC-1 (mucin 1)
Cytokines: Leukaemia inhibitory factor (LIF)
Endometrial growth factors:
- Heparin-binding epidermal growth factor (HB-EGF)
- Insulin-like growth factor-binding protein-1 (IGFBP-1)
- Endometrial immune markers

21.5.3 Genetic Markers

Hoxa10 gene expression in the endometrium rises at the time of ovulation and has been shown to be essential for human implantation [6]. The uterine sensitization-associated gene-1 (USAG-1) is preferentially expressed in the maximal duration of endometrial receptivity [7]. Endometrial bleeding associated factor (EBAF) is found to be expressed in the late secretory and menstrual phase of the endometrium [8].

21.6 Hormonal Interplay

During stimulation, both gonadotropin (LH and FSH) and steroid hormone (oestrodiol and progesterone) levels vary. This may negatively or positively impact the endometrium according to the rise and timing of rise of these hormones.

21.6.1 Effect of Oestradiol on the Endometrium

A study by Basir et al. [9] in 2001 studied the effect of oestradiol in high and low responders in patients undergoing ovarian stimulation. They found that there was a much greater endometrial glandular volume in natural cycles as compared to stimulated cycles. The glands were more tortuous and numerous and occupied a greater area at the time of implantation.

In high responders, they observed a decline in glandular volume and an increase in the diameter of the glands, which was in direct proportion to the rise in oestradiol levels. This led to prolonged retention of glandular secretions and retarded emptying. This caused asynchronous secretory transformation of the endometrium due to reduced volume and insufficient secretions, leading to reduced endometrial receptivity. They also observed that stromal oedema was marked in such cases.

21.6.2 Effect of Progesterone

A study conducted by Bell et al. [10] has demonstrated the changes occurring in the endometrium due to the effects of progesterone. They divided the proteins that are secreted and synthesized by the endometrium into three groups, depending on their response to external stimulation.

Group 1: EP6, EP12
Group 2: EP 13, EP14, EP15
Group 3: EP 9, EP11

Of these three groups, group 2 is the one that is dependent on the histological endometrial type and is unaffected by short-term changes in levels of progesterone. According to the study, EP 14 and 15 are the two major proteins of pregnancy. EP 15 is associated with the decidua spongiosa region of the decidua parietalis during pregnancy and originates in the secretory glandular epithelium. During the menstrual cycle, it is said to be present in the uterine lumen and thus play a role in the implantation of the blastocyst. Whereas

EP14 is not secreted in significant amounts during the menstrual cycle, in pregnancy, it is the major secretory protein of the decidua compacta layer of the decidua parietalis. Both these proteins are mainly progesterone dependent because their synthesis depends on the stage of differentiation of the endometrium. The rate of synthesis of group 3 endometrial proteins is dependent on variations in the progesterone levels, independent of the stage of differentiation. The study suggests that its presence in the peripheral sera may be a way to examine the response of the endometrium to progesterone.

21.7 Effect of Various Stimulation Protocols

Controlled ovarian stimulation interrupts natural physiological processes. It affects the levels of oestrogen and progesterone, the timing of their expression and their ratios as well as the endometrial expression of their receptors. All this is likely to alter the extent and timing of endometrial receptivity [11]. COH also has a profound effect on endometrial gene expression. The pattern of expression depends upon the type of protocol used (agonist or antagonist). On a genomic level, implantation is affected due to dysregulation of genes in response to changing hormone levels, especially progesterone.

A study by Laberta et al. [12] showed that in patients of COH with high progesterone levels, there is dysregulation of 140 and 370 genes respectively (depending on the method used), which has an impact on the biological functions they represent, mainly cell adhesion, immune system and organ development. High progesterone also has a secondary impact on the E2 receptors and can lead to desensitization of the receptors to E2. The higher the E2 levels rise, the higher the progesterone levels appear to be, indicating a dependency on the number of follicles formed with COH. The study also noted that in cases with high progesterone, where the endometrium was out of phase on day 3, no pregnancies were obtained. However, if a day 5 transfer was done in these same patients, the pregnancy rates were decent. This gave credence to the theory that given enough time, the endometrium recovers from the effects of high steroid hormones.

21.7.1 Clomiphene Citrate and Endometrium

Clomiphene is an oestrogen receptor blocker that acts by competitively binding to oestrogen receptors [13]. It remains in the bound form for a longer duration than oestrogen and thus reduces the receptor concentrations. This in turn reduces the effect of the negative feedback mechanism on gonadotropin production. However, this competitive binding also leads to endometrial thinning in 15–50 % patients. The mechanism responsible for this is the ER down-regulation that leads to suppression of pinopode formation. It has been observed that this endometrial suppression is not dose dependent and recurred in repeat cycles in the same woman.

21.7.2 Letrozole and Endometrium

Letrozole has some advantages over clomiphene in its action over the endometrium. This aromatase inhibitor works on an enzymatic level, without affecting or blocking the oestrogen receptors. Thus, endometrial thickness remains unaltered. It was especially preferred in patients in which clomiphene caused endometrial thinning. However, this has now been banned by the FDA for use in ovulation induction.

21.7.3 Gonadotropins and Endometrium

A study conducted by Kolibianakis et al. in 2002 [14] studied the effects of gonadotropins on the endometrium in COH cycles. They found that endometrium advancement was noted at the time of oocyte pickup which was directly related to the level of LH at the initiation of treatment and the duration of FSH stimulation before addition of the antagonist. This is explained on the basis

of the two-cell–two-gonadotropin theory [14]: the higher LH level at the start of treatment leads to increased androgen production in the theca cells, which in turn will cause increased oestrogen production by the granulosa cells, due to FSH stimulation. The higher circulating oestrogen levels in turn cause earlier appearance of progesterone receptors in the endometrium, thus leading to endometrial advancement.

21.7.4 GnRH Agonists and Antagonists

The prolonged pituitary suppression caused by administration of agonists appears to affect the implantation window as well, causing it to shift forwards. A study by Hernandez [15] demonstrated that antagonist decreases the oestradiol production by the granulosa cells, which in turn affects endometrial development by affecting the mitosis of endometrial cells. In antagonist cycles, there is increased frequency of endometrial advancement, probably due to the fact that unlike agonist cycles, complete pituitary suppression does not occur in antagonist cycles, leading to a higher starting LH level, as described above, leading to increased oestradiol levels at an earlier stage [14]. Extreme endometrial advancement, of more than 3 days, is seen in higher frequency in antagonist cycles, leading to lower pregnancy rates in these cases. On a molecular level, a study by Rackow et al. [16] showed that HOXA 10 gene expression in endometrial stromal cells was impaired in antagonist cycles, as compared to agonists, thereby affecting endometrial receptivity.

As a counterview to this, a study by Saadat et al. [17] demonstrated that endometrial advancement takes place in all COH cycles, irrespective of the protocol used. They proved this both ultra-structurally, as well as by electron microscopy. According to them, the main cause appears to be due to increased progesterone levels leading to premature luteinization. A study by Simon et al. [18] has compared the standard step-up stimulation protocols with a step-down regimen. Their study was based on the theory that lower oestradiol levels during COH help to improve endometrial receptivity in patients undergoing IVF. They showed that oestradiol levels on the day of hCG trigger were significantly lower in the step-down regimes as compared to the step-up protocols. The implantation and pregnancy rates were also higher in these patients.

Early rise in progesterone levels is another factor seen in COH cycles. Rise in progesterone takes place especially in high responders, and although this does not affect oocyte quality, a level above 1.5 mg/dl [10] has a negative impact on endometrial receptivity, with precocious secretory endometrium formation and an out-of-phase endometrium on the day of implantation.

21.8 The Luteal Phase

Even though the agonist or antagonist treatment stops on the day of the hCG trigger, their effect on the suppression of endogenous LH continues, lasting for as long as 10 days after stopping stimulation [19]. Abnormally low LH levels may be insufficient to stimulate and maintain the corpus luteal function, leading to a luteal phase defect.

In high responders on antagonist protocol, an agonist trigger is often used to induce the LH surge for final oocyte maturation. Because of the agonist's longer-lasting action, LH insufficiency is common, leading to an out-of-phase endometrium, which is prematurely secretory in nature. This reduces implantation and pregnancy rates drastically. To bypass this, modified luteal support regimes have come into practice. Also, many clinicians prefer to vitrify the embryos formed and transfer them in a subsequent natural or hormone replacement cycle [20].

Conclusion

This chapter summarizes the physiology and functioning of the endometrium, the interplay of hormones that takes place on a day-to-day basis, how this differs when it is exposed to exogenous hormones and what can be done to optimize its functioning. This knowledge can help to improve pregnancy rates and increase the live birth rates in patients being treated for

infertility. Numerous tests and diagnostic methods are now being derived based on this, and treatment of the endometrial factor in infertility is now the wave of the future.

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Luteal Support: What to Use When?

22

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Abstract

Supraphysiological hormonal profiles are the cause of luteal defect observed in stimulated IVF cycles. Hence it is essential to support the luteal phase in stimulated cycles for which different forms of support are available. Progesterone and human chorionic gonadotropins (hCGs) so far have been the ideal supports for pregnancy. HCG results in higher incidence of ovarian hyperstimulation. Luteal phase support with progesterone results in increase in implantation and pregnancy rates. For now, progesterone seems to be the best option as luteal phase support. Oral progesterone is associated with reduced bioavailablity. Vaginal progesterone is associated with increased at-site concentration.

Keywords

Luteal phase • Progesterone • hCG • Luteal support

22.1 Introduction

The menstrual cycle starts with rise in level of FSH (follicle-stimulating hormone). Rise of FSH co-relates with the rise of oestrogen. Simultaneously there is first recruitment of follicles and then selection of the dominant follicle. In the middle of menstrual phase there is rise of LH (leutinizing hormone). This rise of LH causes ovulation. After ovulation the dominant follicle gets transformed into a corpus luteum. Corpus luteum secretes progesterone and oestrogen of which progesterone is the dominant hormone.

If conception takes place, then developing blastocyst secretes hCG (human chorionic gonadotropin). The role of hCG is to maintain the corpus luteum and support it. If conception does not take place, then corpus luteum regresses and the level of progesterone and oestrogen falls, which is then followed by onset of menstruation.

The luteal phase forms a bridge between the ovulatory phase and the beginning of the menstrual cycle.

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Along with the hormonal changes there are changes in the endometrium, which starts growing after menstruation under the influence of rise of oestrogen. After ovulation, there is rise of progesterone, which transforms it into a secretory endometrium. Progesterone prepares the endometrium for pregnancy by stimulating proliferation in response to hCG, which is produced by the corpus luteum. This occurs in the luteal phase of the menstrual cycle. Progesterone also promotes local vasodilatation and uterine musculature quiescence by inducing nitric oxide synthesis in the decidua.

The length of the luteal phase varies, the average being 14 days. Corpus luteum and the hormones secreted by it support the ongoing pregnancy initially for 8–12 weeks. This function is then taken over by the placenta.

22.2 Effect of Luteal-Phase Support on Endometrial microRNA Expression Following Controlled Ovarian Stimulation

It has been suggested that during ovarian stimulation for IVF, the endometrium becomes receptive after oocyte retrieval. Prior to and during the implantation process, the expression of multiple endometrial genes and gene products is highly regulated. The role of mRNAs in regulating cellular processes during the endometrial transition has recently attracted a great deal of attention. Neo-angiogenesis is a pivotal process in reproductive function where it regulates endometrial regeneration, corpus luteum formation and finally placentation. The regulatory function of mRNAs in the process of neo-angiogenesis has been illustrated in several in vitro and in vivo models.

The array-based study has revealed that there is an expression of a unique set of mRNAs in the endometrium following controlled ovarian stimulation. The level of expression for these mRNAs undergoes significant changes during the peri-implantation period. This expression is influenced by ovarian steroids. Expression of mRNAs may be associated with target genes and gene pathways. The mRNAs found to have enriched or depleted transcript load during the luteal phase may have specific roles in the control of endometrial receptivity during the peri-implantation period through regulation of their target genes [1].

22.3 Aetiology of Luteal Phase Defect (LPD) in ART Cycles

Removal of large quantities of granulosa cells during oocyte retrieval leads to diminished production of progesterone by corpora lutea, resulting in defect in the luteal phase. HCG administration for final oocyte maturation in stimulated IVF cycle could cause LPD by suppressing LH production via short-loop feedback mechanism [2].

Supraphysiological levels of steroids secreted by number of corpora lutea directly inhibit LH release via negative feedback actions at the hypothalamo-pituitary level. Corpus luteum requires consistent LH stimulus to perform its physiological function. LH support during luteal phase is responsible for maintenance and normal steroidogenic activity of the corpus luteum. As a result, unnecessary withdrawal of LH causes premature luteolysis [3, 4]. LPD is also seen equally in stimulated cycles with use of GnRh antagonists [5]. Luteal phase support is thus an integral part of ART cycles.

22.4 Option for Luteal Support

Luteal support may be given as

1. hCG: 1,500–2,000 I.U I/M starting from oocyte retrieval
2. Progesterone: from day of oocyte retrieval to 7–10 weeks after pregnancy injectable progesterone in oil: 25–100 mg I/m, oral progesterone, vaginal progesterone 200 mg tds or q.i.d, gel: 90 mg once or twice daily
3. Oestradiol valerate: 2 mg b.d. starting from oocyte retrieval to 7–10 weeks of pregnancy
4. GnRha for luteal support

22.4.1 Progesterone

Progesterone is a naturally occurring hormone secreted by the corpus luteum. In the presence of oestrogen, it transforms a proliferative endometrium into a secretory endometrium for implantation of the embryo. Progesterone also promotes local vasodilatation and uterine musculature quiescence by inducing nitric oxide synthesis in the decidua [6]. Once the embryo is implanted it acts to maintain the pregnancy.

22.4.1.1 Route of Administration

Oral Ingestion

Oral progesterone is extensively metabolized and has systemic side effects. There is reduced bioavailability. Micronized progesterone formulations initially used orally are now used vaginally [7]. Dehydrogesterone (DG), an oral progesterone, is a retroprogesterone with good oral bioavailability. According to Chakravarty et al., comparing oral DG vs. vaginal micronized progesterone for luteal support, both are associated with similar rates of successful pregnancies [8].

Intramuscular Injection

It is the most reliable route to achieve desired concentration of progesterone. It is rapidly absorbed, and peak level is reached in 8 h. Serum progesterone levels remain sustained compared to other routes as it is administered in an oil vehicle. It has the disadvantage of inconvenience of daily injections and pain or abscess formation at injection site. Allergic reactions may be seen. However, results are similar with intramuscular and vaginal progesterone.

The doses of IM progesterone used for LPS vary between 50 and 100 mg/day without any significant difference concerning the outcome [9]. Despite the conclusion of Pritts and Atwood's meta-analysis, vaginal administration of progesterone is a viable alternative to the IM injections of progesterone, as parenteral administration is associated with a high number of side effects [9]. On the basis of presented evidence, IM progesterone is not recommended as a first-choice LPS method in stimulated IVF cycles.

Rectal Administration

This route of progesterone administration is not widely accepted; there are minimal clinical trials on this method.

Vaginal Administration

Advantages of vaginal progesterone are patient comfort and effectiveness; there is high at-site concentration with low serum concentration. It does not cause drowsiness or sleepiness but is inconvenient because of vaginal discharge. Following intravaginal administration of progesterone, high uterine progesterone concentrations with low peripheral serum values are observed, due to counter-current exchange in progesterone transport between anatomically close blood vessels [10] and due to the uterine first-pass effect, where liver metabolization is absent [11]. It is a standard choice for luteal support. Vaginal and intramuscular progesterone have similar efficacy with comparable implantation and clinical pregnancy rates [12].

Vaginal delivery options are

- Vaginal pessaries can be given in t.d.s or b.d dose. Patients have to lie flat for 30 min following insertion. Pessaries are messy and are associated with vaginal discharge. Occasionally the insertion can be associated with vaginal itching and perineal irritation.
- Progesterone gel administration is delivered comfortably. There is no need to lie flat after insertion. The dose is 90 mg daily or B.D.

22.4.2 hCG

hCG is an indirect form of luteal support which acts by stimulating corpora lutea to produce progesterone. It is ineffective in the presence of inadequate number of LH receptors or a malfunctioning corpus luteum, which is hypo-responsive to hCG. hCG is effective if there is a specific defect in post-ovulatory LH secretion or in trophoblastic hCG production. It raises oestradiol and progesterone concentration, thus rescuing failing corpora lutea in stimulated IVF cycles. hCG administration increases concentration

of placental protein [13], integrin and relaxin, which have been shown to increase at time of implantation [14]. The disadvantage of using hCG for luteal support stems from its potential for increasing rates of ovarian hyperstimulation syndrome (OHSS) when compared with other treatments or no treatment at all. With regard to OHSS, one should therefore be cautious with the administration of hCG for luteal supplementation in stimulated IVF cycles [13].

Luteal support with hCG should be avoided if E2 levels are >2,500–2,700 pg/ml on the day of hCG administration [15], or if the number of follicles is >10 [16]. According to the 2011 Cochrane database systematic review [17], luteal phase support with hCG provided significant benefit as compared to placebo or no treatment in terms of increased pregnancy rates and decreased miscarriage rates, but only when GnRha were used.

22.4.3 Oestradiol

The quality of endometrium on which implantation depends is affected by both progesterone and oestradiol. The role of progesterone for luteal support in stimulated cycles is clear. The role of oestradiol is not clear. There is a drop in oestrogen concentration in the luteal phase. There are some patients who could benefit from oestrogen addition during progesterone support. According to a meta-analysis by Kolibianakis et al. [18], the difference in pregnancy rates between two regimens, i.e. progesterone only and progesterone plus oestrogen, is very small.

According to a recent meta-analysis of 10 randomized controlled trials, 7 on GnRha and 3 on GnRh antagonists, the addition of oestrogen to progesterone for luteal phase support does not improve IVF outcomes [19].

A study conducted and approved by the Johns Hopkins Hospital Institutional Review Board to evaluate the expression of miRNAs during the luteal phase following controlled ovarian stimulation for IVF and the influence of different luteal phase support protocols on miRNA profiles showed that after luteal phase support the miRNAs are up-regulated or down-regulated. Hence, luteal support following controlled ovarian stimulation has a profound influence on miRNA profiles. Up- or down-regulation of miRNAs after progesterone, or progesterone and oestrogen, suggests a role for luteal support in the peri-implantation uterus in IVF cycles through the regulation of associated target genes [20].

22.4.4 GnRh Agonist

GnRh agonist may support corpus luteum by stimulating secretion of LH by the pituitary, by acting on endometrium by locally expressed GnRh receptors, a direct effect on the embryos or by some combination of these possibilities. GnRh agonist also increased luteal phase hCG, E2 and progesterone in both stimulation regimens. It could be given as single dose or multiple dose.

22.4.4.1 Single Dose

The exact mechanism is still not known. It was suggested that GnRH-agonist can help in the maintenance of the corpus luteum, acting directly on the endometrium via local receptors, a direct effect on the embryos or by some combination of these possibilities. A single dose of GnRH agonist (0.5 mg leuprolide acetate) administered subcutaneously on day 6 after ICSI in both agonist and antagonist cycles enhanced pregnancy rates [21]. A meta-analysis showed that the luteal-phase single-dose GnRH-agonist administration can increase implantation rate in all cycles and clinical pregnancy rate and ongoing pregnancy rate in cycles with GnRH antagonist ovarian stimulation protocol [22]. GnRH agonist addition during the luteal phase significantly increases the probability of live birth rates [23].

22.4.4.2 Multiple Dose

In multiple-dose protocol, 200 μg intranasal buserelin followed by 100 μg every day or alternate day up to day 14 of the luteal phase is given. Intranasal administration of buserelin could be effective in triggering ovulation and in providing luteal support. This treatment was associated with a good pregnancy rate (28 %) with IUI [24].

22.5 Co-treatment Schemes

Besides support with progesterone, oestradiol and hCG co-treatment with ascorbic acid, aspirin, steroids or sildenafil is done to improve endometrial blood flow and receptivity.

22.5.1 Ascorbic Acid

Luteal regression is associated with ascorbate depletion and generation of reactive oxidative substances that inhibit LH action and block steroidogenesis. Griesinger conducted a prospective randomized study to evaluate impact of ascorbic acid as an addition to luteal support. They made the following observation: the addition of ascorbic acid provided no additional benefit in stimulated IVF cycles, regardless of dose used [25].

22.5.2 Steroids

There is a hypothesis that immunosuppression by exogenous steroids as a co-treatment for luteal phase support can be used to improve rates of embryo implantation and pregnancy. The rationale behind the use of steroids is that the embryos might be exposed to bacterial or leucocyte infiltration if the protective coating of zona pellucida is breached. In a prospective randomized control study involving routine ICSI patients, Ubaldi et al. did not find any beneficial effect of adding low-dose prednisolone to progesterone during the luteal phase [26].

22.5.3 Aspirin

Aspirin inhibits cyclooxygenase, thus avoiding prostaglandin synthesis. Luteal regression is caused by a pulsatile release of prostaglandins from the uterus in the late luteal phase [27]. Aspirin increases uterine blood flow; hence, it was postulated that it would increase endometrial receptivity, thereby increasing implantation [28]. Recent studies are unable to find benefit with routine use of aspirin during IVF cycles [29]. Aspirin may improve pregnancy rates in auto-antibody/seropositive patients in repeated IVF failures [30].

22.5.4 Sildenafil

Sildenafil improves the uterine artery blood flow [31]. It acts as type 5 specific phosphodiesterase inhibitor by enhancing the vasodilatory effect of nitric oxide by preventing degradation of cyclic GMP. Sher et al. studied the effect of vaginal sildenafil on the outcome of in vitro fertilization (IVF) after multiple IVF failures attributed to poor endometrial development with a cohort of 105 infertile women aged less than 40 years, with normal ovarian reserve and at least two consecutive prior IVF failures attributed to inadequate endometrial development. Patients underwent IVF using long GnRha protocol with addition of sildenafil vaginal suppositories (25 mg, 4 times a day) for 3–10 days. Vaginal administration of sildenafil enhanced endometrial development in 70 % of patients studied. High implantation and ongoing pregnancy rates were achieved in a cohort with a poor prognosis for success [32].

22.6 Timing of Luteal Support

The timing of LPS should not be later than day 3 after OR. The hCG administered for final oocyte maturation covers the luteal phase for a maximum of 8 days. However, taking the uterolytic effect of progesterone into account, it is recommended to start treating the patients with progesterone at least as early as the day of embryo transfer [33, 34].

22.7 Duration of Luteal Support

Luteal support is continued until early pregnancy. There are no studies to either support or contest the generally accepted practice of prolonging progesterone supplementation during early pregnancy. Schmidt et al. (2001) were the first to publish a retrospective study to compare the delivery rate with IVF or ICSI in women who received

progesterone supplementation with those who did not during the first weeks of pregnancy. The results showed no difference in the delivery rate [35].

Subsequently, a prospective randomized controlled trial was conducted by Nyobe et al. [36]. They evaluated whether or not the prolongation of luteal support during early pregnancy had any influence on the delivery rate after IVF. Results indicated that prolongation of progesterone supplementation in early pregnancy had no influence on the miscarriage rate, and thus no effect on the delivery rate. It would appear that the increase in endogenous hCG level during early pregnancy makes up for any possible lack of endogenous LH that has been caused by stimulated IVF cycles.

22.8 Results of the 2011 Cochrane Database Systematic Review Comparing Different Routes of Progesterone Supplementation [17]

Oral route is associated with reduction in pregnancy rates compared to intramuscular or vaginal but was not statistically significant. There is evidence of benefit of intramuscular over vaginal route in terms of outcome of ongoing pregnancy and live birth rate. There is no significant difference in pregnancy rate between vaginal progesterone gel and other types of vaginal progesterone. Luteal support with hCG provided significant benefit with increased pregnancy rates. There was no significant difference between progesterone and hCG or between progesterone and progesterone plus hCG or oestrogen in terms of pregnancy and miscarriage rates.

This review showed a significant effect in favour of progesterone for luteal phase support, favouring synthetic progesterone over micronized progesterone. Overall, the addition of other substances such as oestrogen or hCG did not seem to improve outcomes. There is no evidence favouring a specific route or duration of administration of progesterone. hCG, or hCG plus progesterone, was associated with a higher risk of OHSS. The use of hCG should therefore be avoided. There were significant results showing a benefit from addition of GnRH agonist to progesterone for the outcomes of live birth, clinical pregnancy and ongoing pregnancy. For now, progesterone seems to be the best option as luteal phase support, with better pregnancy results when synthetic progesterone is used.

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Endometrial Support Beyond Hormones

23

Mala Arora and Shilpa Gulati

Abstract

Implantation is a very complex process, which is controlled by a number of molecules like hormones, cytokines and growth factors and their cross-talk. During the implantation period, the endometrium acquires an appropriate morphological and functional state under the influence of ovarian steroids and molecular mediators. Assisted reproductive technology protocols continue to evolve with the aim of achieving higher pregnancy rates; however, despite these advances, implantation rates are still relatively low and have not increased sufficiently in the last decade to allow widespread adoption of single-embryo transfer.

A number of empirical treatment modalities have been tried but with limited success rates, since the pathological processes are poorly understood. Endometrial stem cells and gene therapy are promising options that can be effective in the future. Use of new tissue profiling technologies at genomic, transcriptomic and proteomic levels will bring new strategies in treating implantation failure and help increase successful pregnancies. This chapter aims to summarize the current knowledge of the mechanism of implantation, molecular and morphological markers of endometrial receptivity and proposed treatment options to improve implantation rate.

Keywords

Endometrium • Receptivity • Implantation • Granulocyte colony stimulating factor • Aspirin • Heparin • Sildinafil

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23.1 Introduction

Embryo implantation is the most critical step of the reproductive process. It consists of a unique biological phenomenon, by which the blastocyst becomes intimately connected to the maternal endometrial surface to form the placenta that will provide an interface between the growing foetus and the maternal circulation [1].

The process of implantation is subdivided into the stages of

- Apposition
- Adhesion
- Invasion

Prior to implantation, the blastocyst shows evidence of polarity, assuming a particular orientation as it approaches the endometrium. Once the blastocyst is oriented correctly (apposition), the zona pellucida is shed. The blastocyst then comes into contact with the endometrial surface and adheres to an endometrial gland opening, drawing nutrition from its secretions (adhesion). Finally, the blastocyst penetrates the surface layer and invades the stroma (invasion) [2].

Successful implantation requires the appropriately timed arrival of a viable blastocyst into a receptive endometrium and a synchronized dialogue between maternal and embryonic tissues [3]. The endometrium is remodelled throughout the menstrual cycle and exhibits only a short period of receptivity, known as the 'implantation window'. In humans, during a natural cycle, the embryo enters the uterine cavity 4 days after ovulation [4]. The endometrium becomes receptive to blastocyst implantation 6–8 days after ovulation and remains receptive for approximately 4 days (cycle days 20–24) [5].

Implantation failure remains an unsolved problem in reproductive medicine and is considered as a major cause of unexplained infertility in otherwise healthy couples. Indeed, the average implantation rate in IVF is around 25 % [6]. Inadequate uterine receptivity is responsible for approximately two-thirds of implantation failures, whereas the embryo itself is responsible for only one-third of these failures [7].

Hence, to improve implantation rates in stimulated cycles, it is important to pinpoint the window of implantation, ensure that the best embryo is selected and synchronize embryo transfer with the time of optimal endometrial receptivity. There is a need to identify ways of evaluating and enhancing endometrial receptivity and embryo quality to maximize implantation rates in ART cycles.

Throughout the menstrual cycle, the human endometrium is primed for blastocyst attachment. Hence, it needs to acquire an accurate morphological and functional state. A large number of molecular mediators, under the influence of ovarian hormones, have been postulated to be involved in this early foeto–maternal interaction. These mediators include a large variety of interrelated molecules including adhesion molecules, cytokines, growth factors, lipids and others [8].

23.2 Immunology of Successful Pregnancy

Immune responses play an important role in embryo implantation. Medawar in 1953 gave the concept that the foetus represents a semi-allograft developing in the potentially hostile environment of the maternal immune system. Immune responses play a very important role, so that the mother accepts a semi-allogeneic foetus [9, 10], The main tissue where maternal allo-recognition of the foetus occurs is in the uterus at the site of placentation, where fetal extra-villous trophoblast cells invade and intermingle with maternal leukocytes. About 40 % of recurrent miscarriages are unexplained and immune dysfunction or alloimmune responses may be responsible.

A number of cytokines and their receptors are expressed at the materno-foetal interface and are thought to play a function in the regulation of placentation. In pregnancy, there is a shift from Th type 1 cytokine production to Th type 2, since type 1 cytokines (e.g. IFN-g and TNF-a) are

harmful for pregnancy as they may inhibit successful implantation [11, 12].

Unexplained recurrent miscarriages could be due to an imbalance between Th1/Th2 systems. If there is increased production of cytotoxic Th1 cytokines (interleukin 2, TNFα), instead of Th2 cytokines (interleukin 4, 6 and 10) which have an immunosuppressant role, it will result in rejection of embryonic allograft. [13] Uterine NK cells account for approximately 70 % of decidual leukocytes and are likely to be involved in the process of placentation. They increase markedly in early pregnancy. To escape lysis by uNK cells, the trophoblast cells express the MHC Ib antigens, HLA-E and HLA-G. Inhibitory KillerIg-like receptors (KIRs) interact with foetal HLA-C in the early weeks of gestation and prevent lysis of the trophoblast cells [14].

The trophoblast invades the decidua to surround and destroy the media of the spiral arteries, transforming them into high-conductance vessels. A role for uNK cells in implantation and placentation is suggested by the findings that high preconceptional NK activity was associated with significantly higher rates of miscarriage [15] and infertility [16]. The uNK cell-derived cytokines influence placentation. Granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF) and leukaemia inhibitory factor (LIF) stimulate growth of the trophoblast; colony-stimulating factors also promote trophoblast cell proliferation and differentiation [17, 18].

23.3 Assessment of Endometrial Receptivity (Morphological and Molecular Markers)

The endometrium is a multilayered, dynamic organ comprising of a functional layer and a basal layer. The cells in the functional layer are shed during menstruation. The basal layer is attached to the myometrium and remains intact during menstruation, serving as a base for endometrial regeneration. The endometrium is composed of several different cell types, including luminal and glandular epithelial cells, stroma with stromal fibroblastic cells, immunocompetent cells and blood vessels.

Noye's criteria for endometrial dating was considered the gold standard approach for evaluating endometrial responsiveness and detecting endometrial abnormalities [19]. Its disadvantages include

- Disruption of normal anatomical layering by endometrial biopsy
- High intra- and inter-observer variability
- Error in endometrial dating for biopsies taken during the 2 days following ovulation as the morphological features of the endometrium do not change significantly during this period

Ultrasonographic evaluation of endometrial thickness and its echogenic pattern is a non-invasive technique to assess the endometrium. Assessment of endometrial blood flow adds a physiological dimension to the anatomical ultrasound parameters. However, the use of endometrial and sub-endometrial blood flow in the prediction of implantation and pregnancy remains unclear.

Pinopods are bleb-like protrusions found on the apical surface of the endometrial epithelium [20]. These structures are several micrometers wide and project into the uterine lumen above the microvilli level. Pinopod expression is limited to a brief period of 48 h in the menstrual cycle corresponding to the putative window of implantation [21, 22]. Others have detected that pinopods may be present throughout the mid to late secretory phase, however, displaying cycle-dependent morphological changes. This suggests that functionality, rather than pinopod presence or absence, is of greater significance.

The appearance of pinopods is progesterone dependent, and association between mid-luteal increase of progesterone level and the first appearance of pinopods in the menstrual cycle was noted [23]. The detection of pinopods, by

Table 23.1 Molecular Markers of Implantation

Molecules	Site	Function
Cell adhesion molecule (CAM) L Selectins Trophinin	Endometrial pinopods,	
Embryo trophoectoderm	Adhesion	
Integrins	Luminal epithelium	Binds to extracellular matrix ligands
E-Cadherins	Luminal epithelium	Down regulated to facilitate trophoblast Invasion
Mucins (MUC-1)	Endometrium	Down regulated on pinopods to expose CAMs thereby selecting a good site for implantation
Cytokines LIF IL-1,6 & 11 CSF-1	Luminal and glandular epithelial cells	Trophoblastic growth and proliferation Role in adhesion and invasive phase
Growth Factors TGF-β EGF family (EGF,TGF-β ,HB-EGF) FGF IGF PDGF	Endometrium Blastocyst (HB-EGF)	Stimulates adhesion Increases invasiveness Promotes decidualization
MMP (Matrix metalloproteinase) MMP-9 TIMP-1	Endometrium & Embryo	Matrix degradation
Prostaglandins		Uterine decidualisation Increased vascular permeability

electron microscopy, during the mid-secretory phase may be a useful test for assessment of endometrial receptivity to optimize implantation rates; however, it is an invasive test.

There are several proposed molecular markers of endometrial receptivity (Table 23.1). *Integrins* are a family of trans-membrane glycoproteins containing extracellular, trans-membranal and intracellular domains. Integrins whose expression is increased in the mid-luteal phase were proposed as markers for the window of implantation [24]. Three cycle-specific integrins are co-expressed by the human endometrium defined histologically on days 20–24 of the human menstrual cycle: α1ß1, α4ß1 and αVß3, but only the ß3 mRNA subunit expression was shown to increase after day 19 and is not detected beforehand [25]. With respect to its expression pattern along with its epithelial localization, aVß3 has been proposed as a potential receptor for embryonic attachment [26].

During the proliferative phase, high oestrogen levels act via the oestrogen receptor-α (ERα) to inhibit integrin expression. The luteal progesterone rise subsequently down-regulates the number of these receptors, thus indirectly suppressing the inhibitory effects of E2 resulting in a net integrin increase. Progesterone, probably, also acts positively by increasing paracrine stromal factors, e.g. epidermal growth factor (EGF) and heparin-binding EGF (HB-EGF) to induce epithelial ß3integrin expression that serves as the rate-limiting step in aVß3 formation.

Aberrant αVß3 integrin expression pattern has been associated with unexplained infertility [27–29], endometriosis [30], hydrosalpinx [31], luteal phase deficiency and, more recently, polycystic ovarian syndrome [32]. Hence, this integrin is a promising marker of implantation process.

Selectins are glycoproteins belonging to the cell adhesion molecule (CAM) family. The human L-selectin is of importance in the implantation

process. On the blastocyst side, strong L-selectin staining has been observed over the entire embryo surface. On the maternal side, the expression of selectin oligosaccharide-based ligands, such as MECA-79 or HECA-452, is up-regulated during the window of implantation [33]. It appears that selectins take part in the very early stages of blastocyst interactions with the uterine wall.

23.4 Recurrent Implantation Failure – Endometrial Receptivity and Thickness

Recurrent implantation failure (RIF) is determined when embryos of good quality fail to implant following several in vitro fertilization (IVF) treatment cycles. A recent definition states that failure to implant in 3 IVF cycles or failure to implant after transfer of 10 good-quality embryos should be categorized as RIF. Implantation failure is related to either maternal factors or embryonic causes. Among the various potential causes of RIF, uterine factors (e.g. thin endometrium, poor endometrial receptivity and immunological incompatibility) have received the most attention in recent years. Assessing the endometrium by uterine artery blood flow indices, sub-endometrial blood flow and endometrial receptivity assay (ERAS) has been suggested (Table 23.2). More recently, endometrial biopsy in a previous cycle with electron microscopic visualization of pinopods to assess the putative implantation window and transfer of good-quality embryos during the window of implantation will improve success rates.

23.5 Treatment Options to Optimize Implantation

The non-hormonal adjuvant treatment of RIF ideally should be targeted to the correction of any potential malfunction that might contribute to the failure of implantation. However, since the pathological processes are poorly understood, a number of empirical treatment modalities have been tried with limited success rates. These are listed below and tabulated in Table 23.3 [34–36].

23.5.1 Sildenafil

Endometrial growth is thought to depend on uterine artery blood flow. Oestrogen-induced endometrial proliferation is in large part dependent upon blood flow to the basal endometrium. Nitric oxide (NO) relaxes the vascular smooth muscle by c-GMP-mediated pathway [37].

Sildenafil citrate, a type-5 phosphodiesterase inhibitor, potentiates the vasodilatory effects of NO by preventing the degradation of c-GMP [38]. Sildenafil citrate can improve the uterine blood flow and, in conjunction with oestrogen, lead to the oestrogen-induced proliferation of the endometrial lining. A good correlation has been found between endometrial thickness and the prevalence of conception. An endometrial thickness of around 9 mm on vaginal ultrasound in the late proliferative phase correlates well with the chance of pregnancy after IVF, whereas a thinner endometrium is associated with poorer implantation rates [39].

Sildenafil citrate improves the uterine artery blood flow and the sonographic endometrial thickening in patients with a poor outcome in a prior assisted reproductive treatment (ART) cycle due to poor endometrial response [40, 41]. The biochemical pregnancy rates are also higher with sildenafil citrate but do not reach statistical significance [41].

Although NO improves uterine blood flow in the proliferative phase, it may have detrimental effects on the endometrium during the implantation window. The NO-mediated release of cytokines like TNFα from the activated natural killer cells has been implicated as a cause of implantation failure [42]. Hence, it may be beneficial to minimize endometrial exposure to NO at the time of embryo transfer by discontinuing sildenafil on or prior to the day of HCG administration.

Nitroglycerine (NTG) patch also improves the endometrial blood flow and lining in IVF patients with a previous poor response but is associated with side effects like headaches and hypotension. The use of sildenafil vaginal suppositories (25 mg) decreases systemic side effects and is preferred over NTG patches.

Table 23.2 Tests for Evaluating Endometrial Receptivity

Test	Marker	Method	Value in Implantation Failure	Relevance
1. Endometrial (a) Molecular markers	αvβ3	Flow cytometric analysis Immunohistochemistry RNA studies on Endometrial Biopsy	Decreased	Research
	LIF		Decreased	
	E-Cadherin		Decreased	
	MECA -79		Absent	
	MUC-1		Decreased	
	IL-10		Decreased	
	VEGF		Decreased	
	eNOS		Decreased	
	HOXA 10,11		Decreased	
	COX-2		Decreased	
(b) Histology	Pinopods	Scanning Electron Microscope	Poorly developed	
(c) Uterine (i) Genomics (ii) Proteomics (iii) Secretomics	Associated genes HOX A10 Proteins IL-1β,TNF-α , IFN-ϒ, MCP-1, Glycodelin, HBEGF, VEGF	Microarray (Endometrial Biopsy) Uterine Flushing (Endometrial secretions)	Decreased	
2. Ultrasonography	Endometrial thickness	2D	<7 mm	Proposed Low PPV, High NPV. Individual parameters not of sufficient accuracy to predict receptivity as compared to Uterine score
	Endometrial pattern		Not Multi layered	
	Myometrial echogenecity		Non homogenous	
	Endometrial volume	3D	<2.5 ml	
	Pulsatility index	Doppler	≥3	
	Vascularisation index			
	Flow index			
	End Diastolic blood flow		Absent	
	Protodiastolic Notch		Present	
3. Hysteroscopy	Synaechae Polyps Myomas		Present	

23.5.2 Aspirin

Low-dose acetylsalicylic acid (aspirin) irreversibly inhibits the cyclo-oxygenase enzyme in platelets, thus preventing the synthesis of thromboxane, which causes vasoconstriction and platelet aggregation [43]. By this mechanism, low-dose aspirin may enhance uterine blood flow and tissue perfusion, thereby improving endometrial receptivity for implantation. Aspirin may also suppress negative effects of prostaglandins on implantation, such as the induction of uterine contractions or inflammatory response.

Table 23.3 Methods Used to Improve Endometrial Receptivity

Molecules	Intervention	Proposed Mechanism	Result
Sildenafil	Vaginal/Oral	Increases the uterine blood flow Increases endometrial thickness	Biochemical pregnancy rates higher but no significant improvement in ongoing pregnancy rates
Aspirin	Oral	Inhibits prostaglandin synthesis Increases uterine blood flow Reduces uterine contractions Reduces inflammation? Attenuates placental apoptosis	No evidence that use of aspirin is effective per Cochrane review 2011 [34]
Low molecular weight heparin	Subcutaneous	Anticoagulant effect Modulates blastocyst apposition, adherence and invasion Enhances trophoblast differentiation and invasion	May benefit but avoid routine use until further research, per Cochrane Review 2013 [35]
Granulocyte colony-stimulating factor	Subcutaneous intrauterine catheter	? Interaction with immune system	Promising role
Intravenous immunoglobulin	IV	Reduces NK cell activity	Lack of evidence in APA negative women
Steroids	Oral	Immunomodulator	No clear evidence per Cochrane review 2012 [36]
Atosiban	IV	Reduces uterine contractility? Priming of endometrium	Further studies required
GnRH agonist in luteal phase	Subcutaenous	Improves corpus luteal function	Further studies required
Local injury to the endometrium	Hysteroscopic procedure	Decidualisation of endometrium Production of cytokines and growth factors	Further studies required

In vitro studies have shown that heparin and aspirin attenuate placental apoptosis, and this could be a possible explanation of how aspirin is beneficial, even in the absence of endometrial or oocyte improvement [44]. This theory along with its low cost, free availability and minimal side effects has popularized the use of low-dose aspirin in ART cycles.

Several studies have shown that aspirin is beneficial in infertility [45]. A non-controlled study found that IVF outcome was significantly improved when aspirin, heparin and intravenous immunoglobulin therapy was administered to women with repeat IVF failures and anti-phospholipid antibodies but not to women with negative anti-phospholipid antibodies [46, 47].

However, Cochrane review of 2011 concluded that there is no evidence that the use of aspirin in women undergoing IVF is effective [34]. A study on the effect of aspirin in uterine haemodynamics among unselected IVF/ICSI women revealed that low-dose aspirin therapy 100 mg/day, when started concomitantly with gonadotropin stimulation, does not significantly affect uterine artery vascular impedance or endometrial thickness on the day of embryo transfer [48].

A recent meta-analysis concluded that use of aspirin does not improve success rates in IVF cycles [49].

23.5.3 Low Molecular Weight Heparin (LMWH)

Many studies have reported congenital and acquired coagulation defects to be more prevalent in women with recurrent implantation failures (RIFs) [50]. This led to the use of anti-coagulants,

mainly heparin, during the course of ART cycles in women with anti-phospholipid antibodies [50–52].

Heparin is a linear polydisperse polysaccharide consisting of 1→4-linked pyranosyluronic acid and 2-amino-deoxyglucopyranose (glucosamine) residues [53]. Due to the highly anionic nature, heparin can bind to a plethora of proteins including anti-thrombin, growth factors, growth factor receptors, viral envelope proteins and extracellular matrix molecules.

The changes in coagulation and fibrinolysis observed during ovarian stimulation are similar to those observed during pregnancy, with the drive for these haemostatic changes potentially being the rapid increase of oestradiol levels, which occur with ovarian stimulation [54]. Excessive coagulation activation was found to be associated with poorer IVF outcomes, despite higher oocyte yields. This suggests that haemostatic mechanisms have an important role in implantation. Heparin can alter the haemostatic response to controlled ovarian stimulation and modify the risk of thrombosis.

Heparin has been proposed to play a role in the process of implantation beyond its anticoagulant effects, through interactions with several adhesion molecules, growth factors, cytokines and enzymes such as matrix metalloproteinases (MMP). It can also modulate many of the fundamental physiological processes required for blastocyst apposition, adherence and invasion. It enhances trophoblast differentiation and invasion and has the potential to improve pregnancy rates and outcomes in ART cycles [53].

E-cadherin expression by the endometrium is decreased by progesterone facilitating trophoblast invasion. Unfractionated heparin (UFH) and enoxaparin, a LMWH, have also been shown to down-regulate decidual E-cadherin expression [55], thereby potentially explaining the observations that UFH and LMWH can promote extravillous trophoblast differentiation [56].

HB-EGF is induced by sex steroids during the secretory phase of the endometrial cycle and persists during early pregnancy [57]. Its expression on the surface of pinopods [58] suggests an early role in blastocyst implantation and placentation. LMWH may potentiate sHB-EGF binding and may also up-regulate sHB-EGF levels via increased MMP activity.

Interleukin -1 (IL-1) increases endometrial epithelial cell β3 integrin expression with an improvement in blastocyst adhesion [59]. LMWH is reported to increase IL-1 expression in activated leukocytes [60]. Modulation of integrin expression by LMWH may be playing a role in improving endometrial receptivity. Enhanced trophoblast migration and invasiveness due to LMWH-induced increase in free insulin-like growth factor I is another proposed mechanism for a beneficial effect of LMWH on the implantation process.

A pilot study on luteal phase empirical LMWH (1 mg/kg/day) a day after oocyte retrieval in RIF patients observed a relative increase by 30 % in live birth rates. Though the difference was not statistically significant, it suggested a potential beneficial effect of LMWH on the clinical outcome of ART in women with RIF. UFH as well as LMWHs are able to modulate the decidualization of human endometrial stromal cells in vitro and therefore might be useful to control endometrial differentiation and receptivity in assisted reproduction [61].

A recent prospective randomized study observed significant differences with regard to pregnancy and implantation rates in ICSI patients treated with combined oral prednisolone and LMWH in unexplained failed implantation [62].

The results of a Cochrane review of three randomized controlled trials with a total of 386 women suggested that peri-implantation LMWH in ART cycles may improve the live birth rate. However, the results were dependent on small low-quality studies with substantial heterogeneity and were sensitive to the choice of statistical model. There are side effects reported with use of heparin, including osteopenia, bruising and bleeding, with no reliable data on long-term effects. Currently, the use of heparin outside well-conducted research trials is not justified [35]. Patients in whom LMWH would be most effective and the appropriate dosing and duration of administration needs to be determined before unselectively exposing women and their embryos to this medication.

23.5.4 Granulocyte Colony-Stimulating Factor

G-CSF is a cytokine with a 177 amino acid polypeptide chain and a molecular weight of 25 kDa. It stimulates neutrophilic granulocyte proliferation and differentiation. It is expressed and produced by the decidual cells, and its receptor, *c-fms*, is expressed on the trophoblastic cells [63].

Scarpellini et al. in 2009 studied the efficacy and safety of G-CSF in women with unexplained recurrent miscarriage with at least four consecutive miscarriages and negative for all clinical investigations. Recombinant G-CSF was administered subcutaneously daily at a dosage of 1 mg (100,000 IU)/kg/day from the sixth day after ovulation until the occurrence of menstruation or to the end of the ninth week of gestation. The number of live births in women treated with G-CSF was significantly higher as compared to controls. Also, elevated levels of beta-hCG were observed during treatment with G-CSF showing thereby that G-CSF may increase the trophoblast growth and metabolism. The side effects included skin rash and leucocyte count higher than 25,000/ml. None of the newborns showed any major or minor abnormalities or malformations [64].

Presence of chronically thin endometrium, resistant to standard treatments, affects a small number of patients undergoing IVF. Endometrial thickness below 7 mm is widely considered suboptimal for transfer and associated with reduced pregnancy chances [65].

Gleicher et al. in 2012 reported the successful use of G-CSF in those who had previously failed to expand their endometria beyond 6.9 mm with the use of standard treatments. Infertile women with endometrial thickness of <7 mm on the day of hCG administration in their first IVF cycles and in whom traditional treatments with oestradiol, sildenafil citrate and beta blockers had been unsuccessful were administered G-CSF by intra-uterine catheter by slow infusion before noon on the day of hCG administration. If the endometrium had not reached at least 7-mm within 48 h, a second infusion was given following oocyte retrieval. A significant improvement in endometrial thickness after G-CSF treatment was reported [66].

Even though there is increasing evidence that G-CSF is not toxic in pregnancy, it should be used very carefully as its safety is still under question and there are not enough women treated with G-CSF in pregnancy to exclude any possible teratogenic effects. There is still little knowledge of the role of G-CSF in human reproduction and its interaction with the immune system, but it has a promising role in those with refractory thin endometrium.

23.5.5 Intravenous Immunoglobulin (IVIg)

Women experiencing implantation failure have a higher frequency of elevated percentage of circulating CD56+ (natural killer) cells (>12 %) than fertile women (3–12 %). IVIg reduces activation of NK cells and NK killing activity both in vitro and in vivo. IVIg in doses of 500 mg/kg prior to embryo transfer significantly improved the pregnancy rates in women with elevated percentage of circulating CD56+ cells [67].

IVIg may be a useful treatment option for patients with previous IVF failure and preconception Th1:Th2 imbalance and/or NK elevation. Preconception immune testing may be a critical tool for determining which patients will benefit from IVIG therapy [68, 69]. IVF outcome was reported to be significantly improved when heparin/aspirin and IVIG was administered to anti-phospholipid antibody (APA)-positive women with repeat IVF failures whereas APA negative women did not seem to benefit from such treatment [47].

IVIg treatment for repeated IVF/ICSI failure and unexplained infertility was reported to significantly increase implantation and pregnancy rates in a systematic review and meta-analysis [70].

Recently, a systematic review of literature on interventions to improve reproductive outcomes in women with elevated natural killer cells undergoing ART does not support the use of prednisolone, IVIg or any other adjuvant treatment in women undergoing ART who are found to have elevated absolute numbers or activity of NK cells, due to the paucity of or poor quality of the evidence [71]. Further research is needed before

NK cell assessment can be recommended as a diagnostic tool in the context of female infertility or recurrent miscarriage.

23.5.6 Steroids

It has been proposed that glucocorticoids may improve the intra-uterine environment by acting as immune modulators to reduce the uterine NK cell count, normalize cytokine expression profile in the endometrium and suppress subclinical endometrial inflammation.

Several studies have reported that immunosuppressive doses of corticosteroids administered for a short period of time to patients undergoing IVF-ET can significantly improve the implantation and pregnancy rates [72], especially in those with associated autoimmune conditions [73]. A study reported that prednisolone reduces preconceptual endometrial NK cells in women with recurrent miscarriage [74]. However, some studies have shown no improvement in implantation and pregnancy rates in glucocorticoid-treated patients [75].

The Cochrane review (2012) concluded that there is no clear evidence that administration of peri-implantation glucocorticoids in ART cycles significantly improves the clinical outcome. The use of glucocorticoids in a sub-group of women undergoing IVF (rather than ICSI) was associated with an improvement in pregnancy rates of borderline statistical significance and should be interpreted with care. These findings were limited to the routine use of glucocorticoids and cannot be extrapolated to women with autoantibodies, unexplained infertility or recurrent implantation failure [36]. Further well-designed randomized studies are required to elucidate the possible role of this therapy in well-defined patient groups.

23.5.7 Atosiban

Uterine contractile activity may adversely affect implantation. Increased contractions have been found in approximately 30 % of patients undergoing embryo transfer. Pharmacological tocolytics may be expected to improve pregnancy rates; however, targeting uterine adrenergic receptors, calcium channels or prostaglandin synthesis has been ineffective.

