The ovary

Anatomy and development

The ovary is a paired gonadal structure that lies suspended between the pelvic wall and the uterus by the infundibulopelvic ligament laterally and the utero-ovarian ligament medially. Inferiorly, the hilar surface of each ovary is attached to the broad ligament by its mesentery (mesovarium), which is dorsal to the mesosalpinx and the fallopian tube. Primary neurovascular structures reach the ovary through the infundibulopelvic ligament and enter via the mesovarium.

The normal ovary varies in size, with measurements up to 5 * 3 *3 cm. Variations in dimension result from endogenous hormonal production, which varies with age and with each menstrual cycle. Exogenous substance, including oral contraceptives, gonadotropin-releasing hormone agonists, or ovulation inducing medication, may either stimulate or suppress ovarian activity and, therefore, affect size.

The ovary consists of a cortex and medulla and is covered by a single layer of flattened cuboidal to low columnar epithelium that is continuous with the peritoneum at the mesovarium. The cortex is composed of specialized stroma and follicles in various stages of development or attrition. The medulla occupies a small portion of the ovary in its hilar region and is composed primarily of fibromuscular tissue and blood vessels.

Blood supply of the ovary comes from the ovarian artery which anastomoses with the uterine artery.

Nerve supply for the ovary comes from the ovarian plexus and the uterovaginal plexus, (Jean et al, 1996).
The ovarian function
The ovary is the maestro that regulates the female hormonal and endocrinal functions
It’s mainly responsible for secretion of sex hormones and steroidogenesis
Which are responsible for the hormonal development of the female breasts and development of other secondary sexual characters.

The other important function is that the ovary is responsible for growth and maturation of ovarian follicles and finally production of mature follicles ready for ovulation, which is a crucial step that should properly take place before any pregnancy occurs.

Normal ovulation is defined by rupture of the ovarian follicle with release of an oocyte (Dunphy et al, 1991).

The only true evidence of subsequent ovulation is the occurrence of pregnancy and regretfully there is no established method to confirm completion of ovulation. The most common means in assessment of ovulation include basal body temperature recordings, determination of serum progesterone levels, detection of LH surge, microscopic examination of cervical mucus and endometrial biopsy. Finally laparoscopy is a diagnostic tool and can detect ovulation and demonstrate ovarian stigma.

Disorders of ovulation will definitely lead to delayed conception and even infertility. They are considered as the most common causes of infertility.

Females with abnormal ovulation are usually characterized by having irregular menstruation and amenorrhea or oligomenorrhea; however, ovulatory dysfunction may occur coincidentally with apparently regular cycles.
The ovulatory disorders are classified into 3 groups by the WHO;
Group I: hypogonadotrophic hypogonadism.
Group II: normogonadotrophic an ovulation.
Group III: hypergonadotrophic hypogonadism.

A blood sample may be taken between cycle days 2 and 6 for: prolactin, FSH, LH and Estradiol to identify the four main causes of ovulatory failure: hypogonadotrophic hypogonadism, normogonadotrophic an ovulation, hypergonadotrophic hypogonadism and hyperprolactinemia.

WHO group I, is usually idiopathic, and is a result of primary hypothalamic or pituitary failure. Other causes include excessive stress or exercise, malnutrition or underweight (Speroff et al, 1989). This group accounts for up to 10% of ovulatory disorders. This condition is best diagnosed by low LH and FSH levels (<5mIU/ml) and a low Estradiol level (<40g/ml) (Rowe PJ, Comhaire FH, Hargrave TB et al., 1997), along with a negative gestagen challenge test (no withdrawal bleeding).

WHO group II, the majority of patients are likely to have polycystic ovary.