Oxytocin antagonist atosiban is being used as a tocolytic to delay premature labour by inhibiting contractions of the uterus. Atosiban given at the time of embryo transfer to women with recurrent implantation failure reduced the number of uterine contractions in these patients and also increased the implantation and pregnancy rates. The pregnancy rate went from zero to 43.7 %. The total dose of atosiban was 36.75 mg [76].

The beneficial effects of atosiban were observed not only in patients who had a high frequency of uterine contractions but also in those who had a low frequency. These findings suggest that atosiban may have other benefits and is effective in priming the uterus for implantation, in addition to its effect on contractions of the uterus [77].

Lower dosage of atosiban (a single bolus dose of 6.75 mg) before Et also improves pregnancy outcomes of patients with RIF. A significantly higher implantation rate was found in those receiving atosiban before ET than in those receiving it immediately after ET [78]. More studies are required to find out exactly how atosiban works and to evaluate its role in patients with RIF undergoing IVF.

23.5.8 Neuromuscular Electrical Stimulation (NMES) Therapy

Thin endometrium at the time of ovulation has been demonstrated to be an important factor in implantation failure. Uterine receptivity in women with thin endometrium may be poor due to the impairment of blood flow impedance through the endometrium. NMES is the application of electrical stimulation to a group of muscles through electrodes placed on the skin. NMES was performed 3–4 times for 20–30 min or once a day consecutively in the follicular phase. Pelvic floor NMES was found to significantly enhance endometrial thickness in patients with thin endometrium. Contraindications to NMES therapy include vaginal wall prolapse,

skin breakdown around the peri-anal region, rectal bleeding, complete denervation of the pelvic floor, presence of cardiac pacemaker, cardiac arrhythmia, unstable seizure disorder, pelvic pain and painful haemorrhoids [79].

It is possible that NMES corrects the impairment of uterine blood flow impedance. Though the exact mechanism by which NMES exerts its effect on the process of angiogenesis and vascularization in the endometrium is unknown, the increased blood supply towards the endometrial and the sub-endometrial regions may be due to the repeated contraction and relaxation of the uterine smooth muscle. NMES being a safe and non-invasive technique is a promising alternative for managing patients with thin endometrium.

23.5.9 Local Injury to the Endometrium

Local inflammatory reactions are necessary for angiogenesis and a successful implantation. This observation led to the hypothesis that endometrial injury might improve implantation in patients with repeated implantation failure as a result of subsequent inflammatory responses and changes in cytokine production in the endometrium.

Studies have shown that prior incidence of hysteroscopic endometrial biopsy is associated with increased rates of implantation, clinical pregnancy and live birth among women who experienced repeated implantation failure but without obvious endometrial defects. This suggests that a hysteroscopic procedure in the preceding cycle could be beneficial for improving pregnancy in subsequent IVF cycles. There were substantial variations in patient selection, timing, number and extent of endometrial injury applied and techniques in these studies [80–84].

In a recent pilot study, it was demonstrated that a site-specific hysteroscopic endometrial injury (a 2 × 2 mm injury at the midline posterior wall about 10–15 mm from the fundus) performed during the ongoing IVF cycle between D2-D7 instead of injuries received during prior cycles significantly improves subsequent embryo implantation in patients with RIF. The endometrium in cycles undergoing endometrial biopsy was found to be thicker; however, the difference did not reach statistical significance [85].

Some of the possible mechanisms by which endometrial injury improves endometrial receptivity include decidualization of endometrium [86] and a wound healing process involving secretion of different cytokines and growth factors beneficial for embryo implantation [87]. Also, synchronization of endometrial and embryo development may play a role as it has been reported that COH cycles result in different structural and functional changes in comparison to natural cycles, including histological advancement, pinopod maturation advancement and steroid receptor down-regulation [88].

However, conflicting results were shown in a Cochrane review, and local injury to the endometrium on the day of oocyte retrieval disrupts the receptive endometrium and has a negative impact on implantation in IVF cycles [89].

23.5.10 GnRH Agonist Injection in Mid Luteal Phase

Administration of a single dose of short-acting GnRH agonist injection 5 or 6 days post ICSI improved the implantation as well as clinical pregnancy rate in all cycles including GnRH antagonist cycles in a meta-analysis of 10 randomized controlled trials, of which 5 trials had usable data for analysis [90]. It has been proposed that GnRH agonist may rescue the corpus luteum by enhancing secretion of pituitary gonadotropins like LH and FSH. However, further studies are required to elucidate the exact mechanism of action.

23.6 Micro RNA Studies

Endometrial receptivity is a complex process involving genetic, morphological and biochemical changes with the expression of numerous molecular mediators. The endometrial gene expression profile changes under the coordinate and sequential action of sex steroid hormones.

Micro RNAs (miRNAs) have emerged as potential regulators of endometrial receptivity and control gene expression at the post-transcriptional level by targeting mRNAs for degradation or translational repression or both. Cell cycle progression, proliferation and differentiation are among the biological processes regulated by miRNAs, processes that are known to occur during the cyclic changes in the endometrium. Luteal support with progesterone and oestrogen + progesterone has a profound effect on endometrial miRNA profiles [91]. Thirteen miRNAs that regulate the expression of 3,800 genes were found to be differentially expressed in secretory endometrium of RIF-IVF patients. Hence, the RIF-associated miRNAs could be exploited as new candidates for diagnosis and treatment of embryo implantation failures [92].

23.7 Embryo Quality

Blastocyst transfer offers higher pregnancy rates and should be offered to all patients with RIF. It is also a well-known fact that in the presence of a receptive endometrium, RIF will occur if the embryos are of poor quality or have aneuploidy [93]. In such cases, pre-implantation genetic diagnosis (PGD) should be offered, and only healthy embryos should be transferred. In the presence of poor endometrial receptivity, the numbers of embryos transferred should be more than single, as the growth factors secreted by one embryo may aid in the implantation of another embryo. In the event of embryos being aneuploid in multiple cycles, the option of donor oocytes may be explored. In the sub-group with normal embryos and non-correctable endometrial compromise, the option of surrogacy may be considered.

Conclusion

The window of implantation is short lived and may be altered in ART cycles due to COH. It may not always coincide with the replacement of a fertilized embryo in the uterine cavity. The process of implantation is complex and as yet poorly understood. It involves two independent variables, the endometrium and the embryo. Both actively secrete integrins, cytokines and growth factors, which are regulated both temporally and spatially in the uterine cavity. The understanding of the regulating mechanisms is still very primitive. Hence, there is a paucity of treatment options available. Most treatment modalities we currently employ are not evidence based, as there is lack of robust randomized controlled trials. The field of genomics, proteomics and metabolomics provides access to a wide variety of genes, miRNA and protein molecules for scrutiny in patients with normal fertility and RIF. However, it has currently not provided the necessary breakthrough in understanding the process of implantation. Research in this field is the need of the day in order to improve the success rates in ART and unexplained recurrent miscarriages.

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Part V

Pathology in Controlled Ovarian Stimulation

Prediction of Poor Responders and Current Concepts in Management

24

Gautam N. Allahbadia and Rubina Merchant

Abstract

Ovarian stimulation is one of the most promising advances in the field of assisted reproduction that has successfully improved pregnancy rates by inducing multifollicular development. The response to ovarian stimulation is a significant predictor of a successful clinical outcome. The ability to predict a poor response to ovarian stimulation equips the clinician with the knowledge to plan and tailor the stimulation protocol to achieve the desired result cost-effectively while avoiding disappointing consequences like cycle cancelation or a failed assisted reproductive technique (ART) cycle. Several markers, such as age, basal (day 3) follicle-stimulating hormone (FSH), inhibin B levels, anti-Müllerian hormone (AMH) levels, and baseline antral follicle counts (AFCs), have been proposed as predictors of an ovarian response; however, no single marker is documented to accurately predict the ovarian response. Several treatment protocols have been formulated to achieve an optimal outcome in poor responders. However, the search for the ideal protocol still eludes clinicians owing to the difficulty in making meaningful comparisons in treatment strategies that stem from wide variations in the definition of a "poor ovarian response" and need for a thorough understanding of the etiologies of a poor response.

Keywords

Poor ovarian response • Poor responder • Management • Ovarian reserve • Predictors of poor ovarian response

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24.1 Introduction: Poor Ovarian Response (POR)

Poor ovarian response (POR) to ovarian stimulation usually indicates a reduction in follicular response, resulting in a reduced number of retrieved oocytes [1]. There have been several definitions and several criteria to define a poor responder to ovarian stimulation. Small numbers of follicles developed or oocytes retrieved, and low estradiol (E2) levels after the use of a standard stimulation protocols have been considered as the most dominant criteria for poor ovarian response [2]. Significantly higher FSH, age, human chorionic gonadotropin (hCG) day luteinizing hormone (LH) level, cycle cancelation rate, total gonadotropin dose but significantly lower AFC, AMH, hCG day E2 level, and number of MII oocytes have been reported in the poor responder group [3].

A poor ovarian response to stimulation creates a significant problem and challenge for the health-care provider, and identification of the etiologies of poor ovarian response constitutes a formidable challenge [4, 5]. Cycle cancelation rates ranging from 9 to 24 % of all stimulated cycles have been reported in poor responders [4]. Low peak estradiol concentrations (<500 ng/L), few dominant follicles on the day of hCG administration (<5 to <2), and therefore, few retrieved mature oocytes (≤4 to ≤6) with resultant lower pregnancy rates have often been reported following stimulation with standard in vitro fertilization (IVF) therapy [6]. Lamazou et al. [7] reported significantly higher cancelation rates (37.8 % vs. 13.3 %, $P<0.004$) and lower pregnancy (22.2 % vs. 35.0 %, $P<0.05$) and live birth rates (11.1 % vs. 26.1 %, $P<0.05$, respectively) following controlled ovarian stimulation (COS) in poor prognosis patients (age >38 years, AFC ≤ 3, and day 3 serum AMH and FSH levels less than 1 ng/mL and more than 10 IU/mL, respectively) compared to good prognosis women [7].

Though several ovarian reserve markers have been used to predict a poor response, there is no ideal predictive test as the poor responder is revealed only during ovulation induction [8].

The "Bologna criteria," presented by the ESHRE working group, is the first realistic attempt by the scientific community to standardize the definition of POR in a simple and reproducible manner that. According to the "Bologna criteria," for a patient to be defined as a poor responder to IVF, at least two of the following three features must be present: advanced maternal age (≥40 years) or any other risk factor for POR (i.e., genetic or acquired conditions, possibly linked to reduced amount of resting follicles), (ii) a previous POR (≤3 oocytes with a conventional stimulation protocol), and (iii) an abnormal ovarian reserve test (ORT) (i.e., AFC <5–7 or AMH <0.5–1.1 ng/mL). One stimulated cycle is considered essential for the diagnosis of POR [1] as a poor ovarian response following maximal stimulation in the first cycle of IVF without any prior testing, provides some information on OR status, and seems to be the preferable strategy [9]. Two episodes of POR after maximal stimulation are sufficient to define a patient as poor responder in the absence of advanced maternal age or abnormal ORT [1]. However, patients of advanced age with an abnormal ORT may be classified as poor responders since both advanced age and an abnormal ORT may indicate reduced ovarian reserve and act as a surrogate of ovarian stimulation cycle outcome. In this case, the patients should be more properly defined as "expected poor responder" [1].

According to the authors, this definition of POR, if uniformly adapted as the "minimal" criteria needed to select patients for future clinical trials, could enable more homogenous populations to be tested for new protocols, reduce bias caused by spurious POR definitions, and also enable comparison of results and reliable conclusions to be drawn. However, the aim of the criteria is not to exclude patients with poor prognosis from IVF programs [1].

According to Polyzos and Devroey [10], although the Bologna criteria aim to define a consistent group of patients, their applicability needs to be tested through clinical trials [10]. Meanwhile, meta-analyses of the currently available trials should be strongly discouraged because they may lead to the adoption of interventions of ambiguous value owing to large variations in the definitions of poor responders within and across

trials, adoption of the criteria in less than 50 % of the trials, and consistently different threshold values [10].

24.2 Predictors of Poor Ovarian Response

The success rates of any assisted reproductive technique (ART) depend on an optimum protocol for ovarian stimulation that must be decided upon by a proper assessment of the ovarian reserve before commencing ovarian stimulation [3]. Over the past two decades, a number of so-called ovarian reserve tests (ORTs), designed to determine the oocyte reserve, have been evaluated for their ability to predict the ovarian response to stimulation and the IVF outcome and have become part of the routine diagnostic procedure for infertility patients undergoing ART [9]. Some of these ORTs include the early follicular-phase FSH, estradiol, inhibin B, AMH levels, AFC, ovarian volume (OVVOL) and ovarian blood flow, clomiphene citrate challenge test (CCCT), exogenous FSH ORT (EFORT), and gonadotropin agonist stimulation test (GAST). Ovarian reserve markers can potentially provide an indirect measure of the cohort of recruitable antral follicles present in the FSH window at the beginning of each menstrual cycle [1]. However, evidence regarding the clinical application of these markers in predicting the outcome of stimulation in poor responders is conflicting.

In this chapter, we aim to assess the clinical accuracy of each of these markers in predicting a poor response and current treatment protocols for poor responders.

24.2.1 Age

Age is one of the most significant markers of the response to ovarian stimulation, an advanced maternal age being proportional to a poor response. Leridon [11] documented that under natural conditions, 75 % of women starting to try to conceive at age 30 years will have a conception ending in a live birth within 1 year, 66 % at age 35 years, and 44 % at age 40 years [11]. The age of patients undergoing assisted reproduction with IVF/GIFT has been inversely related to the pregnancy rate and directly related to the miscarriage rate. In women of 40 years or over, the overall pregnancy and live birth rates were significantly higher, and the miscarriage rate was significantly lower in the group receiving donated oocytes compared to the group using their own oocytes suggesting that the age-related decline in fecundity is associated with the age of the oocytes rather than the age of the uterus [12]. Studies on the ovarian sensitivity to gonadotropin stimulation suggest that the biological age is not equivocal to chronological age and is of greater importance in predicting the outcomes of assisted reproduction [13]. Serum and urinary markers of ovarian reserve, follicular-phase inhibin B, FSH, and AMH levels, have physiologically been associated with ovarian aging and can be used to predict low oocyte yield and treatment failure in infertile women undergoing IVF [14].

Female age and the number of oocytes retrieved have been shown to modulate the chances for pregnancy in current and subsequent cycles, the application which will allow the identification of couples with a reasonable prognosis and balanced decision-making on the management of poor responders. A systematic review of ten studies indicated that older poor responders had a lower range of pregnancy rates compared with younger poor responders (1.5–12.7 vs. 13.0–35 %, respectively) [15]. Though higher gonadotropin doses (225 IU rFSH) have proven more efficacious than 150 IU in younger women despite the higher total dose requirement, they failed to give a higher oocyte yield in older women, suggesting that a higher gonadotropin dose does not compensate for the age-related decline in the number of follicles available for stimulation [16]. Significantly fewer follicles ($p<0.05$) have been reported in women >42 years, while those >39 years had significantly fewer oocytes ($p<0.01$) compared to those <35 years. Live births declined with increasing age, when age was assessed as a continuous variable ($p=0.023$) [17]. Age has been demonstrated as the only independent predictor of

pregnancy in IVF as compared to hormonal and ultrasound indices of ovarian reserve [18]. Though it is a significant predictor of non-conception, it has a low predictive accuracy [19].

24.2.2 Number of Oocytes Received

Patients have been categorized into three groups according to the number of oocytes retrieved: 0–3 oocytes (poor responders), 4–15 oocytes (normo-responders), and >16 oocytes (hyper-responders). AMH and AFC were the best markers for the prediction of total oocyte count, independent of age, FSH, and LH levels and without any significant effects on pregnancy rates [3]. A systematic review of four studies on poor responders undergoing IVF showed that pregnancy prospects are reduced when fewer oocytes are retrieved (0–7 % with 1 oocyte vs. 11.5–18.6 % with 4 oocytes), while five studies concerning pregnancy rates in subsequent cycles suggested a more favorable outcome in unexpected poor responders and if ≥2 oocytes were retrieved [15].

24.2.3 Endocrine Markers

24.2.3.1 Anti-Müllerian Hormone (AMH)

The anti-Müllerian hormone (AMH) is exclusively produced by granulosa cells of ovarian follicles during the early stages of follicle development [20]. Plasma levels of AMH reflect the continuous noncyclic growth of small follicles, thereby mirroring the size of the resting primordial follicle pool and, thus, acting as a useful marker of ovarian reserve [21]. The clinical applications of the measurement of circulating AMH are mainly based on its ability to reflect the number of antral and pre-antral follicles present in the ovaries. It has also been proposed as a surrogate for AFC in the diagnosis of polycystic ovary syndrome (PCOS) and to indicate iatrogenic damage to the ovarian follicle reserve [22]. Women with low AMH levels have a high probability of treatment cancelation and failure to proceed to embryo transfer and a low chance of achieving a viable pregnancy [17].

Advantages of the use of AMH levels as a marker ovarian reserve are as follows: (i) they are among the best endocrine markers for assessing the age-related decline of the ovarian pool in healthy women; (ii) they are a reliable predictor of ovarian reserve, especially when combined with age with a sensitivity and specificity of 72–97 % and 41–93 %, respectively, positive predictive values between 30 and 79 % but higher negative predictive values, cycle stability, and operator independency; (iii) they are predictive of both poor and hyper ovarian responses prior to an in vitro fertilization (IVF) cycle, in a variety of ovarian pathological conditions, including PCOS, premature ovarian failure (POF) [21], and endometriosis [23]; (v) they can predict the number of oocytes retrieved [10]; (vi) they may help to individualize dosing for ovarian stimulation, thereby improving the efficiency and safety of IVF [20]; and (vii) they exhibit no intracycle fluctuations and are negligibly affected by hormonal changes, such as those that occur during pregnancy or under oral contraceptives. Patients with AMH levels ≤0.5 and ≤1 ng/mL have a poor response to ovarian stimulation, a significantly higher total dosage of gonadotropins used and day 3 FSH levels, and lower maximum E2 levels and clinical pregnancy rates suggesting that AMH could be an acceptable screening test in prediction of ovarian reserve [24]. Moreover, day 5 follicular-phase AMH concentrations have been reported as better predictors of the ovarian response following FSH stimulation under pituitary desensitization compared to basal (day 3) AMH levels, but the predictive capacity of day 5 AMH was not better than that provided by day 5 estradiol levels [25].

However, AMH levels have limited value in the prediction of ongoing pregnancies following IVF as a number of poor responders and older patients (>40 years) have been reported to achieve pregnancy in spite of very low AMH levels, with a better prognosis for young poor responders [21, 26]. An AMH cutoff value ≤1 ng/ml may predict poor ovarian reserve, poor ovarian response to stimulation [24], and whether patients could have an embryo transfer but had no power to predict

the achievement of pregnancy [26]. Though some authors have suggested an influence of AMH in predicting live birth after assisted conception independently of age and its use in counseling couples before undergoing fertility treatment, its predictive accuracy is poor [27].

Despite its limitations, AMH, however, is the best current available measure of ovarian reserve for different clinical conditions [20]. The widespread clinical application of AMH levels as an ORT emphasizes the need for an international standard for AMH and improved assay validity, so that results using future assays can be reliably compared [20–22]. Prospective well-powered studies comparing different infertility treatment strategies, based on initial AMH levels using appropriate end points (live birth and cost-effectiveness), and that could represent a true step forward in rendering counseling and infertility care more patient tailored are urgently awaited [20].

24.2.3.2 FSH Levels

Significantly higher FSH levels but significantly lower AFC, AMH, hCG day E2 level, and number of MII oocytes have been reported in poor responders [3]. Studies have reported significantly higher cancelation rates (37.8 % vs. 13.3 %, $P<0.004$), lower pregnancy (22.2 % vs. 35.0 %, $P<0.05$), and live birth rates (11.1 % vs. 26.1 %, $P<0.05$, respectively) following controlled ovarian stimulation (COS) in poor prognosis patients (>38 years) with day 3 FSH levels >10 IU/mL, AFC≤3, and day 3 serum AMH levels <1 ng/mL compared to good prognosis women [6]. Increased levels of day 3 FSH and decreased levels of inhibin B can be used to assess ovarian reserve [8].

Basal and clomiphene citrate (CC)-induced FSH and inhibin B levels have also been correlated with mean ovarian volume (MOV) and mean antral follicle counts (MFC). Erdem et al. [17] reported significantly higher basal FSH ($p<0.05$), lower basal and induced inhibin B levels ($p<0.05$), and lower MOV and MFC ($p<0.01$) following IVF/ICSI in poor responders compared to normal responders [18]. Maman et al. [28], however, failed to show an association between patients with a history of high basal FSH (15.0±3.6 IU/l) or those with low basal FSH (9.0±3.0 IU/l) and a reduced ovarian reserve with the IVF outcome and further suggested that ovarian stimulation need not be delayed until FSH declines [28].

24.2.3.3 Inhibin Levels

Early follicular-phase inhibin B concentrations, obtained following ovarian stimulation under pituitary suppression for assisted reproductive treatment, have been reported to be highly predictive of the ovarian response [29]. Significantly lower day 3 and day 10 inhibin B levels ($p<0.001$) have been demonstrated in women with diminished ovarian reserve. Peñarrubia et al. [29] demonstrated significantly lower day 5 inhibin A and inhibin B levels following gonadotropin therapy in patients with a canceled cycle compared to the control group. They demonstrated a significant association between day 5 inhibin B levels and the cancelation rate (with a predictive value of ovarian response of 91.03 %) that was independent of and stronger than the effects of any other hormone variable investigated. However, day 5 inhibin B was not a better predictor of pregnancy than the other hormone variables studied on this day [29].

24.2.4 Clomiphene Citrate Challenge Test (CCCT)

An abnormal CCC test has been identified as a better predictor of diminished ovarian reserve than basal (day 3) FSH concentrations or other hormonal and sonographic tests and the only independent significant factor in predicting ovarian response to stimulation in IVF cycles [16, 17] that provides valuable information for both patients as to their chances of achieving a pregnancy and also for the medical team deciding on options for stimulation protocols [16]. Yong et al. [16] reported significantly lower estradiol values on hCG day, number of retrieved and metaphase II oocytes, and rate of transfer cycles in women with an abnormal CCC test, while cycle cancelation rates (36.8 % vs. 19.8 %; $P<0.05$) were significantly higher, and pregnancy rates per

embryo transfer (13.3 vs. 21.5 %, respectively) were lower in women with a poor response and an abnormal CCC test than in those with a normal test. The sensitivity, specificity, and positive and negative predictive values of the CCC test for cycle cancelation were found to be 43 %, 76 %, 37 %, and 80 %, respectively, while those for non-conception were 93 %, 31 %, 84 %, and 15.6 %, respectively. In patients with an elevated day 10 or 11 FSH level, which could not be detected using only basal FSH screening (43.8 %), the cancelation rate (48 vs. 19.8 %, $P<0.01$), the rate of transfer cycles (48 % vs. 72.3 %, $P<0.05$), and the mean number of retrieved oocytes (4.9 ± 2.5 vs. 6.4 ± 3.1, $P<0.01$) were all significantly different from the normal test group [16]. The results of Yong et al. [16] were in sharp contrast to a previous study [30] comparing basal FSH and the full CCCT demonstrating that the CCCT showed a poor specificity in predicting poor response and nonpregnancy and has hardly any additional value [30].

24.2.5 Ultrasound Indices of Ovarian Reserve

Transvaginal ultrasonography is an easy-to-perform and noninvasive method that provides essential predictive information on ovarian responsiveness [19]. Ultrasound measurements of ovarian volume, baseline AFC, and Doppler measurements of ovarian stromal blood flow now make it possible to predict low response to IVF therapy. Low response can be expected if the ovary has a volume <3 cm^3, the mean ovarian diameter in the two longest planes is <20 mm, or with AFC ≤3 in each ovary [5]. Significantly lower ultrasound indices of ovarian reserve, such as mean ovarian volume (MOV) and mean follicle count (MFC) ($p<0.001$) have been demonstrated in women with diminished ovarian reserve. The lower MOV and MFC values correlated with significantly higher basal FSH ($p<0.05$) and lower basal and induced inhibin B levels ($p<0.05$) in poor responders undergoing IVF/ICSI compared to normal responders. Ovarian volume alone was reported to be better than age and basal hormones in predicting poor ovarian response [18] Data on ovarian stromal blood flow are still unclear, but an ovarian peak systolic velocity of <10 cm/s is associated with low response. If low response is anticipated based on baseline ultrasound scan, effective stimulation protocols that can reduce cancelation rates and improve pregnancy rates should be used for IVF [5].

24.2.5.1 Antral Follicle Counts (AFCs)

Estimation of the antral follicle numbers with a diameter of 2–5 mm by transvaginal ultrasonography on the first or second day of menstruation, or just before the administration of exogenous gonadotropins, enables the prediction of the ovarian response and pregnancy results of patients undergoing ARTs. Antral follicle counts have been significantly correlated with patient age, day 3 serum FSH level, use of gonadotropins, serum estradiol concentration, number of oocytes retrieved, and, later, number of oocytes or embryos transferred. Significantly higher cycle cancelation rates (68.8 % vs. 5.3 % and 0, respectively) and no pregnancies (0, 23.7 %, and 36.8 %, respectively) have been reported in patients with a low AFC (≤3) compared with patients with AFCs = 4–10 or ≥11 [31]. AFC evaluation has been considered as a first choice test in the assessment of ovarian reserve prior to IVF, more accurate than basal FSH [32].

A comparative study between the three-dimensional ultrasound parameters (AFC, ovarian volume, and ovarian vascularity indices) with AMH and other conventional endocrine markers for the prediction of poor response following controlled ovarian hyperstimulation (COH) during assisted reproduction demonstrated that AFC and AMH were the most significant predictors of poor response to ovarian stimulation. The sensitivity and specificity for prediction of poor ovarian response were 93 % and 88 % for AFC and 100 % and 73 % for AMH (at optimum cutoff values of ≤10 and ≤0.99 ng/mL, respectively). While AMH and AFC had a similar predictive accuracy either alone or in combination, they were not shown to be predictive of non-conception, which is dependent on the woman's age [19].

24.2.6 Combined Predictors

A newly devised ovarian response prediction index (ORPI) [ORPI = AMH (ng/ml) × AFC (2–9 mm)/patient age] exhibited an excellent ability to predict a low ovarian response and a good ability to predict the retrieval of greater than or equal to 4 MII oocytes, an excessive ovarian response, and the occurrence of pregnancy in infertile women. The ORPI might be used to improve the cost-benefit ratio of ovarian stimulation regimens by guiding the selection of medications and by modulating the doses and regimens according to the actual needs of the patients [33]. Predictors of ovarian reserve, such as the woman's age, AMH, and FSH, also serve to predict the FSH dosage nomogram for ovarian stimulation, which clinicians could apply during their daily clinical practice. They could predict a starting FSH dose <225 IU in 55.1 and 25.9 % of women younger and older than 35 years, respectively [34].

24.3 Limitations of Ovarian Reserve Markers

However, certain drawbacks of the use of ovarian reserve markers to predict ovarian response to stimulation are as follows: (i) they seem to be affected by common ovarian toxicants, such as smoking, which advance the age at menopause; (ii) the clinical use of these markers is limited by the variety of assays, lack of definitive thresholds, and their intercycle variability in older women [14]; (iii) they do not necessarily reflect the extent and quality of the primordial follicle pool or accurately predict ovarian response to hormonal stimulation [1]; and (iv) most ORTs evaluated have only modest-to-poor predictive properties for the occurrence of poor ovarian response owing to a modest test accuracy and a poor pregnancy prediction accuracy and are, therefore, far from suitable for relevant clinical use [9]. Whether the a priori identification of actual poor responders in the first IVF cycle has any prognostic value for their chances of conception in the course of a series of IVF cycles remains to be established. High thresholds used to prevent couples from wrongly being refused IVF result in a very small minority of IVF-indicated cases (approximately 3 %), identified as having unfavorable prospects in an IVF treatment cycle [9]. Hence, results should be conveyed with caution when highly discrepant with age, in the obese, and in women with irregular menstrual cycles. Further research is needed to assess their predictive value for determining fertility in the general population [14].

24.4 Management of Poor Responders

The management of poor ovarian response remains one of the most significant challenges posed to clinicians practicing assisted conception [5, 8], the most important problems in evaluating the available evidence being the lack of a sufficiently homogenous population despite attempts at a consensus on the definition of a poor responder and a poorly understood etiology [35, 36]. As a result, much controversy exists on how to manage a poor responder in assisted conception and every new suggestion has proved contentious [36]. Very few large prospective randomized trials have compared different protocols [6].

Ovarian stimulation is a significant step in the management of poor responders. Numerous interventions, including high doses of gonadotropins, recombinant FSH, flare-up GnRH agonist protocols, luteal initiation GnRH agonist "stop" protocols, GnRH antagonist protocols, adjuvant therapy with growth hormone (GH) or GH-releasing factors, use of corticosteroids, and pretreatment with combined oral contraceptives and natural cycle IVF, have been proposed to improve the ovarian response to stimulation in poor responders. However, few have been shown to be beneficial for all such patients [35]. Controversies exist regarding the selection of gonadotropin preparation, choice of adjuvant therapy with GnRH analogs, and use of oral contraceptive pills, and results in low responders have remained suboptimal both in terms of ovarian response and oocyte/embryo quality in spite of a variety of stimulation regimens used [5].

The evidence regarding the clinical efficacy of these protocols in improving the ovarian response in poor responders is detailed below.

24.4.1 Types of Gonadotropins

The few available relevant studies do not indicate that recombinant FSH (rFSH) improves the outcome of ovarian stimulation in poor responders [8]. Recombinant FSH has no advantage over urinary human menopausal gonadotropin (hMG) on ovarian performance or the outcome of IVF-ET in poor responders' IVF cycles [37]. Comparable results have been observed in poor responders (>37 years) when rFSH was used alone or in combination with hMG, except for the quality and the number of embryos transferred, which were better in the rFSH + hMG group [38].

24.4.2 Increased Gonadotropin Doses

Although high doses of gonadotropins have been used by the vast majority of authors, results have been controversial and prospective randomized studies have shown little or no benefit [8]. Despite the maximum gonadotropin dose (≥300 IU), poor responders defined by the Bologna criteria, undergoing ovarian stimulation cycles for IVF/ICSI, demonstrated very low per cycle (7.1 versus 5.2 %) and per patient (11.6 versus 8.8 %) live birth rates, irrespective of age (<40 or ≥ 40 years) and the treatment protocol used. An increase in the number of oocytes retrieved was an independent variable related to live birth rates (OR 1.92, 95 % CI, 1.03–3.55 for >3 versus 1–3 oocytes) [39].

There is no significant improvement in oocyte and embryo yield or pregnancy rates in 122 patients (<36 years) with a low predicted ovarian reserve based on a serum AMH measurement (<1.4 pmol/l) following an upward adjustment of the standard FSH starting dose above 150 IU/day. On the contrary, an extra 1,100 IU of FSH per IVF cycle was consumed in patients who received a higher starting dose of 200–300 IU/day FSH, suggesting that the upward FSH dose adjustment in anticipation of low ovarian reserve cannot be advocated as it is both expensive and of no proven clinical value [40]. No significant differences have been observed in the age, peak serum E [2] concentration, days of stimulation with rFSH, total number of M2 oocytes retrieved, number of embryos transferred, clinical pregnancy rates, and cancelation rates of stimulation and embryo transfer between patients administered with a GnRH agonist microdose protocol with daily fixed doses of 300 IU of rFSH, 450 IU of rFSH, or 600 IU of rFSH except for the total rFSH dosage. Doses above 300 IU of rFSH show no benefit in increasing the pregnancy rate in microdose cycles. On the contrary, because the duration of stimulation did not differ between the groups, the usage of 300 IU rFSH in microdose cycles resulted in a lower requirement of rFSH consumed in a cycle compared with higher dosages and proved to be a more cost-friendly option [41]. In patients with a diminished egg reserve, the very poor pregnancy rates found with high-dosage gonadotropins were comparable to those in women with a normal egg reserve stimulated with low-dose gonadotropin regimens, suggesting that low-dose gonadotropin regimens have the benefit of reducing costs and risks of ovarian hyperstimulation without reducing efficacy and, in some cases, actually increasing pregnancy rates [42].

24.4.3 GnRH Agonist (GnRHa) and Antagonist (GnRH-ant) Protocols

A meta-analysis of six randomized controlled trials that compared the efficacy of GnRH-ant versus GnRH agonists as coadjuvant therapy for ovarian stimulation in poor ovarian responders observed no difference between GnRH-ant and GnRHa (long and flare-up protocols) with respect to the cycle cancelation rate, number of mature oocytes, and clinical pregnancy rate per cycle initiated, per oocyte retrieval, and per embryo transfer. The GnRH-ant protocol proved to be superior to the GnRHa long protocols in terms of the number of retrieved oocytes ($P = 0.018$) when the meta-analysis was narrowed to the two trials, but

GnRHa flare protocol proved to be superior to GnRH-ant protocol in terms of the number of retrieved oocytes ($P=0.032$) when the meta-analysis was narrowed to the four trials that compared the two protocols. The authors, however, emphasized the need for additional randomized controlled trials with better planning to confirm these results [43].

Schoolcraft et al. [44] observed no significant differences in mean age, number of oocytes, fertilization rates, number of embryos transferred, or embryo score between the microdose GnRH agonist flare (ML) and a GnRH antagonist/letrozole (AL) protocol in 534 poor responders classified as past or potential poor responders based on clinic-specific criteria. Peak E 2 levels were significantly lower in the AL group and ongoing pregnancy rates were significantly higher in the ML group (52 % vs. 37 %, respectively). Observing a higher ongoing pregnancy rates and a trend toward superior implantation rates with the ML protocol, they suggested that the ML protocol represents a preferred approach for the poor responder compared to the AL protocol; however, an increased sample size would be necessary to verify these findings [44]. DiLuigi et al. [45], on the other hand, in a later but smaller study showed no differences in the number of oocytes retrieved, clinical pregnancy rates (PR), and ongoing PRs between the microdose GnRH agonist (leuprolide acetate) protocol versus luteal phase GnRH antagonist (ganirelix) protocol in predicted poor responders [45].

24.4.3.1 GnRH Agonist Flare Versus GnRH Agonist Long Protocol

No improvement in the ovarian response has been reported in previous poor responders stimulated with the GnRH "microdose flare" protocol (leuprolide acetate, 0.5 mg/day on day 2 and at least 6 ampules of gonadotropins on day 3) compared to the GnRH agonist long protocol despite a 39.6 % increase in the dose of gonadotropins. The pregnancy rates/cycle and live birth rates following the GnRHa flare protocol in patients who responded but failed to conceive with the GnRHa long protocol and the poor responders, respectively, were 18.1 % versus 9.9 %, respectively, and 0 % versus 2.5 %, respectively. Poor response was defined as <3 developing follicles on day 7, with an estradiol level <200 pg/mL. Patients with a cycle day 2 follicle-stimulating hormone (FSH) level >15 mIU/mL before initiating GnRHa were not included in the flare-up protocol. The authors concluded that although the flare-up protocol after an unsuccessful luteal phase long protocol increases the pregnancy rate per cycle slightly, the live birth rate is not improved in poor responders [46].

24.4.3.2 GnRH Agonist Flare Versus GnRH Agonist Stop Protocol

Surrey [47] observed that traditional GnRHa flare and luteal phase long protocols do not appear to be beneficial in the management of the poor responder. Reduction of GnRHa doses, "stop" protocols, and microdose GnRHa flare regimes all appeared to enhance outcomes, although the relative benefit of one approach over another has not been conclusively demonstrated [47]. Detti et al. [48], however, demonstrated a trend toward higher delivery rates with microdose flare protocol (GnRHa 20 microg administered twice daily with gonadotropins from day 2 to the day of hCG administration) for poor responders when compared to the stop protocol (GnRHa 500 microg/day from the midluteal phase to the start of menses, then gonadotropins from day 2 of cycle) or the regular dose flare (gonadotropins beginning with GnRHa on day 2 at 1 mg/d for 3 days, followed by 250 microg/day until the day of hCG administration). The authors once again indicated the need for a larger prospective study to determine if this trend leads to a significant finding in this patient population [48].

24.4.3.3 GnRH Agonist Flare Protocol

Though the GnRH "microdose flare" protocol has shown promising results in poor responders [38], Karande [49] reported low clinical pregnancy rates (11.5 %/retrieval and 13.4 %/embryo transfer) and implantation rates (3.5 %) and a high cycle cancelation rate (23.8 %) despite adequate ovarian response with the retrieval of 10 ± 6.6 (range, 1–37) in poor responders undergoing COH with the "flare" protocol in cycles with low basal FSH [49]. Though the flare-up protocol

after an unsuccessful luteal phase long protocol increases the pregnancy rate per cycle slightly, the live birth rate is not improved in poor responders [46].

24.4.3.4 Luteal Initiation GnRH Agonist "Stop" Protocols

Luteal initiation GnRH agonist "stop" protocols were shown to improve ovarian response according to prospective studies with historical controls, but this was not confirmed by well-designed prospective, randomized, controlled studies [8]. Garcia-Velasco et al. [50] reported a similar cancelation rate (with no cycles canceled due to ovulation), pregnancy rate, and implantation rate in 70 low responder patients (<3 mature follicles in a previous cycle, normal basal FSH levels, and a previous canceled IVF cycle) who underwent a GnRH stop protocol (GnRHa stopped with the onset of menses with high gonadotropin doses) and the nonstop protocol (long GnRHa suppression with high doses of gonadotropins), respectively. Despite similar doses of gonadotropins in both the groups, a significantly higher number of mature oocytes (8.7 ± 0.9 versus 6.2 ± 0.7, *P*: = 0. 027) and a significant reduction in the gonadotropin ampules required (56.6 ± 2.7 versus 68.0 ± 3.5, *P*: = 0. 013) were observed in the stop protocol compared to the nonstop protocol, suggesting that early cessation of GnRHa combined with high doses of gonadotropins could improve the oocyte retrieval rate in poor responders [50].

24.4.3.5 GnRH Antagonist "Delayed-Start" Protocol

Poor responders to the conventional estrogen priming antagonist IVF protocol have been shown to benefit from a delayed-start antagonist protocol (estrogen priming followed by early follicular-phase GnRH antagonist treatment for 7 days before ovarian stimulation) with improved ovarian response by promoting and synchronizing follicle development without impairing oocyte developmental competence. A significantly higher number of dominant follicles (4.2 ± 2.7 vs. 2.4 ± 1.3), shorter duration of ovarian stimulation (9.4 ± 1.4 days vs. 11.1 ± 2.0 days), higher number of mature oocytes retrieved (4.9 ± 2.0 vs. 2.2 ± 1.1), and trend toward increased fertilization rates with ICSI (86 ± 17 % vs. 69 ± 21 %) were observed following the delayed-start antagonist compared to the conventional GnRH antagonist protocol, respectively. After delayed start, the average number of embryos transferred was 2.8 ± 1.4 with implantation rate of 9.8 % and clinical pregnancy rate of 23.8 % [51].

24.4.4 Growth Hormone

Growth hormone (GH) augments the effect of gonadotropins on granulosa and theca cells and plays an essential role in ovarian function, including follicular development, estrogen synthesis, and oocyte maturation [52]. In an effort to improve outcomes of IVF cycles, the use of GH has been considered. Although the use of GH in previous poor responders has been found to show a significant improvement in the live birth rate, this result was only just significant [53]. Co-treatment with GH failed to show an increase in pregnancy rates in patients with a history of poor response in previous IVF-ET cycles stimulated with an hMG/GnRH antagonist protocol [52] or GnRHa long protocol [54] despite a significantly higher number of oocytes retrieved [39]. A Cochrane database review of six small trials suggested that in women with no previous poor response, GH augmentation does not improve the rate of pregnancy. There was no difference in the total dose of gonadotropin and number of oocytes retrieved between normal and poor responders. In previous poor responders, a trend toward improved outcome with GH treatment deserves further study [55]. A following Cochrane database review suggested no evidence that the routine use of GH affected the outcome of live birth. In previous poor responders to IVF, there was no significant difference in live birth when combining trials of GH and GRF. However, when trials using GH were analyzed separately, there was an increase in live births. Since this data was from just three small trials, the authors concluded that before recommending GH in IVF, further research is necessary to fully define its role and GH, in the

meanwhile, should only be considered in the context of a clinical trial [53].

24.4.5 Transdermal Testosterone Application

No significant improvement in the AFC or in the main parameters of the ovarian response (numbers of preovulatory follicles, total and mature oocytes and embryos) has been observed between patients randomized to receive either a transdermal testosterone application or placebo gel for 15 days before FSH treatment. No significant beneficial effects of androgen administration on the ovarian response to FSH could be demonstrated. However, subsequent clinical trials are needed to determine whether an optimal dose and/or a longer duration of testosterone administration may be helpful [56].

24.4.6 Corifollitropin α

Polyzos et al. [57] reported low pregnancy rates following treatment of Bologna poor ovarian responders with corifollitropin alpha (150 μg) followed by 300 IU recombinant follicle-stimulating hormone (rFSH) in a GnRH antagonist protocol similar to conventional stimulation with a short agonist protocol. Cycle cancelation rate was 32.6 %, and embryo transfer rate was 53.3 % with an ongoing pregnancy rate of 7 %. There was no significant difference in the ongoing pregnancy rates between patients treated with the standard short agonist-hMG protocol and the corifollitropin alpha protocol for poor responders [57].

24.4.6.1 Corifollitropin α Followed by Menotropin for Poor Ovarian Responders' Trial (COMPORT)

Corifollitropin α followed by menotropin for poor ovarian responders' trial (COMPORT) is a randomized trial that aimed to investigate whether this novel protocol is superior to treatment with rFSH in an antagonist setting for young poor responders [58]. Although preliminary studies in the Bologna poor responders demonstrated very low pregnancy rates, a recent pilot study on 150 Bologna poor responders (<40 years) has shown promising results with corifollitropin α (150 μg on day 2 of the menstrual cycle) followed by highly purified menotropin (hpHMG; daily fixed dose of 300 IU from day 9 till ovulation trigger) in a GnRH antagonist setting compared to rFSH in a GnRH antagonist (fixed daily dose of 0.25 mg of GnRH antagonist from day 7 onward) protocol. There was a difference of 19.5 % in ongoing pregnancy rates between the corifollitropin α and FSH groups (28 % vs. 8.5 %, respectively; $p=0.05$) with a power of 85 % [58]. A previous study by the same authors showed that age had a significant impact on the results with the COMPORT; ongoing pregnancy rates in women <40 years were 28 % versus 0 % in patients ≥40 years of age ($P=0.017$). Corifollitropin alpha followed by hphMG in a GnRH antagonist protocol, thus, yields very promising pregnancy rates, albeit only in young (<40 years old) poor ovarian responders fulfilling the Bologna criteria [59].

24.4.7 Natural Cycle IVF

Previous trials have shown that natural cycle IVF is an effective treatment for the general infertile population and might be an option for poor ovarian responders [60]. In an analysis of 500 consecutive natural cycle IVFs, Schimberni et al. [61] demonstrated that natural cycle IVF is an effective treatment, especially in young poor responders, and yields a pregnancy rate of 9.8 % per cycle, 17.1 % per transfer, and 16.7 % per patient. There was an age-related decline in the pregnancy rates, the per cycle, per transfer, and per patient pregnancy rates being 18.1 %, 29.2 %, and 31.7 %, respectively, in patients ≤35 years; 11.7 %, 20.6 %, and 20.3 %, respectively, in women aged between 36 and 39; and 5.8 %, 10.5 %, and 9.7 %, respectively, in women aged ≥40 years [61].

However, a recent study by Polyzos et al. [60] demonstrated consistently and significantly lower live birth rates per cycle (2.6 versus 8.9 %, $P=0.006$) and per treated patient (7.4 versus 25 %, $P=0.005$) following natural cycle IVF in

Bologna poor responders compared to normal responders (control group) who did not fulfill the Bologna criteria. The live birth rates in poor responders did not differ among the different age groups (≤35 years, 36–39 years, and ≥40 years). The study concluded that though natural cycle IVF is a promising treatment option for younger normal responders, its potential is very limited to poor ovarian responders as described by the Bologna criteria, irrespective of the patient's age. This highlights the very poor prognosis of these women and, therefore, the urgent need for future trials to examine the effect of ovarian stimulation protocols in women with poor ovarian response as described by the Bologna criteria. Though the analysis was limited by its retrospective design, since only consecutive patients were treated with exactly the same protocol, the likelihood of selection bias might be considerably limited [60]. The relatively high cancelation rate in patients with poor prognosis raises the question of the use of modified natural cycle IVF in this group [7].

While the use of corticosteroids reduces the incidence of poor ovarian response in women undergoing IVF treatment, limited data obtained with nitric oxide donors are encouraging. Pretreatment with combined oral contraceptives prior to stimulation may help ovarian response. No benefit was observed with standard use of ICSI or assisted hatching of zona pellucida [8].

24.5 Is There an Ideal Protocol for Poor Responders?

Vollenhoven et al. [62] demonstrated no significant differences in fertilization rate, utilization rate, or pregnancy rate/initiated cycle irrespective of age and stimulation regimen among three stimulation regimes in a retrospective analysis of 1,608 cycles in poor responders and 8,489 cycles in the nonpoor responders. Normal responders had a significantly greater pregnancy rate/initiated cycle for all stimulation regimens in both age groups compared with "poor" responders [62]. No statistically significant differences have been observed in the duration of stimulation, gonadotropin consumption, number of oocytes retrieved, number and quality of embryos transferred, and clinical pregnancy rates between the flare GnRH agonist and GnRH antagonist protocols in 220 patients with poor prognosis for ovarian response, based on previous cycles or clinical criteria [63]. The relative superiority of the reduction of GnRHa doses, "stop" protocols, and microdose GnRHa flare regimes has not been conclusively demonstrated though all three protocols have been shown to enhance the outcomes [47]. A meta-analysis of six randomized controlled trials also demonstrated no difference in the clinical outcomes between GnRH-ant and GnRHa (long and flare-up protocols) for ovarian stimulation in poor ovarian responders [43].

There is no one controlled ovarian hyperstimulation (COH) protocol which is best suited for all poor responders. Prediction of compromised response prior to cycle initiation by a thorough assessment of ovarian reserve as well as a careful review of past response should allow for selection of an appropriate COH protocol for each individual patient [47].

24.6 Oocyte Donation

Assisted conception with donor oocytes may be offered as a last resort to poor responders to ovarian stimulation or those with a severely decreased ovarian reserve after appropriate informed and implication counseling and an informed consent.

24.7 Future Prospects

Optimistic data have been presented by the use of high doses of gonadotropins, flare-up GnRH agonist protocols (standard or microdose), stop protocols, luteal onset of gonadotropin-releasing hormone agonist, and short protocols and natural cycle also seems to be an appropriate strategy to be considered [64]. However, the lack of a uniformly applied definition of the poor responder and dearth of prospective randomized trials make data analysis difficult [47]. Though the Bologna criteria for a unified definition for POR aims to

provide a relatively homogenous population to enable future research, the population described by the Bologna criteria might not be sufficiently homogenous necessitating stratified randomization in relevant randomized controlled trials (RCTs). Unless evidence necessitating stratified randomization is not available, interventions aimed at evaluating for POR according to the Bologna criteria should use RCTs of sufficient sample size, with proper allocation concealment and masking [35]. The response to stimulation varies substantially among women and is difficult to predict. Several predictive markers of COH outcome have been proposed (e.g., maternal age and ovarian reserve), but the search for optimal predictors is ongoing [55]. Although data are accumulating with evidence suggesting that the ovarian response to COH and differences in COH and IVF outcomes are mediated by various FSH gene polymorphisms involved in FSH signaling, estrogen biosynthesis, folliculogenesis, folate metabolism, and other aspects that influence the response to exogenous gonadotropin administration, the optimal biomarkers and the efficacy of the tests still remain to be evaluated [55]. Clinical trials to look for algorithms integrating the N680S missense variant genotype, associated with poor response during COH, and to test if such clinical protocols can optimize the rFSH dose and detect women at risk of a poor response during a COH cycle should be conducted [65].

Despite the numerous protocols proposed, it must be borne in mind that individualization of management is essential and depends on assessment of the ovarian reserve [5]. More data from good quality, well-designed, large-scale, randomized, controlled trials are needed to assess the efficacy of the different management strategies [8, 64].

Conclusion

Though there is no ideal predictor for a poor ovarian response and no ideal protocol for the efficacious management of a poor responder, it rests upon the clinician to thoroughly evaluate the etiology of a poor ovarian response in each individual patient or, alternatively, use the relevant endocrine and ultrasound indices to detect a potential poor responder before embarking on treatment to ensure cost-effective management. AMH and AFC represent significant predictors of the ovarian reserve; however, their predictive accuracy with regard to live birth rates is low. Age remains the only significant independent predictor of live births, whose effect cannot be diminished by an increased dose of gonadotropins in poor responders. In the meantime, the Bologna criteria for defining a "poor responder" that encompasses age, ultrasound, and endocrine predictors could be used to identify the "poor responder," while the integrated ovarian response prediction index (ORPI) could be used to guide the selection of a particular stimulation regimen and modulate the dose in poor ovarian responders. Until well-designed large-scale studies on a homogenous population are not available to streamline management, the management for poor responders would have to be individually tailored.

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Premature Rise of Progesterone During Ovarian Stimulation

25

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Abstract

Premature rise of progesterone in controlled ovarian stimulation cycles influences IVF outcome. Several authors failed to demonstrate any negative impact while others reported the detrimental effect associated with progesterone rise (pre-ovulatory). It seems that P rise >1.5 ng/ml may have deleterious effect on endometrial receptivity, accelerating the endometrial maturation process that desynchronizes the crosstalk between the embryo and endometrium during implantation. This decreases the pregnancy rate. Progesterone elevations on the day of hCG in GnRH analogue cycles are the result of the ovarian stimulation itself, driven by high follicle-stimulating hormone dosages, high oestradiol levels, the increased number of follicles and oocytes, increased sensitivity of LH receptor of the granulosa cells to FSH or poor ovarian response with increased LH sensitivity. To prevent the premature rise of progesterone in COS, we should use milder stimulation protocols, earlier trigger of ovulation in high responders and single-blastocyst transfer on day 5. The optimal GnRH analogue protocols during the entire stimulation period appear to be the long agonist as well as 'long' and long GnRH antagonist regimens (oral contraceptive pre-treated fixed antagonist regime). The most appropriate choice to avoid the negative effects of follicular progesterone elevations is to cancel fresh embryo transfer and to transfer frozen-thawed embryos in natural cycles.

Premature luteinization (PL) refers to a rise in serum progesterone (P) levels on the day of hCG administration. Most studies used an absolute P level on the day of hCG administration as an indicator of PL, and the cut-

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off level differed from 0.8 to 2 ng/mL. Some authors defined PL as a P/E2 ratio of >1. There is a marked variation in the incidence (13–71 %) of PL due to discrepancies in definition, population characteristics and/or treatment protocols. The pathogenesis of PL in COH is still poorly understood. Several hypotheses may be considered to explain this phenomenon: elevation of follicular LH levels, serum accumulation of HCG from HMG, increased LH receptor sensitivity of the granulosa cells to FSH or poor ovarian response with increased LH sensitivity. The consequences of this premature elevation of serum P on IVF outcome remain controversial. Attempts to prevent COH include use of low-dose hCG alone in the late COH stages, flexible antagonist protocol, use of mifepristone, aspiration of a single leading follicle and hCG administration when the levels of serum P exceeded 1.0 ng/mL.

Keywords
Premature rise of progesterone • Ovulation induction • Endometrium • Pregnancy rate • Premature luetinization • Ovarian stimulation • LH

25.1 Introduction

The incidence of premature luteinizing hormone (LH) surge has significantly decreased by the introduction of gonadotropin-releasing hormone (GnRH) analogues for pituitary suppression in in vitro fertilization (IVF) [1]. Despite pituitary down-regulation, however, several researchers have described a phenomenon reported as premature rise in serum progesterone levels on the day of human chorionic gonadotropin (hCG) administration or late follicular phase [2]. Decreased implantation and pregnancy rates have been reported with this phenomenon. Its pathogenesis is still poorly understood. One of the major reasons for the controversy has been the diverse definitions of P rise in literature.

25.2 Definition

In past, an absolute progesterone concentration on the day of HCG administration was taken as an indicator of progesterone elevation with arbitrarily set cut-off concentrations ranging from 0.8 to 2 ng/ml [3–7]. In recently published studies, using new methods for serum progesterone assessment, this cut-off concentration is usually set at 1.5 ng/ml [8]. This cut-off is supported by the presence of a marked difference in endometrial gene expression profile between patients with a progesterone serum concentration above and below the threshold of 1.5 ng/ml on the day of HCG administration [8, 9].

More follicles produce more serum P. It would, therefore, be better to take into account the ovarian response, rather than the serum P levels only. Progesterone >1.5 ng/mL and P/E(2) >0.55 affect the clinical pregnancy rate. P/E(2) ratio is the only independent prognosticator for cycle outcome [10].

25.3 Incidence

There is a marked variation in the incidence of premature rise of progesterone due to discrepancies in definition, population characteristics and/or treatment protocols among the studies. Although the frequency of elevated serum progesterone concentrations varies, incidences as high as 35 % of stimulated cycles in women treated with GnRH agonists [3, 6] and 38 % of cycles in women treated with GnRH antagonists [7, 11] have been reported. However, in a large retrospective analysis of over 4,000 cycles, the

incidence of progesterone rise (above 1.5 ng/ml) on the day of HCG administration was estimated to be 8.4 % in agonist and antagonist cycles [12].

25.4 Pathogenesis

The pathogenesis of P elevation in COS is still poorly understood. But it has become certain that it is multi-factorial. Several hypotheses may be considered to explain this phenomenon:

1. In GnRH agonist cycles, P elevation is a magnitude response to FSH rather than LH [12, 13]. P elevation is positively correlated with (a) high FSH daily doses and total FSH doses, (b) prolongation of follicular phase, e.g. in rFSH/GnRH antagonist cycle delaying hCG administration 2 days after presence of >3 follicles (>17 mm) [15], (c) high oestradiol concentrations, (d) increased steroidogenic activity, (e) increased number of retrieved ocytes, (f) increased number of follicles. In a study [14], patients with P >1.5 ng/ML were found to have high concentration of oestradiol and increased number of follicles [2].
2. Increased follicular steroidogenic activity: An excessive amount of progesterone is produced by granulosa cells as part of early luteinization. In COS cycles, there are excess number of follicles, each one producing a normal amount of progesterone consistent with the late follicular phase [2]. Early increase in progesterone levels that result from an initial intense FSH stimulation leads to increased granulosa cell steroidogenic activity [11] (mature granulosa cell response to high FSH exposure).
3. Increased follicular phase LH activity: No relationship exists between LH and progesterone levels at the end of the follicular phase since the observed increases in progesterone were not accompanied by increases in LH [11].
4. Serum accumulation of HCG from HMG [15]: A systematic review shows that providing LH activity supplementation in combination with FSH during ovarian stimulation does not have a consistent effect on serum progesterone concentrations at the time of hCG administration. However, these data also suggest that in accordance with physiological concepts, the timing of LH activity administration could influence the impact on serum progesterone level. Progesterone rise was even higher in recombinant FSH as compared with HMG ovarian stimulation [16, 17] supporting the fact that LH reduces progesterone level rather than contributing to progesterone rise. In a prospective study, LH rise was not found on the day of hCG stimulation in GnRH analogue cycles.
5. Increased sensitivity of LH receptors of the granulosa cells to FSH: LH acts on granulosa cells when LH receptors have been induced by FSH at the later stage of follicular phase. *In vitro* experiments have clearly demonstrated that LH has a synergistic effect with FSH on granulosa cells to stimulate progesterone production [18, 19] and that LH is far more potent than FSH on granulosa cells to produce steroids as assessed by cAMP accumulation [19].

As the granulosa cells respond to FSH, proliferation and growth are associated with an increase in FSH receptors. The theca cells are characterized by steroidogenic activity in response to LH, converting pregnenolone into androgens. Aromatization of androgens to oestrogens is a distinct activity within the granulosa cell layer induced by FSH by activation of the P450 aromatase gene. Androgens produced in the theca layer diffuse into the granulosa layer, where they are converted to oestrogens that are released into the follicular fluid and from here into the peripheral circulation. Prior to ovulation, the granulosa cell layer is characterized by aromatization activity and conversion of theca androgens to oestrogens, an FSH-mediated response.

Factors that are associated with progesterone rise are the prolongation of the follicular phase (by delaying HCG administration) [20] and the oestradiol concentrations [14]. A study [20] reported that if the follicular phase is prolonged by 2 days after the presence of >3 follicles >17 mm is confirmed at ultrasound scan in recombinant FSH/GnRH antagonist stimulated cycles, a lower probability of ongoing pregnancy

rate can be expected, probably through prolonged exposure of the endometrium to raised concentrations of progesterone. Hence, prolongation of stimulation is an important factor to be considered. Prolongation of follicular phase is related to the rise of oestradiol. Moreover, the rise in oestradiol concentration is associated with high risk of premature progesterone rise [21].

The adrenal is a secretory source of circulating progesterone during early follicular phase. This was demonstrated by the rapid rise of progesterone after administration of ACTH during suppression of endogenous gonadotropin secretion with triptorelin acetate. ACTH stimulates the conversion of cholesterol to pregnenolone in the adrenal cortex which is rapidly converted to progesterone. Moreover, it seems that the source of progesterone shifts towards the ovaries just prior to the ovulation [22].

Poor ovarian response with increased LH sensitivity. In poor ovarian responders, premature rise as defined by the P/E2 ratio was more prevalent. It was associated with poor ovarian response with increased LH sensitivity, similar to the report by Younis et al., who concluded that neither the LH nor the hCG content of the recombinant preparations is responsible for this elevation of P/E2 ratio level and suggested that P elevation is not necessarily an LH-dependent event and may be primarily related to an adversely affected cumulus–oocyte complex [23]. When considering P rise, ovarian response or reserve may be of critical importance [24]. The main factors associated with increased risk of progesterone rise during COS cycles are ovarian parameters, including the total FSH dose, the intensity of the ovarian response, and excess number of follicles or oocytes [15].

Recently emerging evidence points to the existence of an oocyte granulosa cell regulatory loop by which complementary signalling and metabolic pathways drive the development and function of both the oocytes and follicular somatic compartments [25, 26]. Growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15) are two well-characterized oocyte-derived growth factors that play crucial roles in follicle growth and ovulation in all mammalian species including humans [25–29]. Spontaneous mutations or genetic targeting of either *Gdf9* or *Bmp15* in mammals affect fertility in females [30]. Disruption of signalling in the ovarian granulosa cells leads to their premature luteinization [31].

25.5 Impact

The impact of premature serum progesterone elevation at the end of the follicular phase under controlled ovarian stimulation (COS) cycle for in vitro fertilization (IVF) is still debated. While several studies reported lower pregnancy rates in patients with high progesterone concentration on the day of human chorionic gonadotropin (hCG) administration [6, 11, 12, 29–32], one found a favourable effect on pregnancy outcome [33], and others failed to demonstrate any association [3, 4, 7].

No significant difference in pregnancy rate was observed by Hofman et al. [33] in patients undergoing IVF/embryo transfer with high or low progesterone concentrations on the day of HCG administration and in patients who received oocytes donated from women with high or low progesterone concentrations. On the contrary, other authors reported that pregnancy rate has been inversely related to serum progesterone levels on the day of HCG administration [3, 4, 6, 11]. The involved endocrinologic mechanism of such an observation, however, is unclear.

Adverse effects of elevated P levels on oocyte maturation, fertilization or early cleavage have been described [6, 11]. On the other hand, no negative impact of progesterone rise on oocyte/embryo quality could be found in several studies [2, 6, 34, 35]. Systematic review and meta-analysis conducted by Venetis C et al. showed that E2 levels (pg/mL) on the day of hCG administration were significantly higher in the group of patients that exhibited progesterone elevation on the day of hCG. No significant difference in the number of COCs retrieved was detected between the patients with and those without progesterone elevation on the day of

hCG administration [2]. These findings suggest that P elevation may influence the endometrium, adversely affecting implantation and subsequent embryo development. Elevated progesterone levels might induce premature endometrial maturation and, as a consequence, earlier opening of the implantation window that leads to asynchronization of the crosstalk between embryo and endometrium. Accelerated endometrial maturation following COS has been clearly demonstrated by histological dating on the day of oocyte retrieval [8], but this is not the case during the implantation window [9]. When the endometrial receptivity was studied, findings pointed to an abnormally accelerated endometrial maturation but only during the pre-receptive secretory phase and not during the implantation window. Consequently, transfer of a day-3 embryo in such too precociously mature endometrium would not allow the proper establishment of the embryo-endometrium crosstalk; this might explain why the pregnancy outcome was impaired when embryo transfer was performed on day 3 (hCG + 5) in patients with high serum [P] on the day of hCG administration [36]. On the other hand, when embryo transfer was performed on day 5 (hCG + 7), no detrimental effect on the pregnancy outcome was observed. The deleterious effect of premature progesterone rise is probably not due to an impact on endometrial receptivity or ovarian parameters but rather to a desynchronized dialogue between embryo and endometrium. [37].

25.6 Prevention

If a negative association between progesterone elevation on the day of hCG administration and the probability of pregnancy exists, it might be worth examining the progesterone level at the beginning of a cycle and on the day of hCG administration, modification of the protocol and timing of triggering of final oocyte maturity, cryopreserving the resulting embryos and their transfer in a subsequent frozen-thawed cycle [6, 34]. A literature search identified several regimens for prevention of P elevation:

25.6.1 Milder Stimulation Protocols

To prevent follicular phase elevations, it might be preferable to use milder stimulation protocols. When comparing the optimal GnRH agonist with antagonist, it was found that with GnRH agonist cycle an early and stable suppression of endogenous FSH led to more synchronized development of follicles compared to fewer follicles and oocyte with fixed GnRH antagonist regime. Several RCTs comparing OC-pre-treated GnRH antagonist with long agonist protocols could not observe significant differences with respect to the number of oocytes retrieved and pregnancy rates [38–40].

25.6.2 Flexible Antagonist Protocol [41]

In IVF-ICSI patients undergoing COS with the antagonist protocol, the antagonist administration was initiated according to at least one of the following patient-specific criteria: (i) at least one follicle measuring >14 mm; (ii) oestradiol levels >600 pg/ml; and (iii) ET > 6 mm. Rapid response, causing earlier antagonist administration initiation, according to the proposed criteria for the prevention of premature LH surges, and the absence of P rise, as evidenced by normal progesterone levels on HCG day, were found to be independent positive predictive factors for favourable IVF outcome. The employment of an algorithm of criteria, aimed at the prevention of premature LH surges in a flexible antagonist protocol, resulted in antagonist initiation earlier than on stimulation day 6 in a significant proportion of patients. A higher pregnancy rate was observed in these patients.

25.6.3 Earlier Trigger for Ovulation [42]

Altering the timing of hCG injection according to serum progesterone concentrations improves embryo quality in cycles with subtle P rise. Serum was obtained daily or every 12 h from day 7 until the administration of hCG. hCG injection was given when the levels of serum P

exceeded 1.0 ng/mL ('rescued' subtle P rise). The mean day of hCG administration in the rescued cycles was 1 day earlier than those of the subtle P rise and no P rise cycles. The rate of embryonic development beyond four-cell stage was increased significantly in the rescued cycles and no P rise cycles versus the subtle P rise cycles. Embryos obtained in the no P rise and rescued cycles were of better morphological quality than those obtained in the P rise cycles. The implantation rate was significantly higher in the rescued cycles than in the P rise cycles. The data suggest that if hCG is administered when a subtle P rise is detected, embryo quality and subsequent implantation rate can be improved.

An earlier hCG trigger in patients stimulated with rFSH/GnRH antagonists for IVF resulted in significant differences between the early-hCG and the late-hCG groups regarding oestradiol (1,388 versus 2,040 pg/mL, respectively) and P (0.8 versus 1.1 ng/mL, respectively) levels on the day of hCG. However, no significant differences were observed between the early-hCG and the late-hCG groups regarding positive hCG (46.2 versus 50 %, respectively) or ongoing pregnancy rates (34.6 versus 40.7 %, respectively). In cycles stimulated with rFSH and GnRH antagonists, an earlier administration of hCG was not associated with an increased probability of pregnancy, but investigators found significant difference in oestradiol and P level in early-hCG and late-hCG groups [43]. An earlier trigger in high responders in order to avoid premature progesterone elevation is feasible.

25.6.4 Cryopreservation and Subsequent Frozen-Embryo Transfer

The most appropriate choice to avoid the negative effects of follicular progesterone elevations is to cancel fresh embryo transfer and to transfer frozen-thawed embryos in natural cycles. Next cycle would have a more synchronized endometrium, thus improving implantation rates.

Conclusion

To prevent follicular phase elevations, it might be preferable to use milder stimulation protocols, earlier trigger of ovulation in high responders and single-blastocyst transfer on day 5. The optimal GnRH analogue protocols during the entire stimulation period appear to be the long agonist as well as 'long' and long GnRH antagonist regimens (oral contraceptive pre-treated fixed antagonist).

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Empty Follicle Syndrome

26

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Abstract

It is possible that the result of stimulation does not end in oocyte retrieval. One of the causes of non-retrieval of oocytes after an apparent normal stimulation cycle is Empty Follicle Syndrome (EFS). It is diagnosed after ruling out the other possible causes that could lead to non-retrieval of oocytes. EFS can be classified as genuine (GEFS) and false (FEFS). It is a highly stressful situation for both the couple undergoing the treatment and the clinical team. Hence, it is necessary to take steps to prevent it and apply a step-by-step formula to minimize it or its occurrence. EFS is a definite entity, and the clinicians would definitely see the cases of the same in the clinical practice.

Keywords

Empty Follicle Syndrome • Genuine Empty Follicle Syndrome • False Empty Follicle Syndrome • Ovarian dysfunction • Oocyte donation

26.1 Introduction

It is not always that the stimulation protocol of an IVF cycle ends up in a favourable result. At times, it is possible that the result of stimulation does not end in oocyte retrieval. One of the causes of non-retrieval of oocytes after an apparent normal stimulation cycle is 'Empty Follicle Syndrome.' It is diagnosed after ruling out the other possible causes that could lead to non-retrieval of oocytes.

The condition of EFS was first described by Coulam et al. in 1986 in a report of five IVF cycles in four patients in whom no oocytes were found in follicular aspirate totaling 30 [1]. The term 'Empty Follicle Syndrome' was then coined. This condition could be very stressful both for the couple as well as the clinical staff and at times lead to unplanned and unforeseen situations [2]. The mechanism for EFS still remains unexplained, though many theories have been put

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forward for it, ranging from pharmacological problems [3–5] to human error [5–8].

There are two types of EFS that exist. In the first, it is the level of beta HCG which is sub-optimal (cause being human error or pharmaceutical reasons) that leads to failure of oocyte retrieval. The other type of EFS is failure to retrieve oocytes despite having achieved optimal levels of beta HCG. Accordingly, EFS can be classified as genuine (GEFS) and false (FEFS), which may offer a better explanation of the condition.

26.2 Definition

26.2.1 Genuine EFS (GEFS)

Genuine EFS can be defined as failure of retrieval of oocytes from mature ovarian follicles after controlled ovarian hyperstimulation for IVF after apparently normal follicular development and steroidogenesis in the presence of optimal beta HCG level on the day of oocyte retrieval.

26.2.2 False EFS (FEFS)

False EFS can be defined as failure of retrieval of oocytes in the presence of sub-optimal beta HCG levels due to an error either in the administration or the bioavailability of beta HCG on the day of oocyte retrieval. This is more commonly encountered than genuine EFS

26.3 Incidence

The incidence of Empty Follicle Syndrome is said to be 0.2 to 5 %, but most of this would fall in the false EFS group.

26.4 Pathophysiology

Many hypotheses propose the possible aetiology for GEFS as a dysfunction in the follicular genesis that causes early oocyte atresia. The hormonal response in the GEFS is apparently normal [9]. It is also postulated that some of these patients may require longer exposure to HCG levels for the detachment of oocyte cumulus complexes from the follicular wall [6]. Onalan et al. [10] challenged the above hypothesis wherein they used a rescue protocol and were able to retrieve oocytes later in the same cycle. This meant that most GEFS were actually FEFS in nature.

Advanced ovarian ageing, granulosa cell dysfunction and altered metabolism in older women have all been proposed as theories to explain EFS [10, 11]. Others have also proposed genetic factors that could be responsible for EFS [10, 11]. However, the first cause should always be kept as low bioavailability of the administered HCG due to either a pharmacological problem or human error [8]. In conclusion, there exists substantial uncertainty surrounding the mechanism and aetiology of EFS.

26.5 Factors Inducing EFS

The following factors have to be studied before labelling a case as GEFS or FEFS:

- Quality of ovarian stimulation
- Technical aspects of oocyte retrieval
- Use of HCG in ovarian maturation
- Shortfalls in administration of HCG
- Interval between HCG administration and oocyte retrieval
- Ovarian dysfunction
- Altered steroidogenesis

26.5.1 Quality of Ovarian Stimulation

Many factors influence the overall quality of ovarian stimulation and directly affect the outcome in terms of oocyte retrieval. Choice of gonadotropin is the foremost factor that affects this quality; recombinant gonadotropin (FSH and LH) is definitely advantageous over urinary preparation because of its higher purity, decreased

batch-to-batch variability and highly specific bioactivity [12].

Higher pregnancy rates were achieved using recombinant FSH than urinary FSH in a meta-analysis conducted by Daya et al. [13]. A study by Balasch and colleagues demonstrated better follicular dynamics and oocyte maturity when recombinant FSH was used as compared to urinary preparations [14].

Monitoring during ovarian stimulation also plays an important role in successful oocyte retrieval. The backbone of follicular monitoring still remains trans-vaginal sonography (TVS). Plasma oestradiol is also set to play an important role, as its levels are directly proportional to follicular size. Both trans-vaginal sonography and plasma oestradiol can predict cycle outcomes [15].

The size of the leading follicle when HCG is administered plays an important role in successful retrieval. As a thumb rule, HCG is given when the leading follicle is around 18cm in diameter. Early or late administration of HCG may result in non-retrieval of oocytes.

26.5.2 Technical Aspects of Oocyte Retrieval

There could be many technical problems that account for unsuccessful oocyte retrieval. Low pressures in the tubings, leakage of pressure, poor-quality disposables and improper assistance during the retrieval procedures may also add to the problem of EFS.

However, technical aspects might not be able to explain EFS single handedly [16].

26.5.3 Use of HCG in Ovarian Maturation

HCG has been used as a substitute to mimic the LH surge that stimulates the final follicular maturation. The use of HCG has been a standard in COH protocols. Poor bioactivity in commercially available HCG preparations could be a leading cause of EFS [3]. Different commercially available HCG preparations have been shown to have different bioactivity. Besides even in the same commercial preparation, there could exist a significant batch-to-batch bioactivity.

EFS has long been claimed 'pharmaceutical industry syndrome' resulting from problems in the manufacture of HCG. This, however, does not satisfactorily explain the solitary incidence of EFS rather than as opposed to a clustered appearance in case of defective drug. Moreover, an unsuccessful oocyte retrieval can be corrected by a rescue dose (second dose) of HCG from the same batch.

26.5.4 Shortfalls in Administration of HCG

HCG administration in an IVF cycle has a pivotal role. Low dosages can lead to poor retrieval rates due to faulty late maturation of the oocyte. Wrong timing can prove fatal in an IVF cycle. Inappropriate dosage may also result in EFS. At times, the patient may also use the wrong route of drug administration. Proper counselling of the patients could minimize these difficulties.

26.5.5 Interval Between HCG Administration and Oocyte Retrieval

Time interval between HCG administration and oocyte retrieval has a remarkable influence on the outcome of the oocyte retrieval in the cycle. Mansour et al. [17] conducted a study on patients undergoing ICSI; he divided the patients into three groups wherein oocyte retrieval was performed 35, 36 and 37 h after HCG administration. He proved that metaphase 2 oocytes were significantly lower in the 35-h group compared with the 36-h group. However, the 36–37 h groups did not show significant difference. The fertilization rates were similar in both the groups. There have been varying results in other similar studies that were performed. Early retrieval has always yielded significantly fewer and more immature oocytes. Hence, the time gap between HCG and oocyte retrieval may contribute to the development of EFS in some cases.

26.5.6 Ovarian Dysfunction

Many authors have suggested ovarian dysfunction as a factor contributing to a failure to obtain oocytes after controlled ovarian hyperstimulation. One of the leading studies conducted by Ben-Shlomo et al. [18] proved that history of poor response to ovarian stimulation had a correlation with poor oocyte retrieval. Advanced stage of ovarian ageing may also contribute to incidence of EFS. Le Sala et al. [16], Zreik et al. [19] and Penarubbia et al. [20] have all suggested EFS as a cause of infertility possibly due to impaired maturation and disrupted growth of oocyte. The failure to retrieve oocytes may be a result of ovarian ageing and altered follicular genesis.

26.5.7 Altered Steroidogenesis

Altered steroidogenesis has been postulated as one of the theories of EFS. The steroid prolife of follicular fluid from a patient of EFS was characterized by increased oestradiol:progesterone ratio and increased follicular concentrate of androstenidione. Non-fertilizable oocytes have also shown a similar composition of steroids in its follicular fluid [10].

Greb et al. [21] conducted a similar study of EFS in perimenopausal patients and arrived at the same conclusion.

Literature also gives studies that are in contrast with the above. The authors, however, regard EFS as a one-time affair rather than a repetitive phenomenon.

26.6 Treatment Options and Clinical Implications

Even though a rare phenomenon, EFS is definitely seen more than once in the life cycle of an infertility specialist. The emotional pressure/stress can be tremendous on both the patient and the treating clinical staff.

26.6.1 Protocol for Management of EFS

The following steps can be taken to avert/minimize this situation (Fig. 26.1).

Step 1: If it is noticed that there is no oocyte in the first ovary, it is prudent to pause the oocyte retrieval and check for any technical problems.

Step 2: If technical problems have been ruled out, the clinician should try to look for premature ovulation, which is evident by presence of fluid in the pouch of Douglas. The clinician can proceed to retrieve the follicular fluid from the other ovary, but the chance of finding an oocyte is minimal. The patient could be counselled for oocyte donation cycle, and if willing, the embryo transfer can be done.

Step 3: If there is no free fluid in the pouch of Douglas, one should proceed to do urine pregnancy test or check levels of beta HCG in serum. Serum levels might take time, and hence the more practical approach would be to do a urine pregnancy test.

Step 4: If the urine test shows negative result or serum beta HCG is low, then the patient should be administered rescue dose of HCG and plan to proceed with the completion of procedure after 24 h.

Step 5: If the urine test is positive or the serum beta HCG levels are normal, then the procedure should be completed in the same sitting. The chances of finding oocytes in the other ovary are minimal. Remedial action in such a case is to proceed to an oocyte donation or change protocols in the next ovarian stimulation cycle.

The following steps would be taken in the next cycle:

1. Shift from antagonist to agonist or vice versa
2. Shift from urinary preparation to recombinant preparations of gonadotropins
3. Use of recombinant HCG for trigger
4. Change the manufacturer of the batch the HCG used

Step 6: In case of genuine EFS, oocyte donation remains the only option.

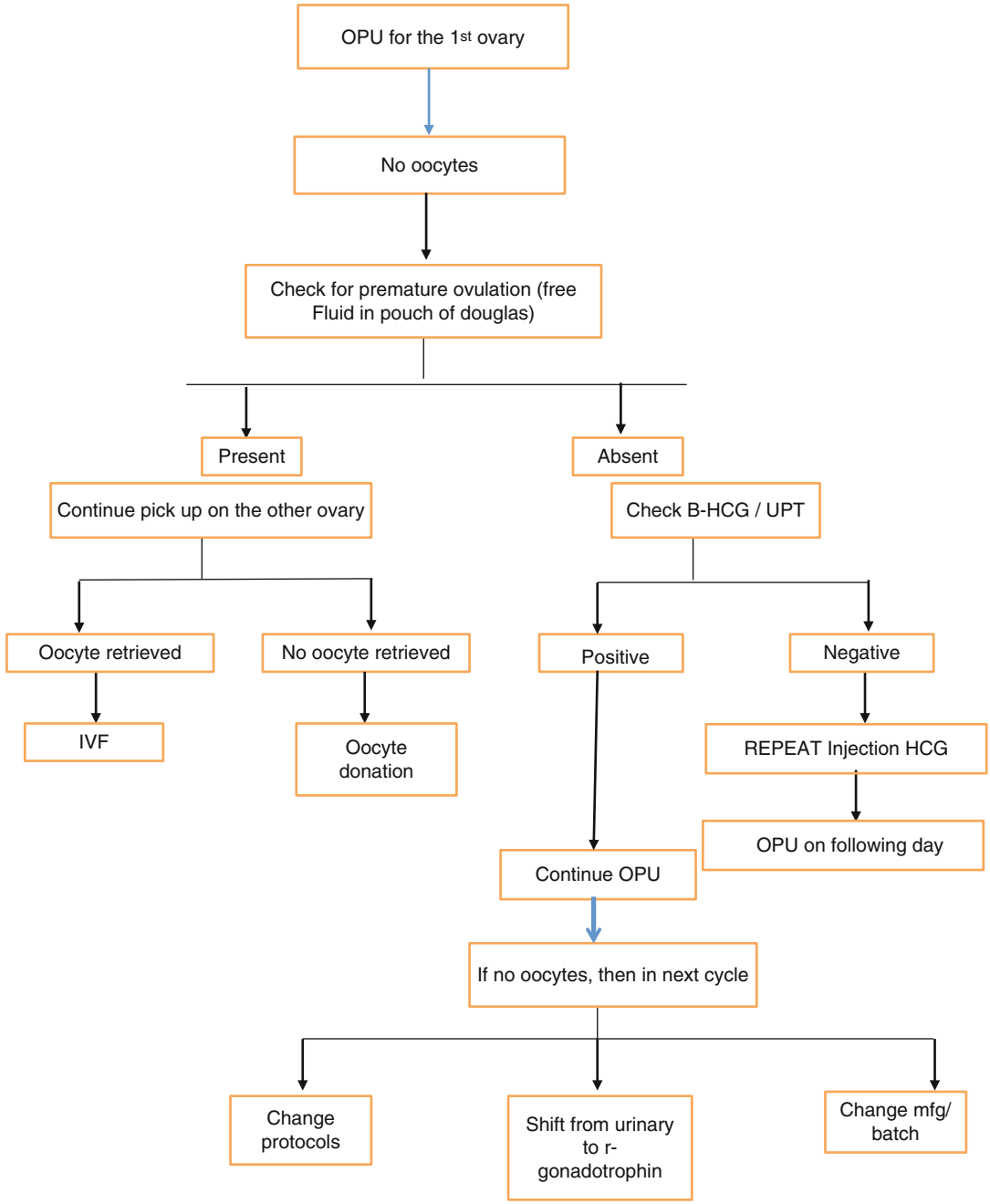

Fig. 26.1 Management of empty follicular syndrome

26.6.2 R-HCG—A Novel Entrant

r-HCG has been an important part in the science of assisted reproduction. Recombinant product has highest purity, increased bioavailability and decreased batch-to-batch variation. With these qualities, r-HCG would go a long way in eliminating the variations caused due to urinary derived

HCG and hence may help us eliminate EFS. There have been reports of successful outcome in cases of previous failures with urinary HCG.

26.6.3 Role of Oocyte Donation

Oocyte donation remains the only hope of treatment in cases of genuine EFS. The couple should be counselled for the same especially after repeated failures. Needless to say, the donor and the recipient should be matched, and the donation should be carried out according to the rules of the land.

Conclusion

EFS is a definite entity, and the clinicians would definitely see the cases of the same in the clinical practice. It is a highly stressful situation for both the couple undergoing the treatment and the clinical team, and hence it is necessary to take steps to prevent it and apply step-by-step formula to minimize it or its occurrence.

Even though EFS has been reported in both natural and unnatural cycles, it is very unlikely that it reoccurs. However, a history of recurrent EFS makes it mandatory to change treatment protocols to prevent it. Due to multiple aetiologies and confounding theories towards development, the definitive treatment of EFS still remains an enigma. Appropriate monitoring, tailoring of drugs and their dosages, managing the time gap interval between HCG dosage and oocyte retrieval and avoiding technical mishaps go a long way in preventing this repetitive situation. Further work and research is needed to identify, diagnose and treat these patients to manage and prevent the EFS and carve a successful path to achieve parenthood.

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Luteinizing Unruptured Follicle

27

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Abstract

Ovulation, the most crucial event of the menstrual cycle, is dependent on an intricate balance between central and ovarian hormones, local growth regulators, enzymes, prostaglandins, steroids, and local connective tissue. Diagnosis of luteinized unruptured follicle (LUF) can be made by direct visualization of ovaries during laparoscopy, serial ultrasounds for follicular monitoring, and measuring peritoneal fluid/serum ratio of estradiol and progesterone during the second half of the cycle. The incidence of LUF seems to be significantly higher in women with unexplained infertility, endometriosis, and pelvic inflammatory disease and in women who have undergone prior ovarian surgery. The pathophysiology, diagnostic modalities, and therapeutic options have been discussed.

Keywords

Luteinized unruptured follicle • Unexplained infertility • Endometriosis

27.1 Definition

Luteinized unruptured follicle (LUF) syndrome is defined as a failure of ovulation in which, despite the absence of follicular rupture and release of the oocyte, the unruptured follicle undergoes luteinization under the action of LH [1].

27.2 Introduction

The term luteinized unruptured follicle syndrome was coined by Jewelewicz in 1975 to describe the condition of infertile women with regular menstruation and presumptive evidence of ovulation without release of ovum. In this condition the egg may have matured properly, but the follicle either fails to burst or the follicle may burst without releasing the egg. In earlier attempts, the LUF was first created by using intrafollicular injection of indomethacin and PGF_{2alpha} antiserum in experimental animals [1]. As compared to fertile women, LUF is more commonly observed in

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women with unexplained infertility, during ovulation induction by clomiphene citrate/hMG [2, 3], endometriosis [4, 5], pelvic inflammatory disease, and after previous ovarian surgery [2].

27.3 Pathophysiology

The mechanism or etiology of LUF is unclear. Many observations regarding etiopathogenesis have been postulated.

It has been observed that lower mean LH peak level occurs in LUF cycles as compared to normal ovulatory cycle [2]. The actual mechanism of attenuated LH response resulting in LUF may be explained as the final follicular maturation, and ovulation is a result of three distinct processes [6] that are dependent on LH:

1. Resumption of meiosis in the oocytes
2. Luteinization of the granulosa cells to form the corpus luteum
3. Digestion and degradation of the follicular wall to release the oocytes

The threshold of LH for the first two steps is lower as compared to the third step of release of the follicle. Therefore, at blunted LH levels, luteinization of the granulosa cells and elevation of progesterone levels may occur without initiation of processes for the breakdown of the follicular wall resulting in trapped follicle.

During the menstrual cycle, cellular growth, differentiation, function, and degeneration of the follicles are under the influence of changing levels of pituitary gonadotropins and local regulators like estrogens, progesterone, prostaglandins, catecholamine, inhibin, and growth factors. One week prior to ovulation, the follicle enters its rapid growing phase when its diameter increases from below 12 mm to 21.8+3.2 mm on the day before ovulation when the steroid hormones and proteoglycans produced by the granulosa cells lead to increase in volume by increasing the intrafollicular colloid osmotic pressure.

Follicle-stimulating hormone (FSH) also stimulates the plasminogen activator to convert plasminogen to its active proteolytic form, plasmin. Plasmin then changes zymogen pro-collagenase to collagenase, which causes degradation of collagen for follicle extrusion. A large number of collagens are known, but the ones of interest in the ovary are the interstitial collagens (type I and III), present in the theca cell layers, and the basement membrane collagen (type IV), present in the basal lamina separating the granulosa from the theca layers. Breakdown of both classes of collagens results in a gradual digestion of the follicle wall, leading to the local protrusion of a conical bleb on the surface of the preovulatory follicle [6]. Enzymatic disorder inhibiting collagen degradation may prevent extrusion of the oocyte. It has been observed that protease inhibitors, which block plasminogen activity, may inhibit ovulation.

The ultimate ovulatory stimulus of LH is mediated by prostaglandins. Both prostaglandin E2 (PGE2) and PGF_{2alpha} have been implicated in ovulation [6]. The preovulatory increase in follicular pro-collagenase is independent of prostaglandin production. As prostaglandin inhibitory drugs like indomethacin block ovulation, it has been observed that the activation of pro-collagenase may be prostaglandin dependent [6–11].

During ovulation induction with clomiphene citrate, hMG or pure FSH incidence of LUF is increased and it may recur in subsequent cycles [3]. During ovulation induction, multiple follicles may be induced which may result in relative deficiency of LH. It has been observed that hCG injection may increase the rate of follicular rupture in some cases although LUF may occur in spite of hCG stimulus.

Mild cases of endometriosis are commonly associated with LUF although the exact mechanism is not clear. Minimal or early endometriosis interferes with the process of ovulation and fertilization. It is postulated that prostaglandins, proteolytic enzymes, and cellular components such as macrophages and lymphocytes in the peritoneal fluid of patients who have minimal or mild endometriosis may individually or jointly affect the ovulation [4]. LUF may be a cause of infertility in endometriosis [5]. In mild endometriosis, women with pelvic inflammatory disease, and women with history of previous ovarian surgeries, the prevalence of LUF is higher and it may be

recurrent as compared to other women. The cause of LUF in these women appears to be mechanical adhesion formation due to subclinical oophoritis, which prevents release of the oocyte.

Hyperprolactinemia associated with psychogenic or stress-related infertility might also be the cause of LUF syndrome [2] as it has been observed that about 30 % of patients conceive spontaneously during the time they are being investigated and few also conceive after they have stopped all treatments.

27.4 Diagnosis

The incidence of LUF varies and depends on the population chosen and on the method for diagnosing ovulation. The incidence of LUF as assessed by laparoscopy varies from 6 to 79 % and by ultrasound is approximately 57 % in unexplained infertility [2]. Incidence of LUF in the infertile patients as compared to fertile patients is 3–8 times greater [2]. Detection of ovulation is made on the basis of visualization of stigma during laparoscopy, serum progesterone levels, LH surge, rise in basal body temperature, and follicular monitoring through ultrasonography and through endometrial dating [2].

27.4.1 Laparoscopy

Direct laparoscopic identification of the ovulation point, the stigma, is used for the diagnosis of LUF. The stigma is clearly identified if laparoscopy is performed within 3–5 days of presumed ovulation. All sides of the ovary should be thoroughly inspected by double-puncture technique and after resting the ovary over the uterine surface. After this period the stigma is healed due to re-epithelization and may not be recognized in the corpus luteum even if the normal ovulation has taken place. However laparoscopic visualization of the stigma is subjective. For confirmation of ovulation, extended laparoscopy may be required to be done consecutively for several days, which make it inconvenient and impractical. It is observed that up to 50 % of patients with no stigma at laparoscopy may actually have ruptured the follicle based on the peritoneal fluid hormonal concentration [2].

27.4.2 Peritoneal Fluid Sampling

The limitations of laparoscopy in diagnosing LUF prompted the development of alternative ways to detect the failure of the follicle to rupture. It has been observed that at the time of ovulation, the peritoneal fluid/serum ratio for progesterone increases from 0.9 on day −2 to 56.4 on day +2 ($P<0.001$) [2, 4]. In the same period of the cycle, the estradiol peritoneal fluid/serum ratio increases from 0.7 to 6.3 ($P<0.001$) [2, 4]. These extreme shifts in the peritoneal fluid/serum ratios result from the discharge of the follicular content, with its high steroid concentrations, into the peritoneal fluid through the rupture in the follicle wall. Therefore, if rupture of the graafian follicle fails to occur, this should be reflected in the peritoneal fluid steroid levels and in the peritoneal fluid/serum ratios [12]. On comparing LUF with non-LUF patients, estradiol and progesterone levels in serum are similar. However, peritoneal fluid concentration of these steroids increases significantly in non-LUF patients [2, 4].

27.4.3 Ultrasound

A reduction in follicle size, combined with the appearance of fluid in the cul-de-sac and changes in the pattern of the endometrium, is considered presumptive evidence of ovulation on ultrasound examination. Ovulation either results in complete disappearance of the follicle, a reduction in its volume with thickening of the follicle wall, or replacement of the follicle by an area of spongy appearance and a crenation pattern [13–15]. Failure of the follicle to rupture is defined as continued growth up to 3 days after the dominant follicle has reached a mean diameter of 30 mm or until no further growth occurs for 3 consecutive days [13–15]. Although serial ultrasonographic scans are fairly accurate in diagnosing LUF,

some authors have made a confirmed diagnosis of LUF by aspirating the follicular fluid under transvaginal ultrasonography in which the degenerated oocyte mass was found [16].

27.5 Treatment

Since the cause of the LUF phenomenon is still not definite, no strict guidelines for treatment can be formulated. In most women with unexplained infertility, LUF represents a biological variable rather than a syndrome, and treatment should only be considered in women with frequent recurrence of LUF.

Marik and Hulka [17] reported that 28 patients with unruptured luteinized follicles at the time of laparoscopy and with no other obvious infertility factor were treated with ovulation induction agents clomiphene and hMG. Fifteen of these patients conceived subsequently. Injection of 5000 IU of hCG intramuscularly to support the natural LH surge may result in normal ovulation in patients with a central cause of LUF. For LUF occurring during ovulation induction cycles, increasing the dosage of hCG to 10,000 IU or addition of human menopausal gonadotropin (hMG) may be the treatment of choice.

Qublan et al. [1] conducted a study to determine the recurrence rates of LUF in three consecutive cycles during clomiphene citrate induction. The results of their study illustrate that the incidence and recurrence rates of LUF syndrome were increased significantly in consecutive cycles. Possible implication of clomiphene citrate in the etiology of the syndrome exists. In such cases, other options like hMG might be justified. However, LUF has been observed during ovulation induction with both clomiphene citrate and hMG. Martinez and coworkers [18] studied 303 cycles in 115 patients with regular ovulatory cycles. They induced ovulation to increase the chances of conception with clomiphene citrate (122 cycles) or hMG (82 cycles). Ninety-nine spontaneous cycles were monitored. LUF occurred in 1 % of spontaneous cycles, 4 % of clomiphene citrate cycles, and 5 % of hMG cycles. These patients respond well to in vitro fertilization as LUF may recur in subsequent cycles.

Bromocriptine is given at mid cycle, in case of transient hyperprolactinemia. Counseling of the patients may alleviate stress and reduce prolactin levels. For patients on nonsteroidal anti-inflammatory drugs, these drugs need to be avoided in the peri-ovulatory period, and for patients with endometriosis, pelvic inflammatory disease, and post-ovarian surgery with recurrent LUF, an early laparoscopic inspection followed by in vitro fertilization should be planned.

Conclusion

The luteinized unruptured follicle syndrome is a form of subtle, anovulatory infertility that cannot be diagnosed by traditional progesterone-dependent ovulation detection methods. Without the use of laparoscopy, steroid hormone concentration in the peritoneal fluid, or ultrasonography, the luteinized unruptured follicle syndrome may go unnoticed and the patient may be falsely diagnosed as ovulatory. The exact mechanism of infertility in LUF is not clear. LUF syndrome occurs statistically more frequently in women with unexplained infertility than in a control group of women. In the absence of standard protocols, different treatment regimens may be used depending on the characteristics of the patient with variable success rates.

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Part VI

Controlled Ovarian Stimulation in Gynecological Disorders

Endometriosis: Surgical Management and Optimal Ovarian Stimulation Protocol for ART

28

Urvashi Prasad Jha, Ramandeep Kaur, Neema Sharma, Ritambhara Agrawal, and Priyata Lal

Abstract

Laparoscopy is the gold standard for the definitive diagnosis and management of endometriosis. The surgical goal in endometriosis is to remove all visible disease and at the same time conserve as much ovarian tissue as possible. Any endometrioma >4 cm should be excised and sent for histopathology to rule out malignancy.

Excision or ablation of endometriotic lesions and adhesiolysis improves fertility and increases pregnancy rates of those continuing more than 20 weeks in minimal to mild endometriosis. Surgical clearance of endometriotic disease improves spontaneous pregnancy rates in moderate to severe endometriosis. Surgery in expert hands decreases chances of loss of normal ovarian reserves. IUI or IVF/ICSI improves pregnancy rates if used after cystectomy. Preoperative treatment with GnRH agonist for 3–4 months before IVF/ICSI in patients with endometriosis improves pregnancy rates.

Keywords

Endometriosis • Laparoscopic ovarian cystectomy • Ovarian reserve • Artificial reproductive techniques

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28.1 Introduction

Endometriosis is defined as the presence of endometrium-like tissue outside the uterus which induces a chronic inflammatory condition. Treatment of endometriosis with infertility consists of either surgical excision of the endometriotic disease along with restoration of normal anatomy or assisted reproduction techniques or a combination of both. The decision about whether to operate or offer assisted reproduction will

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depend on the patient's symptoms, the presence of any complex masses on ultrasound, the ovarian reserve and ovarian access for IVF, risk of surgery, and the financial cost involved. Some women with infertility and endometriosis may benefit from a combination of assisted reproduction and surgery.

The gold standard for definitive diagnosis of endometriosis currently is laparoscopic visualization. The laparoscopy need not be timed and performed at a particular part of the menstrual cycle. To avoid underdiagnosis, laparoscopy should not be performed during or within 3 months of hormonal treatment [1] because of the resultant temporary suppression of endometriosis with these hormones.

The surgical goals in endometriosis are to remove all visible endometriotic disease, conserve as much ovarian tissue as possible, lyse all adhesions particularly in patients complaining of painful symptoms, restore anatomy to as near normal as possible, and avoid unnecessary tissue trauma.

Biopsy of a lesion with histopathological confirmation of endometriosis is ideal in peritoneal disease, but negative histopathology does not exclude it [1]. However, in ovarian endometriomas >4 cm, it is mandatory to have a histopathology to exclude malignancy.

28.2 Laparoscopic Treatment for Minimal and Mild Endometriosis (AFS Stage I or II)

The gold standard for surgical management (visualization, excision, ablation, and adhesiolysis) in early endometriosis is laparoscopy with its advantage of lower morbidity, greater magnification, and visualization of early lesions (Fig. 28.1a, b).

"Excision or ablation of endometriotic lesions plus adhesiolysis to improve fertility in minimal-mild endometriosis is effective compared to diagnostic laparoscopy alone to improve ongoing pregnancy rates" [2] (evidence level 1a). CO2 laser vaporization of endometriosis can be considered instead of monopolar electrocoagulation, since laser vaporization is associated with higher cumulative spontaneous pregnancy rates (grade C recommendation). Excision of the cyst wall rather than drainage and coagulation increases spontaneous pregnancy rates (ESHRE 2014 guidelines, grade A recommendation) [2]

The Cochrane Collaboration meta-analysis suggests an improved pregnancy rate (6R 1.66, 95 %; CI 1.01–2.51) and a pregnancy continuing beyond 20 weeks (OR 1.64, 95 %; CI 1.05–2.57), with laparoscopy in these patients [3]. Hence, 3–100 laparoscopies would be required to achieve

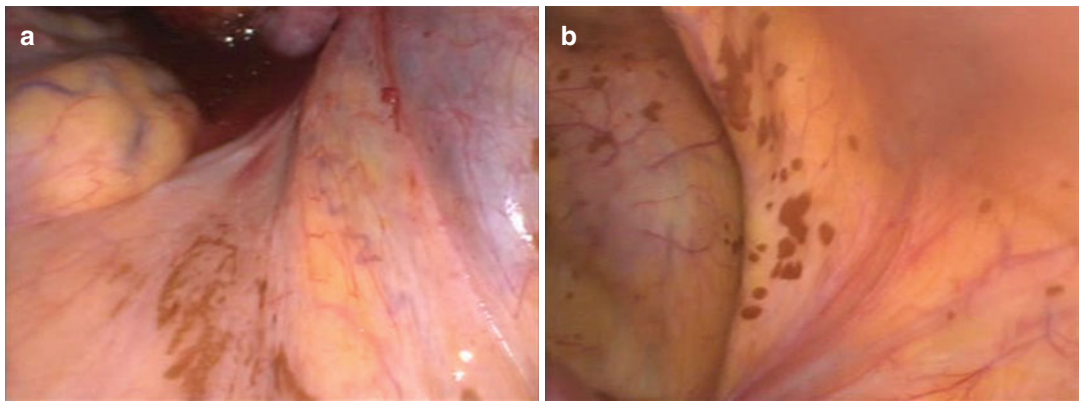

Fig. 28.1 (**a**) Case of right infundibulopelvic ligament with surface endometriosis. (**b**) Brownish multiple areas of superficial peritoneal endometriosis in the pelvis

one extra pregnancy. The risk of low complications of laparoscopy has to be weighed against this advantage while making an informed decision with the patient.

28.3 Surgical Treatment in Moderate or Severe Endometriosis and Endometriomas: AFS Stage III, IV, and Above

In patients with moderate or severe endometriosis, the chances of spontaneous conception are low at a monthly fecundity rate of < 3 %. In those with pain additionally as a symptom, surgical clearance of endometriotic disease has resulted in significant pain relief.

As per the ESHRE 2014 guidelines, "no RCTs or meta-analyses are available to answer the question whether surgical excision of moderate to severe endometriosis enhances pregnancy rate." Two prospective cohort studies showed crude spontaneous pregnancy rates of 57–69 % (moderate endometriosis) and 52–68 % (severe endometriosis) after laparoscopic surgery, which are much higher than the crude pregnancy rates of 33 % (moderate) and 0 % (severe) after expectant management. So, operative laparoscopy can be considered instead of expectant management to increase spontaneous pregnancy rates (grade B recommendation) [2].

A good practice point made is that laparoscopic ovarian cystectomy is recommended:

- If an ovarian endometrioma ≥3 cm in diameter is present to confirm the diagnosis histopathologically
- To reduce the risk of infection
- To improve access to follicles
- To improve ovarian response

The woman should be counseled regarding the risks of reduced ovarian function after surgery and the loss of the ovary/ovaries. The decision may be reconsidered if she has had previous ovarian surgery.

28.4 Effect of Ovarian Cystectomy on Ovarian Reserve

It has been shown that previous cystectomy did not predispose to the risk of removing normal ovarian tissue and compromising ovarian function [4, 5] suggesting that "multiple cystectomies might not be a risk factor for the removal of normal ovarian tissue provided the procedures are performed by experienced surgeons" (Figs. 28.2a–c and 28.3a–c) [5].

28.5 Role of Hormonal Treatment in Infertility

According to ESHRE 2014 guidelines [2], ovarian suppression using OCs, GnRH agonists, danazol, or progesterone is not recommended to improve infertility (grade A recommendation).

28.6 Role of Hormonal Therapy as an Adjunct to Surgery

Preoperative medical treatment with any of the hormonal therapies increased, independently, the risk of removal of normal ovarian tissue during laparoscopic cystectomy [6] containing primordial, primary, and secondary follicles as seen in normal ovaries. Hence, "pre-operative medical treatment may actually be detrimental for patients with ovarian endometriosis" [6]. The Guideline Development Group (GDG) [2] recommends not to prescribe adjunctive hormonal treatment before surgery to improve spontaneous pregnancy rates, as suitable evidence is lacking. Cochrane review [7] also demonstrated insufficient evidence of benefit of such preoperative medical treatment.

Adjunctive hormonal treatment after surgery is not recommended to improve spontaneous pregnancy rates (grade A recommendation) [2].

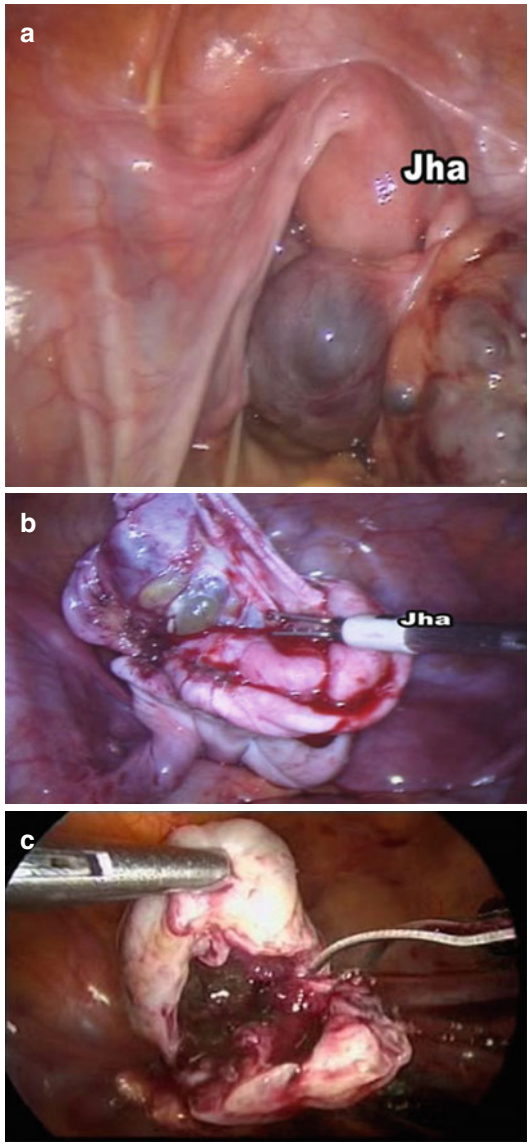

Fig. 28.2 (**a**) Endometriomas with adhesions in pouch of Douglas. (**b**) Small multiple endometria dissected; use sharp dissection during cyst excision where possible and in the adherent areas. (**c**) Peeling of large endometriotic cyst wall in progress; cauterize vessels on the side of the cyst wall rather than on the ovarian surface

28.7 Role of Assisted Reproductive Techniques

Assisted reproductive techniques used for these patients would be IUI or IVF/ICSI. After surgery it is recommended that one of these techniques is used depending on tubal status, ovarian reserve, severity of disease, and presence of other factors like male factor infertility.

28.7.1 Role of IUI

In mild to moderate endometriosis, IUI with controlled ovarian stimulation using gonadotropins increases live birth rates instead of expectant management or IUI alone (5.6 and 5.1 times higher live birth rates, respectively). Best results are within 6 months after surgical treatment, since pregnancy rates are similar to those achieved in unexplained infertility [2] (ESHRE grade C recommendations).

28.7.2 Role of IVF/ICSI

The pregnancy rates after IVF/ICSI can be lower in patients with stage III and IV endometriosis as compared to those with tubal factor infertility. Soon after surgical correction of advanced endometriosis, IUI or IVF should be considered for good results. As per ESHRE 2014 guidelines [2], "IVF is appropriate treatment especially if tubal function is compromised, if there is also male factor infertility and/or other treatments have failed" (evidence level 2b, grade B recommendation).

"Risk of recurrence is no reason to withhold IVF therapy after surgery for endometriosis in stage III or IV, since cumulative endometriosis recurrence rates are not increased after ovarian hyperstimulation for IVF" (evidence level 2a, grade C recommendation). Antibiotic coverage may be used at the time of oocyte retrieval though chances of abscess formation are low.

"Treatment with a GnRH agonist for 3–4 months before IVF or ICSI should be considered in women with endometriosis as it increases the odds of clinical pregnancy four fold." However, the authors of Cochrane review called for further research (evidence level 1b, grade A recommendation) [8].

In infertile women with endometrioma larger than 3 cm, there is no evidence that cystectomy

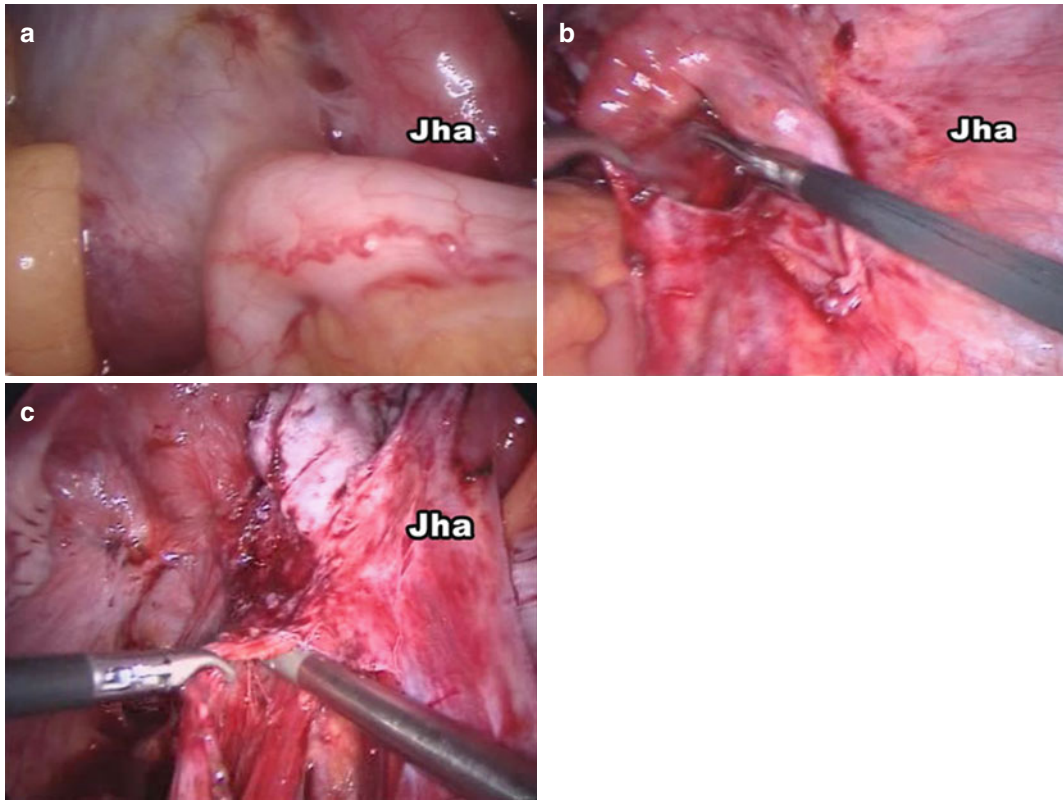

Fig. 28.3 (**a**) Adherent appendix to endometrioma. (**b**, **c**) Retroperitoneal dissection of ureter enables safer excision of endometriotic disease in the area

prior to treatment with ART improves pregnancy and it is recommended only to consider cystectomy prior to ART for improving endometriosis-associated pain or the accessibility of follicles. (Grade A Recommendation ESHRE 2014). Women with endometrioma should be counseled regarding the risks of reduced ovarian function after surgery and the possible loss of the ovary. The decision to proceed with surgery should be considered carefully if the woman has had previous ovarian surgery.

According to Cochrane review [9], aspiration was associated with a greater ovarian response than expectant management (a wait-and-see approach). Further randomized controlled trials of interventions for the management of endometrioma in women undergoing ART are required.

The effectiveness of surgical excision of deep nodular lesions before treatment with ART in women with endometriosis-associated infertility is not well established with regard to reproductive outcome, but generally women request surgery due to pain.

It would be prudent to measure pre- and postoperative ovarian reserve by measuring reliable and sensitive markers like AMH (antimullerian hormone) (Fig. 28.4).

Conclusion

Laparoscopy is the gold standard for treatment of endometriosis. Pre- or postsurgery hormonal therapy is not recommended. Artificial reproductive techniques in the form of IUI or IVF/ICSI are recommended to improve pregnancy rates depending on severity of disease. Preoperative use of GnRH agonists for 3–4 months increases pregnancy rates.

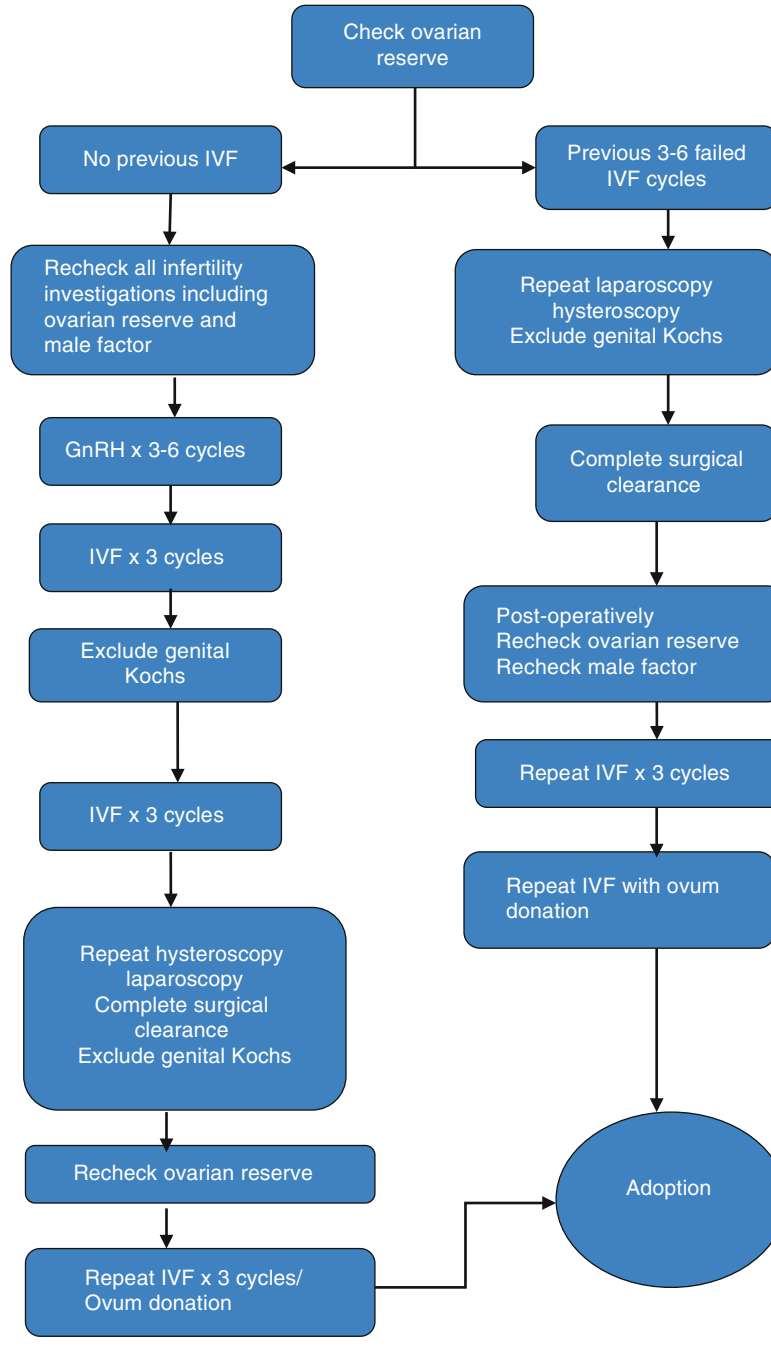

Fig. 28.4 Dr. Jha's algorithm for management of endometriosis

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Hyperprolactinemia

29

Abha Majumdar and Nisha Sharma Mangal

Abstract

Prolactin is a polypeptide hormone that is synthesized in the anterior pituitary gland and secreted in a pulsatile manner. It plays central role in a variety of reproductive functions and lactation. Prolactin release in humans depends on physiological state and varies in response to different stimuli.

Hyperprolactinaemia is a common endocrinological disorder; it could be physiological, pathological or idiopathic in origin. The predominant physiological consequence of hyperprolactinaemia is suppression of pulsatile GnRH. The clinical manifestations of conditions vary significantly depending on the age and the sex of the patient. In women, it frequently leads to gonadal dysfunction including ovulatory disorder, menstrual disturbances, galactorrhoea and infertility.

Problem in diagnosing and treating hyperprolactinaemia is the occurrence of the macro-prolactin molecule, which is, although biologically inactive, yet detected as hyperprolactinaemia in most immune assays. The management of anovulatory infertility due to hyperprolactinaemia requires establishing high prolactin levels as the cause of anovulation. Dopamine agonist is the mainstay of treatment. Gonadotropins can be used as substitution therapy to induce ovulation. Resistant cases of pituitary macro-adenoma may require surgical or radiological management.

Keywords

Prolactin • Hyperprolactinemia • Galactorrhea • Infertility

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29.1 Introduction

Prolactin is an anterior pituitary hormone, which has its principal physiological action in initiation and maintenance of lactation. Hyperprolactinaemia is a condition of elevated

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prolactin levels in blood that could be physiological, pathological, or idiopathic in origin. Elevated prolactin levels could be associated with severe clinical manifestations on one side or be completely asymptomatic on the other side of the spectrum. It plays a central role in a variety of reproductive functions. In female reproduction, pathological hyperprolactinaemia most commonly presents as an ovulatory disorder and is often associated with secondary oligomenorrhoea or amenorrhoea.

29.2 Prevalence

It is a common endocrine disorder of the hypothalamic-pituitary axis. It occurs more commonly in women [1]. The prevalence of hyperprolactinaemia ranges from less than 1 % in an unselected adult population to as high as 9–17 % in women with reproductive diseases [2].

29.3 Prolactin Molecule

Prolactin is a 23 kDa polypeptide hormone (199 amino acids) synthesized in the lactotroph cells of the anterior pituitary gland. Its secretion is pulsatile and increases with sleep, stress, food ingestion, pregnancy, chest wall stimulation and trauma. The primary source of prolactin is anterior pituitary. Other sites include the endometrium in luteal phase and the decidua [3, 4].

29.3.1 Macro-Prolactin

Monomeric 23 kDa form is the predominant form of prolactin molecule also known as 'little prolactin', but it is also present in other molecular forms which differ in their bioactivity (Table 29.1). These big variants of prolactin molecule are of 50 and 150 kDa and are also known as 'big prolactin' and the 'big-big prolactin', which have high immunogenic properties but poor or no biological effect. These 'big prolactin' or macroprolactin represents dimers, trimers, polymers of prolactin or prolactin-immunoglobulin immune complexes. When these big variants circulate in large amounts, the condition is referred to as 'macro-prolactinaemia', identified as hyperprolactinaemia by the commonly used immunoassays. These forms rarely show any biological activity but unfortunately are detected as hyperprolactineamia in most prolactin assays [2]. In these situations, even with high levels of circulating prolactin hormone, the individual may remain clinically asymptomatic [5, 6]. Commonly used commercial assays do not detect macro-prolactin separately. Polyethylene glycol precipitation is an inexpensive way to detect the presence of macro-prolactin in the serum [1].

Table 29.1 Major forms of the prolactin molecule (the little, big, and big-big)

Little prolactin	Macroprolactin
Secretion is pulsatile	Dimers, trimers or polymers/ or structural modification
Biologically active form	Poor or no biological action
Monomer, molecular wt. 23 kDa	Molecular wt. 50–150 kDa
	Highly immunogenic

29.4 Biological Action

Prolactin plays a central role in variety of reproductive functions. The main biological action of prolactin is in mammary development and in inducing as well as maintaining lactation [7]. In addition, it also stimulates immune responsiveness and exerts metabolic effects [8]. It binds to specific receptors in the gonads, lymphoid cells and liver [9]. Plenty of mediators of central and peripheral origin take part in regulating prolactin secretion through a direct or indirect effect on lactotroph cells [5].

Prolactin secretion is under dual regulation by hypothalamic hormones, but the predominant signal is tonic inhibitory control of hypothalamic dopamine, which acts upon pituitary lactotroph D2 receptors. Factors affecting prolactin secretion are listed in Table 29.2 [10].

Table 29.2 Factors affecting prolactin secretion

Prolactin inhibiting factors	Prolactin stimulatory factors
Dopamine	Hypothalamic peptides
Gama amino butyric acid (GABA)	Dopamine receptor antagonist
Somatostatins	Thyrotropin releasing hormone
Acetylcholine	Vasoactive intestinal peptide (VIP)
Norepinephrine	Epidermal growth factor (EGF)
	Histamine
	Serotonin

29.4.1 Effect of Hyperprolactinaemia on Ovulatory Function

In females, elevated prolactin levels cause ovulatory disturbances and menstrual irregularities. The main cause of anovulation is impaired gonadotropin pulsatility and derangement of the oestrogen-positive feedback effect on LH secretion. But ovarian response to gonadotropin is well maintained in these patients [11]. It also has direct action on ovaries in regulating ovarian steroidogenesis [12]. The action of prolactin on ovaries varies in different phases of a menstrual cycle:

1. Follicular phase – elevated prolactin disrupts normal follicular development, causes atresia of dominant follicle and inhibits ovulation.
2. Luteal phase – elevated prolactin inhibits progesterone synthesis by the corpus luteum and causes premature destruction of the corpus luteum [13].

29.5 Aetiology

Hyperprolactinaemia can be physiological, pharmacological, pathological or idiopathic in origin. Physiological hyperprolactinaemia is usually mild or moderate and may cause temporary episodes of hyperprolactinaemia that do not warrant any treatment because repeat assays generally show normal prolactin levels. There are a number of pharmacological agents that may lead to hyperprolactinaemia, and discontinuation of the drug readily restores prolactin level to normal. During normal pregnancy, serum prolactin rises progressively to around 10–20 fold. Prolactinomas (prolactin-secreting adenomas) are the most frequent cause of chronic pathological hyperprolactinaemia and account for 25–30 % of functioning pituitary tumours [14]. Prolactinomas are of two types depending on their size: micro-adenomas (smaller than 10 mm) and macro-adenomas (10 mm or larger). Smaller tumours are generally very slow growing or static, but the larger ones require to be followed up regularly, and if their growth causes compression of surrounding neuronal tissue, surgical intervention may be required. A number of chronic systemic diseases also cause moderate rise in prolactin levels leading to disturbed reproductive function or galactorrhoea. Apart from these known reasons for high circulating prolactin levels, a large proportion of women presenting with symptomatic hyper-prolactinaemia are idiopathic in origin (Table 29.3) [15].

29.6 Clinical Presentation

The predominant physiological consequence of hyperprolactinaemia is suppression of pulsatile GnRH. The clinical manifestations of conditions vary significantly depending on the age and the sex of the patient and the magnitude of prolactin excess. Clinical presentation in women is more obvious and occurs earlier than in men (Table 29.4). Presenting symptoms in women are manifold and range from those arising due to hypogonadism (oligo-ovulation, anovulation, menstrual irregularities, symptoms of hypo-oestrogenism) to those that occur by lactotroph stimulation of breasts causing galactorrhoea. In addition, these women can also present with neurological symptoms caused by mass effects of the tumour within or around the pituitary. Symptoms include headache, visual field loss, cranial neuropathy, hypo-pituitarism, seizures and cerebrospinal fluid rhinorrhoea [16].

Table 29.3 Causes of hyperprolactinemia

I. Physiologic conditions
Sleep
Food ingestion
Stress
Pregnancy and lactation
Chest wall stimulation
II. Idiopathic hyperprolactinemia (30–40 % of cases)
III. Hypothalamic-pituitary stalk damage
Tumors: Craniopharyngioma, Meningioma, Dysgerminoma, Pineal gland tumors
Empty sella syndrome
Lymphocytic hypophysitis
Pituitary stalk section
Suprasellar surgery
Irradiation
Trauma
IV. Pituitary hypersecretion
Prolactinoma (Microadenoma and Macroadenoma)
Metastatic tumors
Infections such as tuberculosis
Cushing disease
Addison's disease
Sarcoidosis
Histiocytosis
Acromegaly
V. Systemic diseases
Chronic renal failure
Hypothyroidism (primary and secondary)
Ectopic production (Hypernephroma, Bronchogenic sarcoma)
Epileptic seizures
Cirrhosis
VI. Drug-induced hyper-secretion
1. Dopamine receptor blocking agents
(i) Phenothiazines- Chlopromazine, Prochloperazine, Thioridazine, Trifluoperazine
(ii) Butyrophenones- Haloperidol, Pimozide
(iii) Benzamides- Metoclopramide, Clebopride
2. Dopamine depleting agents – Reserpine, Alpha- methyldopa, Opiates
3. Histamine receptor antagonist - Cimetidine, Ranitidine
4. Serotonin reuptake inhibitors- Fluoxetine
5. Stimulator of serotonergic pathway- Amphetamine and Hallucinogens
6. Estrogens, Antiandrogens.
7. Calcium channel blockers- Verapamil

Table 29.4 Clinical presentation in female

Delayed puberty
Amenorrhea
Oligomenorrhea
Luteal phase defects
Infertility
Galactorrhea
Decreased libido
Decreased bone mass density
Signs of chronic hyperandrogenism
Symptoms of hypothyroidism
Symptoms related to pitutary adenoma

It is worth noting that many women with hyperprolactinaemia do not have galactorrhoea, and many with galactorrhoea do not have hyperprolactinaemia. This is because galactorrhoea requires adequate oestrogen or progesterone priming of breasts. Conversely, isolated galactorrhoea with normal prolactin levels occurs due to increased sensitivity of the breast to the lactotrophic stimulus [17].

Approximately 3–10 % women with PCOS have co-existent hyperprolactinaemia [18]. Ovulatory dysfunction and hyper-androgenism both are commonly existing clinical presentations in patients with hyperprolactinaemia as well as in PCOS. Persistently elevated oestrogen levels are often found in women with PCOS which could result in prolactin elevation. However, it is still controversial whether they have cause-effect relationship or share a common mechanism or it is just a coincidental finding.

Prolonged hypo-oestrogenism secondary to hyperprolactinaemia may result in osteopenia [19]. Spinal bone mineral density (BMD) is decreased by approximately 25 % in such women and is not necessarily restored with normalization of prolactin levels [20, 21]. Hyperprolactinaemic women may present with signs of chronic hyperandrogenism such as hirsutism and acne, possibly due to increased dehydro-epiandro-sterone sulfate secretion from the adrenals [22], as well as reduced sex hormone-binding globulin leading to high free testosterone levels.

29.7 Diagnostic Evaluation

29.7.1 Serum Prolactin Estimation

Prolactin is a very dynamic hormone, so caution must be taken while diagnosing women to have hyperprolactinaemia warranting treatment. Careful history of drug ingestion, any stressful condition preceding sample collection including history of sexual intercourse, breast stimulation and chest wall injury should be noted. Chronic renal disease and thyroid disorder also needs to be ruled out. Normal serum prolactin levels in females vary between 5 and 25 ng/ml, with physiological and diurnal variations [23]. Serum prolactin levels are higher in the afternoon and hence should preferably be measured in the fasting morning sample [24]. Hyperprolactinaemia is usually defined as fasting levels of above 20 ng/ml in men and above 25 ng/ml in women [8]. Unless the prolactin levels are markedly elevated (>50 ng/ml), the investigation should be repeated before labeling the patient as hyperprolactinaemic. Even one normal value should be considered as normal, and an isolated raised one should be discarded as spurious (Fig. 29.1).

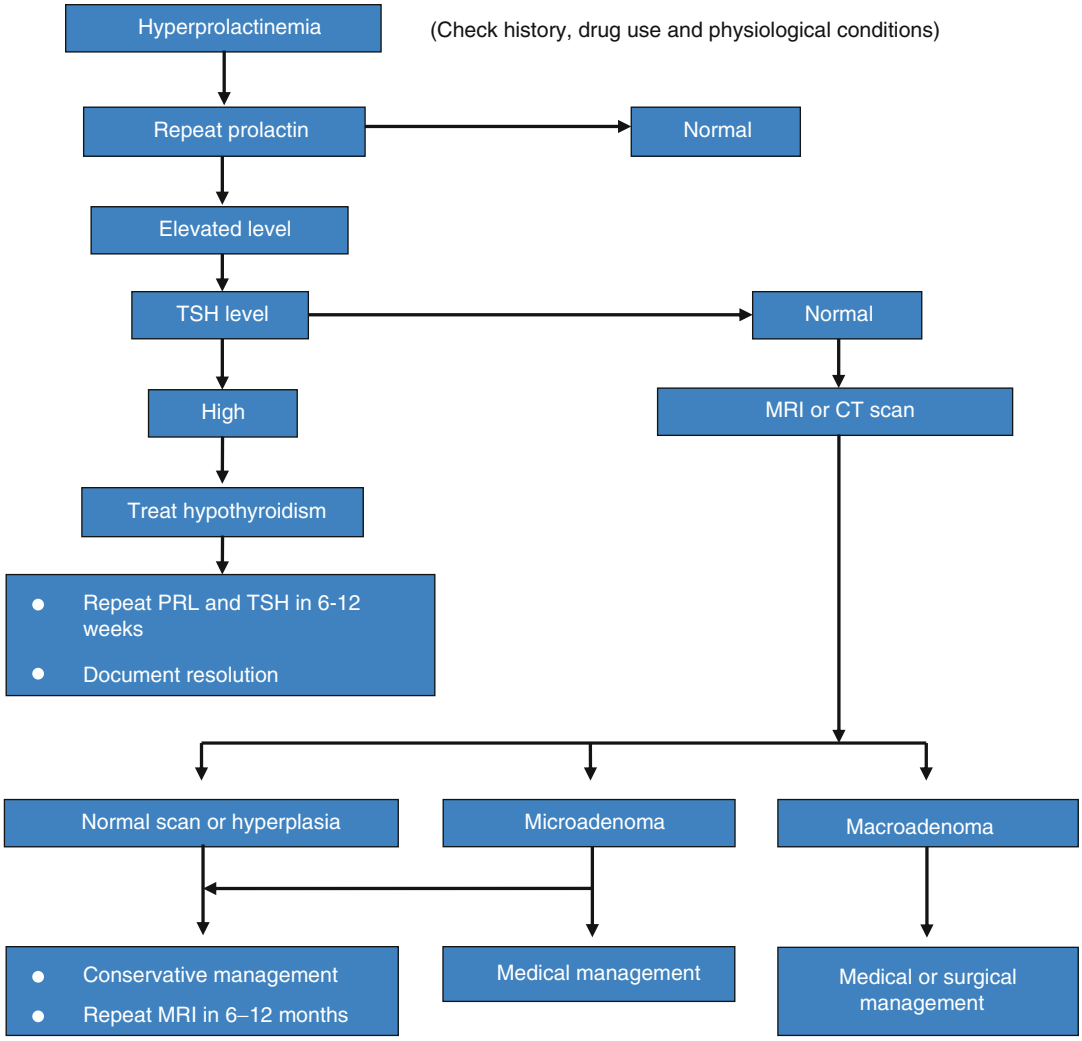

Fig. 29.1 Approach to management of patient with hyperprolactinemia

29.7.2 Radiological Imaging

This should only be advised judiciously to patients with confirmed diagnosis of hyperprolactinaemia. A mildly elevated serum prolactin level may be due to a non-functioning pituitary adenoma or craniopharyngioma compressing the pituitary stalk, but high prolactin levels are commonly associated with prolactinomas [2]. Increased use of CT scan and MRI may reveal other silent pituitary masses which neither pose any mass effect nor elevation of prolactin levels and are called 'incidentaloma' [25]. A prolactinoma is likely if the prolactin level is greater than 250 ng/ml [26], and a level of 500 ng/ml or greater is usually diagnostic of a macroprolactinoma [1]. Drug intake usually elevates prolactin levels up to 100 ng/ml. But few drugs including risperidone and metoclopramide may cause prolactin elevations above 200 ng/ml [27]. In cases where other causes of hyperprolactinaemia have been excluded and no adenoma can be visualized with MRI, the hyperprolactinaemia is referred to as 'idiopathic' and should be treated on the merit of the symptoms caused or whether fertility is desired.

29.8 Treatment Approach

The management of hyperprolactinaemia should be individualized on the basis of clinical findings, underlying cause and the presence of hypogonadism or infertility. Asymptomatic women with hyperprolactinaemia and/or micro-adenomas, who are not concerned about fertility, may be followed without active intervention. Annual clinical review and prolactin assay might be sufficient if the clinical condition remains stable.

29.9 Treatment of Hyperprolactinaemia With Anovulatory Infertility

In the absence of other factors, anovulatory infertility due to hyperprolactinaemia is successfully treated with dopamine agonists. Exogenous gonadotropin stimulation can be added along with dopamine agonist to achieve optimal ovulation. However, if an underlying treatable cause is identified, it should be eliminated first.

29.9.1 Elimination of Known Cause

When the problem is drug induced, we should consider whether the medication can be discontinued or replaced with another agent. Medication should be discontinued if it is safe to do so and serum prolactin level repeated. If the drug is essential for the patient's health (e.g. psychotropic agent) but is causing symptoms of hyperprolactinaemia, treatment with a dopamine agonist should be avoided, since it might compromise the effectiveness of the essential drug. Such patients should simply be treated with gonadotropins for the purpose of ovulation induction or replacement of sex steroids for hypogonadism.

Treatment of hypothyroidism with thyroid replacement therapy often restores the elevated prolactin level to normal. If the prolactin levels do not respond to adequate thyroid replacement, dopamine agonist may be required to treat the hyperprolactinaemia. If hyperprolactinaemia is associated with hypo-adrenalism, replacement treatment with corticosteroids lowers prolactin levels [28]. In case of a prolactin-secreting tumour, surgical therapies should be considered only when they are resistant to medical treatment or based on the symptoms caused due to their location or size [29].

29.9.2 Drug Therapy

29.9.2.1 Dopamine Agonist

Dopamine agonist is the mainstay of medical management and has revolutionized the treatment of idiopathic hyperprolactinaemia, as well as prolactinomas. Dopamine agonists have been in clinical use for many years and remain the cornerstone for therapy of hyperprolactinaemia [1, 30]. Most commonly used dopamine agonists are bromocriptine and cabergoline. Others are lisuride, pergolide, quinagolide, terguride and

metergoline. Patients who are intolerant or fail to respond to one agent may do well with another.

In patients with idiopathic and micro-adenoma-associated hyperprolactinaemia, prolactin levels may reduce in a week, but ovulation and menstruation require few weeks to normalize. Weekly assessment of progesterone is the most popular method to confirm resumption of ovulatory function. Restoration of prolactin levels to normal after using dopamine agonist results in ovulation with a pregnancy rate of approximately 70 % [31].

Bromocriptine Bromocriptine is a lysergic acid derivative with a bromine substitute at position 2 [32]. It is a strong dopamine agonist, binds to dopamine receptor and inhibits prolactin secretion. It decreases prolactin synthesis, DNA synthesis, cell multiplication and overall size of prolactinoma. It has a short half-life and so requires 2–3 times daily administration to maintain optimal suppression of prolactin levels. A daily dose of 5.0 mg is effective in about two thirds of the cases, but to save time one can commence with 7.5 mg/day in divided doses. Only 10 % of cases will need a higher dose than that, but it is usually ineffective to raise the dose above 20–30 mg/day. The drug may also cause mild drowsiness, hence one should avoid taking it prior to driving and preferably take it before sleep.

Intolerance to bromocriptine is common and the main indication of using an alternative drug. To avoid intolerance, it may be better to start with the lowest possible dose of 1.25 mg/day in the evening with food. If side effects are not too troublesome, a second dose of 1.25 mg with food in the morning is added next week. Thus, we gradually increase the dose by 1.25–2.5 mg/day each week till optimal dose is reached. Serum progesterone should be estimated weekly or in luteal phase periodically to check ovulation.

Vaginal usage of the same drug is better tolerable and causes lesser gastritis, nausea as well as sedation. Vaginal absorption is nearly complete, and avoidance of the liver first-pass metabolism allows lower therapeutic dosing [33]. Studies are also there regarding efficacy and safety of its long-acting form (depot-bromocriptine and slow release forms) [34, 35], but because of the availability of better tolerable drugs like cabergoline, these forms are not in routine clinical use. Bromocriptine has good treatment results, but the problem is that prolactin returns to elevated levels in 75 % of patients after discontinuation of treatment and there is no clinical or laboratory assessment that can predict those patients who will have long-term beneficial results [36].

Side effects associated with this drug are nausea, vomiting, headache, constipation, dizziness, faintness, depression, postural hypotension, digital vasospasm and nasal stuffiness. These symptoms are most likely to occur with initiation of treatment or when the dose is increased. One rare but notable side effect is neuropsychiatric symptoms like auditory hallucinations, delusion and mood changes. This may be due to hydrolysis of the lysergic acid part of the molecule. It quickly resolves with discontinuation of drug [37].

Cabergoline Cabergoline shares many characteristics and adverse effects of bromocriptine but has a very long half-life allowing weekly dosing. This is more effective in suppressing prolactin levels and reducing tumour size [38]. The lower incidence of side effects and the weekly dosage makes cabergoline a better choice for initial treatment. It can also be given vaginally if intolerable in oral administration [39]. A dose of 0.25 mg twice per week is usually adequate for hyperprolactinaemia. Maximum dose that can be given is 1 mg twice a week. Once pregnancy is established, one can discontinue the dopamine agonist.

29.9.2.2 Ovulation-Inducing Agents

Pulsatile Gonadotropin-Releasing Hormone (GnRH) Therapy As the main cause of anovulation is impaired gonadotropin pulsatility and derangement of the oestrogen-positive feedback effect on LH secretion, so pulsatile GnRH therapy combined with human chorionic gonadotropin (hCG) can be used in these patients [11]. Administration of GnRH in pulsatile mode restores the periodic release of FSH and LH from the pituitary, which corrects anovulation.

It is administered by a computerized minipump via a chronic indwelling intravenous or

subcutaneous catheter. Subcutaneous route is preferred for its convenience and lack of invasiveness, but intravenous administration yields more predictable response and a higher rate of ovulatory cycle. Lower dose should be used initially in order to minimize the likelihood of multiple pregnancies due to hyper-stimulation of the ovaries. Pulsatile GnRH administration may be discontinued after ovulation, and the corpus luteum is supported by exogenous hCG. Adverse effects include infection and haematoma at cannula site, antibody formation, hyper-stimulation and rarely desensitization due to inadequate dose or frequency [40].

Human Gonadotropins as Substitution Therapy If hyperprolactinaemia does not respond to treatment with dopamine agonist alone to cause ovulation or a patient is unable to tolerate the doses required to suppress high prolactin levels effectively, ovulation induction can be done by gonadotropins.

Gonadotropins used for ovulation induction in women are either urinary or recombinant products. If a patient has amenorrhoea, it is similar to that of hypogonadotropic hypogonadism; therefore, effective folliculogenesis and steroidogenesis require administration of combination of both FSH and LH. Human menopausal gonadotropin, which is derived from postmenopausal urine, contains both LH and FSH in a ratio of 1:1 and appears appropriate to be used for such cases. However, patients with oligomenorrhoea can also be treated with recombinant FSH alone as some LH continues to be secreted in such women. Widespread availability, safety and consistency of recombinant gonadotropin make it more suitable for ovulation induction. Ovarian stimulation is started with gonadotropins, and the response is monitored by ultrasound along with rising serum oestradiol levels. When a dominant follicle attains a size of 18 mm, hCG is given to promote final stage of oocyte maturation and ovulation. Post-ovulatory luteal support is mandatory till pregnancy is achieved and continued for the first 8–9 weeks of gestation. However, if menstruation ensues, luteal support is discontinued and a fresh cycle of gonadotropin stimulation can be planned. In these cases which are treated with gonadotropins rather than dopamine agonist, high prolactin levels may coexist.

Clomiphene Citrate After complete normalization of prolactin levels with dopamine agonist with no evidence of ovulation by weekly estimation of progesterone in the following 6–8 weeks, it is important to rule out underlying PCOS. These patients often show polycystic ovaries with increasing oestradiol levels and can be effectively treated by combining clomiphene citrate to the dopamine agonist.

29.9.3 Surgical Excision of Prolactinomas

Response with medicines is variable as some prolactinomas show prompt shrinkage with low dose, while others may require prolonged treatment with higher dosage [14, 41]. Even in macroprolactinomas, surgery is reserved for refractory and medication-intolerant patients [42]. Common indications of surgery are very large tumours, those with suprasellar and frontal extension, major chiasmal compression and visual impairment persisting after medications. Besides the usual surgical risks, hypo-pituitarism is a potential long-term effect of surgery and should be discussed with patients as part of the decision-making process. Unfortunately, excision is often incomplete and therefore relapse occurs although prolactin levels are lower than before.

29.9.4 External Radiation Therapy

External radiation therapy is only reserved for residual tumour in patients who have undergone surgery. It is of very limited benefit in the treatment of these patients, since the response is typically quite modest and delayed [43]. Patients should be counseled that such treatment carries a risk of developing hypo-pituitarism.

29.10 Pregnancy and Lactation

In idiopathic hyperprolactinaemia, dopamine agonist is stopped as soon as pregnancy is confirmed. As the risk of tumour expansion is low in micro-adenomas during pregnancy,

dopamine-agonist therapy is stopped even in these cases. In cases of macro-prolactinoma, it is essential to monitor visual fields from time to time during pregnancy to detect tumour expansion, and if it is suspected, MRI can confirm the need for medical or surgical intervention. Serial prolactin assays during pregnancy are not helpful in predicting tumour expansion during gestation [44]. If symptomatic tumour enlargement (headache, visual field defect or diabetes insipidus) is detected, the treatment of choice is to reinstitute dopamine agonist [29, 45] with monthly follow-up. Though both drugs have been found to be safe in pregnancy, the number of reports studying bromocriptine's use in pregnancy far exceeds that of cabergoline. Indications for surgery during pregnancy are severe chiasmal compression by a tumour unresponsive to drug therapy and the rare occurrence of pituitary apoplexy. Whenever possible, defer the operation until after delivery. Breastfeeding does not pose any demonstrable risk to the mother with a prolactinoma.

Conclusion

Prolactin is a very dynamic hormone and eludes diagnosis because of multiple physiological factors interfering in its estimation. Therefore, it is pertinent to establish the pathological relevance of hyperprolactinaemia as the main cause of ovulatory dysfunction. Dopamine agonist is the mainstay of treating anovulatory hyperprolactinaemia. Gonadotropins can be used as substitution therapy to induce ovulation in women who do not respond to or tolerate dopamine agonist. However, if the underlying cause of hyperprolactinaemia warrants treatment on its own merit, it needs to be addressed separately.

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Polycystic Ovarian Syndrome and Response to Stimulation

30

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Abstract

Polycystic ovary syndrome (PCOS) is a polygenic, multifactorial heterogeneous disorder of uncertain etiology. It is one of the most common endocrine disorders affecting females. The prevalence of PCOS is around 6–8 % in reproductive age group females. PCOS remains an enigmatic disorder, the etiology of which is still unclear. The evidence that PCOS is dependent on genetic factors is very strong. The features of PCOS can be seen in early childhood as premature adrenarche, adolescent PCOS, hirsutism, and acne. In a reproductive age group female, PCOS can present as menstrual irregularities like amenorrhea, oligomenorrhea, and infertility. Obesity is common in patients with PCOS. The optimal treatment for infertility with PCOS is yet to be contemplated. Various regimens have been developed for the treatment ranging from lifestyle modification to clomiphene to IVF with no consensus.

Lifestyle modification is the first line in the management of PCOS. Weight loss as little as 5 % will lead into normalization of menses and ovulation. Clomiphene citrate is the first choice for induction of ovulation in most anovulatory women with PCOS with a conception rate of 22 % and ovulation rate of 75 %. If clomiphene citrate fails to

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result in pregnancy, then the second-line treatment is either gonadotrophins or laparoscopic ovarian drilling. In vitro fertilization can be offered to those couples that do not conceive after 3–6 cycles of IUI. Also there is advantage of doing a single embryo transfer in a young good prognosis patient. Use of GnRH trigger in patients with PCOS has lead to dramatic decrease of OHSS.

Keywords
PCOS • Ovulation induction • Anovulation • Clomiphene citrate

30.1 Introduction

Polycystic ovary syndrome (PCOS) is a polygenic, multifactorial heterogeneous disorder of uncertain etiology. It is one of the most common endocrine disorders affecting females. The prevalence of PCOS is around 6–8 % in reproductive age group females [1]. It has many different clinical manifestations like infertility, menstrual irregularities, and hirsutism. Stein and Leventhal in 1935 described a symptom complex associated with anovulation. They reported a subset of patients having amenorrhea, hirsutism, and enlarged ovaries who resumed menstruation after bilateral wedge resection of ovaries [2].

PCOS remains an enigmatic disorder whose etiology is still unclear. The evidence that PCOS is dependent on genetic factors is very strong. Familial clustering of cases is the most important evidence, as features of PCOS are seen in identical twins than in nonidentical twin. The mode of inheritance of PCOS is unclear; given the clinical and biochemical heterogenecity of the syndrome, it is unlikely to be a single-gene disorder.

The origin of PCOS can be traced to in utero exposure of excess testosterone. Frank et al. (2012) [3] stated that genetic factors have an important part to play and that the disorder has its origins in early, possibly prenatal, life. They stated that polycystic ovary syndrome is a genetically determined, primary ovarian disorder resulting in excess androgen production. The capacity to hypersecrete androgens begins in fetal life and that the typical clinical and biochemical features of PCOS are "downstream" effects of exposure to androgen excess at or before puberty [3].

30.2 Etiopathogenesis of PCOS

To understand the pathogenesis of PCOS, the physiology of normal menstrual cycle should be understood. Normal menstrual cycle is a complex process of events involving the hypothalamus, pituitary, ovary, and uterus. During the follicular phase, there is a rise in the level of FSH hormone, which causes an increase in E2 levels and LH. Increase in LH causes androgen production in theca cell that with the help of aromatase enzyme gets converted to E2. As the follicle grows and E2 levels rise, it causes negative feedback on FSH so that the growth of smaller follicle stops and the follicle having maximum E2 levels and highest number of FSH and LH receptors emerges as the dominant follicle. E2 level rises, causing a surge, which is followed by an LH surge that leads to ovulation. Corpus luteum is formed and this causes rise in progesterone and E2. The LH and FSH levels reach nadir during late secretory phase, which is followed by menstruation if the corpus luteum is not rescued by pregnancy.

In patients of PCOS, there is a steady state of hormone levels with no fluctuation as compared to normal fluctuations observed in normal menstruation cycle. The level of FSH is usually low or normal due to increased inhibition by estrogen and inhibin B levels. Also, the level of LH is raised. It is the frequency, not amplitude, that is increased, but there is no cyclic variation as seen in normal menstrual cycle. The bioavailability of LH is also increased due to glycosylation, which causes more basic forms leading to increased bioactivity. Also there is abnormality in GnRH

pulse generator and sensitivity of GnRH to progesterone is decreased There is decreased dopamine neuronal activity because of lack of progesterone [4].Since level of LH is increased, there is increased production of androgen which does not get converted to estrogen, and there is increased intraovarian androgen. So, new follicles are recruited each cycle, but due to lack of estrogen dominance, no dominant follicle is selected. Anovulation and multiple small follicles of 2–10 mm are present in the ovary giving the PCO morphology on ultrasound (USG).

Around 40–50 % of PCOS females suffer from insulin resistance and hyperandrogenemia. Insulin resistance is a condition where endogenous and exogenous insulin has less than normal effect on the muscle, fat, and liver. Hyperinsulinemia causes increased androgen by two mechanisms. It acts through its receptor in ovarian theca cell and IGF-1 receptor in ovarian theca cell. Also it acts on the liver and causes decreased production of SHBG leading to increased level of free androgen. Insulin potentiates level of LH on theca cells. So a self-propitiatory cycle is formed where increased insulin leads to increased androgen, which causes hyperinsulinemia.

30.3 Definition of PCOS

Many systems of classification have been proposed for defining PCOS (Fig. 30.1):

1. NICHD (1990) [5]
2. ESHRE/ASRM (2003) [6]
3. AEPCOS (2009) [7]

30.4 Clinical Presentation of PCOS

The features of PCOS can be seen in early childhood as premature adrenarche, adolescent PCOS, hirsutism, and acne. In a reproductive age group female, PCOS can present as menstrual irregularities like amenorrhea, oligomenorrhea, infertility, hirsutism, and metabolic syndrome and in postmenopausal women, endometrial hyperplasia (Fig. 30.2).

30.4.1 Laboratory Investigations and Differential Diagnosis

There are many conditions that can present as PCOS like androgen-producing tumor, Cushing syndrome, and nonclassical congenital adrenal hyperplasia. So it is important to distinguish these conditions from each other. There are many tests available. Table 30.1 mentions the relevant tests needed for distinguishing the above conditions.

30.4.2 Antimüllerian Hormone (AMH) and PCOS

In PCOS women AMH levels are often raised due to increased number of follicles and granulosa cells. Women with hyperandrogenemia tend to have higher AMH levels. It is found that high AMH levels inhibit folliculogenesis. Thus, there is a subgroup of women with PCOS who have a high AMH and will not respond to ovarian stimulation. There are some women who respond to treatment with lowering of AMH levels.

30.5 Management

The main focus of management will be in the context of infertility, ovulation induction, and response to ovulation (Fig. 30.3).

The optimal treatment for infertility with PCOS is yet to be contemplated. Various regimens have been developed for the treatment ranging from lifestyle modification to clomiphene to IVF with no consensus. Due to varied etiology and symptomatology different therapies go side by side like lifestyle modification and ovulation induction. Also, different forms of therapies are complementary to each other like weight loss leading to a better response in ovulation induction.

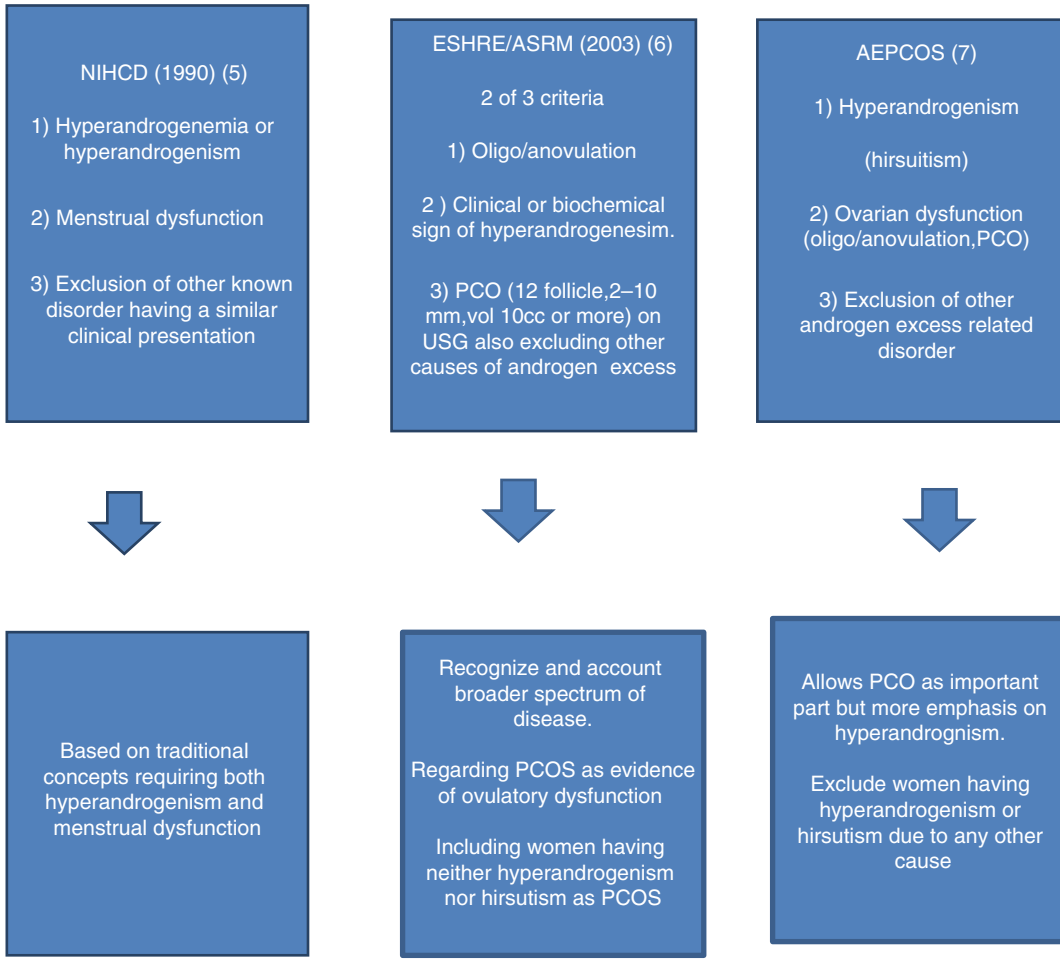

Fig. 30.1 Criterion for diagnosis of PCOS

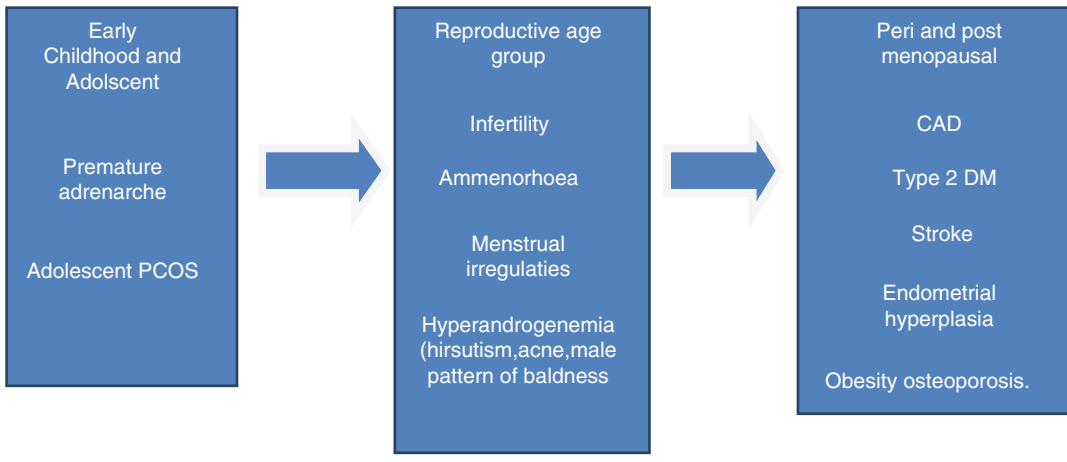

Fig. 30.2 Clinical picture of PCOS

Table 30.1 Differential diagnosis of PCOS and laboratory tests

Condition	Test	Range
PCOS	Total testosterone DHEA LH/FSH	<200 ng/ml Increased >2:1
Hypothyroidism	Free T3 Free T4 TSH	Decreased Decreased Increased
Hyperprolactinemia	Serum prolactin	Increased
Late-onset CAH	17-hydroxyprogesterone	>200 ng/ml (>800 diagnostic)
Androgen-secreting ovarian tumor	Testosterone	>200 ng/ml
Androgen-secreting adrenal tumor	DHEAS	>700 μg/dl
Cushing syndrome	Cortisol	Increased
Severe insulin resistance	OGTT S. insulin (2 h) B. sugar (fasting) Postprandial (2 h)	>80 μU/ml >126 mg/dl >200 mg/dl
Idiopathic hirsutism	Menstrual history Serum progesterone Serum testosterone	Oligo-/amenorrhea Decreased Normal to increased

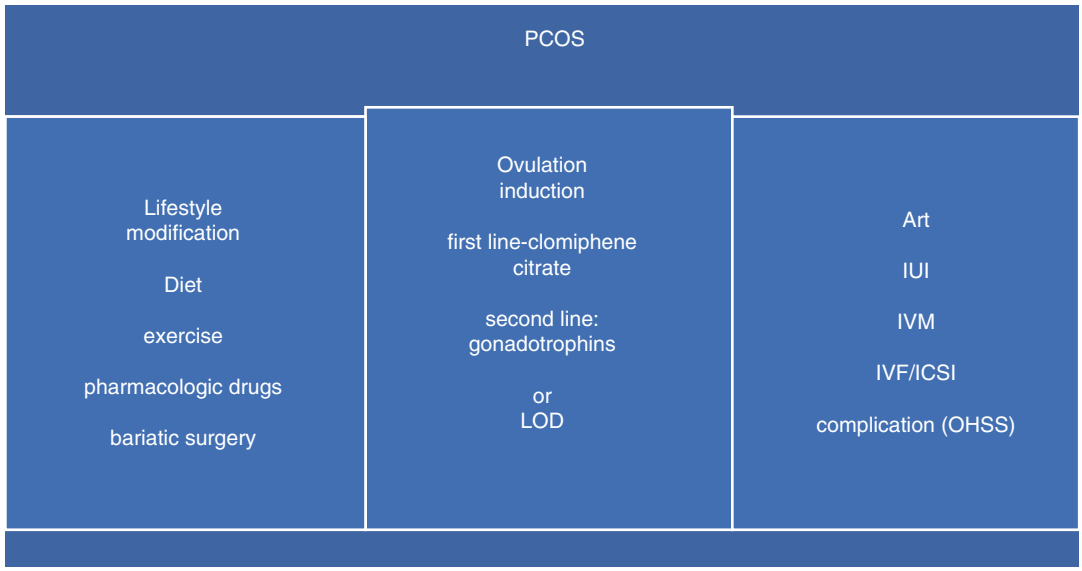

Fig. 30.3 Management of PCOS

30.5.1 Preconceptional Counseling

The treatment of a PCOS woman planning pregnancy is started preconceptionally. The patient is started with tablet folate 5 mg daily starting 3 months before planning pregnancy as it decreases the incidence of congenital malformations in the offspring. The patient is recommended to stop smoking and illicit drug usage.

The main part of counseling is weight loss as it is well known that obesity is associated with multiple pregnancy complications like miscarriage,

gestational diabetes mellitus (GDM), and preeclampsia. Losing as much as 5 % weight is associated with resumption of menstruation, ovulation, and pregnancy [8].

30.5.2 Lifestyle Modification and Weight Loss

Obesity is common in patients with PCOS. The obesity per se decreases the chance in getting pregnant and decreases the response to ovulation induction with drugs and ART. The obesity is centripetal in distribution with increase in visceral fat; even in the case of lean PCOS, there is a tendency of weight gain in abdominal area.

Therefore, the first line of management in the case of PCOS is weight loss and more important is the maintenance of the weight loss. Even a loss of 5 % weight can lead to decrease in irregularities of period and in some cases resumption of menstruation [9]. Weight loss decreases the complication like miscarriage rate, preeclampsia, and gestational diabetes mellitus (GDM) in patient of PCOS planning pregnancy. The treatment of obesity is multidisciplinary and involves behavioral counseling, diet, exercise, and pharmacological therapy. The intervention should be started in preconceptional period (see Fig. 30.3). Weight loss leads to decrease in free testosterone levels by increasing SHBG levels [8].

It can be achieved by the following means:

1. Diet
2. Exercise
3. Pharmacological treatment
4. Behavioral treatment
5. Bariatric surgery

30.5.2.1 Diet

This is one of the most important aspects as calorie restriction is the key to weight loss. Many researchers have suggested different types of diets like the Atkins diet, high protein diet, and no carbohydrate diet, but without much results. There is increased evidence in favor of diets utilizing food having reduced glycemic load and high glycemic index. In the absence of good level of evidence, calorie restriction of 500 kcal/day is presently recommended for PCOS female [8]. An overall decrease in calorie intake is more important than any specific composition. Lifestyle treatment leads to weight loss, decrease in free androgen, abdominal obesity, and surrogate marker of insulin resistance and an improved quality of life in PCOS.

30.5.2.2 Exercise

Daily exercise is one of the key factors for weight loss. Exercise reduces the risk of having DM type 2 and cardiovascular disease in a PCOS. Moderate activity that is sustained is better than vigorous activity that is not regular. The aim is to develop a healthy lifestyle that is continuously followed. Moran et al. [10] described that climbing 8000 steps a day along with change in diet pattern decreases the testosterone level by 57 %. Insufficient physical activity is one of the reasons obese PCOS women put on weight. Patients who are morbidly obese should be advised rigorous weight loss under supervision because of possible orthopedic and cardiovascular risk involved in unsupervised exercise. Hoeger et al. [11] advised weekly exercise for 150 min/week along with dietary restriction with the goal of 5–7 % weight loss leading to decrease in SHBG and insulin resistance.

30.5.2.3 Pharmacological Management

Drugs are used either to suppress appetite or those which have an antiobesity effect. Antiobesity drugs include orlistat which acts by decreasing intestinal absorption of fat [12]. Appetite suppressant like sibutramine acts by decreasing the appetite and has dose-dependent action [13]. Statins act by inhibiting HMG-CoA reductase enzyme, which is the rate-limiting step in cholesterol pathway. There is decrease in levels of testosterone along with dyslipidemia and therefore PCOS patients are at risk for developing diabetes and cardiovascular disorder. They are teratogenic in pregnancy. According to Cochrane (2011), statins are effective in reducing serum androgen levels and LDL, but statins are

not effective in reducing fasting insulin or insulin resistance. There is no good evidence available on the long-term use of statins alone or in combination for management of PCOS [14].

30.5.3 Behavioral Therapy

PCOS women have increased chances of having depression due to obesity and infertility. Counseling is very helpful for these PCOS women.

30.5.3.1 Bariatric Surgery

Bariatric surgery can be offered to morbidly obese women with BMI of >35 kg/m^2 [15]. With surgery the weight loss is maintained. The pregnancy per se becomes high risk, as there is increased chance of IUGR and decreased weight gain in these females.

30.5.4 Insulin Sensitizers

30.5.4.1 Metformin

Metformin is a biguanide, oral insulin-sensitizing agent used in the treatment for diabetes mellitus type 2. It acts by increasing the peripheral uptake of glucose in the muscle and intestine [16], decreases hepatic glucose uptake, and inhibits lipolysis, thereby decreasing the circulating levels of free fatty acids and increasing the insulin sensitivity. It thus helps in decreasing weight and LDL cholesterol. The tablet is available in both regular and sustained release form starting with a minimum dose of 500 mg per day along with meals to a maximum level of 1500–2000 mg per day [3].

Metformin should be given to women having impaired glucose tolerance, diabetes mellitus type 2, and severe insulin resistance. It is also given where there is metabolic syndrome like dyslipidemia and central obesity. In adolescent PCOS girls, it has been shown that metformin treatment can result in decrease in hyperandrogenemia and hyperinsulinemia. Cochrane analysis (2013) has found that metformin decreases the incidence of OHSS in females undergoing ovulation induction, but it does not increase the chance of having a live birth [17]. The drug is prescribed only in patients having glucose intolerance [8]. Also in cases of ovulation induction, there is no benefit of prescribing metformin alone or with clomiphene citrate except in cases where patient has BMI [18] of >35 kg/m^2.

Precautions Metformin can cause lactic acidosis in 1:33,000 cases. It is a serious condition with a mortality of 50 %, mainly occurring in women with renal impairment. Symptoms are often nonspecific like fatigue, myalgia, abdominal distension, vomiting, and respiratory depression. Immediate cessation of the drug is indicated on observing any of the symptoms. Serum electrolytes, blood glucose, ketones, pH, serum lactate level, and serum metformin levels, if possible, should be checked. To take precautions against this condition, metformin should be discontinued 48 h before any planned surgery or any radiographic study utilizing intravenous contrast dye. Ethanol potentiates the effect of metformin and patients should be warned against high alcohol intake. Hemodialysis may be needed to resolve the situation.

Minor side effects like nausea, vomiting, diarrhea, bloating, flatulence, and metallic taste occur in 20 % of patients. It resolves if drug is taken with food. Since this effect is dose dependent, the dose of metformin should be increased in an incremental fashion. If discomfort is significant, the drug should be discontinued. There may be weight loss associated with the nausea and vomiting accompanying the drug. Megaloblastic anemia may occur in some patients because of subnormal B_{12} levels. Before starting metformin, renal and liver functions should be tested.

Hypoglycemia does not occur with metformin in euglycemic patients. It may be seen in special cases where there is deficient caloric intake and concomitant use with sulfonylureas and strenuous exercise is not compensated with adequate intake or excessive alcohol consumption. It is contraindicated in renal disease and myocardial infarction. Drug interaction occurs with diuretics, oral contraceptives, and phenytoin.

Metformin induces regular cycles in some women treated for 4–6 months. It improved ovulation, hirsutism, hyperandrogenemia, and insulin

resistance. Lowering of fasting insulin levels is seen in 2–3 months. A repeat test is required only after this period. If amenorrhea persists, clomiphene or rosiglitazone is added. Ovulation rates are higher when combined with clomiphene. Patients with elevated pretreatment levels of testosterone show the best results in resumption of ovulation with significant reduction in testosterone. Those with raised fasting insulin responded less and those with normal testosterone showed no effect.

30.5.4.2 Thiazolidinediones

Thiazolidinediones include rosiglitazone and pioglitazone. They are less effective than metformin in decreasing insulin resistance and lead to weight gain and are category C drug in pregnancy [4]. They are synthetic agonists for peroxisome proliferator-activated receptor gamma (PPAR), which serves as a regulator gene for metabolism of carbohydrate, fats, and lipids [3].

30.5.4.3 Myoinositol and D-Chiro-Inositol

Myoinositol positively modulates insulin sensitivity in nonobese PCOS patients without compensatory hyperinsulinemia, improving hormonal parameters. Thus, myoinositol improves reproductive axis functioning in PCOS patients. Menstrual cyclicity was restored in all ammenorrheic and oligomenorrheic patients.

30.5.5 Ovulation Induction

Lifestyle modification forms an important part of PCOS, but around 40–50 % of these females have anovulation and suffer from infertility. The first-line drug for ovulation induction is clomiphene citrate (Fig. 30.4).

Problems in Ovulation Induction in PCOS

1. Disturbed folliculogenesis leading to poor response to induction
2. Large number of antral follicles sensitive to FSH leading to multiple follicular development, OHSS, and multiple pregnancy

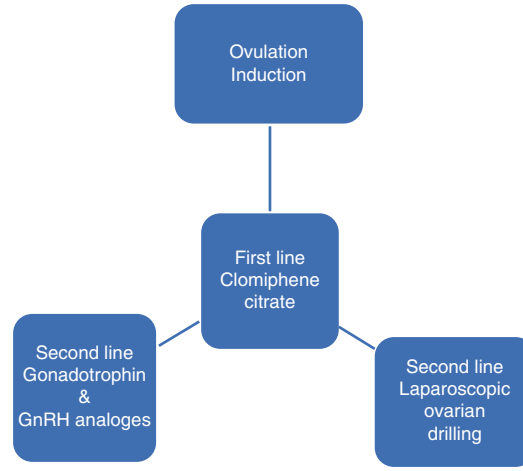

Fig. 30.4 Ovulation induction in PCOS

3. Tonically elevated serum LH levels leading to premature luteinization, low pregnancy rates, and high miscarriage rate

30.5.5.1 Clomiphene

Clomiphene citrate is a nonsteroidal triphenylethylene derivative [3]. It is a selective estrogen receptor modulator which normally acts as estrogen receptor antagonist, but when the level of estrogen in the body is very low, it acts as an agonist [4]. It is available as two racemic isomers En (62 %) and Zu (38 %). Clomiphene is excreted in stools and around 85 % is excreted in 6 days. En clomiphene is more potent and responsible for the action of clomiphene for ovulatory induction. Zu clomiphene is less potent and stays in circulation for a longer time. It accumulates over series of time and is probably responsible for adverse effect of clomiphene on endometrium and cervix [19].

Indications for Usage of Clomiphene Citrate [20] It is useful in anovulatory females who have PCOS, obesity, thyroid disorder, luteal phase defect or in some cases of hypothalamic dysfunction related to eating disorders, extreme weight loss, hyperprolactinemia, and pituitary tumors. Before starting clomiphene, thyroid dysfunction and hyperprolactinemia if present, should first be

corrected for underlying cause. According to Cochrane meta-analysis, clomiphene is effective in inducing ovulation in PCOS patients [21].

The efficacy of clomiphene citrate in unexplained infertility is by inducing superovulation of more than a single ovum. But studies have found out that only clomiphene citrate with timed intercourse is no better than no intervention as there is no improvement in only clomiphene citrate group. IUI along with clomiphene citrate is more useful in patients with unexplained infertility as it leads to increase in pregnancy rate [22]. At least three cycles of clomiphene citrate should be offered.

Treatment Regimen Standard therapy: clomiphene citrate is started from day 2 to day 5 after onset of spontaneous menstruation or progesterone-induced menstruation, for 5 days.

The dosing of clomiphene citrate should be based upon BMI, age, AMH, antral follicle count, response to previous stimulation, and day 2 FSH [8]. The dose of the tablet is 50 mg per day, but in the case of lean PCOS, the dose is as less as 25 mg/day. The maximum response is obtained with 150 mg/day. The maximum dose that can be safely used is 250 mg/day, but that is rarely required. Higher dose may be useful in patients with higher BMI. In obese, anovulatory women with at least 2 years of infertility, success rates generally are lower, with 16 % achieving live birth in women with BMI >35 kg/m^2 compared with 28 % for women with BMI 14 < 30 kg/m^2.

If clomiphene citrate is used for ovulation induction, then it must be given for a maximum of 3–6 cycles. The likelihood of pregnancy is very low after this period. The cumulative pregnancy rate after six cycle of CC is 50–60 %[9]. If no pregnancy occurs after six cycles, then the second line of therapy with gonadotrophins or laparoscopic ovarian drilling should be offered.

Efficacy Approximately 75 % of patients of PCOS will ovulate with clomiphene citrate, but the pregnancy rate is only 22 %. This could be because of the negative effect of clomiphene citrate on endometrium and cervix [23, 24].

Monitoring Monitoring is done with ultrasound (USG). The baseline scan is done on the second day of the cycle to see for any ovarian cyst and endometrial thickness. The patient is started on tab clomiphene citrate and advised USG from day 9 onward. Patient is advised to have intercourse on alternate days starting from day 10 of the cycle. Whether to give HCG or not is optional [25]. If there is unruptured follicle in the previous cycle, the patient is advised HCG in the next cycle. Many investigators do a baseline scan and then start using LH kit to predict ovulation rather than doing repeated USG.

Adverse Effect Adverse effects of clomiphene are hot flushes, vaginal dryness, visual disturbances, headache, mood swings [26], blurring of vision, double vision, and scotoma [27] (<2 %). The drug is stopped in case of visual side effect.

Complications Complications include *multiple* pregnancy which occur in 7 % of the patient's most common being twin [28]. There is no evidence of any risk of anomalies in baby born after treatment with clomiphene citrate or of a higher miscarriage rate. There is a slight increase in ovarian hyperstimulation syndrome (OHSS). Risk of ovarian cancer is not increased.

30.5.5.2 Combined Therapy

Metformin Metformin was prescribed along with clomiphene citrate in cases of PCOS. Evidence has shown that it is useful only in patients having BMI >35 kg/m^2 and in patients with clomiphene citrate resistance [18]. However, Moll et al. [29] showed no added benefit of metformin along with clomiphene citrate in ovulation induction.

Glucocorticoids Some of the women with PCOS show involvement of an adrenal component with raised DHEAS. In these women glucocorticoids may be given. The desired effect should be to normalize without suppressing the adrenal component, with dexamethasone (0.25–0.5 mg/day). Dose of 0.25 mg/day is seen

to suppress 50 % of patients. Dexamethasone may be started with clomiphene and stopped when ovulation is documented. Dexamethasone gives best results when administered at night, as adrenals are most active early morning. DHEAS and testosterone levels are monitored after 1 month. They are found to produce good results in clomiphene resistance with raised DHEA.

30.5.5.3 Alternative Therapy

Tamoxiphen Tamoxiphen is a selective estrogen receptor modulator that is useful in breast cancer and appears to be as effective as clomiphene citrate. It has an agonist action on the uterus, bone, and pituitary and an antagonist on the breasts and blood vessels. It can be offered to females who are intolerant to clomiphene citrate. It is started with a dose of 40 mg per day from day 2 to day 6 of cycle and can be increased to 80 mg per day. According to a Cochrane review, it is as effective as clomiphene in inducing ovulation induction [21].

Aromatase Inhibitor Letrozole is an aromatase inhibitor. It acts by inhibiting the action of the aromatase enzyme, which converts androgens to estrogens. The drug is started with 2.5 mg from day 2 of menses for 5 days. The use of this drug is prohibited in India.

30.5.6 Second Line of Treatment

30.5.6.1 Laparoscopic Ovarian Drilling (LOD)

There are two categories of treatment that can be offered to a patient with PCOS not responding to clomiphene citrate: laparoscopic ovarian drilling and gonadotrophin with GnRH analogues [8]. Laparoscopic ovarian drilling achieves unifollicular ovulation without the risk of OHSS and multiple pregnancy [30]. Four to ten punctures are made in the ovarian stroma either by monopolar electrocautery or laser. Forty percent of the patients start ovulating, but the rest will require other form of ovulation induction after LOD [31, 32].

The indications are:

1. Clomiphene citrate resistance
2. Patients who are unable to do frequent ultrasound monitoring especially during gonadotrophin therapy
3. Patients who hypersecrete LH
4. Patients who need laparoscopy for other gynecological conditions
5. To decrease OHSS in response to gonadotrophins in patients at high risk

Predictor of Success Normal BMI, LH >10 IU/ML, and shorter duration of therapy are predictors of success [33].

Side Effects There could be adhesion formation due to trauma to ovarian surface. Sometimes it may lead to a decreased ovarian reserve if too much drilling is done. According to Cochrane [34], LOD is an effective method for inducing ovulation alone with additional advantage of a decreased rate of multiple birth.

30.5.6.2 Gonadotrophin and GnRH Analogues

Gonadotrophins are used in induction of ovulation as the second line to clomiphene citrate in a patient of PCOS. The goal of treatment in PCOS is unifollicular development as PCOS patients are at increased risk of OHSS. The gonadotrophins increase the duration and threshold of FSH window so that there is growth of multiple follicles in the same menstrual cycle [35]. Patients with PCOS have normal to low FSH levels with high LH levels. Gonadotrophin stimulation is indicated in patients who are resistant to clomiphene. Ideally as the levels of LH are raised in PCOS, FSH only preparations are used, but recent Cochrane database has shown that HMG is as effective as FSH for ovulation induction [36]. Gonadotrophins are useful in clomiphene citrate-resistant cases. With the help of GnRH analogues, LH surge can be prevented. It causes an increase in cost of therapy and an increased risk of OHSS and multiple pregnancy.

Table 30.2 Regimes for ovulation induction

	Step-up (From Balasch [37])	Step-down (From Balasch [37])
Dose	Starting dose: 37.5 IU. After one week, increment of 37.5 is made until follicular growth starts	Start with higher dose of 150–225 IU. On ultrasound if follicle grows up to 10–12 mm, decrease by increments of 37.5 IU to 112.5 IU. If follicle continues to grow, reduce dose to 75 IU
Advantages	Encourages monofollicular growth and hence risk of OHSS and multiple pregnancy much less	Length of cycle is shorter and it is more like a natural cycle
Disadvantages	Length of cycle increased	Frequent and intensive monitoring, as high doses can lead to hyperstimulation Increased chances of multiple pregnancy

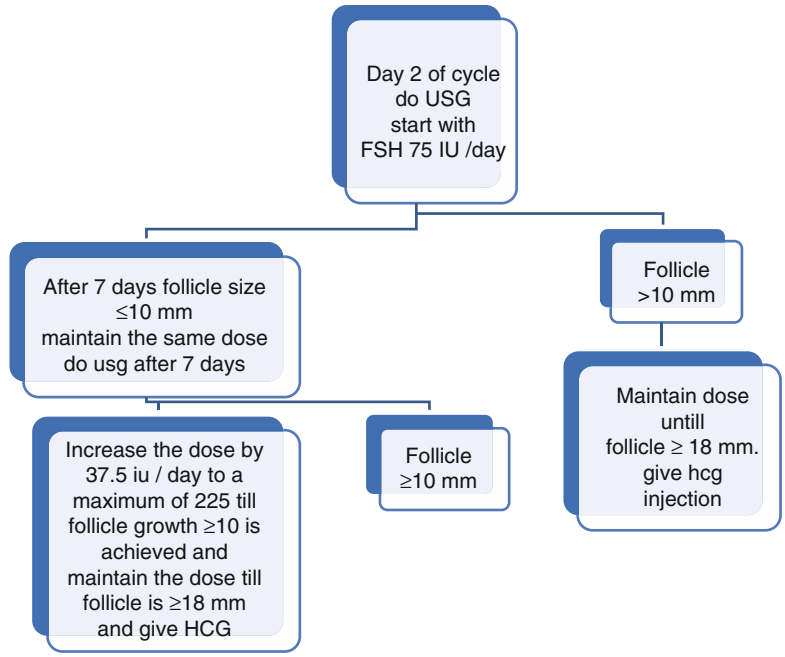

Fig. 30.5 Chronic step-up regime

The challenges in the case of PCOS are:

1. Increased risk of hyperstimulation
2. Increased risk of multiple pregnancy
3. Premature rise of LH
4. Increased chance of cycle cancellation

It is important to evaluate the women completely before starting gonadotrophins. Uterine cavity evaluation for myomas and adhesions, HSG (hysterosalpingography) for tubal obstruction, semen analysis, and complete endocrinal workup including prolactin and thyroid function should be done. Ovarian reserve assessment by AMH and antral follicle count should be evaluated.

30.5.7 Regimes for Ovulation Induction

There are two types of stimulation protocols used: chronic step-up and chronic step-down (Table 30.2) [37] (Figs. 30.5 and 30.6).

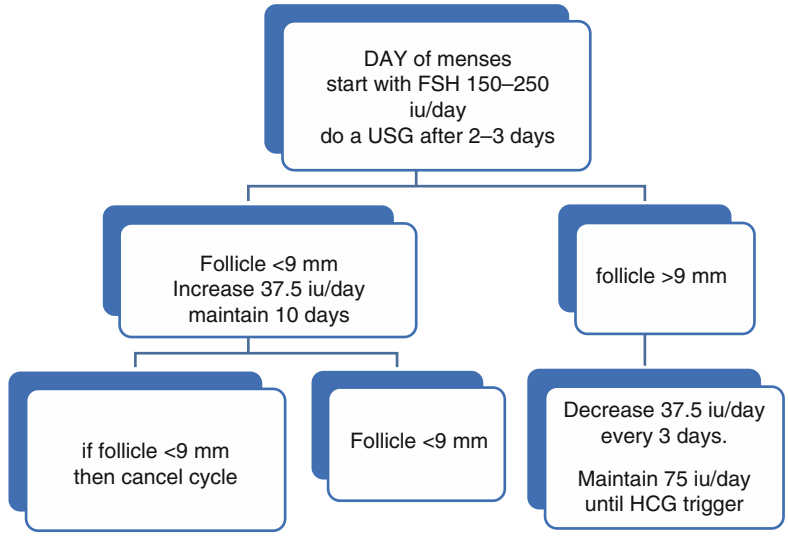

Fig. 30.6 Chronic step-down regime

30.5.7.1 Monitoring

Patient should be counseled before starting the gonadotrophins of the risk of hyperstimulation and chances of cancellation and multiple pregnancy. Monitoring can be done by ultrasound and blood estradiol levels. Ultrasound is one of the best measures to monitor the cycle. According to ASRM guidelines [38], cancellation is advised when there are two follicles of 16 mm or one follicle of 16 mm and two follicles of 14 mm in women less than 38 years, with blood E2 levels >2500 ng/ml. Some studies have kept the values to 1000 ng/ml. Ovulation can be triggered with HCG either urinary 5000 IU or recombinant 250 µgm. According to a Cochrane review, both have been found to be equally efficacious [39].

30.5.7.2 GnRH Analogues

There is an increase in LH in PCOS. It is postulated that increase in LH can cause premature luteinization as well as deleterious effect on oocyte quality. So the GnRH analogues came into vogue as they can prevent premature LH rise. However, there is no significant benefit and the use of analogues leads to higher hyperstimulation, increased rate of multiple pregnancy, and increased cost of therapy. Currently, the use of analogues is not recommended during ovulation induction in a patient of PCOS [40].

Gonadotrophins give a 70 % ovulation rate, with a 20 % pregnancy rate and 5–6 % multiple pregnancy rate. Intensive monitoring is required for gonadotrophin cycle. The patient is started with a minimum dose of 37.5–75 IU/day and monitored strictly. The goal of the therapy is unifollicular development. Patient should be counseled beforehand for chances of cancellation.

30.5.7.3 ART (IUI, IVF, and IVM)

ART will be required in around 20 % of the patients. In vitro fertilization is a reasonable option for prevention of higher-order births especially in PCOS patients who are prone for multiple pregnancy. Patients can start with simple treatment like IUI with gonadotrophins and then proceed to IVF.

Intrauterine Insemination Ovulation induction with clomiphene or gonadotrophins is followed with ultrasound monitoring. Pregnancy rate of 11–20 % is observed. It has been recommended in some studies that IUI with gonadotrophins is better than the use of gonadotrophins alone [41]. Patient can be offered double IUI with one at 12 h and the other at 36 h of HCG injection. However, studies have not shown increased results with double IUI [42].

Table 30.3 Difference in GnRH agonist and antagonist protocols in PCOS

Feature	Agonist	Antagonist
Acceptance	Less	Patient friendly
OHSS	More	Less
Multiple pregnancy	Same	Same
GnRH agonist trigger	Cannot be used	Can be used
Oocyte yield	More	Slightly less
Pregnancy rate	Slightly high	5 % less [47]

In Vitro Fertilization In vitro fertilization is the third line of therapy in the case of PCOS patients. It is indicated in the following subset of patients:

1. Severe male factor infertility
2. Multiple failed IUI
3. Persistently raised LH
4. Bilateral tubal block
5. Severe endometriosis
6. Patient desiring PGD/PGS

Patient can be offered multiple regimens ranging from classical long protocol to antagonist protocol to mild stimulation protocol. Classical long protocol is still the most commonly practiced (Table 30.3).

It has been suggested that increased luteinizing hormone (LH) secretion in PCOS may interfere with fertility. The mechanisms include premature oocyte maturation and deleterious LH effect on granulosa cell steroidogenesis. In addition, elevated LH levels may be associated with an increased pregnancy loss, as early secretion of progesterone makes endometrium unreceptive for implantation. Keeping this view in mind, long agonist protocol which first causes downregulation of pituitary followed by stimulation with gonadotrophin came into vogue. However, due to triggering with HCG, there was increased incidence of OHSS. So options were sought and antagonist protocol came into use.

This is a shorter protocol than agonist where stimulation begins on day 2 or day 3 of natural menstrual cycle. There is no downregulation of the pituitary, and premature LH surge is prevented by adding GnRH antagonist when follicle is 14 mm (flexible protocol) or on day 5 (fixed protocol) of stimulation when there is high probability of LH surge. According to Cochrane (2011) [43], the antagonist protocol in comparison to the long GnRH agonist was associated with a large reduction in OHSS without a difference in live birth rates.

In Vitro Maturation (IVM) As PCOS women are prone to hyperstimulation, there is an option in high-risk women to retrieve immature oocytes without stimulating the ovary and mature them in vitro following which they are fertilized. Since the ovaries are not stimulated, there is no chance of OHSS. The pregnancy rate is lower in IVM.

30.5.7.4 Triggering Ovulation in PCOS

Once there are three follicles of more than 18 mm, a trigger with HCG can be given. With antagonist protocol, there is a possibility of triggering follicle with agonist trigger, which can lead to decrease in incidence of OHSS. GnRH agonist acts at the level of the pituitary, displaces GnRH antagonist, and activates GnRH receptor, which causes a surge of gonadotrophin LH, and FSH which leads to ovulation. It is as close to natural cycle as possible. There is first FSH surge as in a natural cycle followed by LH surge, which leads to resumption of meiosis. Unlike natural cycle LH surge, GnRH agonist surge is a short surge lasting from 24 to 36 h with only two phases unlike three phases of LH surge [44]. So less amount of gonadotrophins are released which leads to early demise of corpus luteum. This is one of the major drawbacks of GnRH triggering. There is deficient corpus luteum leading to deficient luteal phase and significantly lower pregnancy rates.

Many strategies have been adopted to correct this luteal phase defect. Dual trigger GnRH agonist along with HCG, which combines the benefit of endogenous release of FSH and LH by agonist trigger and small bolus of HCG to cover early luteal phase insufficiency caused by agonist trigger, has been used. Peter Humaidan [45] in their study gave 1500 IU of HCG along with GnRH agonist to correct this luteal defect at the time of egg retrieval and got good pregnancy rates. Engmann et al. [46] supplemented the luteal phase with intramuscular progesterone and estradiol valerate as modified luteal phase supports post GnRH agonist trigger. According

to Cochrane database, a comparison of GnRH agonist with HCG trigger concluded that agonist should not be routinely used for triggering ovulation in normal responder as it leads to significantly lower live birth rates (Table 30.4) [47].

Indication for use of agonist trigger is PCOS patients having high risk for OHSS or previous history of OHSS. GnRH agonist trigger could be Decapeptyl in the dose of 0.2 mg subcutaneously or leuprolide acetate 1 mg subcutaneously.

30.5.8 PCOS and OHSS

PCOS patients have very high chances of going into OHSS. Since they have high AMH levels and even with very small dosage of gonadotrophins, they may land up in growth of excessive follicles leading to OHSS.

Table 30.4 Comparison of GnRH agonist with HCG trigger

Comparison	GnRH agonist trigger	HCG trigger
OHSS	Very rare	High
Cost	Cost of GnRH trigger is minimal	Expensive
Total duration of action	Short (24–36 h)	Long (half-life is 48–73 h)
Luteal phase	Deficient	Adequate
FSH surge	Present	Absent
Pregnancy rate	Lower	Higher

Ovarian hyperstimulation syndrome (OHSS) is an iatrogenic complication. Regarding moderate cases, it has an incidence of 5 %. The incidence of cases requiring hospitalization is up to 2 %. With the increasing use of antagonist protocol, the incidence of OHSS has come down drastically. The cases of OHSS severe enough to get admitted are relatively few and occur most commonly with HCG trigger. To overcome this drawback of HCG trigger, GnRH agonist triggering is recommended in high responders and women having history of OHSS.

30.5.8.1 OHSS-Free Clinic

With the advent of GnRH agonist triggering, the concept of OHSS-free clinic has come. It is based on a three-segment approach to prevent OHSS.

Segment A: It consists of optimization of the ovarian stimulation including GnRH agonist triggering in a GnRH antagonist cycle.

Segment B: It consists of optimum cryopreservation methods for oocyte or embryo vitrification.

Segment C: It includes embryo replacement in a receptive, non-stimulated endometrium in a natural cycle or with artificial endometrial preparation (Fig. 30.7) [48].

30.6 Summary

Lifestyle modification is the first line in the management of PCOS. Weight loss as little as 5 % will lead into normalization of menses and ovulation.

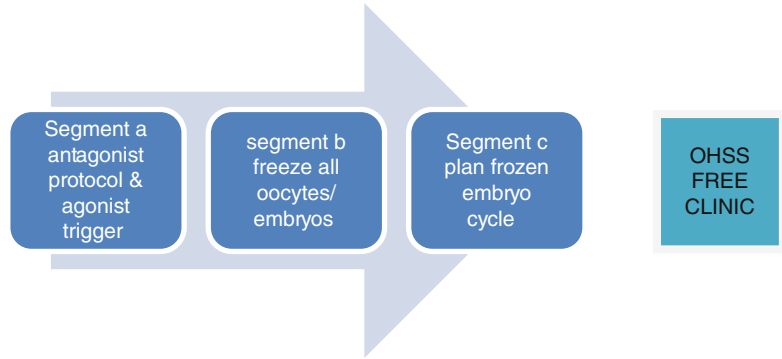

Fig. 30.7 OHSS-free clinic three-segment approach to prevent OHSS

Clomiphene citrate is the first choice for induction of ovulation in most anovulatory women with PCOS with a conception rate of 22 % and ovulation rate of 75 %. Use of metformin in PCOS should be restricted to those patients with glucose intolerance. There seems to be no advantage to adding metformin to clomiphene citrate in women with PCOS. If clomiphene citrate fails to result in pregnancy, then the second-line treatment is either gonadotrophins or laparoscopic ovarian drilling. Low-dose FSH protocols are effective in achieving ovulation with starting dose of 37.5 IU/day. Intensive monitoring is required in patients taking gonadotrophin. There is higher risk of OHSS and multiple pregnancy with gonadotrophin therapy. Laparoscopic ovarian surgery is an alternative to gonadotrophin therapy for clomiphene citrate-resistant anovulatory PCOS. Surgery can achieve unifollicular ovulation with no risk of OHSS or high-order multiples. Induction of ovulation in combination with IUI is indicated in women with PCOS and associated male factor infertility. In vitro fertilization can be offered to those couples that do not conceive after 3–6 cycles of IUI. Also there is advantage of doing a single embryo transfer in a young good prognosis patient. Use of GnRH trigger in patients with PCOS has lead to dramatic decrease of OHSS.

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Role of Laparoscopic Ovarian Drilling in Polycystic Ovarian Syndrome and Its Effect on ART

31

Pankaj Talwar, Ashok K. Pillai, and Falahunisa Shaikh

Abstract

Surgical ovarian wedge resection was the first reputable treatment for women with anovulation, amenorrhea, and polycystic ovary syndrome (PCOS), but was largely discarded both due to the risk of postsurgical adhesions and the introduction of medical ovulation induction treatment. However, women with PCOS who are treated with medical ovulation induction, with drugs such as gonadotropins, often have an excess formation of follicles, which may result in ovarian hyperstimulation syndrome and multiple pregnancies. Moreover, gonadotropins, even though are effective, are expensive and time-consuming and their use requires exhaustive monitoring. Surgical therapy with laparoscopic ovarian "drilling" (LOD) may avoid or reduce the need for medical ovulation induction or may facilitate its usefulness.

Keywords

Anovulation • Ovarian wedge resection • Laparoscopic ovarian drilling • Electrocauterization • Monopolar cautery

31.1 Introduction

The year 1935 saw a remarkable discovery of an endocrine disease in the field of gynecology. Two famous gynecologists from Chicago, who graduated from Rush Medical College and thereafter practiced at Michael Reese Hospital, Irving F. Stein and Michael Leventhal, described a symptom complex associated with anovulation. Stein and Leventhal described seven patients with hirsutism, amenorrhea, and enlarged polycystic

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ovaries, four of whom were obese. They reported the results of bilateral wedge resection, removing one-half to three-fourths of each ovary; all seven patients resumed regular menses, and two became pregnant. Stein and Leventhal developed the wedge resection after they observed that several of their amenorrheic patients menstruated after ovarian biopsies. The association of amenorrhea and polycystic ovaries thus has been known now for decades [1].

Subsequently, it is now recognized that PCOS is a disorder that is characterized principally by oligomenorrhea or amenorrhea with clinical or laboratory evidence of hyperandrogenemia. Furthermore, it is now recognized that a significant proportion of overweight women with PCOS have hyperinsulinemia.

31.2 Pathophysiology

The hyperandrogenism and anovulation that accompany PCOS may be caused by abnormalities in four endocrinologically active compartments:

1. The ovaries
2. The adrenal glands
3. The periphery (fat)
4. The hypothalamic–pituitary compartment [2]

In patients with PCOS, the *ovarian compartment* is the most consistent contributor of androgens. Dysregulation of CYP17, the androgen-forming enzyme in both the adrenals and the ovaries, may be one of the central pathogenetic mechanisms underlying hyperandrogenism in PCOS. The ovarian stroma, theca, and granulosa contribute to ovarian hyperandrogenism and are stimulated by LH. The increased testosterone levels that occur in patients with PCOS are considered ovarian in origin [2].

The *peripheral compartment*, defined as the skin and the adipose tissue, manifests its contribution to the development of PCOS in several ways: the presence and activity of 5α-reductase in the skin largely determine the presence or absence of hirsutism. Aromatase and 17ß-hydroxyl steroid dehydrogenase activities are increased in fat cells, and peripheral aromatization is increased with body weight. With obesity, the metabolism of estrogens, by way of reduced 2-hydroxylation and 17α-oxidation, is decreased, and metabolism via estrogen-active 16-hydroxyestrogens (estriol) is increased. Whereas E2 is at a follicular phase level in patients with PCOS, E1 levels are increased as a result of peripheral aromatization of androstenedione. A chronic hyperestrogenic state, with reversal of the E1:E2 ratio, results and is unopposed by progesterone.

The hypothalamic–pituitary compartment also participates in aspects critical to the development of PCOS. An increase in LH pulse frequency relative to those in the normal follicular phase is the result of increased GnRH pulse frequency. This increase in LH pulse frequency explains the frequent observation of elevated LH and LH:FSH ratios. FSH is not increased with LH, which may result from the combination of increased gonadotropin pulse frequency and the synergistic negative feedback of chronically elevated estrogen levels and normal follicular inhibin. About 25 % of patients with PCOS exhibit mildly elevated prolactin levels, which may result from abnormal estrogen feedback to the pituitary gland [2].

The basic problem lies in persistent anovulation. In contrast to the characteristic picture of fluctuating hormone levels in the normal cycle, a steady state of gonadotropins and sex steroids can be depicted in association with persistent anovulation. This steady state is only relative. In patients with persistent anovulation, the average daily production of estrogen and androgens is both increased and dependent on LH stimulation [3].

The ovary does not secrete increased amounts of estrogen, and estradiol levels are equivalent to early follicular phase concentrations. Circulating estrone levels are slightly elevated. The increased total estrogen is due to peripheral conversion of the increased amounts of androstenedione to estrone [3].

31.3 Diagnostic Criteria

Following are diagnostic criteria based on the modified consensus of the National Institutes of Health [2]. For most of the twentieth century, PCOS was a

poorly understood condition. In 1990, the National Institutes of Health (NIH) held a conference on PCOS to create both a working definition of the disorder and diagnostic criteria. The outcome of this conference, the NIH criteria, served as a standard for researchers and clinicians for more than a decade. The criterion is as follows:

31.3.1 Major Diagnostic Criteria

- Chronic anovulation
- Hyperandrogenemia
- Clinical signs of hyperandrogenism
- Other etiologies excluded

31.3.2 Minor Diagnostic Criteria

- Insulin resistance
- Perimenarchal onset of hirsutism and obesity
- Elevated LH:FSH ratio

In this scheme, there are only two major criteria for the diagnosis of PCOS: anovulation and the presence of hyperandrogenism as established by clinical or laboratory means. These features alone are sufficient for the diagnosis in the absence of other pathologies accounting for hyperandrogenism (i.e., AOAH [adult-onset adrenal hyperplasia], adrenal or ovarian neoplasm, Cushing syndrome) or anovulation (i.e., hypogonadotropic or hypergonadotropic disorders, hyperprolactinemia, and thyroid disease) [3].

In 2003, a consensus workshop in Rotterdam in the Netherlands developed new diagnostic criteria, the Rotterdam Criteria [4]. According to this, the American Society for Reproductive Medicine and the European Society for Human Reproduction and Embryology agreed that two of the following criteria must be met once other endocrinopathies have been ruled out (i.e., Cushing, adrenal hyperplasia):

- Oligomenorrhea
- Clinical and/or biochemical evidence for hyperandrogenemia
- Polycystic-appearing ovaries on ultrasound

31.4 Modality of Management

Treatment of PCOS depends on the patients' complaints and thus their goals. Some complain of ovulatory dysfunction, some of hirsutism, and some of infertility.

31.4.1 Weight Reduction

Weight reduction is the initial recommendation for patients with accompanying obesity because it promotes health; reduces insulin resistance, sex hormone-binding globulin (SHBG), and androgen levels; and may restore ovulation either used alone or in combination with ovulation induction agents. Weight loss of as little as 5–7 % over a 6-month period can reduce the bioavailable or calculated free testosterone level significantly and restore ovulation and fertility in more than 75 % of women. Exercise involving large muscle groups reduces insulin resistance and can be an important component of nonpharmacologic, lifestyle-modifying management [3].

31.4.2 Medical Management

Hormonal suppression: They decrease adrenal and ovarian androgen production. Progestin component suppresses LH; estrogen component increases hepatic production of sex hormone-binding globulin and also decreases conversion of testosterone to DHT (e.g., oral contraceptives, medroxyprogesterone, gonadotropin-releasing hormone analogs, glucocorticoids).

Antiandrogens: Antiandrogens decrease production of DHT and thus treat hirsutism as well (e.g., ketoconazole, finasteride, spironolactone, cyproterone acetate, flutamide).

Insulin sensitizers: Insulin sensitizers decrease hyperinsulinemia seen in PCOS and also improve peripheral insulin sensitization (e.g., metformin).

31.4.3 Surgical Management

The concept of ovarian wedge resection was introduced to us by Stein and Leventhal in 1935 [1]. It was abandoned due to postsurgical adhesions. Thereafter, as ovulation-inducing medical agents became available, the medical induction of ovulation became the dominant form of treatment. Clomiphene citrate (CC) was used for a long time as a first line of treatment. However, 15–20 % of women remain anovulatory despite receiving incremental dose of CC. Furthermore, there was a discrepancy between the ovulation and conception rates. Gonadotropin therapy is usually the next step following failure with clomiphene. However, Setji et al. [5] stated that because of the peculiarly high sensitivity of polycystic ovaries to gonadotropin stimulation, it was plagued by an unacceptable rate of multiple pregnancies and ovarian hyperstimulation syndrome. An alternative to the medical approach is surgical treatment. The most widely used surgical treatment is laparoscopic ovarian drilling. Laparoscopic ovarian drilling was first described by Gjonnaess in 1984 [6]. Both laparoscopic ovarian cautery and laser vaporization using carbon dioxide (CO2), argon, or neodymium-doped yttrium aluminum garnet (Nd:YAG; Nd:Y3Al5O12) crystal lasers have been used to create multiple perforations (approximately 10 holes per ovary) in the ovarian surface and stroma (inner area of the ovary) [7].

Cleemann et al. [8] stated that laparoscopic ovarian drilling (LOD) can resolve infertility within 4–6 months in 50–60 % of couples. So a strategy with LOD in women with PCOS will shorten the time to pregnancy, reduce the need for medical ovulation induction, and enable diagnosis of those women with anatomic infertility who can achieve pregnancy only by IVF treatment [4].

31.4.3.1 Selection of Candidate

- Altered LH:FSH ratio.
- Poor response to ovulation induction drugs.
- Rule out other causes of infertility.
- If LH:FSH ratio is too low, avoid too many punctures as it may lead to ovarian failure.

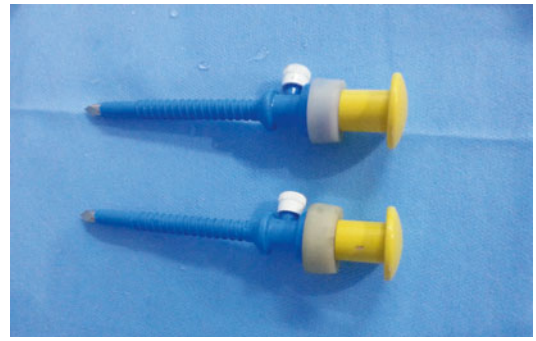


Fig 31.1 Trocar 10 mm

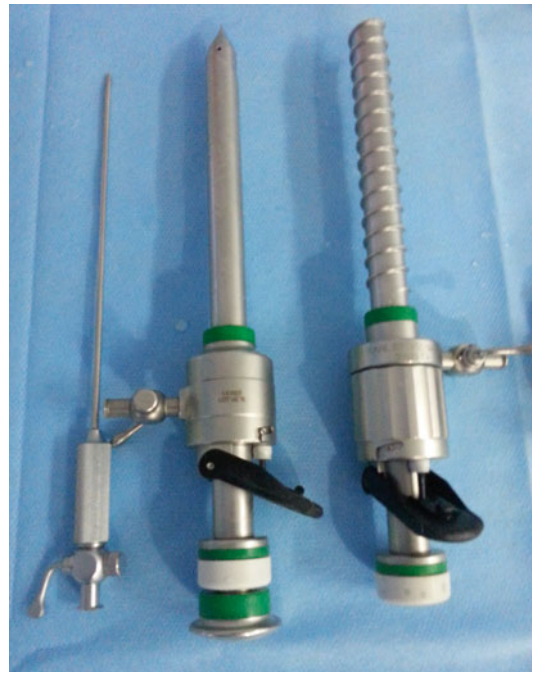


Fig. 31.2 Trocar 10 mm, Veress needle

31.4.3.2 Technique

- The laparoscopic procedure is performed in the follicular phase of natural cycle, the patient is painted with antiseptic solution and draped, and the light cable, insufflation tube, electrosurgical cautery wire, suction irrigation tube and Veress needle, and trocar (Figs. 31.1 and 31.2) should be checked while focusing and balancing the telescope.
- Laparoscopy is performed via three ports of entry after insufflation of the peritoneal cavity by electronic high-flow pneumoperitoneal

insufflators with carbon dioxide gas. A 10-mm laparoscope is inserted through 10-mm port in the inferior crease of the umbilicus into the abdominal cavity with two additional 5-mm ports (Fig. 31.3) in the left and right iliac fossae, and a diagnostic laparoscopy with chromotubation for tubal patency is done.

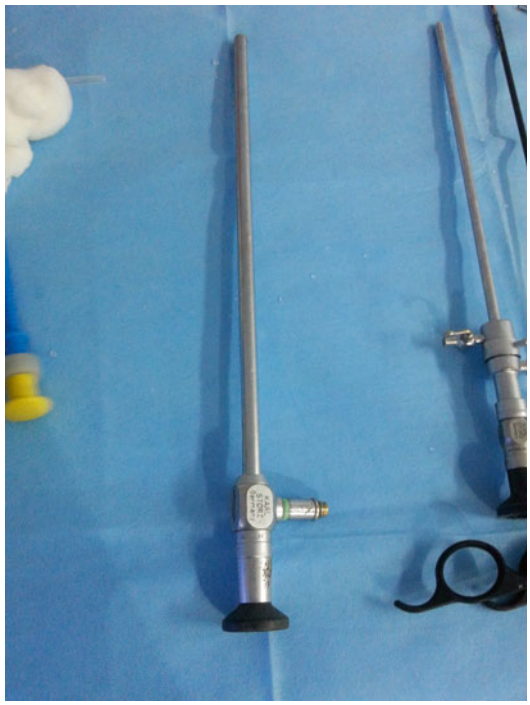


Fig. 31.3 10-mm and 5-mm laparoscope

- An atraumatic grasping forceps (Fig. 31.4a, b) is used to hold the ovarian ligament to stabilize the ovary and to perform the ovarian drilling using an insulated unipolar electrocautery needle electrode, on both ovaries.
- The uninsulated part of the needle is 8 mm long and its diameter is <1 mm (Fig. 31.5a, b). The needle is inserted into the ovarian surface as close to and as perpendicularly as possible. A short duration of a cutting current of 100 W is used to aid the entry of the needle. The whole length of the needle is inserted into the ovary and is activated for 2–3 s with 40 W of coagulating current at each point. A total of 4–5 punctures per ovary are created, depending upon the size of the ovary (Fig. 31.6a–d).
- After drilling, the ovary is allowed to cool in a pool of saline to prevent excessive heat trauma. The abdominal cavity is then rinsed with 500–1,000 cc of sterile saline with suction and irrigation cannula (Fig. 31.7) to remove blood and coagulated tissue and minimize postoperative adhesion.

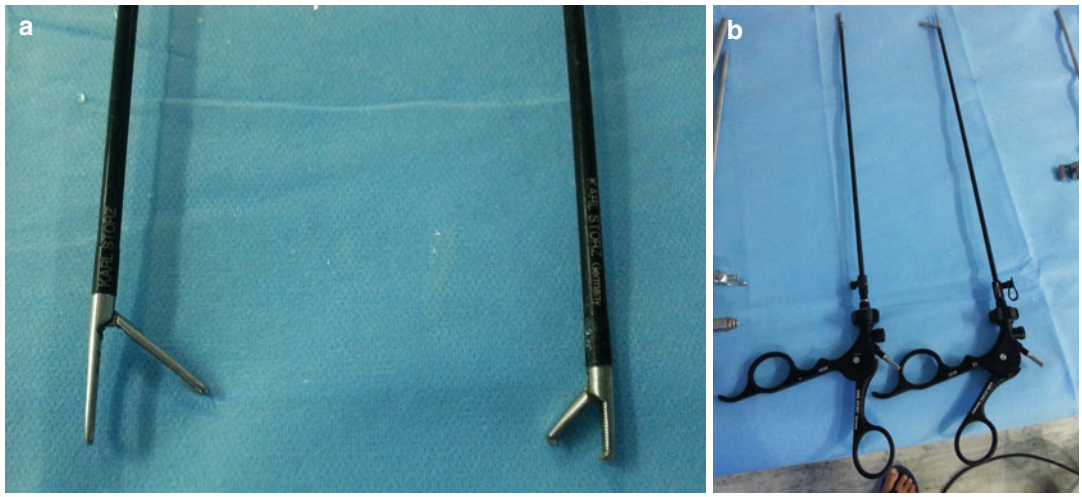


Fig. 31.4 Plain (**a**) and toothed (**b**) grasper (plain grasper should be used as it is atraumatic)

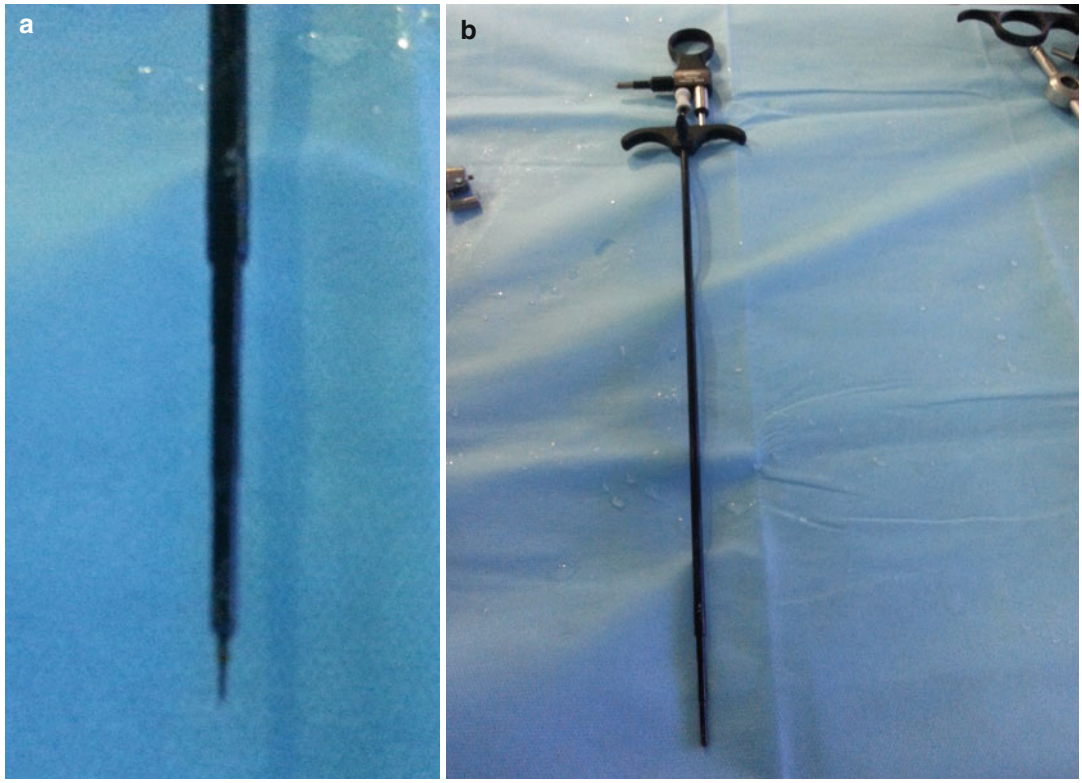


Fig. 31.5 (**a**, **b**) Monopolar needle has an open end 8 mm long, which can be inserted in ovaries

31.4.3.3 Mechanism of Action

The mechanism of action of LOD is thought to be similar to that of ovarian wedge resection. Both procedures may destroy ovarian androgen-producing tissue and reduce the peripheral conversion of androgens to estrogens (one of the many disturbances of endocrine physiology that occur in women with polycystic ovarian syndrome).

A fall in the serum levels of androgens and luteinizing hormone (LH) and an increase in follicle-stimulating hormone (FSH) levels have been demonstrated after LOD [9, 10]. The endocrine changes following the surgery are thought to convert the adverse androgen-dominant intrafollicular environment to an estrogenic one and to restore the hormonal environment to normal by correcting disturbances of the ovarian–pituitary feedback mechanism. Thus, both local and systemic effects are thought to promote follicular recruitment, maturation, and subsequent ovulation [11].

31.4.3.4 Advantages

With ovarian drilling, studies have shown (Table 31.1) [6, 9, 12–17] that there has been decrease in pregnancy loss, in subsequent ovulation induction ovaries become more responsive, and less ovulation induction drugs or gonadotropins are needed [6, 7, 9].

Cohort studies report ovulatory rates of 70–90 % and pregnancy rates of 40–70 %. The response is influenced by body weight. Abdel Gadir et al. [18] have an important evaluation of results of gonadotropin treatment and ovarian drilling with electrocautery. The pregnancy rate was comparably the same but the abortion rate in ovarian drilling was 6–7 % as compared to 26–28 % in gonadotropin-only treatment. Miscarriage is due to persistently high levels of LH levels, which is reduced by ovarian drilling [12].

After 6–12 ovulatory cycles if pregnancy has not occurred, then these women need assisted reproductive techniques.

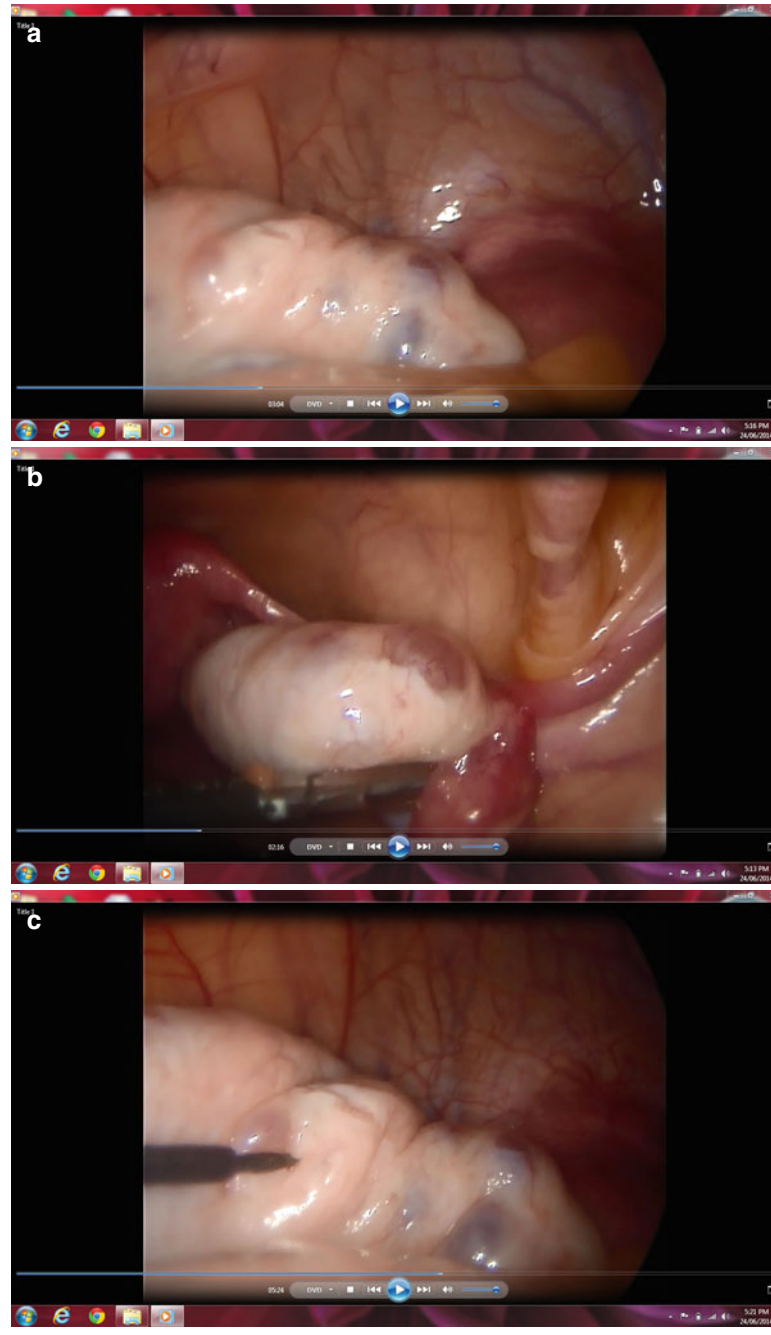
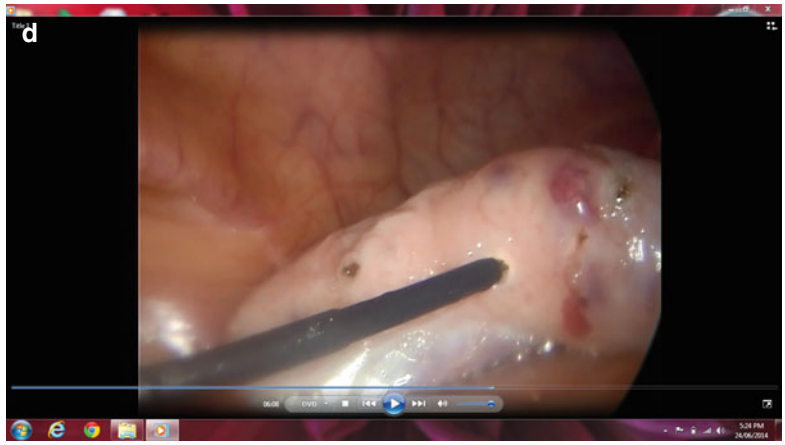


Fig. 31.6 (**a**–**d**) Laparoscopic ovarian drilling. (**a**) Bulky ovaries with multiple cysts lying in the ovarian fossa. (**b**) Ovaries being held with plain grasper. (**c**) Monopolar cautery brought near the ovaries for ovarian drilling. (**d**) 4–5 punctures made in ovary with monopolar cautery with a 100-W current

Fig. 31.6 (continued)



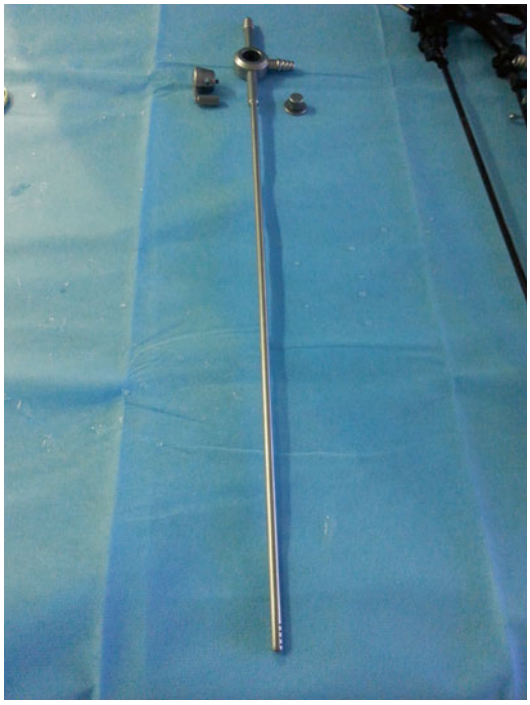


Fig. 31.7 Suction and irrigation cannula, after ovarian drilling to wash the ovaries with normal saline

Table 31.1 Various studies showing the effect of LOD on pregnancies

Author	Year	Technique	Number of cases	Spontaneous ovulation (%)	Pregnancies (%)
Gjønnaess [6]	1984	Cauterization	62	92	84
Greenblatt and Casper [9]	1987	Cauterization	6	71	56
Cohen [12]	1989	Cauterization	778		31.8
Daniel and Miller [13]	1989	Laser	85	83.8	66.7
Kovacs et al. [14]	1991	Cauterization	10	70	20
Pellicer and Remohi [15]	1992	Cauterization	131	67.1	52.6
Gjønnaess [16]	1999	Cauterization	252	92	84

31.5 Summary

Infertility is a common problem faced by women with PCOS. Medical treatment is the first line of management certainly. But if patients do not respond to it, then laparoscopic ovarian drilling has been found to be successful in terms of ovulation, pregnancy, and reduction of abortion. It can be thus concluded that PCOS requires a balanced approach.

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Ovulation Induction in Hypogonadotropic Hypogonadism

32

Umesh Nandani Jindal and Sheetal Jindal

Abstract

Hypogonadotropic hypogonadism (HH) is a heterogenous disorder in which the testes in males and ovaries in females fail to function because of the lack of gonadotropin drive from the pituitary, despite the presence of complete functional competence. The pituitary by itself may be at fault due to some lesion or there may be deficiency of gonadotropin-releasing hormone pulses from the hypothalamus. A variety of genetic functional and acquired lesions can affect the functioning of the hypothalamus and pituitary. The affected individuals are severely hypogonadic, i.e., the deficiency of estrogens in females and the testosterone in the males.

Despite such severe hypogonadism, normal steroid oogenesis, gametogenesis, and fertility are achievable with appropriate hormone therapy. Pulsatile GnRH therapy can be used in functional and other hypothalamic amenorrheas. In pituitary causes, exogenous gonadotropins are required. Both FSH and LH need to be given. Ovulation and pregnancy rates reach 70–80 % with appropriate therapy.

Keywords

Hypogonadotropic hypogonadism • Ovulation induction • Kallmann syndrome • Anorexia nervosa • Pulsatile GnRH • Gonadotropins • Leptin • HPO axis • Stress-induced amenorrhea

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32.1 Introduction

Hypogonadotropic hypogonadism (HH) is a distinct medical condition. There is hypofunction of the gonads which results because of the absence of gonadotropin drive. The hallmark of the disorder is extremely low serum levels of gonadotropins, i.e., follicle-stimulating hormone (FSH) and

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luteinizing hormone (LH) which are secreted by the pituitary gland. Pituitary gland may itself have some disease or disorder, or there may be loss of gonadotropin-releasing hormone (GnRH) pulses from the hypothalamus [1]. There is a complete dissociation of hypothalamus-pituitary-ovarian (HPO) axis. The gonads, i.e., the ovaries in the female and the testes in the male, are unable to function despite the presence of full functional competence. There is neither gametogenesis nor steroidogenesis.

The condition of HH has fascinated and intrigued reproductive scientists for years. It has provided a natural experimental model to unravel the mysteries of reproductive physiology. With the administration of purified exogenous gonadotropins (LH and FSH), the individual roles played by every player in the hormonal symphony of HPO axis could be elucidated. The neuroendocrine control of menstruation and ovulation in females and spermatogenesis in males could be better understood. Moreover, pharmacological interventions in HH women formed the basis for superovulation strategies for assisted reproduction techniques (ART) with a special reference to the role of LH and its impact on oocyte and embryo quality [2].

32.2 Etiopathogenesis

The World Health Organization (WHO) classified ovulatory dysfunction into three classes depending upon the level of gonadotropins and estrogens in the blood [3]. Hypogonadotropic hypogonadal anovulation is classified as class I, characterized by decreased levels of gonadotropins (i.e., FSH and LH), resulting in low levels of estrogens (E) and anovulation.

There is an exhaustive list of diseases (genetic, infiltrative, neoplastic, and traumatic) which may affect the pituitary or the hypothalamus (Fig. 32.1). In addition to the organic lesions, the hypothalamic pulse generator is responsive to many systemic influences, e.g., stress, exercise, weight loss, and systemic illnesses. There is functional and reversible HH in these situations [4].

The role of leptin and adipokines in the adipocyte and fat metabolism and energy homeostasis and their impact on HPO axis are being increasingly recognized. Leptin, a 167-amino acid hormone, is secreted by adipocytes and its levels are positively correlated with body fat. Earlier, leptin was thought to be a solution for the obesity problem. Slowly, the role of leptin was better defined in energy-deficient states. In response to acute energy deprivation, the reproductive hormones are decreased in order to avoid high metabolic demands and pregnancy. This reduction is mediated through leptin [5]. Kisspeptin is another recently discovered neuromodulator that acts upstream of the GnRH to control pulsatile GnRH release. Kisspeptin is the main mediator which relays the negative and positive steroid feedbacks and information regarding body energy stores to hypothalamus. It has a key role to play in the onset of puberty. Kisspeptin may be the main mediator of metabolic and other factors affecting the hypothalamus [6]. Better elucidation of the role of leptin and kisspeptin in the neuroendocrine control of ovulation is likely to introduce newer therapeutic options in the management of HH and anovulation [6–8].

32.3 Clinical Presentation

The incidence of HH varies according to the population studied. In infertility clinics, the estimates vary from 1 to 3 % of all infertility and approximately 5 % of anovulatory infertility. The prevalence of congenital or idiopathic HH varies from one in 3,000 to 4,000 population and is two to five times more common in males [9].

Women with HH may present with delayed menarche, primary amenorrhea, or secondary amenorrhea. Genetic etiologies, e.g., Kallmann syndrome, present with primary amenorrhea. Sudden onset of secondary amenorrhea may antedate an event, e.g., parturition, irradiation, and surgery. It may have a more gradual onset when amenorrhea is associated with other systemic illnesses. The diagnosis of functional hypothalamic amenorrhea is quite obvious due to the presence

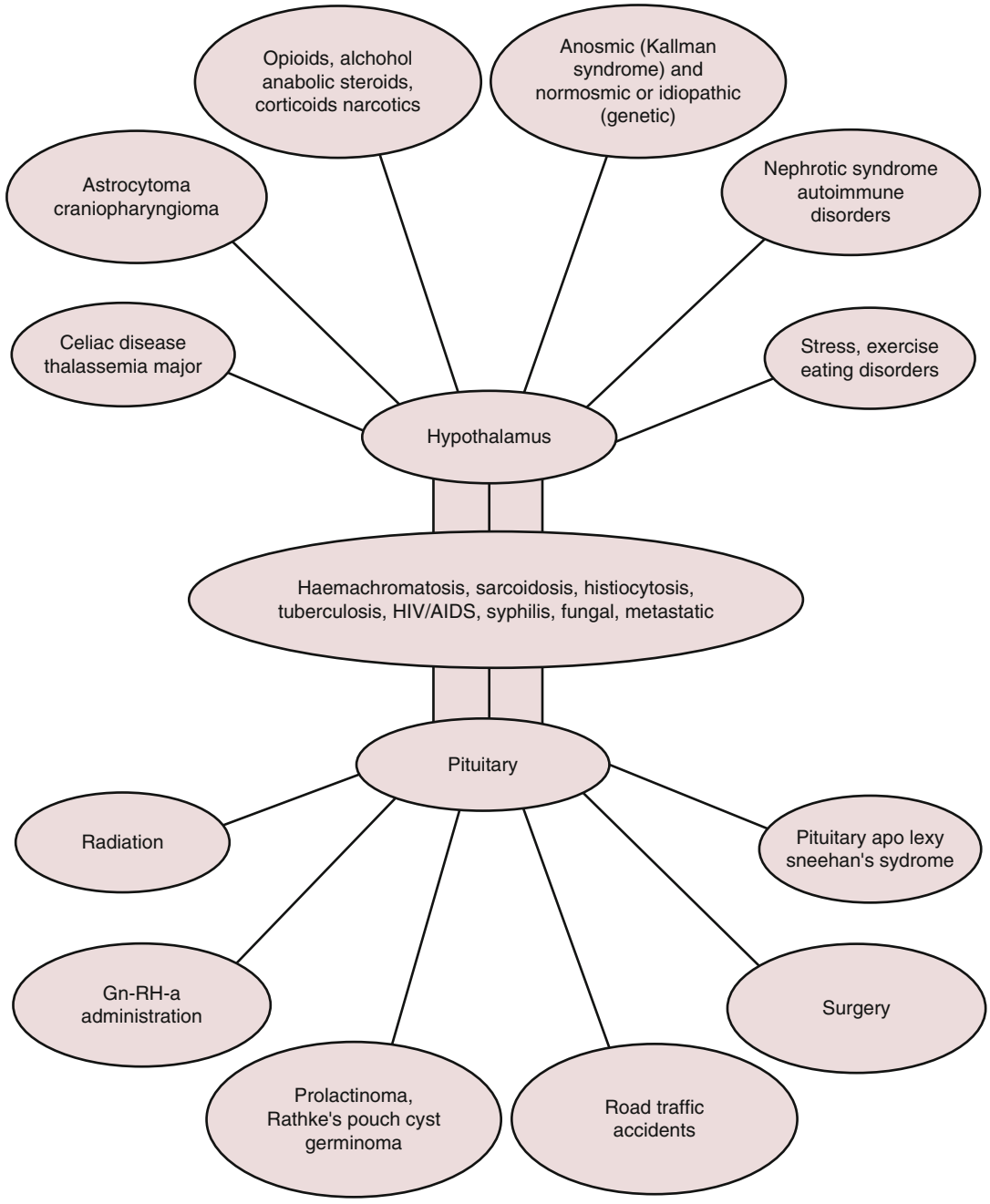

Fig. 32.1 Etiology of hypogonadotropic hypogonadism

of a significant history. History of weight loss and excessive exercise or dietary restriction in an anxious and stressed female presenting with amenorrhea are enough to indicate the diagnosis. The hypogonadotropic hypogonadism related to obesity is only recently recognized. Obesity affects establishment of a healthy HPO axis. In metabolically active obese women, obesity leads to hypogonadism [1] and in women with insulin resistance to PCOS.

The following case history is an illustrative example: a 30-year-old woman presented with progesterone withdrawal negative secondary amenorrhoea for 14 months. Her previous menstrual cycles were regular. She was married for 38 months and gave history of weight gain of (30 kg) since marriage. Her serum hormone levels were FSH 1.2 mIU/ml, LH 0.5 mIU/ml, and E2 15 pg/ml. Her ultrasound examination revealed a small uterus with linear hyperechoic endometrium. Both ovaries were very small but had 5–6 antral follicles of 1–2 mm diameter each. There was negligible stroma around the follicles. Her thyroid and adrenal functions were normal. She responded to ovulation induction with gonadotropins.

32.4 Ovulation Induction

Irrespective of the etiology of HH, the treatment to achieve fertility is very straightforward, i.e., ovulation induction (OI). Despite such profound hypoestrogenism and amenorrhea, these women respond very well to OI and achieve significantly high pregnancy rate. We discuss the topic under the following headings.

32.4.1 Confirmation of Diagnosis and the Underlying Etiology

Before treatment is started, it is mandatory to confirm the diagnosis. Amenorrheic women in whom progesterone (P) withdrawal is negative and combined estrogen (E) and progesterone (E+P) withdrawal is positive can have either hypergonadotropic amenorrhea or hypogonadotropic amenorrhea. Values in very low (i.e.,1 mIU) or low normal range of serum LH and FSH done during amenorrhea are enough to confirm the diagnosis. In case of any doubt, the test may be repeated to rule out a laboratory error. It is counterproductive to induce menstruation with E+P and then do the LH and FSH levels. One must take care to give a gap of at least 1 month between the test and the administration of estrogens or E+P preparations. Exogenous E or E+P preparations downregulate the pituitary and hypothalamus and may lead to false low serum levels of FSH and LH.

In cases of HH, serum E levels are extremely low. The absence of P withdrawal is enough for diagnosis and serum E level testing is not mandatory. Transvaginal ultrasound reveals a very small infantile type of uterus with thin, linear hyperechoic single-layer endometrium. In case the woman has been on E+P for hormone replacement therapy, the uterus may be of normal size and endometrium is better delineated. The ovaries are very small and difficult to locate, sometimes not even seen properly. No assessment of antral follicle count (AFC) is possible in a majority of cases.

A variant of HH with ovaries having PCOS morphology has been described [10, 11]. In such a woman, the ovaries are small but show multiple, small, one to two millimeter-sized follicles. These ovaries also respond to stimulation similar to that in HH but have a high risk of ovarian hyperstimulation syndrome (OHSS) [10, 11].

The case described below is a typical example: a 25-year-old woman presented with history of primary amenorrhea and primary infertility for three years. Her husband had severe oligoasthenozoospermia. She gave history of one abandoned in vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI) cycle. This was done to avoid OHSS because of the multifollicular development and high risk of OHSS. On reevaluation, her serum FSH level was 0.5mIU/ml and serum LH 0.1 mIU/ml. She gave history of induced menstrual bleeding with E+P, not with progesterone only. On ultrasound examination, her ovaries were medium sized and revealed 10–12 antral follicles of 1–6 mm size on each side and were devoid of any surrounding stroma. She was diagnosed as HH with polycystic ovaries. Second cycle of superovulation for IVF-ICSI was undertaken with recombinant FSH by default. The cycle had to be abandoned. There was follicular development of 8–10 follicles but her E_2 levels remained very low. A third cycle with urinary gonadotropins containing both LH and FSH resulted in adequate stimulation for IVF-ICSI and twin live births.

After confirmation of the diagnosis, it is important to diagnose the underlying etiology.

History of primary amenorrhea associated with anosmia is a clear pointer toward Kallmann syndrome. In the absence of anosmia, primary HH is labeled as idiopathic HH. A variety of genes have been identified for the idiopathic HH [12].

In case of suspected organic lesion, the history is very important. The diagnosis is easier in cases where there is a suggestive history, e.g., of accident, surgery, drugs, and systemic illness. In case a tumor or an infiltrative lesion of the brain is suspected, magnetic resonance imaging (MRI) of the pituitary region clinches the diagnosis. Despite all the tests, an idiopathic cause remains the most common diagnosis.

32.4.2 Distinguishing Between Hypothalamic and Pituitary Causes

It is important to distinguish the hypothalamic and the pituitary causes in case of HH because of 3 reasons. Firstly, pituitary lesions may have other concomitant endocrinopathies related to adrenal, thyroid, osmoregulation, or somatotropic axis. The importance of diagnosing other deficiencies is quite obvious. Secondly, any lesion in the pituitary may increase during the pregnancy and cause further complications due to the pressure effects. Thirdly, the method of OI may differ. While hypothalamic amenorrhea can be treated with both pulsatile GnRH and exogenous gonadotropins, HH of pituitary origin has only one option, i.e., the exogenous gonadotropins.

Genetic, systemic, functional, and idiopathic categories are generally hypothalamic in origin (see Fig 32.1). In these cases, the pituitary in addition to ovaries also remains responsive to exogenous GnRH. The pituitary origin of HH is mostly pathological. Neoplastic, infiltrative, vascular, or pituitary stalk lesions need to be ruled out (Fig. 32.1).

Appropriate history, blood tests to rule out comittant endocrinopathies, and an MRI of the brain are sufficient to arrive at the diagnosis. Adequacy of thyroid function can be assessed by serum thyroid-stimulating hormone (TSH) and triiodothyronine (T3) and thyroxine (T4) levels. Serum levels of growth hormone (GH), prolactin, cortisol, and dehydroepiandrosterone give reasonable estimate of other functions of pituitary, thyroid, and adrenal glands.

GnRH stimulation test is used to assess the responsiveness of pituitary. Serial samples of LH and FSH are taken after a bolus administration of native GnRH or GnRH analogue. An intact pituitary should respond with spurt of LH and FSH. Although widely used earlier, it does not give much additional information regarding pituitary adrenal axis or pituitary thyroid axis over the baseline hormone testing. An MRI of the sella is more useful. The role of GnRH stimulation test is to be reserved for those cases where basal hormone measurements are not helpful and where there is a strong clinical evidence of pituitary deficiency [1].

32.4.3 Exclusion of Other Infertility Factors

Before starting OI in these women, the couple must be evaluated to rule out other causes of infertility (infertility factors). The minimum required tests include the semen analysis and a hysterosalpingography (HSG) for tubal evaluation. Endometrial biopsy after an E+P-induced menstruation is indicated only if an infective pathology (e.g., genital tuberculosis) is suspected, such as in high-prevalent countries. There is no requirement of diagnostic laparoscopy or hysteroscopy.

Adequacy of the endometrial response may be assessed with a trial cycle of E administration. Estradiol valerate (E_2) may be given twice or thrice a day. Transvaginal ultrasound done after 10 days of E_2 reflects the endometrial response. This step can be omitted if there is history of good menstrual flow with E+P.

32.4.4 Physiological Basis of Ovulation Induction in Hypogonadotropic Hypogonadism

In women who do not desire pregnancy, the goal of therapy is to maintain adequate menstrual function with cyclic replacement of E+P. This

will ensure general well-being and bone health. OI has to be undertaken in women desirous of pregnancy.

In HH of the pituitary origin, the only option available is to stimulate the ovary directly with the help of exogenous FSH and LH. This therapy is a substitution therapy, i.e., replacement of the deficient hormones. There is another option in hypothalamic amenorrhea. Pituitary can be stimulated with exogenous, native GnRH given in pulsatile manner mimicking the natural pulses. These GnRH pulses stimulate the pituitary to release LH and FSH, which is a more physiological technique.

FSH is the main stimulator of the ovarian follicles and granulosa cells and is indispensable. The role of LH is however not well understood. HH is one naturally occurring experimental model which has clarified the role of LH in ovulation induction. LH is essential for theca cell function. The synergistic but different effect of FSH and LH on granulosa and theca cells, respectively, is the basis of two cell-two gonadotropin theory. Estrogen is produced by granulosa cells under the influence of FSH from the androgen substrate, produced and supplied by theca cells under the influence of LH. LH is not only essential for steroid production but also for the maturation of oocyte and target tissue responses [13, 14]. It has been estimated that levels of 1–10 IU/L of LH should be sufficient to achieve these effects [13]. Moreover, a higher dose of LH may be detrimental and causes atresia of follicles—the LH ceiling effect [14].

Ovarian stimulation with recombinant FSH or FSH alone in women with HH results in follicular growth but E levels remain low [15, 16]. This is in contrast to successful stimulation with FSH alone in pituitary downregulated, normogonadotropic women. In these women, some residual LH activity remains despite downregulation; LH is essential in HH women who are devoid of any endogenous LH activity.

32.4.5 Pharmacological Agents

1. Native GnRh
2. Gonadotropins—urinary or recombinant

32.4.5.1 GnRh: Pharmacology

Native GnRh available in vials is to be given by subcutaneous (SC) or intravenous (IV) routes. A continuous infusion pump has to be used to deliver the precise dose at timely intervals. Local injection site irritation and visibility of the pump are the main disadvantages. Once very popular, pulsatile GnRh is used uncommonly these days. GnRh is not available in India for use.

32.4.5.2 Gonadotropins: Urinary or Recombinant

Either urinary or recombinant gonadotropins are used for ovulation induction. Each ampule of human menopausal gonadotropin (HMG) obtained from the urine of menopausal women contains 75 IU FSH and 75 IU of LH. These are available as HMG or highly purified HMG (HP-HMG) preparations containing 75 or 150 IU of FSH and LH per ampule as lyophilized powder. Urinary HMG can be given only by intramuscular route, while HP-HMG preparations can be given by subcutaneous SC route.

HMG factually contains some LH and mostly LH-like activity which is derived from variable amount of LH and mainly human chorionic gonadotropin (hCG). Urinary LH is highly unstable and has a variable potency. Thus, the LH-like activity is achieved by adding hCG [14].

Recombinant FSH (rFSH) and LH (rLH) are manufactured with genetic engineering technology using Chinese hamster ovary cell lines. Recombinant hormones are highly purified products with consistent batch to batch activity as compared to urinary products. Urinary products are a mixture of various bioisoforms of FSH, while rFSH contains only one isoform and also differs in terminal sialic acid content. Despite extensive purification, urinary FSH preparations retain some LH activity, while rFSH is devoid of any LH activity [16]. Recombinant FSH is available as single- or multiple-dose vials and pens which deliver a very small volume of solution with precision. Two products follitropin alpha and follitropin beta are currently available in the market. In India, many other companies have started marketing recombinant FSH products.

Recombinant LH is available only as 75 IU lyophilized powder. Both rFSH and rLH products are extremely well tolerated and user friendly in administration, although costlier than urinary products. Both products can be used through IM and SC routes. Following single administration, follitropin alpha has terminal half-life of 37 h and 74 % bioavailability [15]. Lutropin alpha has a half-life of about 18 h and bioavailability of 56 % [15].

Conventionally, rLH is given as a single daily injection. Twice daily regimen may have better endocrine profile in the stimulation cycles in HH women [17]. Recently, a mixture of follitropin alpha and lutropin alpha (follitropin alpha/lutropin alpha 150 IU/75 IU) has become available [18]. Early dose finding studies concluded that 75 IU rLH was effective in 94 % of women to achieve adequate follicular maturation and only a few would require a higher dose [19].

The choice between HMG and recombinant preparations depends on the cost and availability. When using high doses, recombinant preparation may have an edge. LH preparation is given separately; dose can be reduced to avoid LH ceiling effect [2]. Urinary products are efficient and cost-effective products. There are no randomized controlled trials comparing the urinary and recombinant products in HH women.

32.4.6 Ovulation Induction Regimens

The objectives of OI in HH are as follows:

1. Monofollicular ovulation
2. Adequate estradiol production
3. Adequate endometrial preparation
4. Timed coitus

32.4.6.1 Pulsatile GnRh

The treatment is suitable for women with intact pituitary, e.g., idiopathic HH or stress-induced amenorrhea [20]). The infusion of GnRh is done with the help of an automated pump at pulse frequency interval between 90 and 120 min [21]. GnRh can be given through IV (5–10 μg/ pulse) and SC (15–20 μg) routes per pulse. IV route is more successful than SC route [21]. Spontaneous LH surge is triggered by rising E levels. An hCG trigger may be given to induce ovulation although it is not mandatory. Intermittent hCG is recommended in the luteal phase since trophic stimulation of corpus luteum from pituitary is lacking in HH cases.

Overall, the treatment results in over 90 % rate of ovulation and a cumulative pregnancy rate of up to 96 % after six cycles [22, 23]. Pulsatile GnRh has also been used successfully for OI in PCOS. In one very large series of 292 anovulatory patients and 900 cycles, there were 130(268) HH women in whom successful ovulation was achieved in 75 % and pregnancy rate in 18 %, per treatment cycle [24]. GnRh was given IV at a dose of 1.25–2.00 micrograms of GnRh every 30–120 min, Maximum cycles required 2.5–5.0 micrograms every 60–90 min. Ovulation and pregnancy rates were higher in all types of HH women as compared to other anovulatory infertilities, like PCOS: only 4 multiple pregnancies occurred (3.8 %) and miscarriage rate was 30 % and even higher in PCOS. No case of OHSS was reported [24].

The main advantage of pulsatile GnRh over gonadotropins is low rate of multiple pregnancy and ovarian hyperstimulation syndrome (OHSS) [22–24]. The main disadvantage is the need to keep the pump connected to the body for quite a long time (2–3 weeks) and the necessity to refill the pump frequently. Once very popular, the treatment is less frequently used in general practice now.

32.4.6.2 Stimulation Regimen with Gonadotropins

Stimulation can be started on any day during amenorrhea or after an E+P withdrawal bleeding. Two to three month priming with sequential E+P treatment may improve response. In author's personal experience, this priming is not required. A novel concept of LH priming has been suggested recently. Pretreatment with 300 IU SC of rLH for 7 days immediately preceding the rFSH significantly decreased the requirement of FSH [25].

Minimum effective dose of FSH is to be selected and a weekly step-up regimen is to be followed.

Table 32.1 Comparison of ovulation induction for anovulation due to hypogonadotropic hypogonadism and polycystic ovary syndrome

Feature	Hypogonadotropic hypogonadism	Polycystic ovary syndrome
Amenorrhea	Always	Oligomenorrhea Amenorrhea
Primary/secondary onset	Both	Both
Pulsatile GnRH therapy	Effective in functional HH only	Effective in all cases
Gonadotropin therapy	Effective	Effective
Oral ovulation-inducing agents	Nil	Clomiphene, letrozole, metformin, etc.
Treatment start	Any day during amenorrhea	After progesterone withdrawal
Starting dose	75–150 IU	37.5 rFSH or even lower
Step-up dose	75 IU	Ultra low-dose step-up
Step-up interval	First step-up 7 days	First step-up 14 days
LH	Mandatory	Avoided
Gonadotropin required	Very high	Low to medium
Duration of stimulation	Longer	Shorter
Multiple pregnancy	High	High
Risk of OHSS	Low	Very high

Most of the ovarian reserve tests, i.e., serum FSH, antral follicle count (AFC), and anti-Müllerian hormone (AMH), do not give a clear idea about the ovarian response. It is best to start with 75 IU and then step up every 5–7 days. In a review article of several studies published from 1966 to 1984, the pregnancy rate varied from 16 to 78 % in different studies [26]. Ovarian response is measured with the help of serum E and transvaginal ultrasound. Triple lining of endometrium on ultrasound is the first sign of ovarian activity and E synthesis. Either of the urinary HMG or recombinant preparations can be used. Coadministration of rLH is mandatory if rFSH used.

The final ovulation trigger is given by hCG injection 10,000 IU when the lead follicle reaches 18 mm in size. This is followed by timed coitus or intrauterine insemination 36–40 h later. Luteal support with hCG decreases the incidence of luteal phase defects and increases the pregnancy rate [27].

There are very few reports in the recent literature on the use of urinary gonadotropins. A combined step-down and step-up approach was also used with the starting dose of 150–225 IU of FSH with 75 IU of LH for 2–3 days, which was then reduced to 75 IU of FSH. Step-up of 75 IU was made every 7 days until follicular growth was achieved [28].

Use of recombinant products gives similar results. Treatment with rFSH and rLH was successful in achieving ovulation in 84 % of cycles, complete luteinization in 80 % of cycles, and pregnancies in 22–24 % of cycles. Cumulative pregnancy rate per patient was 39.5 % [29].

There is no consensus on which is a better regimen—pulsatile GnRh or exogenous gonadotropins. In those cases where pituitary is not functional, the only choice is exogenous gonadotropin. In women with intact pituitary function, the choice depends upon availability, cost, convenience, patient, and physician preference.

32.4.7 Differences Between OI in HH and Polycystic Ovarian Syndrome (PCOS)

The principles of OI are similar in both conditions. PCOS is a very common condition. Most physicians handling OI and infertility get enough experience in managing PCOS. However, one does not see HH cases very often as it is an uncommon condition. There are few important similarities and differences between the OI of PCOS and HH (Table 32.1).

32.4.8 Prognostic Factors

Prognosis of OI and fertility outcome is related to age and the presence or absence of other fertility factors. AMH is generally in the low normal range in HH due to partial gonadotropin-dependent regulation of AMH [30].

The traditional tests, i.e., FSH and serum E levels, remain the preferred choice for the diagnosis of HH However AMH appears to be a promising marker of ovarian reserve and response even in women with HH [31].

The cumulative pregnancy rate in HH with various treatment modalities after 6 cycles reaches up to near 60–70 %. In the remaining women, other incidental infertility factors may be present. In these cases, in vitro fertilization provides excellent results.

32.4.9 Treatment of Functional Disorder: Stress-Induced Amenorrhea

Stress-induced amenorrhea is related to the classic triad of stress, exercise, and dietary restriction. In severe weight loss even without exercise, the hypothalamic pulse generator may be disturbed. The condition is called anorexia nervosa. With increasing participation of women in competitive sports, the effects of strenuous exercise along with dietary restriction, on female reproductive system, are increasingly being stipulated [32]. Even recreational sports have an impact on the highly sensitive HPO axis [33]. Moreover, there is an ever increasing stress of competition with balancing of family and professional lives. In addition to the HPO axis, stress induces disturbance of hypothalamic-pituitary-adrenal axis (HPA axis) and also of thyroid function. Appropriate cognitive behavioral therapy directed toward reducing stress, improving nutrition, and decreasing exercise level may be able to resume ovulation in many such women [34, 35].

Women with functional amenorrhea are hypolepitinemic. Leptin replacement in these women may restore ovulatory menstruation besides correcting metabolic and endocrine derangement [5]. Many women with weight loss-related amenorrhea do not ovulate even after body weight is near normal. In one study, leptin administration resulted in ovulatory menses in three and there was evidence of ovulation in two out of two women [7]. At present, such treatment remains experimental but does hold promise for the future.

32.4.10 Thalassemia Major and Hypogonadotropic Hypogonadism

With the major developments in the field of transfusion medicine and iron chelation therapy, the prognosis for thalassemia major and intermedia has improved significantly, and life expectancy has increased. It is natural to expect the demand for pregnancy from women reaching reproductive years. Besides problems of managing iron overload and need to stop chelation therapy during pregnancy, conception is also a problem. Hypogonadotropic hypogonadism is a common problem in thalassemia major because of the widespread iron deposits. Although fertility can be restored in these women with appropriate OI, it requires a multidisciplinary team to look after these pregnancies. Favorable pregnancy was reported in a large study [36]. Of 46 women of thalassemia major who conceived, 33 did so with gonadotropin OI; others with milder forms of menstrual disturbances conceived spontaneously; 91 % of pregnancies resulted in successful deliveries with appropriate medical management [36].

32.5 Induction of Spermatogenesis in Males with HH

The etiopathogenesis of HH in males is similar to that in females; the congenital form is three to four times more common in males as compared to females [4]. These cases present with delayed puberty erectile dysfunction, loss of libido, and regression of secondary sex characteristics like the testicular size or the facial hair growth. Serum LH, FSH, and testosterone levels are in extremely low range. Fertility is generally not of immediate

concern in adolescent age. However, long-term fertility potential is definitely required. Men present late in life or who have been earlier treated with androgens require induction of spermatogenesis for fertility.

Testosterone replacement is the standard therapy for induction of puberty. This is a cost effective and safe and effective way of inducing puberty and secondary sexual characteristics. Long-term therapy maintains libido and erectile function for long time. Testosterone therapy is a replacement but suppressive therapy. hCG and FSH have been used for spermatogenesis. There is some evidence that induction of puberty and spermatogenesis with gonadotropins or GnRH before testosterone therapy may have an edge over testosterone therapy in preserving fertility potential [37, 38].

Both pulsatile GnRh and gonadotropins can be used. Pulsatile GnRh has to be given through the infusion pump; pulses are given at the rate of 100–400 μng/kg every 2 h.

The duration of therapy is at least 4 months. The main disadvantages are the inconvenience and high cost; this treatment is followed by only few centers now [39, 40].

The standard regimen for induction of spermatogenesis is to give hCG injection 1,500–,000 IV twice or thrice a week. With this treatment, Leydig cells are stimulated to produce testosterone. Puberty can be induced with hCG by itself. It can also induce spermatogenesis but is less efficient for this purpose. FSH in the dose of 150 IV twice or thrice a week after 3 months of hCG priming or alternatively started simultaneously restores spermatogenesis early and with more reliability. The combined treatment may have to be given for up to 2 years [41, 42].

Regardless of the hormones used, the total number of sperms remains below the normal threshold, but 50–80 % pregnancy rate achieved even with sperm concentration of 5 million or less [39, 40] The prognostic factors include higher pretreatment testicular volume, the absence of cryptorchidism, and no previous testosterone therapy [37].

Spontaneous pregnancies occur 6–9 months after start of FSH but can require up to 2 years of treatment. If a pregnancy does not occur after a reasonable time after appearance of sperm in the ejaculate, the couple should be advised intrauterine insemination or ICSI as the case may be. The success of ICSI is similar to that in other cases of male infertility [43, 44].

Failure of gonad therapy is almost unknown. In appropriately diagnosed cases of HH, if sperms do not appear in ejaculate after 3–6 months of therapy, then an additional cause for azoospermia need to be ruled out. There may be congenital or acquired obstruction in the vas deferens, seminal vesicles, or ejaculatory ducts. In these cases, the efficacy of gonadotropin therapy can be judged by increase in testicular volume, vascularity, testosterone levels, and fine needle aspiration cytology from testes.

Hypogonadotropic hypogonadism is a challenging disorder in both males and females with appropriate management; the result is very dramatic and rewarding.

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Ovarian Stimulation Protocols in Fertility Preservation

33

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Abstract

Established fertility preservation options in the postpubertal female patient with cancer include embryo and oocyte cryopreservation. Controlled ovarian hyperstimulation (COH) is required for both of these procedures, which may (conventional) or may not (random start) coincide with the menstrual cycle. Understanding of the folliculogenesis has led to establishment for random-start protocols that are now being extensively utilized prior to embryo or oocyte cryopreservation. Correct identification of the phase of the menstrual cycle is essential prior to using random-start protocols.

Keywords

Cryopreservation • Fertility preservation • Gonadotropins • Infertility • Neoplasms • Ovarian stimulation

33.1 Cancer and Fertility

Improvement in cancer therapies has led to improvement in cancer survival rates. This has not only led to increase in life expectancy but also highlighted issues affecting long-term survivors. The toxic effects of some of the chemotherapeutic agents like alkylating agents (e.g., cyclophosphamide, busulfan, and ifosfamide), which are common components of chemotherapy for breast cancer, lymphomas, leukemia, and sarcomas, are well documented. Pelvic radiation therapy is also known to cause follicular destruction, and exposure to 5–10 Gy pelvic radiation appears to be toxic to oocytes, resulting in premature ovarian insufficiency in

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many women. These effects are dependent on age of the women at the time of treatment and the baseline ovarian reserve prior to starting the treatment for cancer. The potential adverse effect of the disease process itself remains unknown.

33.2 Options of Fertility Preservation in Cancer Patients

There is importance of reproduction for many cancer patients especially those who are young. In a survey conducted on young women affected by breast cancer [1], more than half of respondents were concerned with infertility as a side effect. As a part of cancer care and counseling, patient should be made aware of the possible long-term consequences of cancer therapy in the form of impairment of fertility. Available fertility preservation options should be discussed as a part of cancer counseling. On the other hand, the discussion with regard to fertility preservation might not be taken that well and can pose a challenge accommodating this treatment in between the treatment of cancer. This should be done in a best possible way jointly with treating oncologist. The time interval available between the diagnosis and the initiation of the cancer therapy is an important aspect as to which available option to be utilized prior to starting cancer treatment. Embryo cryopreservation and oocyte cryopreservation are the standard options available at present. Embryo cryopreservation and oocyte cryopreservation both need ovarian stimulation prior to oocyte recovery. Other options such as cortical tissue cryopreservation, in vitro maturation of the immature oocytes, and ovarian cryopreservation are very promising but at this stage are experimental and need further trials. Use of medical therapies like gonadotropin-releasing hormone (GnRH agonist) is controversial and needs further robust randomized controlled trials.

33.3 Effect of Cancer on Ovarian Stimulation

Controlled ovarian hyperstimulation (COH) is essential for embryo and oocyte cryopreservation. This can be achieved with either conventional methods of ovarian stimulation or with random-start ovarian stimulation protocols. Recent studies [2, 3] have demonstrated no significant change in ovarian reserve or response to gonadotropins in patients with different cancers who are undergoing IVF treatment. By contrast others [4, 5] have found a decline in ovarian response in cancer patients who are undergoing ovarian stimulation protocols to preserve their fertility prior to cancer therapy. A recent meta-analysis [6] found a reduced number of oocytes in patients with malignancies undergoing COH for fertility preservation. One of the theories behind this varied response to ovarian stimulation is put down to fact that cancer is typically a catabolic state increasing stress hormone levels and altering the hypothalamic pituitary axis, thereby impairing reproductive capacity. Another study [7] to evaluate COH in women with cancer compared with healthy women found no significant differences in the number of oocytes retrieved, number of mature oocytes retrieved, and the number of oocytes fertilized. However, the patients with cancer had longer duration of stimulation and higher total dose of gonadotropin, which could be explained by some element of hypothalamic dysfunction. There is also varied stimulation response seen in cancer patients positive for BRCA mutations. Mutations in BRCA genes are associated with an increased risk of breast and ovarian cancers. A low response to ovarian stimulation in BRCA mutation positive breast cancer patients was seen in another study [8].

33.4 Ovarian Stimulation Protocols in Cancer Patients

Proper understanding of the reproductive endocrinology is essential when planning ovarian stimulation protocols for such group of women as they may

present in any part of their menstrual cycle, and achieving optimal reproductive outcome is of paramount importance in such group of women as they may not get another chance of considering their reproductive potential. Protocols for ovarian stimulation can be broadly divided into conventional and random-start ovarian stimulation protocols.

33.4.1 Conventional Ovarian Stimulation Protocols (COS)

Conventional methods of ovarian stimulation are related with onset of menstrual cycle. It involves initiation of the gonadotropins for ovarian stimulation in the follicular phase of the menstrual cycle, as this has been thought to improve the clinical outcomes. The suppression of LH surge for spontaneous ovulation has been successfully achieved either with gonadotropin-releasing hormone (GnRH) agonist started in the preceding luteal phase or with gonadotropin-releasing hormone (GnRH) antagonist started when the dominant follicle has attained diameter of 14 mm or on day 6 of ovarian stimulation. GnRH antagonist may offer advantage in ovarian stimulation for cancer patients by shortening the time frame from patient presentation to embryo/oocyte cryopreservation, but this protocol still requires onset of menses before initiating ovarian stimulation. Awaiting for onset of menstrual cycle prior to starting ovarian stimulation for fertility preservation prior to initiation of cancer treatment can increase the stress and anxiety of patient and treating oncologist with regard to outcome of cancer therapies and hence prevent patients from forgoing fertility preservation treatment. This has been overcome by random-start ovarian stimulation protocols.

33.4.2 Random-Start Ovarian Stimulation Protocols (ROS)

With better understanding of the reproductive physiology, endocrinology, and ovarian folliculogenesis, random-start ovarian stimulation protocols have been developed to minimize the time interval needed for initiation of ovarian stimulation and hence with a hope to increase the uptake of these fertility-preserving strategies. It is no longer felt that there is a single wave of folliculogenesis initiating in the follicular phase but there are two to three waves in one interovulatory cycle. Studies on changes in ovarian follicular dynamics during the human menstrual cycle have now well documented that there are wavelike changes in follicle number rather than a single cohort of antral follicles occurring only during the follicular phase of menstrual cycle. This concept has been used in providing ovarian stimulation in any part of menstrual cycle and has been extrapolated to providing ovarian stimulation prior to cancer therapies as this subgroup of women can present in any part of menstrual cycle. The rest of the pattern of stimulation with regard to dose and type of gonadotropins is very much similar as in conventional protocols.

Depending on the timing of presentation in relation to menstrual cycle, random-start protocols can be divided into starting controlled ovarian stimulation in early follicular phase, late follicular phase, early luteal phase, and late luteal phase (Fig. 33.1) [9]. Early follicular phase is defined as presentation within first 7 days of onset of menstruation. Late follicular phase is primarily defined as presentation after 7 days of onset of menstrual cycle or the presence of dominant follicle of >12 mm diameter or progesterone level <2 ng/ml. Early luteal phase is defined as presentation within first 7 days after ovulation. Late luteal phase is presentation within last 7 days of the last menstrual cycle. Late follicular phase and early luteal phase are the most challenging time for random-start ovarian stimulation protocols.

33.4.2.1 Early Follicular Phase Protocol

Presentation in early follicular phase can very similarly be treated like convention stimulation regimens with GnRH antagonist protocol provided there is no dominant follicle more than 12 mm. If there is already dominant follicle more than 12 mm, it can then be treated as being in late follicular phase, which will be discussed subsequently (Fig. 33.1).

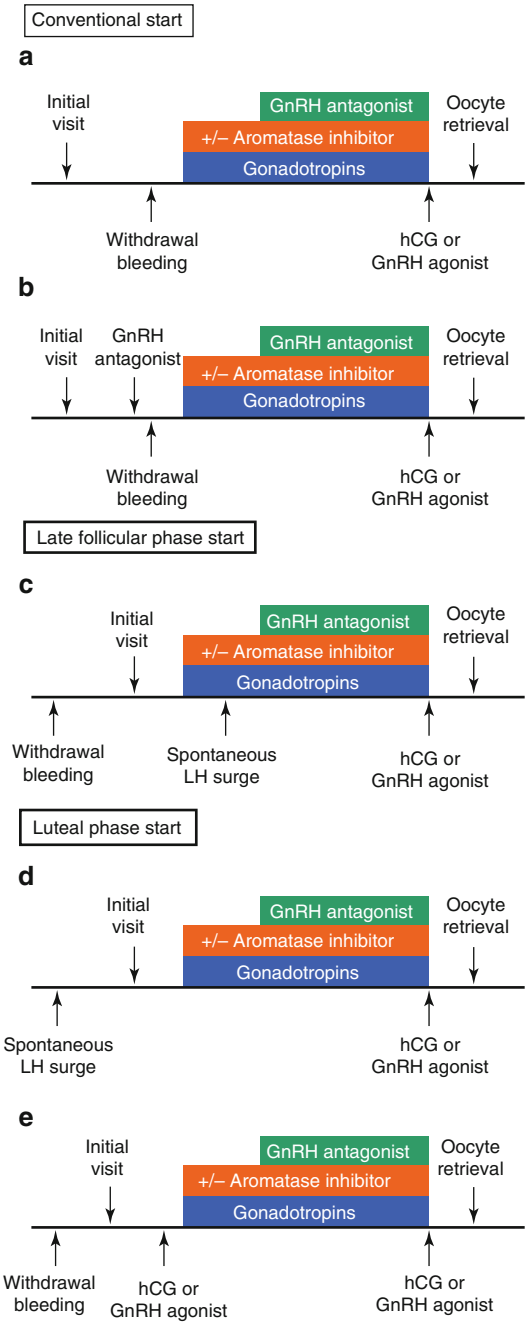

Fig. 33.1 Conventional and random-start antagonist IVF protocols for cancer patients undergoing fertility preservation. COS can be started with spontaneous menses (**a**) or with menses following luteolysis induced by GnRH antagonist (**b**). COS can also be initiated in the late follicular (**c**) or luteal phase following spontaneous LH surge (**d**) or after ovulation induction with hCG or GnRH agonist (**e**) (From Cakmak and Rosen [9])

33.4.2.2 Late Follicular Phase Protocol

Late follicular ovarian stimulation involves assessment of the ovaries for the presence or absence of dominant follicles. If the ovary contains dominant follicle more than 12 mm, further management would involve luteolysis of the dominant follicle with either GnRH antagonist or progesterone followed by stimulation for the rest of the follicles. Prior to luteolysis, triggering of the already grown follicle by HCG or GnRH agonist may be considered. This can then be followed 36–40 h later by luteolysis with GnRH antagonist for 3–4 days. Depending on the ovarian reserve of the patient, ovarian stimulation can then be started by appropriate gonadotropins based on age and ovarian reserve parameters (AMH, antral follicular count) (Fig. 33.1).

33.4.2.3 Early and Late Luteal Phase Protocol

Early luteal phase is defined as the completion of spontaneous LH surge and the presence of corpus luteum in the ovaries. Early and late luteal phase can be essentially treated in the same way. Luteolysis of the corpus luteum can be achieved by GnRH antagonist 3 mg stat or in multiple doses of 0.25 mg. This can be followed by gonadotropin stimulation in conventional way preferably with recombinant FSH avoiding exogenous LH activity, which might prevent luteolysis. Pituitary suppression for LH surge by GnRH antagonist can be initiated in usual way when the dominant follicle reaches 14 mm and use of either HCG or GnRH agonist for trigger of the final maturation of the oocyte. (Fig. 33.1)

The dose of gonadotropin should be individualized depending on age, ovarian reserve, and weight of the patient. Using higher doses of gonadotropins can be one of the strategies to increase the embryo and oocyte yield per cycle. In a study comparing a low-dose antagonist IVF protocol (150 IU FSH) and a higher-dose antagonist IVF protocol (>150 UI) in cancer patients, although the number of follicles > 17 mm was greater in the higher-dose group, there was no difference in the number of oocytes generated between the two groups [10]. That study suggests

that the use of higher doses of gonadotropins may not necessarily result in higher oocyte/embryo yield consistent with the theory that higher doses of gonadotropins may stimulate the recruitment of chromosomally abnormal or incompetent oocytes [11]. However, in patients with decreased ovarian reserve as assessed with the use of AFC and/or AMH, higher doses of gonadotropins may be required.

The studies have suggested that these protocols have been able to provide reasonable number of mature eggs able to fertilize but the exact implantation potential and subsequent pregnancy rates of embryos and oocytes retrieved through these protocols are still unanswered. Being relatively new and being tried mainly in cases in cancer patients, these have been reported in very few studies.

33.4.2.4 Controlled Ovarian Stimulation with Estrogen-Sensitive Tumors

Controlled ovarian stimulation in women with estrogen-sensitive tumors is another challenge and should be dealt with caution. This can be achieved with addition of letrozole or tamoxifen to the above protocols to nullify the effect of rising estradiol levels during stimulation. Study by Oktay et al. [12] concluded that stimulation protocols using letrozole alongside gonadotropins are currently preferred over tamoxifen protocols as treatment with letrozole results in a higher number of oocytes obtained and fertilized when compared to tamoxifen protocols.

There has been case report by Reichamn et al. [13] of using these ovarian stimulation protocols for oocyte vitrification in prepubertal girls diagnosed with cancer that have not yet attained menarche and hence got no gonadotropin recruitable follicles. This has been reported as having successful ovarian stimulation and oocyte retrieval. This approach needs further robust evidence before it can be widely applicable to such group of cancer-affected females.

The goal of superovulation for this group of patients represents a compromise between obtaining a relatively large number of oocytes for vitrification, to maximize chances of later pregnancy, and the absolute need for avoidance of ovarian hyperstimulation in a patient who will shortly begin chemotherapy.

33.5 Complications of Controlled Ovarian Stimulation in Cancer Patients

The patients referred for fertility preservation may not necessarily represent the typical population of subfertile patients treated in IVF units. Cancer may affect multiple tissues throughout the body and can result in variety of complications during ovarian stimulation. Cancer may induce hypercoagulable state, and when this is combined with increased serum estradiol levels, it may put cancer patients undergoing controlled ovarian stimulation at an increased risk of thromboembolic events. Therefore, consideration should be given for commencement of anticoagulation therapy around the time of ovarian stimulation. The other strategy of preventing thromboembolic events is to use letrozole during ovarian stimulation as in women undergoing ovarian stimulation with estrogen-sensitive malignancies to keep estradiol levels close to <500 pg/ml. Letrozole at 2.5 or 5 mg/day can be started with ovarian stimulation and can be titrated up to 10 mg/day depending on the estradiol levels. Letrozole or GnRH antagonist should be continued even after oocyte retrieval for up to a week depending on the estradiol levels at the time of ovulation induction.

Some blood-borne malignancies may alter the hemostatic function and may create a tendency toward bleeding during oocyte retrieval owing to thrombocytopenia, platelet dysfunction, or defective coagulation factor synthesis. Collaborative team approach involving hematologist and anesthetist should be preferred to prevent complication of bleeding in these patients. Pelvic infection after oocyte retrieval can be a problem especially in neutropenic patients. In the case of neutropenia, consultation from the patient's oncologist for the use of granulocyte colony-stimulating factor to increase the neutrophil count should be obtained, and prophylactic antibiotics should be given before oocyte retrieval to decrease the risk of

infection. Cancers involving tracheal compression, or large pleural effusion, may preclude safe administration of anesthesia for oocyte pickup. This should be discussed with anesthetists prior to starting ovarian stimulation.

In cancer patients, antagonist protocols should be preferred and where possible trigger for final maturation to be planned with GnRH agonist to prevent the risk of OHSS. The impact of OHSS can be profound and all measures should be taken to prevent it. In cancer patients, its effect can be more profound as it may result in delaying or complicating planned life-saving cancer therapy.

Another aspect of ovarian stimulation, which needs further consideration, is the fact that success in vitrification has been seen in nonmalignant healthy infertile population, and little information is obtained about vitrification in cancer patients though studies have shown no difference in oocytes and embryos derived from patients with cancer and in nonmalignant conditions.

Conclusion

Successful implementation of fertility preservation services requires establishing liaisons with reproductive specialists and oncology colleagues. While survival is clearly the most important issue facing a young woman with cancer, it is clear that future fertility is also important. A rapid and efficient referral system with minimal delay can bring good results, and this can be established in close collaboration and effective communication with colleagues in medical, surgical, and radiation oncology.

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Ovarian Stimulation in Cancer Patient

34

Sohani Verma

Abstract

Ovarian stimulation in cancer patients is a relatively new and challenging concept. A large number of cancer patients in the reproductive-age group are now expected to survive and lead a normal life. However, various therapies responsible for this markedly improved prognosis can also cause a significant detrimental effect on the reproductive ability. It is important that all young patients diagnosed with cancer are fully informed and offered the option of various fertility preservation strategies. Ovarian stimulation followed by either embryo or mature oocyte cryopreservation is currently the most successful option. Special considerations are required while performing ovarian stimulation in cancer patients. A detailed pretreatment assessment, counseling, and proper selection of ovarian stimulation protocol within the available short time period with minimum side effects are crucial to maximize the success of fertility preservation.

Keywords

Ovarian stimulation • Cancer patient • Current cancer • Previous/past history of cancer

34.1 Introduction

With significant improvement in survival rates for various types of malignancies, mainly due to the recent advancements in early diagnosis and treatment, more and more patients with either current or past diagnosis of cancer are now seeking infertility treatment.

The other important reason for assisted reproduction specialists to come across these patients more often is that infertile patients are reported to have higher incidence of cancer [1].

The infertile nulliparous women especially those with unexplained infertility have been associated with an increased risk of developing ovarian and uterine cancers (standardized incidence

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ratio [SIR] 2.64 [95 % CI 1.10–6.35] and 4.59 [95 % CI 1.91–11.0] respectively) [2].

Several confounding factors such as infertility itself rather than the medications may well be the reason for the increased incidence of cancer among infertile women [1].

Infertility practitioners can be faced with two different subgroups of cancer patients. The first group consists of those women recently diagnosed with cancer and have been recommended either surgery or chemotherapy/radiotherapy with potential to cause significant compromise to their reproductive ability. These women may not have had subfertility issues as such, but they wish to preserve their chance of having a child in the future. The treatment of this group presents several unique challenges. Cancer may affect multiple tissues throughout the body and can result in a variety of complications during controlled ovarian stimulation [3]. A multidisciplinary approach including the oncologist, psychologist, and reproductive medicine specialist is imperative to counsel and help the patient to make an informed choice. The task is especially difficult as the patient and family are still going through the shock and acute distress of being diagnosed with cancer and their maximum focus is to get the treatment started at the earliest.

Multiple strategies have emerged in the recent times aiming to preserve fertility in such women. These include embryo and oocyte (both mature and immature) cryopreservation, cortical and whole ovary cryopreservation, ovarian transplantation, ovarian transposition, and GnRH agonist protection [4]. Recent advances in the technology of vitrification of human oocytes and embryos have increased the opportunities for this group of women [5]. Current statistics of chances of live birth from cryopreserved ovarian tissue are depressingly poor, with a handful of births reported worldwide [5]. Currently, embryo and mature oocyte cryopreservation following in vitro fertilization (IVF) are the only techniques endorsed by the American Society of Reproductive Medicine, and all other methods are still considered to be investigational [6, 7]. Ovarian stimulation for these patients in order to retrieve good quality sufficient number of oocytes under the time pressure with minimum side effects is extremely important and remains a challenging task.

The second group of patients is those who had suffered and survived the cancer either during their childhood or reproductive age. More than 7,90,000 new female cancer cases were estimated to be diagnosed in 2012 in the United States [8]. Substantial improvements in cancer treatment have greatly increased 5-year survival rates in these women. From 2002 to 2012, 83 % of women younger than 45 years diagnosed with cancer survived [9]. The treatment for most of the cancer types in reproductive-age women involves either removal of the reproductive organs or cytotoxic treatment (chemotherapy and/or radiotherapy that may partially or definitively affect reproductive function) [10]. Infertility is reported to be a major concern as a long-term effect of these treatments, especially in female cancer survivors [11, 12].

Quantification of the risk of reproductive dysfunction after cancer treatment (radiotherapy to pelvic organs and chemotherapy regimens containing alkylating agents) is a major challenge [13]. Menstruation is not a sensitive way to identify the gonadotoxic effects of treatment [14]. Barton et al [13] noted an increased risk of infertility in cancer survivors at very young ages, even though many resumed menstruation, showing that the menstrual function does not equate to normal fecundity. The fertility preservation should be considered in these cancer survivors if they are not ready to attempt conception [13].

34.2 Pre-ovarian Stimulation Assessment and Counseling

Controlled ovarian stimulation (COS) is the key step for embryo or mature oocyte cryopreservation. The number of oocytes retrieved and their quality are imperative factors to predict the potential efficacy of the fertility preservation program [3].

The antral follicle count (AFC) and measurement of anti-Mullerian hormone (AMH) are the

two most important tests to assess the ovarian reserve and counsel the patient about predicted response. This information is also valuable to decide the COS protocol and starting dose of gonadotropins. The ovarian response will also be influenced by the patient-specific factors, most importantly female age. A detailed clinical history including previous pregnancies and menstrual and gynecological history and a physical examination to assess pelvic anatomy as in any other case for ovarian stimulation are important and must be carried out. A complete medical assessment of patient's health and professional counseling are essential prerequisites for the treatment in cancer patients. Many women may be systemically unwell with contraindications to anesthesia or surgical oocyte collections [5].

There are mixed reports about the response of cancer patients to COS protocols: some reporting no significant change [15–17] and others demonstrating worse ovarian response in cancer patients compared with age-matched healthy women [18–20]. Both the malignancy and the patient's multisystemic condition may have an impact on the response to ovarian stimulation [20]. It has been reported that in patients with BRCA-1 mutations, oocytes may be more prone to DNA damage, clinically manifesting as diminished ovarian reserve or earlier menopause [21]. These patients should be informed that the expected number of oocytes retrieved after COS may be lower compared with healthy patients of similar age. However, more studies are needed to confirm these findings [3].

In one study, AMH was found to be significantly lower in patients with lymphoma before chemotherapy compared with healthy control subjects [22]. Ebbel et al. [23] demonstrated that women with cancer before gonadotoxic therapy may have significantly lower AFC compared with healthy women aged 25–40 years.

Some women may have estrogen-sensitive tumors, which may be stimulated during superovulation with gonadotropins [5]. The question of the possible posthumous utilization of their gametes or embryos needs to be addressed in a sensitive but clear manner, with a written declaration of intent [5].

34.3 Ovarian Stimulation Protocols for the Cancer Patients

The main objective of COS in women with current cancer is to retrieve sufficient number of good quality oocytes within the shortest possible time with minimal risks. There is absolute need for avoidance of ovarian hyperstimulation syndrome (OHSS) in a patient who will shortly begin chemotherapy [5]. The choice of the protocol is influenced by the time frame available, potential side effects, tumor biology, and the menstrual cycle phase.

There is a potential risk that the supraphysiological E_2 levels during COS with gonadotropins may promote the growth of estrogen-sensitive tumors such as endometrial and estrogen receptor-positive breast cancers [24]. The rise in E_2 is directly proportional to the number of follicles recruited to grow. Therefore, alternative and potentially safer protocols have been suggested for this group of patients.

34.3.1 Choice of COS Protocols for Cancer Patients

- Natural-cycle protocol
- Tamoxifen alone
- Tamoxifen combined with gonadotropins
- Aromatase inhibitors (letrozole)
- Aromatase inhibitors combined with gonadotropins
- Gonadotropins
- Long agonist protocol
- Short agonist protocol
- Antagonist protocol
- Conventional-start protocol
- Random-start/any phase stimulation protocol

34.3.2 Natural-Cycle IVF/ICSI Protocol

Since the elevation of estradiol levels is undesirable in estrogen receptor-positive cancer women, these patients have been offered natural-cycle IVF,

which resulted in a single embryo in approximately 60 % of the preservation cycles [25].

However, for those patients diagnosed with current cancer, they usually have a single cycle opportunity owing to the time constraints. Maximizing the number of oocytes and embryos is extremely important; therefore, natural-cycle IVF giving only one or two oocytes and high rate of cycle cancelation is ineffective and not recommended for the purpose of fertility preservation [3].

34.3.3 Tamoxifen

It is a nonsteroidal triphenylethylene compound related to clomiphene and known to have an antiestrogenic action on breast tissue. This acts by inhibiting the growth of breast tumors by competitive antagonism of estrogen at its receptor site and accepted as first-line drug in hormonal prevention and treatment of estrogen receptor-possible breast cancer [26]. The selective antagonist action of tamoxifen on the estrogen receptors in the central nervous system (similar to that of clomiphene) leads to an increase in GnRH secretion from the hypothalamus and a subsequent release of FSH from the pituitary, resulting in the stimulation of ovarian follicular development [3].

Tamoxifen can be used for COS alone starting on day 2–5 of the menstrual cycle in doses of 20–60 mg/day or in combination with gonadotropins, similar to the use of clomiphene [27]. Its usage has been suggested in estrogen receptor-positive breast cancer patients and shown to increase the mature oocyte and embryo yield compared with natural-cycle IVF (1.6 vs. 0.7 and 1.6 vs. 0.6, respectively) and reduce cycle cancelations [25]. When combined with gonadotropins, there is further increase in the number of oocytes (5.1 vs. 1.5) and embryos (3.8 vs. 1.3) [28]. As tamoxifen has stimulatory effect on the endometrium, it cannot be used in women with endometrial cancer for ovarian stimulation.

34.3.4 Aromatase Inhibitors: Letrozole

The third-generation aromatase inhibitors such as letrozole significantly reduce the risk of recurrence in postmenopausal women with hormone receptor-positive breast cancer. Centrally, these release the hypothalamic-pituitary axis from estrogenic negative feedback, increase the secretion of FSH by pituitary gland, and thereby stimulate follicle growth [29]. Stimulation protocols using letrozole with gonadotropins are currently preferred over tamoxifen protocols due to higher number of oocytes obtained and fertilized when compared to tamoxifen protocols [28]. The main advantage of adding daily letrozole to gonadotropins COS protocols is to decrease serum E_2 levels to be closer to that observed in natural cycle which is <500 pg/ml, without affecting oocyte or embryo yield [30, 31].

In a study [31] comparing the letrozole plus gonadotropin protocol in breast cancer patients and the standard IVF protocols in age-matched noncancer patients with tubal-factor infertility, letrozole 5 mg/day was started on day 2 or 3 of cycle, and FSH 150–300 IU/ day was added 2 days later. All medications were discontinued on the day of hCG trigger. The letrozole was reinitiated after oocyte retrieval and continued until E_2 level fell to <50 pg/ml. The results were similar in terms of the number of total oocytes retrieved and length of COS in both groups [31]. The recommended dosage of letrozole is 2.5–10 mg/day [3]. Oktay et al. [31] demonstrated that the best maturity and fertilization results are achieved when hCG is given at 19.5–20.5 mm rather than traditional criteria of 17–18 mm.

34.3.5 Gonadotropins

34.3.5.1 GnRH Agonist Protocols

Traditional long downregulation GnRH agonist-based COS protocol requires 3–4 weeks preparation before oocyte retrieval. As there is usually considerable time constrains and patient would like to start chemo- or radiotherapy at the earliest possible, the long protocol is not a preferred option in the cancer patients.

Short "flare-up" protocol using GnRH agonist from day 1 of the cycle and gonadotropins from day 2 or 3 of the cycle can be used if these patients present at the appropriate phase of the menstrual cycle. No significant difference in pregnancy rates has been reported between short "flare-up" and antagonist cycles [31].

34.3.5.2 GnRH Antagonist Based COS Protocols

The majority of patients are treated with a GnRH antagonist-based protocol, which allows the shortest deferral of the initiation of radio-/chemotherapy [3].

Conventional-Start Antagonist-Based COS Protocol Gonadotropins are started on day 2 of the menstrual cycle. As GnRH antagonists immediately suppress the pituitary release of FSH and LH and thereby prevent premature LH surge, these are initiated when the size of the lead follicle reaches 12–14 mm or from day 6 of gonadotropins stimulation.

This approach still requires awaiting menses before initiating gonadotropins, but it decreases the interval to oocyte retrieval compared to conventional long agonist protocols.

34.3.5.3 Random-Start/Any Phase COS Protocol

Random-start protocols are stimulation protocols, which can start on any day of the cycle as these patients do not have much time before chemotherapy (See Fig. 33.1)

Luteal phase-start protocol. The use of GnRH antagonists during the preceding luteal phase was explored originally for cancer patients and then for poor IVF responders as a method to improve ovarian stimulation by inducing corpus luteum breakdown and synchronizing the development of the next wave of follicles [32]. If a GnRH antagonist (single dose of 3 mg cetrorelix) or 250 mcg once daily for 2–3 days subcutaneously) is given during the midluteal phase, menses ensues a few days later [32]. This would minimize the potential delay in oocyte retrieval and thereafter starting the cancer treatment.

Late follicular phase protocol. The late follicular phase has been defined as after day 7 of the menstrual cycle with emergence of a dominant follicle (>13 mm) and/or progesterone level < 2 ng/ml. In a study reported by Cakmak et al. [33], if the cancer patient presented in the late follicular phase, then one of the following treatment plans were employed:

- COS started without GnRH antagonist after the LH surge. GnRH antagonist was started later in the cycle when the secondary follicle cohort reached 12 mm.
- Ovulation was induced with hCG or GnRH antagonist followed by start of the COS in 2–3 days in the luteal phase.

The authors reported that the numbers of total and mature oocytes retrieved and fertilization rates were similar between groups. However, the length of COS was 2 days longer, and therefore, the total dose of gonadotropin used was significantly higher in late follicular and luteal phase-start groups compared with the conventional-start group [33].

This approach overall provides a significant advantage by decreasing total time for the IVF cycle [3]. This is consistent with a newer concept of ovarian physiology, which indicates that there are multiple waves of follicle recruitment during each menstrual cycle [34]. Further clinical studies are needed to assess the efficacy of this strategy.

34.4 Final Oocyte Maturation and Prevention of OHSS

Ovarian hyperstimulation syndrome (OHSS) is the most serious complication of COS; it is important to balance the risk of OHSS and obtain sufficient number of oocytes or embryos to maximize the chances for a successful pregnancy in the future [3]. The impact of OHSS can be even more serious in cancer patients as it would further delay or complicate their planned cancer treatment.

As hCG trigger is well known to be associated with risk of including OHSS, it has been suggested to use GnRH agonist instead in GnRH antagonist-based cycles. The dosage between 1 and 4 mg leuprolide acetate has been recommended in the studies [3, 35].

Intracytoplasmic sperm injection (ICSI) on retrieved oocytes rather than simple IVF is recommended to avoid risk of failed fertilization. Depending on the patient's age, a survival rate of the embryos following thawing of 35–90 %, an implantation rate of up to 30 %, and a cumulative pregnancy rate of 30–40 % can be achieved [36, 37]. With vitrification freeze-thaw protocols,

promising results with more than 60 % of mature oocytes surviving after thawing and subsequent fertilization have been reported [38, 39].

Conclusion

With significant improvement in the survival rates, quality of life issues- especially the reproduction has become an important aspect. A detailed and professional counseling regarding fertility preservation is an essential part of comprehensive cancer care [3]. Assessment of ovarian reserve and prediction of ovarian response to COS are important to decide right COS protocol. While the main objective is to retrieve maximum number of good quality oocytes, the potential risks must be avoided. Letrozole plus gonadotropins COS protocol is an effective and safe option in patients with estrogen-sensitive cancers undergoing fertility preservation [3]. A random-start or any phase protocol is an emerging new strategy. Further studies with long-term follow-up to evaluate the pregnancy and live birth rates are necessary to prove the efficiency of newer protocols.

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Part VII

Negative Impact of Controlled Ovarian Stimulation

Epigenetics and Ovarian Stimulation

35

Jayant G. Mehta

Abstract

In recent years, a number of reports have linked epigenetics and ovarian stimulation to human-assisted reproductive techniques (ART). These reports have alluded to the fact that the pathological causes of diseases such as Beckwith-Wiedemann syndrome (BWS-OMM #130650) and Angelman syndrome (AS-OMIM #105830) may be associated with epigenetic disruption of chromosomal regions, or epimutations, as a consequence of defective DNA methylation status of imprinted genes. The acquisition of a unique epigenetic profile in a small subset of genes in the male and female germlines is time specific during the development of gametes. It involves a well-orchestrated expression of enzymes. Three important mechanisms that are involved in the imprinting process include DNA methylation, post-translational modification of histone proteins, and remodeling of chromatin and RNA-based mechanisms. Genomic imprinting once established in the germline must remain unaltered following fertilization of these gametes and throughout the life of the offspring. This observation raises possibility for ART-induced epigenetic disturbance during the maintenance of these imprints in early embryonic life. How genomic imprinting may be influenced by ovarian stimulation is explored here. These differential epigenetic marks in the gametes result in a parent-of-origin-specific expression of these imprinted genes in the offspring. Based on the mouse model and limited ART human observations, the consequences of dose-dependent hormonal superovulation and how it may affect the genomic imprinting are discussed. The mechanisms involved in epigenetic deregulation and reprogramming of gametes as well as early embryos are also considered.

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Keywords

Epigenetics • Ovulation induction • DNA methylation • CpG • Genomic imprinting • Ovarian stimulation

35.1 Introduction

The birth of Louise Brown, in 1978, heralded an era of assisted reproduction. The number of children born with the help of assisted reproductive technologies (ART) has been steadily growing, approaching almost 2–3 % of the total births in developed countries [1, 2]. Although the use of ART continues to grow exponentially, animal studies have suggested the possibilities of superovulation and embryo culture media affecting non-genomic inheritance, lending biological plausibility to the concern that ART may increase the risk of imprinting disorders [3–5]. In the early 2000s, a number of reports were published that have linked human-ART by alluding to the fact that the pathological causes of diseases such as Beckwith-Wiedemann syndrome (BWS-OMM #130650) [6–10] and Angelman syndrome (AS-OMIM #105830) [9, 11, 12] are a direct result of epigenetically based defective DNA methylation status of imprinted genes. Realization and understanding of the causes and the mechanisms involved in epimutations have emerged as an urgent task for all specialists in genetics and reproductive medicine and have triggered efforts aimed at ensuring safety of ART. The aim of this chapter is to review the evidence associated with abnormal epigenetic modification of the genome, primarily defective DNA methylation, within ART processes and possible contribution of ovarian stimulation to these epigenetic modifications.

35.2 Epigenetics, DNA Methylation, and Genomic Imprinting

35.2.1 Epigenetics

Epigenetics (epi- Greek: epí- over, outside of, around, above -genetics), a term coined by Conrad Waddington (1942), describes changes in the gene expression, gene activity, and phenotype caused by mechanisms, not involving base pair changes in the underlying DNA sequence, during the development of an individual. However, the classical definition describes epigenetics as a science-investigating mechanism by means of which a genotype produces the phenotype [13, 14]. Jean-Baptiste Lamarck (1744–1829) formulated the theory that the environment shapes genes, and these changes are passed on to the offspring. It was Charles Darwin (1809–1882) who postulated that genes are not changed by the environment but formulated through natural selection. Epigenetic phenomena, therefore, manifest upon any changes in the realization of hereditary information from DNA transcription to RNA translation and processing of the protein molecule. It is understood that epigenetic modifications are inherited over cell generations and express long-term position effect in the genome. These modifications are reversible [15, 16]. Epigenetics and biological processes are known to influence reprogramming in early development, X-chromosome inactivation, cancer, obesity, and biobehavioral reproduction. It is therefore not surprising that ART interventions may have the potential to affect epigenetic processes at multiple stages of gametogenesis and embryo development. The mechanisms and control of the epigenetic alternations of DNA are still being unraveled. However, the three mechanisms that have been identified so far include:

1. DNA methylation
2. Posttranslational modification of histone proteins and remodeling of chromatin
3. RNA-based mechanisms—small interfering RNAs (e.g., miRNAs)

35.2.2 DNA Methylation and ART Procedures

One of the most intensely studied and first identified epigenetic modification in mammals is

DNA methylation. In normal cells, it ensures the proper regulation of gene expression and stable gene silencing. During DNA methylation, a methyl group (−CH3) is transferred from S-adenosylmethionine (SAM) and covalently added to the fifth carbon of a cytosine base on a cytosine guanine (CG) dinucleotide [17]. This process is further associated with histone modifications (acetylation/deacetylation and methylation). Functionally, significant DNA methylation in the mammalian genome occurs mainly at cytosine bases included in CpG dinucleotides (p indicates the phosphate group linking the two bases).

The interplay of these epigenetic modifications is crucial in regulating the functioning of the genome by changing chromatin architecture, and it is sometimes associated with antisense RNAs [18, 19]. Generally speaking, methylation silences gene transcription through structural blocking of transcriptional factor which binds to DNA by the presence of a methyl group [20]. While DNA methylation is responsible for silencing of imprinted genes and inactivation of X-chromosome, few genes, such as IGF2 and IGF2R, are activated by methylation [21]. DNA methyltransferase (DNMT) catalyzes the methylation process and acts in conjunction with methyl-CpG-binding proteins, interacting corepressors, and transcription factors. Moreover, DNMT1 acts as a sequence-independent methyltransferase to conserve the patterns of methylation status in the process of DNA replication and cell division in somatic cells [22]. During the process of gametogenesis and early life, de novo methylation is regulated by two "constituent" isoforms, DNMT3a and DNMT3b. DNMT1 is a "maintaining" methylase, is specific to hemimethylated sequences, and provides reproduction of the DNA methylation pattern on the daughter strand after replication. Inheritance of the methylation status is provided by this enzyme which ensures gene expression in cell generations.

The most critical role of DNA methylation in the gene expression is "genomic imprinting." This activity differentially marks maternal and paternal gene in the individual genome through epigenetic process. Furthermore, this marking further ensures that monoallelic expression of imprinted genes depends on their maternal or paternal origin. The monoallelic expression of imprinted genes is provided by supramolecular chromatin modifications, which are differentially responsible for marking parental alleles. The main regulator of this process is allele-specific DNA methylation established upon germline cell maturation [23].

35.2.3 Differentially Methylated Regions

Differentially methylated regions (DMRs) have been identified in all imprinted genes studied. These are CpG sequences, whose methylation status considerably differs from that of the parental homologs. It has to be appreciated that not all the CpG dinucleotides are methylated. An area of the genome (usually at least 200 base pairs (BP) long) with high incidence of CpGs (exceeding 50 %) is normally referred to as "CpG Island." Approximately half of the CpG islands are located near the transcription start site of genes, particularly for housekeeping genes; these CpGs are generally not methylated nor have low levels of methylation. The remaining 50 % of CpG islands are intragenic or intergenic and are believed to represent the transcription start site of noncoding RNAs [24]; these CpG islands are usually methylated [25].

The methylation pattern established during germline cell development for these regions is strictly specific. It has been observed that in some imprinted loci, methylation occurs exclusively in oogenesis but not during spermatogenesis. In contrast, in some other loci, DMRs are methylated during spermatogenesis rather than in oogenesis. After fertilization, differential methylation is preserved in somatic cells under the influence of DNA methyltransferase activity. Although some DMRs regulate the activity of a particular imprinted gene (micro-imprinted domains), others are known to coordinate the expression of a whole gene cluster. An important feature of imprinted genes in the mammals is the presence of organized clusters in the genome. The presence of DMRs within a cluster of imprinted genes permits the establishment and maintenance of monoallelic expression of a gene

group. In the human genome, three large clusters of imprinted genes with their own imprinting centers have been identified and are located in distinct regions: two in region 11p15.5 and one in region 15q11–q13 [26].

Moreover, the allele-specific methylation of primary DMRs provides a heritable "memory" that is maintained throughout fertilization and embryo development [26]. However, abnormal expression of imprinted genes can also result from genetic disorders (deletion or duplication, mutation or uniparental disomy) and epimutations (methylation anomalies). While gene activation is driven by gradients of signaling molecules and transcription factors, gene silencing is supported by DNA methylation and chromatin modifications carried out by specialized enzymatic activities. It is therefore highly probable that mutations in many components of the epigenetic gene silencing machinery could lead to a variety of human disorders as observed after ART treatment.

35.2.3.1 Posttranslational Modification of Histone Tails

Posttranslational modifications of histone tails involve addition of an acetyl, methyl, and phosphate group or, more rarely, ubiquitination, sumoylation, ADP-ribosylation, deamination, and non-covalent proline isomerization. It has been observed that histone modifications work often in conjunction or independently of DNA methylation [25]. During the histone tail modifications, the affinity of the basic histone proteins to the acidic DNA is changed. Positive charge of lysine is neutralized by acetylation, facilitating the disassociation of the histone protein from the negatively charged DNA, thus allowing chromatin conformation to open up more.

It has also been observed that DNA is least transcriptionally active when it is methylated and when bound with unacetylated histones. The reality is that the "histone code" is extremely complex and is still being deciphered. As an example, arginine residues can be mono- or dimethylated, while lysine residues can accept one, two, or three methyl residues [27–29]. Additionally, the epigenetic control at the chromatin level is regulated by two chromatin remodeling complexes. The Brahma/SWI/SNF complex changes the position of nucleosomes along the DNA, whereas the SNF2H/ISWI complex mobilizes nucleosomes.

35.2.3.2 Noncoding RNAs (ncRNA)

Mattick and colleagues [30] have reviewed the third model of epigenetic control which involves noncoding RNAs (ncRNA). These ncRNAs play a central role in genetic and epigenetic processes and are generated in abundance by vast nonprotein-coding segments of DNA. In fact, transcription factors and proteins involved in epigenetic modification (e.g., DNMTs and methyl DNA-binding domain proteins) are known to bind RNA, which in turn contributes to chromatin structural organization [30].

One of the prominent features of the epigenome is its plasticity. Although epigenetic marks are maintained through cellular mitosis and are considered to be stable, they have been known to be modified reversibly by the environment. Moreover, important epigenetic changes are associated with aging and involve global DNA hypomethylation and isolated hypermethylation of specific loci [31]. Furthermore, it has also been established that epigenetic programming can change during specific windows of sensitivity [31].

35.2.4 Genomic Imprinting

Genomic imprinting is an epigenetic process that is responsible for regulating gene transcription and is known to lead to the expression of only one allele of a particular gene in a parental-specific manner, in other words, parental inheritance. Mouse embryos created with two female pronuclei or two male pronuclei failed to survive in utero [32–34]. These experiments elegantly demonstrated the concept of genomic imprinting. In these studies, embryos derived from female pronuclei showed near-normal embryos, with abnormal extra embryonic tissue. However, embryos derived from male pronuclei demonstrated poorly developed embryonic tissue [32, 34–36]. These studies imply that for normal embryogenesis to occur, both maternal and paternal genomes were required.

The two rare disorders of imprinting associated with ART raised questions about the effect of ART on early development [37, 38]. Because humans contain two sets of autosomal genes, a copy is inherited from each parental gamete. During classical Mendelian inheritance, independent of parental origin, the genes from each parent are expressed in the offspring in equal measures. Therefore, the disease phenotype is highly dependent on uniparental expression as observed in a small handful of known genetic disorders.

Currently, more than 150 imprinted genes have been identified in mice and humans [39–41]. They play crucial roles in embryonic growth and development, placental function [26], postnatal metabolic pathways, and behavior associated with the control resources [42].

Recently, few studies in humans have reported widespread methylation patterns in children born after ART and have suggested the notion of biologic plausibility (reviewed in Ref. [43]).

35.3 ART and Epigenetics

As discussed earlier, gene imprinting studies using mouse nuclear transplants have demonstrated that for normal embryogenesis to occur, both maternal and paternal genomes are required [32–34]. As epigenetic reprogramming of DNA methylation occurs during germ cell and preimplantation development, it is highly probable that ART manipulations of conception, which include ovarian stimulation, in vitro maturation of oocytes, the use of ICSI, the use of immature sperm, in vitro culture of embryos, and cryopreservation of both embryos and gametes, could theoretically disturb the normal conception process leading to epigenetic errors [22, 44, 45]. It is therefore not surprising that ART interventions have the potential to influence epigenetic processes at various stages of gametogenesis and embryo development.

Although it has not been established which ART procedures are involved in epigenetic anomalies, the "timing" of the manipulation relative to the erasure and establishment of imprinting marks is a key contributory factor. Furthermore, because oocytes are vulnerable to reestablishment of methylation marks, which occurs just before ovulation, the artificial induction of ovulation in the course of an ART cycle could conceivably affect oocyte imprinting [9, 46, 47].

Furthermore, one can argue in favor of hormonal influence on epigenetic processes disrupting imprinting marks laid down asynchronously in both gametogenesis. In the male haplogenome, imprinting marks undergo erasure actively and are initiated during prenatal stages of spermatogenesis and are completed at postnatal stage. While, in the female haplogenome, the erasure is passive and begins after puberty in growing oocytes from primordial to antral follicles [45]. During this phase, the entire PGC genome, including imprinted genes, is demethylated and then remethylated in a sex-specific manner. This ensures that the egg and sperm have the appropriate respective imprinted marks. The time course for remethylation of gametes is different for the two sexes. While the imprinting process progresses over time with increasing oocyte diameter, different imprinted genes complete the process at different times and, for several imprinted genes, the process is not completed until ovulation [48, 49]. Therefore, possible disruption at different points during this process may influence and result in varying degrees of epigenetic aberrations. In addition, incomplete erasure of the imprints can result in epigenetic inheritance to the next generation. More specifically, any epigenetic modification may result in transmission to the offspring even if one epigenetic mark is not erased in the parental germ cells.

As discussed in earlier chapters, females with low ovarian reserves or advanced maternal age have very poor prognosis of a successful outcome. It is therefore likely that use of high doses of exogenous hormones during this period may disrupt the acquisition of imprints in oocyte maturation. This forced oocyte maturation may also lead to the loss of maternal-specific expression and the development of imprinting disorders in some or all of the oocytes that are normally non-ovulated.

It is also necessary to consider that some cells in the embryo appear to be more susceptible to alteration in imprint marks during in vitro manip-

ulation. It has been reported that in mouse model in vitro culture, the placenta exhibited a greater loss of imprinting than did the embryo [50, 51]. Although the placenta epigenetic marks might seem irrelevant since the placenta is discarded at birth, this observation is noteworthy when considering the transgenerational effects of altered epigenetic marks in the placenta in animals [51, 52] and their relevance to human diseases [42, 53, 54] and how altered placental function theoretically could affect future generations [43].

35.4 Epigenetic Reprogramming (EP) of Oocytes

As patient is exposed to different procedures during ART treatment, it is difficult to determine which individual techniques might perturb epigenetic events in oocytes and embryos. The availability of large numbers of oocytes and preimplantation embryos from a known genetic strain of mouse, at precise stages of development, has allowed mechanisms responsible for aberrant genomic imprinting to be investigated [55].

35.4.1 Epigenetic Programming in Mice Oocytes

Seki et al. [56] observed in mice that around embryonic day 7.25 (E7.25), EP starts with germ cell development from epiblast cells, continues after the primordial germ cells (PGCs) have reached the genital ridge at E10.5, and lasts until E13.5 (42–44 days in humans). They further observed a marked genome-wide DNA demethylation once the PGCs had migrated into the developing gonads, as high as 73.2–85 % in embryonic stem cells compared with less than 10 % in female PGCs at E13.5. At this stage, both imprinted and nonimprinted genomic loci are demethylated. This erasure is necessary to maintain the totipotency of germline. Moreover, it has been suggested that demethylation provides erasure of the accumulated aberrant epigenetic modifications (epimutations). Although the chromatin rearrangements are very transient (from E11.5 to E12.5) [57], it is worth noting that the erasure of differential DNA demethylation of imprinted genes persists until new imprints are imposed later in the embryo in a sex-specific manner. Realistically, demethylated chromatin state is maintained during arrest of meiosis in female germline cells. In spermatogonia, methylation is initiated on resumption of mitotic divisions and is completely terminated at meiosis I pachytene. By contrast, in oocytes, methylation is established only during their maturation and terminates at metaphase II [23, 58]. It has been confirmed that the de novo methylase DNMT3 in collaboration with DNMT3L is responsible for establishing a new DNA methylation state at repeated sequences and developmental genes [59, 60] and for resetting the sex-specific germline DMR imprint [61, 62]. As a consequence, sex-dependent methylation of imprinted genes is observed in germline cells during their maturation. Mature oocytes and spermatozoa significantly differ in their epigenetic organization: the sperm genome is more methylated and its chromatin is more condensed as compared to that in oocytes, owing to replacement of histones by protamines. Interestingly, most regulatory imprinted domains in the human genome are methylated in oocytes. So far, only three imprinted domains (H19, MEG3, and GNAS) are known whose methylation is established in the male germline. This marked difference of methylation of imprinted genes in different parental chromosomes may have appeared during evolution as an additional protection against active demethylation of the paternal genome, which is switched immediately after fertilization [62].

Because the maternal imprint appears to be established at the same time for all the analyzed imprinted genes, methylation dynamics seem to be more progressive during adult mouse follicle growth, as compared with those in neonatal period [63].

35.4.2 Epigenetic Programming in Human Oocytes

Sato and colleagues [63], studying the human oocyte development competence by cumulus cell morphology and circulating hormone profile, observed that the timing of maternal imprinting appears to be identical to the mouse model. They further observed that

in late antral follicle stage oocytes, DNA methylation on maternally methylated DMRS was completed. This was in contrast to only 50 % DNA methylation in early follicle stages (primordial and primary follicle stages). Interestingly, the paternally methylated DMRs remained unmethylated at all the stage of development.

Exploring this further, it was observed that the de novo methylation of KCNQ1OT1 DMR (KvDR1) occurred very slowly with the meiosis II progression [64], and only about two thirds of alleles were observed to be methylated on this DMR in fully grown germinal vesicle (GV) oocytes. Contrary to this observation, Geuns and colleagues [65], looking at a different region within KvDR1, reported an overall methylation pattern for this imprinted gene as early as GV stage. It is likely that the discrepancy observed by the two groups can be attributed to methylation acquisition dissociated between the two different regions of this DMR.

It should be noted that, of the 20 identified gDMRs/ICRs, 17 maternal ICRs are methylated in the oocyte, whereas only three are methylated in the sperm (paternal gDMRs). Because of the high number of maternal ICRs being methylated in the oocytes, it is highly feasible that the frequency of imprinting errors during maternal epigenetic reprogramming could be statistically higher than in the sperm. Moreover, maternal ICRs are CpG island promoters, whereas paternal ICRs are relatively CpG poor and intergenic. It begs to ask the questions whether these sexual discrepancies observed is linked to the different developmental kinetics of male and female gametogenesis and does it suggest evolutionary reasons for this observation [66, 67]. Studies have also highlighted the crucial role of maternal reprogramming. Studies demonstrated that maternal ICRs play a dominant role in early development regulating the biologic pathways related to the establishment of the fetomaternal interface [67].

35.5 Epigenetic Effects of Hormonal Superovulation

It is a common practice in in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) treatment cycles to stimulate ovaries using exogenous gonadotropins. Depending on the age and ovarian reserves of the patient, different stimulation protocols have been employed (discussed in earlier chapters). Due to lack of sufficient clinical material, most of the observed epigenetic changes come from animal studies. A comparative study of two cell mouse embryos from superovulated mice revealed a higher incidence of methylation abnormalities when compared with nonsuperovulated mice [68]. Furthermore, the loss of methylation observed on the maternal allele at SNRPN, PEG3, and KCNQ1OT1 was dose dependent and statistically significant at the higher dosage of hormone stimulation. The authors concluded that hormonal stimulation of the ovaries may affect gene imprinting [68].

To understand the EP and consequences of superovulation, Sato's group [69] studied the methylation of three maternally methylated genes (Peg1, Zac, and Kcnq1ot1) and one paternally methylated gene (H19) in pooled superovulated MII oocytes from two different strains of mice (ICR and BDF). The group observed that all of the maternally methylated genes had normal methylation after superovulation; however, H19 had gained methylation. These results suggested that the acquisition of maternal methylation imprints is unaffected but that oocyte quality was affected such that abnormal methylation occurred on H19.

In a separate study, Fauque and colleagues [70] reported decreased levels of expression of H19 in blastocysts after superovulation. The possible explanation for these results could be an alteration in oocyte quality affecting H19 EP marking or a delay in embryo development. This is feasible as H19 is normally expressed first at the blastocyst stage [71].

The effects of superovulation were further examined at different times in the development. Using a low-dose superovulation protocol, Fortier et al. [72] demonstrated alteration in the expression of maternally and paternally methylated imprinted genes in the midgestation mouse placenta, suggesting that trophectoderm-derived tissues may be more susceptible to disruption of imprinted genes than the embryo proper.

Based on these studies, the authors concluded that superovulation could possibly have two distinct effects, one to disrupt the acquisition of methylation

imprints during oocyte growth and the second to impair the proper maintenance of imprints during preimplantation development. These observations further confirmed that imprinting errors occur in a dose-dependent manner, with more frequent disturbances at the higher dosage of hormone stimulation than low hormone doses. Furthermore, both the maternal and paternal H19 alleles were perturbed by superovulation suggesting that maintenance of imprinting after fertilization may be affected. Consistent with the above observation, in a human study, the authors confirmed that hormonal stimulation of the ovary affects gene imprinting, leading to a loss of methylation at PEG1 and a gain of methylation at H19 in superovulated immature human oocytes [69].

Based on both the animal and limited human studies, it is highly probable that superovulation may be responsible for modifications in maternal-effected gene products that are later required for imprinting maintenance in developing embryos [73].

35.6 Use of Immature Gametes: In Vitro Growth (IVG) and In Vitro Maturation (IVM)

As clinics attempt to use immature gametes by culturing them in media with different concentrations of gonadotropins, growth factors, and other proteins, there has been a concern that the use of immature gametes may be associated with imprinting defects in the offspring. The processes of IVG and IVM of oocytes are fairly complex and with different efficiency in different species (see for review) [74]. Although live offspring have been obtained following IVG/IVM of mice primordial follicles [75], only isolated preantral follicles have been grown in non-rodent species [74].

In contrast, the process of IVM in humans involves the maturation of germinal vesicle (GV) oocytes to metaphase II (MII), with primary clinical indications for women with PCOS or fertility preservation in patients preparing to undergo cytotoxic cancer therapy [76].

Although a mouse study has reported significant histone acetylation changes in MII oocytes and early cleavage embryos, after IVM [77], in human, no increase was detected in congenital malformation in children conceived by IVM [78].

However, Khoueiry and colleagues [64] observed that the KCNQ10T1 DMR (KvDMR) is more methylated in the GV and metaphase I (MI) oocytes of natural cycles than those from stimulated cycles (62.55 vs. 67.8 % for GV and 70.3 % vs. 63.6 % for the MI, respectively). This observations implied that gonadotropin stimulation is likely to modify the dynamics of de novo methylation during oocyte maturation or/and may be responsible for recruiting too young follicle. It is therefore probable that these imprinting disruptions observed may be due to the developmental delay in the oocytes preventing imprint establishment at the right time or by ovarian stimulation or in vitro culture interfering with the imprint acquisition in the oocytes.

Moreover, evidence exists that additional manipulations may also be associated with altered methylation of imprinted genes. IVG of mouse oocytes has been reported to result in hypomethylation of IGF2 and PEG1/MEST and hypermethylation of H19 [79]. In contrast, Anckaert and colleagues [80], using a well-established follicle culture system with controlled titrating exposure to follicle stimulating hormone, detected no abnormalities in the methylation of a number of imprinted genes.

Because IVG and IVM are either being evaluated for or are clinically used, it is necessary that more controlled studies should be undertaken, allowing better understanding of how EP influences the clinical use of immature oocytes. It will be important for future studies to establish optimal culture techniques in order to pursue the physiological significance of any defects detected in oocytes by closely examining the offspring and their placentae at later times in gestation.

From a genetic perspective, it is reassuring that in a transgenic mouse model, various ART techniques including IVF, ICSI, round spermatid injection (ROSI), and cell culture have not been associated with an increase in the frequency or spectrum of point mutations [81].

Conclusion

Diseases such as Beckwith-Wiedemann syndrome (BWS-OMM #130650) and Angelman syndrome (AS-OMIM #105830) have been reported to be associated with epigenetic disruption of chromosomal regions, or epimutations, as a consequence of defective DNA methylation status of imprinted genes. Three important mechanisms that are involved in the imprinting process include DNA methylation, post-translational modification of histone proteins, and remodeling of chromatin and RNA-based mechanisms. The acquisition of a unique epigenetic profile in the male and female germlines is time specific during the development of gametes. It involves a well-orchestrated expression of enzymes. Furthermore, genomic imprinting once established in the germline must remain unaltered following fertilization of these gametes and throughout the life of the offspring.

It is highly probable that epimutations found in babies conceived using ART may be a consequence of the ART procedures rather than an inherent condition in the infertile population. No studies to date have identified at which stage(s) during the ART procedures the epigenetic alterations could arise. However, tangible evidence from animal and limited human studies points to the fact that superovulation is responsible for an increased incidence of epimutations. Because acquisition of maternal imprints extends over a relatively long period of time, there is a danger of these imprints being exposed to disturbances. Furthermore, it has been demonstrated that the exogenous gonadotropins influence the kinetics of oocyte maturation by inducing accelerated follicular growth in some cases [82]. This ovarian stimulation has profound effects on the gene imprinting resulting in poor development of the embryos, placentation, and possibly leading to failed implantation(s). As discussed previously, the acquisition of methylation in the oocyte is a complex phenomenon requiring proper oocyte growth. Human oocytes are more prone to epigenetic errors as they encounter more stressors—such as multiple hormone administration, advanced maternal age, environmental factors, or inherent infertility [83]. In contrast, animal studies excluding infertility effects have highlighted the negative impact of ovulation induction per se in this critical period.

One major disadvantage of using exogenous hormones in ART is the release of some MII oocytes with incomplete or labile imprints. Considering several studies in humans and mice, the administration of exogenous gonadotropins has been shown to induce molecular changes in the oocyte with a negative impact on the maintenance of genomic imprints during subsequent embryogenesis. As suggested, ovarian stimulation may have a greater adverse impact on the maternal factors required for imprint maintenance than on imprint acquisition [84]. It is therefore likely that exogenous hormone treatments may be responsible for the epigenetic reprogramming of the gametes as well as the early embryos. The scope of this chapter has restricted in-depth discussion on the EP contributions to implantation, endometrial receptivity, and placentation. Collectively, it is likely that deleterious effects of EP may also alter the physiologic environment of the uterus.

Due to limited human data being available, there is a need for well-designed and controlled study to better understand the mechanisms involved in epimutations. Finally, because superovulation may have deleterious effects on imprinting maintenance, research in humans needs to be performed not only on oocytes but also on embryos. Better insight will improve risk assessment among ART practitioners of the impact of ovarian induction protocols on epigenetic control of the genome.

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Effect of Ovarian Stimulation Protocols on Oocyte and Embryo Quality

36

James Catt

Abstract

A review of oocyte and embryo quality suggested that the usual parameters of pregnancy are not sensitive enough to determine differences possibly caused by ovarian stimulation. The dual methodology of time to syngamy and appropriate on time embryo development was investigated to determine whether they would be useful methodology for oocyte and embryo quality. We could determine no differences between pituitary suppression, follicular stimulation, and follicular maturation with "standard" stimulation protocols for "normal" responders. Differences were found with "out of protocol" stimulations and possibly among adjuvants used. We contend that the syngamy early development model should be used with more conventional outcomes to help determine any benefits of new protocols.

Keywords

Ovarian stimulation for IVF • Oocyte quality • Embryo quality • Quantitative outcomes • Syngamy • Alpha/ESHRE consensus

36.1 Introduction

The potential effect of stimulation protocols can be very important in IVF as the maxim "you cannot make good embryos with bad eggs" still holds today. An extension to this maxim would be that we can make "bad" embryos from "good" oocytes using inappropriate materials and methods. In other words, embryo quality is dependent on many more variables than oocyte quality, and since this chapter is about the effects of stimulation, the discussion will be largely restricted to oocyte quality.

There are few central problems with assessing the effect of stimulation protocols on outcomes, and these include the drugs themselves, their inappropriate use in terms of dosage and duration of stimulation, or the unproven "mixing and matching" of various protocols. Another difficulty we face when assessing stimulation protocols is the objective, quantitative measurement of

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oocyte, and embryo quality. The literature abounds with publications investigating the efficaciousness of protocols, but the outcome measures are often unsatisfactory. In addition, the effect of stimulation on the endometrium itself has rarely been addressed in the literature. This chapter concerns the effects of stimulation on oocytes and embryos and the effects on the endometrium, and implantation is considered in other chapters in this book.

This chapter attempts to unravel some of the data concerning the effect of stimulation protocols on oocyte and embryo quality by examining quantitative outcome measures.

36.2 Oocyte Quality

To define "good" quality oocyte is, essentially, very difficult. The best quality oocytes are those which are most likely to implant, if fertilized in an appropriate fashion. The road to implantation however is dependent not solely on oocyte quality but a host of other variables inherent in fertilization, growth of a subsequent embryo, and a receptive endometrium. The number of variables is huge; well over a 100 have been identified. Two other points indicate that implantation potential of an oocyte is not a useful guide to oocyte quality. One is that the time taken for an oocyte to form an embryo, with ultrasound evidence of a fetal heart, is about 7 weeks post-oocyte retrieval which is too long a time for practitioners to make decisions about protocols to improve oocyte quality. The second point is that implantation potential only takes into account those embryos which are transferred and gives us no idea about the overall oocyte quality. What are needed are functional tests of oocytes which can be related back to overall quality.

Over the years there have been numerous publications that have claimed that various methodologies can be used to determine oocyte quality. These can be categorized into two areas, oocyte morphology/physiology and functional tests of follicular fluid and cumulus cells.

36.3 Oocyte Morphology

The morphology of collected oocytes and associated vestments can be readily viewed and subjectively measured. Such features as cumulus expansion and atresia have been suggested as markers of oocyte quality but are often used with other perceived physiopathologies such as zona pellucida defects, size of the first polar body, width of the perivitelline space, as well as cytoplasmic features such as vacuoles, refractile bodies, and cytoplasmic granulation. However, despite many observations, few pathologies [1, 2] have been associated with poor outcomes with the exception of smooth endoplasmic reticulum condensates (SER) [3, 4]. Even though SER condensates are reported to be deleterious, others report normal outcomes [5, 6].

In conclusion, as scientists we attempt to relate our observations with a measurable outcome, but, in reality, these correlations often do not stand the test of time.

36.4 Follicular Functional Tests

Similar to morphology, a functional test to measure oocyte quality is attractive and has been investigated over the past decade. A review of the use of single biochemical markers and the more recent "omics" approach suggest that the methodology has promise but has not resulted in anything of clinical use, as yet [7].

36.5 Zygote Physiology

A novel functional test based on the time to the first zygote division has received little attention (except as an embryo selection tool). The time to syngamy is defined as the time from insemination (either conventional IVF or ICSI) to pronuclear membrane dissolution or division to the two-cell stage. It has been suggested by a number of investigators that the timing of syngamy is a reflection of embryo quality [8, 9] although one recent report failed to find a correlation [10].

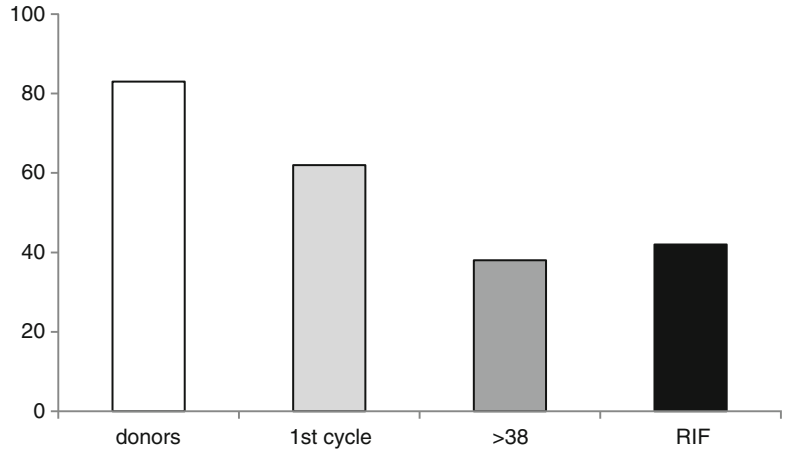

Fig. 36.1 Percent of zygotes entering syngamy at 25 hpi for donor oocytes (42 cycles/462 zygotes), standard IVF patient <38 (94 cycles/864 zygotes), advanced maternal age >38 (76 cycles/479 zygotes), and recurrent implantation failure patients (62 cycles/484 zygotes)

We believe that there is good circumstantial evidence to support the contention that entry into syngamy is indeed related to oocyte quality and by extension embryo quality. By definition, patients of advanced maternal age (>38), poor responders, and repeat implantation failure (RIF) have poor quality oocytes, and this is reflected in their rates of syngamy (Fig. 36.1). Two controls were used for this dataset, first cycle patients aged less than 39 and oocyte donors. In this graph we have plotted the percent of zygotes entering syngamy at 25 h post insemination [3]. It is clear from this graph that a much higher proportion of zygotes from first cycle patients and oocyte donors enter syngamy compared with either advanced aged (39–42) or RIF patients. Indeed the donor oocyte group shows a very high proportion of zygotes entering syngamy compared with the infertile group, possibly a reflection of their infertility.

For the purposes of this chapter, we will use entry into syngamy as a measure of oocyte quality.

36.6 Embryo Quality

While the maxim stated at the beginning of this chapter that only good quality oocytes can make good quality embryos is true, it is easily derailed and that we can make poor quality embryos from good quality oocytes using inappropriate conditions.

What defines a "good" embryo? There are as many embryo scoring and grading systems as there are IVF units, and some of the most commonly used criteria of fragmentation and overall morphology are currently being reexamined in the light of several observations, originally based on specific observation times and, more recently, the advent of time lapse recording systems [3]. Even with the most stringent scoring systems, the "best" embryos have only a 50 % chance of implantation. This suggests that the use of such systems is limited as to its efficacy. More recently, the term "on time, appropriate development," as defined by the Alpha/ESHRE consensus, has proved to be at least as effective as the more complex methods. Again, time lapse measurements of early development are adding more precise information to appropriate development [11].

On time, appropriate development and the effect on implantation rates are illustrated in Fig. 36.2, where the same dataset used for Fig. 36.1 was used. It is quite clear from this graph that the embryos from the poorer prognosis groups develop at a slower rate, and these differences increase with extended culture.

The differences between the groups are further illustrated in Fig. 36.3, where the ratio of blastocysts forming on day 5 was greatest for the "good" prognosis groups (donor and first cycle patients) compared with the "poor" prognosis groups (advanced maternal age and RIF patients).

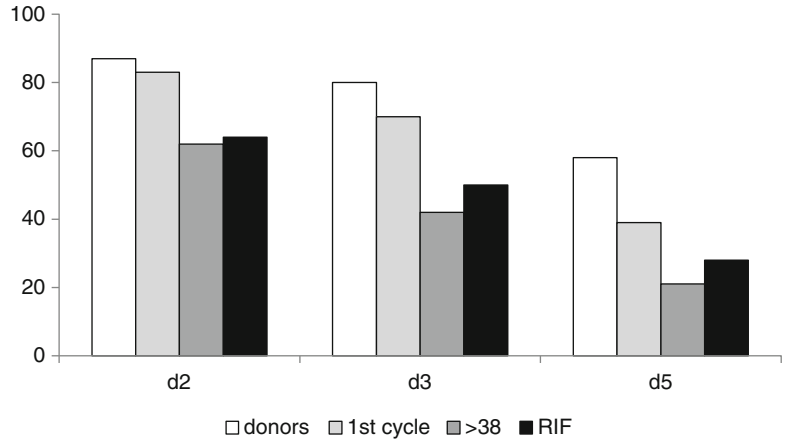

Fig. 36.2 The percentage of zygotes from each group meeting their developmental milestones. These milestones were 4-cell embryos at 43 h post insemination (hpi) on day 2, >7 cells at 67 hpi for day 3, and blastocoel formation at 115 hpi for day 5

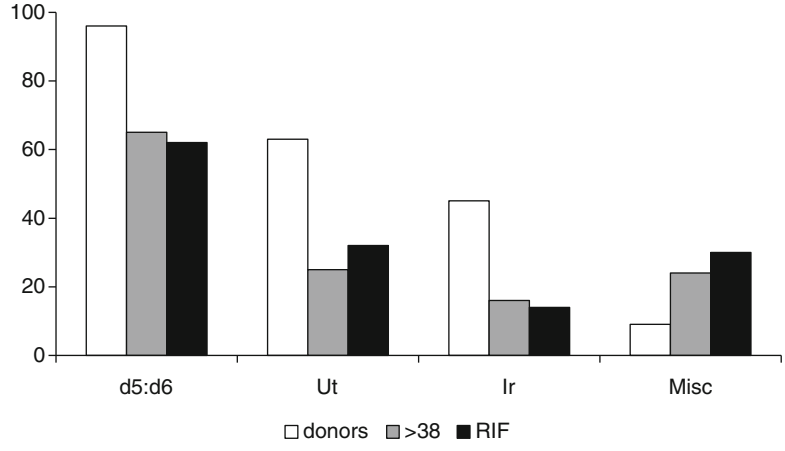

Fig. 36.3 The ratio of blastocysts forming on day compared with day 6 (d5:d6), the percent of zygotes that were transferred or frozen (utilized, ut), the implantation rate (measured as a fetal heart) per embryo that was transferred fresh (Ir), and the miscarriage rate after fetal heart detection

The overall utilization rates (defined as the percent of zygotes that were either transferred or frozen) and implantation rates per embryos transferred fresh follow the same trends. The apparent decrease in miscarriage rates suggests that "good" prognosis embryos are less liable to fail in pregnancy maintenance.

Using the "on time, appropriate development" methods should now give us more quantitative methods to look at the effect of stimulation regimes on embryonic development. However, can we make the contention that if only "good" oocytes make "good" embryos, will the measurement of zygote syngamy be sufficient by itself to investigate differences in stimulation regimens?

36.7 Evidence

There are three main areas of ovarian stimulation which can potentially affect the quality of oocytes and embryos. These are suppression of pituitary functions using GNRH agonists and antagonists, stimulation of follicular development with FSH, and the "trigger" used to initiate oocyte maturation and release. Various adjuvants have also been used to attempt to mediate all of these processes.

36.7.1 Pituitary Suppression

Data for agonist and antagonist pituitary suppression have been extensively studied, and the data is

Table 36.1 Comparison of early development events between agonist and antagonist pituitary suppression

Stage	Hours post insemination (hpi ± 1)	Agonist (4534 zygotes) (% of zygotes)	Antagonist (4823 zygotes) (% of zygotes)
Syngamy	25	58 %	59 %
>3 cell d2	43	68 %	66 %
>7 cell d3	67	50 %	54 %

Syngamy, day 2 and day 3 measurements were at the Alpha/ESHRE consensus times post insemination. Patients were aged less than 39 and having their first IVF cycle

Table 36.2 Comparison of early development events using either rFSH alone or using a combination of rFSH and uFSH

Stage	Hours post insemination (hpi ± 1)	rFSH (876 zygotes)	rFSH and uFSH (642 zygotes)
Syngamy	25	56 %	64 %
>3 cell d2	43	65 %	70 %
>7 cell d3	67	47 %	49 %

Measurements and patient segmentation were as in Table 36.1

consistent with little or no difference between their outcomes when using relatively non-quantitative outcome measures such as live births [12, 13]. We have used syngamy and early embryonic development to compare agonist and antagonist for a substantial number of cycles in several IVF units, both in Australia and other countries. The results are summarized in Table 36.1. No statistical difference could be found with the agonist versus antagonist using non-inferiority testing with a statistical power of 95 % (>4000 zygotes in each arm). The data adds further evidence that, under normal "standard" protocols, there are no statistical differences between agonist and antagonist pituitary suppression.

36.7.2 Follicular Stimulation

Stimulation of follicles is usually achieved using FSH either recombinant (rFSH), extracted from urine (uFSH) or a mix of both. Again the literature is rife with reports as to the benefit of one regimen over another. It is very interesting to analyze two Cochrane reviews, the first [14] suggesting a small increase in live take-home baby rate with rFSH and the second suggesting no difference [15]. I think this underlines one of the problems using pregnancy and live birth data, as outlined above, the data having inadequate resolving power with too many confounders.

In Australia, we have not been able to use urinary-derived FSH until recently, so we have not been able to derive a comparison between urinary and recombinant products. The comparison we have been able to make is between antagonist and rFSH and antagonist and a mix of rFSH and uFSH (from an associated clinic not in Australia). The results are shown in Table 36.2. While the differences are significant ($p = 0.0066$), the dataset needs deriving under more stringent conditions.

36.7.3 Follicular Maturation

Maturation of follicles has usually been achieved with hCG (either recombinant or extracted), but more recently the use of antagonist to suppress the pituitary has given us the opportunity to use agonist to mature the follicles.

The majority of studies have shown that using pregnancy rates and live births, there are no differences between the triggers between recombinant and urinary hCG [16], but there has been a small reported difference between rhCG and agonist trigger [17]. This was reported as a decrease in the pregnancy rate.

Our early development data comparing uhCG and rhCG indicated no significant differences (data not shown). There has been one report using time lapse measurement of early development comparing agonist and rhCG versus antagonist and agonist trigger [18]. There was a reported increase in syngamy rates with rhCG, but this was not reflected in subsequent divisions.

It would appear from the above discussion that stimulation protocols have little measurable effect on oocyte and early embryo quality. It is important to bear in mind that the data used to produce these results was from "good" prognosis patients

Table 36.3 Effect of delaying trigger beyond a lead follicle diameter of 24 mm

Stage	Hours post insemination (hpi ± 1)	Lead follicles <20 mm 242 cycles	Lead follicles >24 mm 42 cycles
Syngamy	25	62 %	42 %
>3 cell d2	43	68 %	58 %
>7 cell d3	67	54 %	42 %

All cycles were antagonist with rhCG trigger

Table 36.4 Comparison between controls (cycle prior to "Colorado") and "Colorado" cycles

Stage	Hours post insemination (hpi ± 1)	Control cycles (before "Colorado"	Colorado cycles
Syngamy	25	45 %	58 %
>3 cell d2	43	54 %	62 %
>7 cell d3	67	38 %	48 %

All cycles were agonist with uhCG trigger

stimulated with "standard" protocols. What happens when these standard protocols are not adhered to or are supplemented with adjuvants?

36.7.4 "Out of Protocol" Stimulations

There are several instances whereby "out of protocol" stimulations occur. A couple of examples of these could be "coasting" to reduce the chances of OHSS, too quick a stimulation (<7 days FSH), too slow a stimulation (>15 days FSH), and inappropriate triggering decisions with discordant follicular cohorts. One might expect that some of these circumstances could reduce oocyte and embryo quality, but substantial data has not been published. We have looked at a couple of inappropriate stimulations using our syngamy model. There was little difference with coasting for a day or two, short and long FSH duration, providing the trigger was administered appropriately (on leading follicles having a diameter of 17–19 mm). If however the trigger was delayed because of discordant follicles of a smaller diameter and the lead follicles reached >24 mm at trigger, then the syngamy and early development were negatively affected (table 36.3).

36.7.5 Adjuvants

Supplements such as growth hormone, LH, Colorado protocol, DHEA, estradiol patches, and heparin have been suggested to benefit outcomes of stimulation for certain subgroups of patients, but the evidence is contradictory. The biggest problem is that the potential beneficiaries are usually few in number, and so it is difficult to get statistical data. The use of the syngamy, early development methodology outlined above, reduces the number of patients (at least fivefold) required to conduct reasonable statistics because the sample size is increased as it uses zygotes instead of patients.

The only data we currently have is in the use of the so-called "Colorado" protocol which uses a mix of antibiotic, immunosuppressant, aspirin, and estrogen during stimulation. This protocol is generally used after several failed cycles (similar to the RIF patients in Fig. 36.1). We have compared "Colorado" cycles and with the patients previous cycle without the "Colorado" protocol. The results are shown in Table 36.4. There is a suggestion that the "Colorado" is of benefit to those patients with repeated implantation failures in terms of an overall oocyte and embryo quality.

Conclusions

So, is our syngamy/early cleavage model of any benefit? We believe it is for two reasons, the first being that used as a laboratory key performance indicator (KPI) one can ensure that stimulations are consistent in producing similar quality oocytes and embryos and the second is that new protocols or deviations of protocols can be monitored quickly and efficiently (as shown in the above examples). As in all investigations, the more data we have, then the more likely we will draw appropriate conclusions. Therefore, previous data as to the effects of stimulation on oocyte and embryo quality should be included in any analysis on the proviso that they are experimentally robust and statistically viable. The use of time lapse systems is proving to be invaluable as a research tool and is backing up

and probably refining the use of our syngamy and early cleavage model. Whether this mandates their routine use or helps us refine our current systems remains to be seen. The take-home message from this chapter is that "standard" protocols when used correctly on those patients who respond "normally" give oocytes and embryos of equivalent quality. As always, our challenge is to broaden our range of protocols to include those who do not respond well to our "standard" ones. A quantitative estimation of oocyte and embryo quality will help us with their design.

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Ovarian Hyperstimulation Syndrome: Can We Eliminate It as a Complication of ART?

37

Mala Arora and Ritika Arora

Abstract

With the explosive increase in in vitro fertilization (IVF) cycles worldwide, the morbidity and mortality associated with ovarian hyperstimulation syndrome (OHSS) cannot be ignored. Researchers and clinicians all over the world are moving toward newer modifications to achieve the goal of OHSS-free clinic and eliminating it as a complication [1]. Segmentation of the IVF cycle with a combination of GnRH-antagonist protocol with GnRH-agonist (GnRHa) trigger followed by embryo/oocyte freezing with subsequent embryo transfer can probably eliminate the risk of OHSS after controlled ovarian stimulation (COS). In case of embryo transfer in COS cycle, combined use of a GnRH-antagonist protocol with GnRHa trigger followed by single embryo transfer (SET), preferably blastocyst, favors reduction in the incidence of OHSS. But due to luteolytic action of agonist trigger, intensive luteal phase support (LPS) should be considered with adequate monitoring for signs of OHSS. Decision making based on patient characteristics, monitoring techniques, clinical experience, laboratory outcomes, and recent evidence is the key to maintain a balance between incidence and severity of OHSS and IVF cycle outcomes in terms of successful healthy pregnancy.

Keywords

GnRH agonist • GnRH antagonist • Frozen-thawed embryo replacement cycle • Agonist triggering • In vitro fertilization (IVF) • Ovarian hyperstimulation syndrome (OHSS)

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37.1 Introduction

OHSS is the most serious iatrogenic complication of assisted reproductive technology (ART). Though use of exogenous gonadotropins for COS/IVF is the main cause, other agents such as clomiphene and gonadotropin-releasing hormone analogs are also responsible in occasional cases. Mild OHSS accounts for up to 33 % of IVF cycles, with the reported incidence of 3 to 6 % for moderate and 0.1 % and 2 % for severe OHSS [2].

Severe OHSS may be complicated by thromboembolism, pleural and pericardial effusion, adult respiratory distress syndrome (ARDS), renal failure, liver dysfunction, or even multiorgan failure. Very few cases of OHSS-related mortality have been reported in the literature [3–5]. In a survey on maternal deaths related to IVF in the Netherlands, 3 deaths per 100,000 IVF cycles were reported [6].

37.2 Pathophysiology of OHSS

OHSS involves marked ovarian enlargement, theca lutein cysts, and increased vascular permeability leading to acute fluid shift from intravascular to extravascular space and its sequelae (Fig. 37.1). Vascular endothelial growth factor (VEGF), a vasoactive cytokine, plays a pivotal role in pathophysiology of OHSS [7, 8].

Numerous factors acting directly or indirectly via VEGF, including interleukins, cytokines, angiotensin II, insulin-like growth factor 1 (IGF-1), etc. may be involved [9–11]. Some studies showed a direct correlation between plasma renin activity and the severity of OHSS [12], but it could probably be the effect and not the cause of OHSS [13]. Mutation of FSH receptor gene may also predispose to OHSS because of abnormally high sensitivity to hCG [14].

The classification of OHSS is beyond the scope of this chapter, but can be found in other sources [15, 16].

37.3 OHSS Prevention: A Stepwise Strategy

37.3.1 Identification of High-Risk Cases

Prediction of high-risk cases prior to COS and individualized approach is the first step in the prevention of OHSS (Tables 37.1 and 37.2) [17–25].

37.3.2 Adjustment of FSH Starting Dose

Starting with the lowest possible dose of gonadotropins and close monitoring during COS with ultrasound and serum estradiol levels reduces the risk of OHSS.

37.3.3 Ovarian Stimulation Protocols Preferably GnRH-Antagonist Protocol

Unlike GnRHa, the GnRH antagonists directly and rapidly inhibit gonadotropin release within several hours through competitive binding to the

Table 37.1 Predictors of OHSS

Pretreatment characteristics	
Young age	<33 y
Lean body weight	Insufficient evidence
Polycystic ovaries on ultrasound/PCOS	
High basal antral follicle count (AFC)	≥12–14
Previous episodes of OHSS or hyperresponse	
High anti-Mullerian hormone (AMH)	Cutoff level of 3.36 ng/ml

From Humaiden et al. [17]

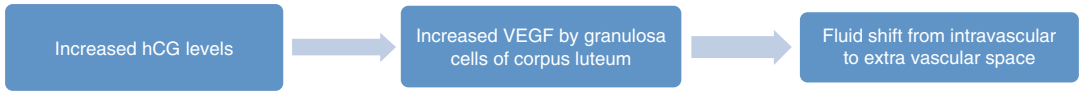

Fig. 37.1 Pathogenesis of OHSS

Table 37.2 Ovarian response parameters

High doses of exogenous gonadotropins in early follicular phase	
High number of growing follicles	>14 follicles with diameter of 11 mm >11 follicles with diameter of 10 mm
High serum estradiol (E2) levels	3500–6,000 pg/ml (better applicable in combination of growing follicles)
Rapidly rising serum E2 levels	
VEGF levels – in follicular fluid	
Number of oocytes retrieved	>15–20
Higher and/or repeated dose of exogenous hCG administration	
Follicular fluid IL-6 and IL-8 levels on the day of embryo transfer	
Pregnancy in fresh cycle	
Multifetal pregnancy	

From Humaiden et al. [17]

pituitary GnRH receptors [26]. GnRH-antagonist treatment protocols are effective and easy to use, allow flexibility of treatment with fewer side effects, and appear to offer a promising alternative to the long-established GnRHa regimens for prevention of a premature LH surge during COS. Flexibility of use of GnRHa as trigger for oocyte maturation is beneficial in reducing OHSS [27]. A Cochrane review in forty-five RCTs comparing the antagonist to the long agonist protocols showed significant lower incidence of OHSS in the antagonist group (29 RCTs; OR 0.43, 95 % CI 0.33–0.57) without any statistically significant difference in rates of live births (9 RCTs; OR 0.86, 95 % CI 0.69–1.08) [28].

37.3.4 Coasting

Coasting involves withholding the gonadotropins and postponing hCG administration until the patient's E2 levels drop to a "safer" level [29]. Prior to the use of GnRH antagonist during COS, it had been widely used in the prevention of severe OHSS. Lower gonadotropin levels increase granulosa cell inhibition and apoptosis leading to lower levels of VEGF and other vasoactive substances involved in the pathogenesis of OHSS [30]. Daily serum E2 levels are monitored in conjunction with follicle tracking until E2 levels decrease to a safe level usually below 3000 pg/ml. A recent Cochrane review showed no evidence of difference in the incidence of moderate and severe OHSS and significantly fewer oocytes retrieved in coasting groups compared with GnRHa (OR −2.44, 95 % CI −4.30 to −0.58; $P=0.01$) or no coasting (OR −3.92, 95 % CI −4.47 to −3.37; $P<0.0001$. But the problem with this review was that four studies which met the inclusion criteria were different as two studies compared coasting with unilateral follicular aspiration, one compared coasting vs. no coasting, and the last study compared coasting with replacement of GnRHa with GnRH antagonist [31]. There is a high risk of cycle cancelation with coasting especially if it is more than 3–4 days or there is >30 % fall in E2 levels [32, 33].

37.3.5 Minimize Use of hCG

Both exogenous and endogenous hCG due to its long half-life and leutrotrophic activity play a key role in the pathophysiology of OHSS [34]. Therefore, decreasing dose of hCG for triggering oocyte maturation, replacing GnRHa for hCG trigger, and avoiding hCG in luteal phase support (LPS) are various methods to prevent delayed-onset OHSS [17].

37.3.5.1 Lowering Dose of hCG Especially in GnRHa Protocol

In high-risk cases, the lowest effective dose of hCG has been proposed, ranging from 5,000 to 2,500 IU [35]. A low dose of hCG appears to reduce the incidence of OHSS but cannot eliminate it.

37.3.5.2 GnRHa Trigger

The role of GnRHa trigger (0.2 mg triptorelin, 0.5 mg buserelin, or 1 mg leuprolide) in elimination

of OHSS in high-risk patients was first suggested in 1988 [36]. GnRHa-induced surge of gonadotropins consists of 24–36 h span with resemblance to physiological mid-cycle LH surge [37]. This is in contrast to hCG-mediated LH surge which spans several days because of its long half-life leading to prolonged levels in circulation. GnRHa can replace hCG trigger in antagonist- or gonadotropin-only stimulated cycles but not in previous downregulation cycles with long-term agonist treatment [38]. In the oocyte donation model studies, avoidance of hCG exposure has been associated with complete elimination of OHSS, while recipient pregnancy rates are equivalent to those observed with hCG triggering [39, 40]. GnRHa trigger is the method of choice in oocyte donors and patients for fertility preservation. A recent Cochrane review of 11 RCTs reported no OHSS events in the GnRHa arm of the study and also concluded that GnRHa should not be routinely used to trigger oocyte maturation due to lower live birth rates and ongoing pregnancy rates, but makes an exception for women at high risk of OHSS, after appropriate counseling. It also concluded that combining GnRHa with embryo vitrification has the potential to provide a good clinical outcome [41]. In an analysis by Humaidan et al. comparing nine RCTs with fresh IVF cycles, no OHSS was reported after GnRHa triggering. Additionally, the delivery rate improved significantly after modified luteal support [6 % risk difference in favor of the hCG group (95 % CI: 20.14–0.2)] when compared with initial studies with conventional luteal phase support (LPS) [18 % risk difference (95 % CI: 20.36–0.01)]. They also reported 0 % incidence of OHSS in oocyte donation cycles (four RCTs). They concluded that GnRHa triggering with modified LPS is a valid alternative to hCG triggering, resulting in an elimination of OHSS [27]. Regarding the LPS after GnRHa triggering in fresh transfers, the majority of studies support supplementation with LH activity in addition to standard LPS with estradiol and progesterone [42]. Some studies showed beneficial effect of intensive LPS with intramuscular progesterone and estradiol patches as well as oral estradiol, but others showed lower pregnancy rates with similar LPS [43–45]. With the dual trigger – agonist followed by hCG (1000–2500 IU) and standard LPS – comparable reproductive outcome with no increased risk of OHSS is reported [44, 46]. Recombinant LH after GnRHa trigger for LPS has also been tried with similar implantation rates as compared to standard luteal progesterone protocol and no cases of OHSS [47].

37.3.6 Deciding Fate of the Stimulation Cycle

37.3.6.1 Cycle Cancelation

Termination of cycle by canceling further stimulation and trigger helps in the prevention of OHSS and associated morbidity. But due to the financial burden and psychological impact on dropout patients, it should only be reserved as a last resort for severe OHSS cases or in cases of total loss of cycle control.

37.3.6.2 Cryopreservation of Oocytes and Embryos

Embryo implantation and positive pregnancy can lead to late-onset OHSS or exacerbation of early OHSS. Therefore, cryopreservation of embryos or oocyte can be an option in the prevention of OHSS. Due to improvement in freezing techniques and culture media, pregnancy rates in the frozen cycles are comparable to the fresh cycles [48].

37.3.6.3 Fresh Cycle with Single Embryo Transfer (SET)

In case of decision of fresh embryo transfer, SET is preferred to decrease chance of multiple pregnancy and associated OHSS especially in younger patient. Adoption of strategies such as blastocyst transfer may permit more time for evaluation and decision regarding cryopreservation in case of aggravation of symptoms.

37.3.7 Other Preventive Regimens for OHSS

37.3.7.1 Intravenous Fluid at the Time of Oocyte Retrieval

Albumin is known to increase the plasma oncotic pressure and decrease the capillary permeability by binding to molecules like VEGF. The role of intravenous human albumin infusion at the time of oocyte retrieval for the prevention of OHSS is

controversial [49, 50]. A recent Cochrane meta-analysis showed a borderline statistically significant decrease in the incidence of severe OHSS with administration of human albumin (eight RCTs, OR 0.67, 95 % CI 0.45–0.99). But with administration of hydroxyethyl starch, a plasma expander, there was a significant decrease in the incidence of severe OHSS (three RCTs, OR 0.12, 95 % CI 0.04–0.40), without any effect on the pregnancy rates [51].

37.3.7.2 Dopamine Agonist

Cabergoline, a dopamine agonist, binds to VEGF receptor-2 and inhibits its phosphorylation leading to decrease in capillary permeability [52, 53]. Oral cabergoline 0.5 mg/day can be administered for at least 8 days in high-risk patients to prevent early OHSS. Cabergoline may reduce the risk of OHSS in high-risk women, especially moderate OHSS. It is unlikely to have a clinically relevant negative impact on clinical pregnancy or on the number of retrieved oocytes [54]. However, impact on live birth, miscarriage, and congenital abnormalities is still uncertain [55]. More recently, quinagolide has been shown to reduce the incidence and severity of OHSS [56].

37.3.7.3 Insulin-Sensitizing Agents: Metformin

Insulin is known to stimulate VEGF protein expression and secretion. Metformin improves insulin sensitivity and reduction of hyperinsulinemia and decreases OHSS. In a Cochrane review on efficacy of metformin treatment in women with PCOS undergoing IVF or ICSI cycles, there was a significant reduction in the risk of OHSS (5.7 % vs. 21.2 %) [57, 58].

37.3.8 Additional Preventive Measures with Limited Evidence

37.3.8.1 In Vitro Maturation of Oocytes (IVM)

In patients with PCOS and in normoovulatory patients at high risk of developing OHSS, IVM of oocytes offers great potential for OHSS prevention. Due to technical difficulties in oocyte retrieval, lower success rate, and reports of high rates of meiotic spindle and chromosomal abnormalities, its practice is limited to very few centers [59, 60].

37.3.8.2 Glucocorticoids

Glucocorticoids are a vasodilator, are an anti-inflammatory, and have inhibitory effect on VEGF gene expression [61, 62]. But sparse evidence and side effects limit its use in the prevention of OHSS.

37.3.8.3 Follicular Aspiration

Timed aspiration of granulosa cells from one ovary prior to hCG administration reduces the production of OHSS mediators while allowing continued contralateral ovarian development [63]. It is not recommended because of cost, invasive procedure, and limited evidence.

37.3.8.4 Aromatase Inhibitors

Luteal phase estradiol suppression by letrozole is suggested with very limited evidence [64, 65].

37.3.8.5 Nonsteroidal Anti-inflammatory Drugs

Low-dose aspirin beginning on the first day of COS has shown to decrease OHSS incidence (0.25 % vs.8.4 %) in a high-risk group [66]. Meloxicam was capable of reducing the OHSS-associated ovarian weight and expression of VEGF in an animal model [67].

37.3.8.6 GnRH-Antagonist Salvage

Luteal phase GnRH-antagonist administration in patients with established severe early OHSS appears to prevent patient hospitalization and results in quick regression of the syndrome [68]. Decision of concomitant administration with embryo transfer requires more studies [69].

37.4 Segmentation for OHSS-Free Clinic by Freeze-All Policy

As stated by Devroey, the strategy to obtain an OHSS-free clinic is closely related to the segmentation concept (Fig. 37.2) [1]. With the advent of vitrification techniques, there has been a major improvement in the embryo survival and subsequent pregnancy rates [70, 71]. For couples

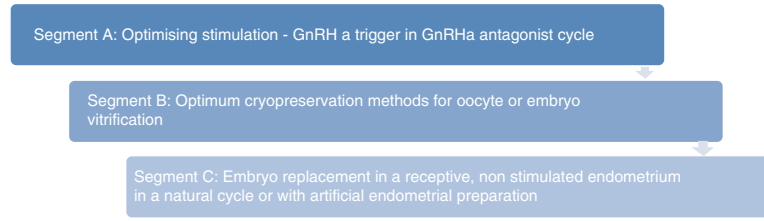

Fig. 37.2 Segmentation of IVF

who do not desire embryo cryopreservation, oocyte vitrification is another option. In a proof-of-concept study by Greisinger et al., 20 high-risk patients (≥20 follicles of ≥10 mm or estradiol ≥4000 pg/ml on trigger day) with GnRHa trigger and cryopreservation of all two pronucleate oocytes reported no case of OHSS and 29.2 % ongoing pregnancy rate in subsequent thaw cycle [38, 72]. Excellent embryo or oocyte survival rates after vitrification support the use of cryopreservation as a routine approach [70]

Advantages of segmentation:

(a) Minimizes late-onset OHSS.
(b) No need of intensive LPS.
(c) No embryo transfer in out-of-phase endometrium, which is common in high responder patients.
(d) Allows embryo transfer in a natural cycle where applicable. With effective cryopreservation that results in little or no damage to embryos, cumulative birth rates per retrieval should, in theory, be highest when embryos are transferred individually [73].
(e) Decrease in multiple pregnancy rate and perinatal and maternal morbidity and mortality associated with it.

37.5 Various Proposals for Decision Making Between Fresh Embryo Transfer or Freeze-All Policy

Although the use of a GnRH-agonist trigger can dramatically reduce the risk of OHSS in high-risk patients, for some patients segmentation of the cycle would still be better option. At present, the optimal threshold for performing a freeze-all after a GnRH-agonist trigger is not clear. Following are the various proposals by different studies (Fig. 37.3):

1. Griffin et al. stratified patients according to their estradiol concentration on the day of triggering final oocyte maturation to add 1000 IU of hCG and GnRHa for patients with peak estradiol <4000 pg/ml and GnRHa alone if peak estradiol is ≥4000 pg/ml [74].
2. In a protocol by Orvieto et al., in patients in whom <20 oocytes were retrieved in the first IVF cycle attempt, and in low responders or patients >40 years old, the COH protocol is individually tailored. In the latter groups, if the tailored COH protocols yield 20 oocytes, or 10 embryos develop, the patient is followed for 5 days after oocyte retrieval for signs of early OHSS (ultrasonographic signs of ascites, hematocrit levels for the degree of hemoconcentration). If early signs develop, ET is withheld and all resulting embryos cryopreserved. If it does not appear, they transferred one blastocyst, with 1500 IU of hCG with the consequent decrease in the risk of multiple pregnancy to almost zero, thereby eliminating the risk of late OHSS [75, 76].
3. According to Kol and Humaidan, ≤25 follicles is a safe threshold for 1500 IU hCG, but above 25 follicles, either a freeze-all policy or intensive luteal phase support with estradiol and progesterone is suggested [77, 78].

Fig. 37.3 Stepwise approach to be individualized and combined to reach the goal of eliminating OHSS

37.6 Investigation and Monitoring of an OHSS Patient

37.6.1 General Condition

General condition is monitored by regular charting of vital signs, weight charts, abdominal girth measurement, and a strict fluid balance record.

37.6.2 Biochemical Tests

A complete biochemical assessment includes hematocrit, electrolytes, liver function tests, kidney function tests, and coagulation profile. Blood gases and acid-base balance are required if there is a respiratory or renal compromise. Serum β-hCG is done to rule out pregnancy. Serum and urinary osmolarity and urinary electrolytes may be needed in more severe forms of the disease. The frequency of these tests is guided by the severity of the disease.

37.6.3 Ultrasonographic Examination

Ultrasound gives important information on ovarian size, amount of ascites, presence of hydrothorax

or pericardial effusion, and detection of pregnancy, whether single or multiple.

37.6.4 Chest X-Ray

A chest X-ray can rule out pleural effusion.

37.6.5 Serum ß-hCG

It is done to confirm pregnancy making the women at a high risk for developing severe disease.

37.6.6 Invasive Hemodynamic Monitoring

When OHSS becomes critical, monitoring of pulmonary artery pressure and central venous pressure may be required.

37.7 Treatment

The condition usually resolves within 10–14 days. Treatment is based on severity of the disease.

37.7.1 Mild OHSS

In mild cases, the treatment is usually conservative and is done at outpatient level with close follow-up. Plenty of fluids is advised. She is advised to avoid exertion and counseled on the warning signs like nausea, vomiting, abdominal pain or distension, and decreased urinary output. Serum electrolytes, hematocrit, and ultrasonography should be done. Analgesics and antiemetics may be used if required. Intake-output monitoring is important.

Drug therapy may be started in an established case.

GnRH antagonists: If given on day 6 after oocyte retrieval in women with OHSS for 4 days, combined with luteal phase support using exogenous estradiol and progesterone, OHSS regressed [69]. In women on antagonist regime, antagonist administration was re-initiated if OHSS developed and continued daily for a week, while all embryos were cryopreserved.

Role of GnRH agonists: This resolved the OHSS. A marked decrease of hematocrit, white blood cell count, ovarian volume, and ascitic fluid has been observed during one week of follow-up [68].

Cabergoline: Cabergoline is given as 0.5 mg/day. It reduces hemoconcentration and ascites in hyperstimulated women undergoing assisted reproduction [79].

37.7.1.1 Reassessment

Reassessment is required if there is increase in weight more than 2 kg or worsening of symptoms.

37.7.1.2 Indication of Hospitalization

Hospitalization should be considered in all severe and critical cases of the disease or if condition worsens. In cases of mild to moderate OHSS, admission is required if a woman is not responding to treatment and if there is intolerable nausea and vomiting, hypotension, signs of pleural effusion or ascites, a hematocrit of more than 48 %, potassium level more than 5.0 mg/l, and serum creatinine more than 1.2 mg.

37.7.2 Severe OHSS

The aim of therapy is correction of circulatory volume, electrolyte imbalance, maintenance of renal function, and prevention of thrombosis.

37.7.2.1 Maintenance of Intravascular Volume and Electrolyte Imbalance

The aim must be to restore normal intravascular volume and preserve adequate renal function. Colloid expander may be used for this purpose, but they have the disadvantage that after a short while, they redistribute into the extravascular space worsening the ascites. Low-salt albumin is the expander of choice and is given in a dose of 50–100 g every 2–12 h. It reverses hematocrit changes, improves renal function, and is safe

from viral contamination. Other options tried are mannitol, dextran, and fresh frozen plasma. Dextran can cause ARDS. Only if there is hyponatremia, normal saline with or without glucose is the crystalloid used for replacement. Up to 1.5–3 l may be needed. Other electrolyte imbalances like hyperkalemia are corrected.

37.7.2.2 Prevention of Thrombosis

Low-dose heparin should be given, as prophylaxis, in cases where there is an altered coagulation profile.

37.7.2.3 Dopamine

Dopamine may help to avoid fluid and salt retention by improving the renal blood flow in oliguric patient.

37.7.2.4 Management of Ascites and Hydrothorax

Paracentesis under ultrasound guidance is done where there is severe discomfort and compromise of venous return leading to a decreased cardiac output and hypotension, renal compromise, respiratory distress, or hemoconcentration unresponsive to medical therapy. Repeat aspiration may be required. This should be done if dyspnea is present because of severe pleural effusion.

37.7.3 Critical OHSS

Critical OHSS causes multisystem failure and requires multidisciplinary intensive care. Renal failure may need to be treated with dopamine, central venous pressure line, and hemodialysis. In case of pulmonary complications, arterial blood gas monitoring, thoracocentesis, or assisted ventilation is required if they do not respond to basic treatment. Patients with thromboembolic episodes require therapeutic anticoagulation with heparin. Laparotomy is required if the cysts undergo torsion, hemorrhage, or rupture. Laparoscopic unwinding can be done in cases of torsion.

Termination of pregnancy: If critical condition does not improve, one may consider termination of pregnancy.

Conclusions

GnRH-agonist trigger with antagonist cycle and freeze-all or fresh transfer with intensive LPS will help in achieving the goal toward elimination of OHSS without compromising pregnancy outcome. The optimal LPS, mode of administration, and length after COH still need to be determined. Currently, the ideal strategy that will eliminate OHSS without compromising ART outcome seems to be cycle segmentation and freezing of all embryos. Continuation of GnRH antagonists for a few days will rapidly regress the ovarian size, and transfer of embryos in subsequent natural cycles will give the best pregnancy outcomes. OHSS-related morbidity and mortality can be reduced by appropriate preventive measures, timely detection of OHSS, and management based on the severity of OHSS.

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Multifetal Pregnancy Reduction

38

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Abstract

Fertility treatments have contributed significantly to the increase in multifetal pregnancies. The first approach to the problem of multifetal pregnancies should be prevention and strategies to limit multifetal pregnancies. The goal of multifetal pregnancy reduction is to increase the chance of a successful, healthy pregnancy. Multifetal pregnancy reduction is usually done early in a pregnancy, between the 9th and 12th weeks. It is most often done when there are three or more fetuses present. Multifetal pregnancy reduction is done using intrathoracic injection of potassium chloride or in early pregnancy by aspiration of gestational sac, by both the transabdominal and the transvaginal approaches. No method has yet been proven to be superior to the others. Advantage of transvaginal procedure is the feasibility of the procedure at an earlier gestational age; however, transabdominal approach between 10 and 12 weeks enables a more detailed USG of the fetuses where nuchal thickness can be assessed and chance of spontaneous reduction of multifetal pregnancy is ruled out. Multifetal pregnancy reduction has been described as a "new moral problem" created by the advancement, but not perfection, of assisted reproductive technologies.

Keywords

Multiple pregnancy • Fetal Reduction • Ultrasound guided • Transabdominal • Transvaginal • Potassium chloride

38.1 Introduction

Multifetal pregnancy reduction (MFPR) is defined as a first-trimester or early second-trimester procedure for reducing by one or more the total number of fetuses in a multifetal pregnancy [1]. In many cases, the involved gestations

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will be "high-order" multifetal pregnancies, defined by the presence of three or more fetuses. Fertility treatments have contributed significantly to the increase in multifetal pregnancies. Between 1980 and 2009, the twin rate increased 76 %, from 18.9 to 33.3 per 1000 live births [2]. The triplet or greater birth rate increased more than 400 % between 1980 and 1998, when it peaked at 1.935 per 1000 births [3]. Between 1998 and 2009, the incidence of high-order multiple deliveries decreased by 29 % [4]. This decrease is the result of both a reduction in the number of embryos transferred with each cycle of in vitro fertilization (IVF) and an increase in the number of multifetal pregnancy reduction procedures being performed. The first approach to the problem of multifetal pregnancies should be prevention, and strategies to limit multifetal pregnancies, especially high-order multifetal pregnancies, should be practiced by all physicians who treat women for infertility.

When a pregnancy involves three or more fetuses (high-order pregnancy), the risks of miscarriage, stillbirth, and lifelong disability increase with each additional fetus. It is known as "selective termination" when it involves a fetus with severe defects or one that is expected to die later in the pregnancy, which would threaten the life of the surviving fetus or fetuses.

The goal of MFPR is to increase the chance of a successful, healthy pregnancy. Multifetal pregnancy reduction is most often done:

Early in a pregnancy, between the 9th and 12th weeks
When there are three or more fetuses present

A couple has several options when faced with a multifetal pregnancy. Counseling should be provided to women with high-order multifetal pregnancies. Resources for providing such counseling include perinatologists, neonatologists, mental health professionals, child development specialists, support groups, and clinicians with expertise in multifetal pregnancy reduction.

They can electively terminate the multifetal pregnancy with the intent to conceive again. Since the pregnancy is most likely wanted, achieved at great psychological and economic cost, and with no guarantee of future conceptions, this option is usually the least desirable.

The couple can attempt to proceed with the pregnancy. Even though there are reports of survival of some or all quadruplets and quintuplets, there is still significant risk of long-term morbidity. Survival with six or seven fetuses, although reported, is extremely rare. There are no reports of any fetal survivals with eight or more fetuses.

The couple can choose selective multifetal pregnancy reduction although it is still controversial with triplets.

The procedure of multifetal pregnancy reduction (MFPR) has, in recent years, become both clinically and ethically accepted as a therapeutic option in pregnancies with four or more fetuses and in multifetal pregnancies in which one or more of the fetuses have congenital abnormalities [5]. MFPR results in better pregnancy outcome, regardless of the initial number of fetuses [6]. In a study of IVF-conceived triplets, selective reduction of the pair to a singleton pregnancy was associated with a significantly greater likelihood of delivery at ≥34 weeks. On average, reduction of the pair was associated with 52 days longer gestation [7]. The pregnancy loss subsequent to fetal reduction has been reported as ranging from 0 to 40 %.

38.2 Methods of Multifetal Pregnancy Reduction [8]

Transcervical aspiration of the gestational sac
Transvaginal puncture and embryo aspiration
Intrathoracic injection of potassium chloride, by both transabdominal and transvaginal approaches

38.2.1 Pre-procedural Preparation

Counseling of the couple regarding the procedure and its possible complications.

Informed written consent.
Prophylactic antibiotic administration.
Patient may be admitted for a day in the hospital.

38.2.2 Embryo Aspiration

38.2.2.1 Transcervical Aspiration

Some authors have used transcervical aspiration of the gestational sac. This method, however, was thought to be associated with an increased incidence of fetal loss due to infection caused by introduction of bacteria from the cervix or due to cervical incompetence brought about by cervical dilatation.

38.2.2.2 Transvaginal Puncture and Embryo Aspiration

Fetal reduction very early in gestation (6 to 8 weeks) by the transvaginal puncture and embryo aspiration has also been reported with fairly good pregnancy outcome [9]. However, this method might have some theoretical limitations, such as:

- Use of general anesthesia
- Possibility of spontaneous fetal reduction at this stage of gestation
- Inability to perform early fetal screening, such as nuchal translucency test, which is done at 10 to 12 weeks of gestation
- Possibility of introducing infections

38.2.3 Intrathoracic Injection of Potassium Chloride by Transabdominal and Transvaginal Approach [10]

Multifetal pregnancy reduction using intrathoracic injection of potassium chloride, by both the transabdominal and the transvaginal approaches, has been reported. No method has yet been proven to be superior to the others. Although several techniques of multifetal reduction have been reported, however, the most popular is the intrathoracic injection of potassium chloride by the transabdominal approach at 10 to 12 weeks' gestation. It is logical to perform a detailed ultrasonographic fetal anomaly scan prior to the reduction. This will allow the reduction to be performed more selectively and will decrease the chance of delivery of a chromosomal or structurally abnormal fetus.

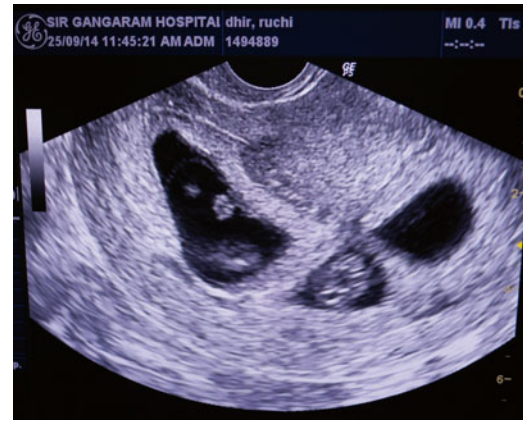

Fig. 38.1 Ultrasonography showing triplets

38.2.3.1 Transvaginal Procedure of Fetal Reduction

This procedure is done between 8 to 9 weeks of gestational age under general anesthesia. Strict aseptic conditions should be maintained throughout the procedure. Patient is placed in dorsal lithotomy position. Cleaning of vagina is done with povidone-iodine solution. Needle guide is attached to the transvaginal probe. Begin with transvaginal ultrasound examination of all the fetuses. Choose the correct path of the needle avoiding the path of the blood vessels. A 35-cm 18-gauge needle with a stylet is introduced through a guide and advanced through the vaginal wall, uterine wall, and into the fetal sac. The stylet is removed and a 21-gauge needle 40 cm long is introduced into the fetal thorax. 0.2–0.5 ml of 2 mEq of potassium chloride is injected. Fetal asystole is observed and needle is removed (Figs. 38.1 and 38.2).

Advantages of transvaginal procedure. The procedure is feasible at an earlier gestational age. However, the physician should be familiar with the procedure before applying it for routine use.

38.2.3.2 Transabdominal Procedure of Fetal Reduction

This procedure is performed between 10 and 12 weeks of gestational age, under local anesthesia. Prior to the procedure, ultrasound examination of all the fetuses is performed.

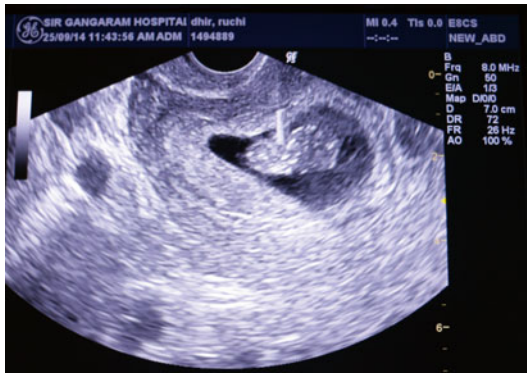

Fig. 38.2 Ultrasonography showing transvaginal fetal reduction by injecting intracardiac potassium chloride

Abdomen is prepared with povidone-iodine solution. Fetus nearest to the ultrasound probe is selected.

Spinal needle no. 21 with stylet is advanced through the abdominal and uterine wall into the fetal sac. Stylet is removed.

Syringe is loaded with 2 ml of 2 mEq potassium chloride.

The needle is visualized on ultrasound and advanced into the fetal thorax.

After the needle is advanced in the fetal thorax, potassium chloride is injected. Needle is removed after confirming fetal cardiac asystole. Cardiac activity of other fetus is confirmed.

Post-procedural second look ultrasound is done after few hours and another scan a few days later.

Advantages of transabdominal route. The advantages of the transabdominal route are as follows:

A more detailed USG of the fetuses can be performed, and nuchal thickness can be assessed as it is measured between 10 and 12 weeks' gestational age.

Chances of spontaneous reduction of multifetal pregnancy are ruled out.

Lower risk of infection.

38.2.4 Intracranial Injection of Potassium Chloride [11]

In certain cases of MFPR, where difficulty is encountered in reaching the thorax due to the fetal position as well as the location of membranes and placenta, an alternative approach may be the insertion of the needle to the fetal cranium. This approach enables a technically easier procedure than the intrathoracic approach. However, this technique should be reserved for selected cases of MFPR and conducted only by experienced operators and centers.

38.2.5 Complications of MFPR

Leaking per vaginum
Bleeding per vaginum
Abortion or loss of remaining fetuses
Infection

38.3 The Dilemma of Multifetal Reduction

Multifetal pregnancy reduction has been described as a "new moral problem" created by the advancement, but not perfection, of assisted reproductive technologies (ART) [12]. It is a moral conflict for recipients (i.e., women, couples, partners, and gestational surrogates) who use ART to build their families or assist others in building theirs and then find themselves at risk because of being "too pregnant" with too many embryos. Recipients are asked to make a decision between sacrificing one for the sake of the other(s) or continuing a pregnancy that will, more than likely, have lifelong consequences for the carrier, the children born, and the intended parents. For those with previous losses or failed fertility treatments, this position of having too many seems like another cruel twist to their already painful childless life. For surrogates who believe that they can carry any pregnancy to a healthy conclusion, the decision by the intended parents is often difficult to comprehend and accept.

38.3.1 Should Fetal Reduction Be Offered to All Triplet Pregnancy? Outcomes of Triplets

Few large-scale datasets include sufficient cases to draw meaningful conclusions about outcome of triplets. Among these is a Canadian study [13] on triplets' birth, which compared the periods 1985–1990 vs. 1991–1996. The stillbirth rate did

not change significantly, whereas the infant mortality declined from 113 to 74/1000 live births, suggesting a temporal reduction in mortality associated with improved perinatal care. Newman compiled data from 12 studies published in the 1980s and 1990s from 471 pregnancies including 1413 triplets [14]. The stillbirth rate was 32/1000, and the neonatal mortality rate was 79/1000 for a perinatal mortality of 109/1000. The perinatal mortality rate for triplets corresponds well with a recent single-center experience of 100 consecutive triplet births [15]. Given the improved outcome and the declining perinatal mortality for triplets with modern perinatal care, it is not anymore certain that MFPR is absolutely indicated in triplet gestations.

Conclusion

No decision in a high-order multiple pregnancy is easy, and parents may understandably review their choices for years afterward, wondering if they should have chosen differently.

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