WHO group III, is defined by raised FSH levels (>20mIU/ml) and low estrogen levels, indicating ovarian failure, and accounts for approximately 5% of ovulatory disorders. It may occur at any age, while in younger women the cause is mostly genetic for example; Turner’s syndrome, in older women near the end of their reproductive life, a raised FSH level is due to aging of the ovary and therefore eminent menopause. If the woman is less than 40 years old, she is classified as suffering from premature ovarian failure, an entity that applies to 1% of the population (Coulam et al, 1986).
Patients younger than 30 years of age should have a karyotype
determination. The incidence of chromosomal abnormalities in patients
with premature ovarian failure with secondary amenorrhea is estimated to
be 2 – 5 %. The presence of a Y chromosome requires surgical removal
of the gonads, as there is a 25 % risk of malignant tumor formation
(Conway et al., 1996). The most common cause of premature ovarian
failure in adults is probably autoimmune failure, and investigations for
autoimmune disorders should take place.

Prolactin measurement, although elevated levels have been found
in up to 11 % of ovulatory infertile women (Varkopoulu et al, 1993),
there was no significant correlation between its levels, progesterone
levels and/or cumulative pregnancy rates (Stratfold et al., 1999).
Therefore, the NICE guideline recommends that the prolactin levels
should not be routinely measured if there is no ovulatory disorder,
galactorrhea or pituitary tumor.
If prolactin level is elevated above 800 mIU/ml (Lenton et al., 1982), then
a repeated estimation is made and a new sample should be sent also for
estimation of thyroid stimulating hormone level. If there is occult
hypothyroidism, then a thyroid function test, including measurement of
thyroid autoantibodies, should be performed.
Although, stress remains the commonest cause of a rise in prolactin level,
polycystic ovaries, psychotherapeutic medication and the premenopausal
state must be considered, as well as the comparatively rare pituitary
prolactinoma. Galactorrhea may be present.
**Introduction**

Evaluation of ovulation is an important part of any infertility investigation. All of the different methods are useful and no one method is the best whereas some are very simple, noninvasive, and inexpensive, others are more complicated, invasive and costly. A few provide the means to determine not only if ovulation occurs but also when it occurs, with varying accuracy. The best choice among the methods available varies with the information required.

**Methods used for ovulation detection include the following**

1. Menstrual history.
2. Recording of the basal body temperature (BBT).
3. Determination of serum progesterone concentration.
4. Determination of the level of urinary LH excretion.
5. Microscopic examination of an endometrial biopsy.
6. Serial transvaginal ultrasonographic examinations (folliculometry).
7. Microscopic examination of cervical mucus.

**Menstrual history**

Menstrual history alone is sufficient to establish a diagnosis of ovulation.

Menses in normally ovulating females has the following criteria.

- Regular.
- Predictable.
• Consistent in volume and in duration.
• Typically accompanied by characteristic premenstrual and menstrual symptoms.

On the contrary in anovulatory women, menses is:
• Irregular.
• Unpredictable.
• Infrequent.
• Vary in flow characteristics.

**Basal body temperature recording (BBT)**

Basal body temperature is body temperature under basal conditions. For this purpose it is measured in the morning, on awakening and before rising. It is measured using an oral glass/mercury thermometer with an expanded scale.

Daily recording of the BBT could be used as a test of ovulation based upon the thermogenic effect of progesterone; as levels of progesterone rise after ovulation, BBT also increases. It would be more beneficial when BBT is plotted on a graph paper (Bates et al, 1990)

In ovulatory women the pattern is usually **biphasic**. The ideal BBT is obviously biphasic and reveals a cycle between 25 and 35 days in length, with menses beginning 12 days or more after the rise in temperature.

BBT recordings also can help to determine the approximate time of ovulation. The thermogenic shift in BBT occurs when progesterone concentrations rise above approximately 5ng/Ml, 1 to 5 days after the midcyclic LH-surge and up to 4 days after ovulation (Luciano et al., 1990).
**Determination of serum progesterone concentration**

Another common method for evaluating ovulation in infertile women is to measure serum progesterone concentration. Levels generally remain below 1 ng/mL during the follicular phase and rise slightly on the day of LH-surge (1-2 ng/ml) and steadily thereafter reach their peak 7 to 8 days after ovulation and then decline over the days preceding menses. Generally any level higher than 3ng/mL provides reliable objective evidence that ovulation has occurred (Wathen et al., 1984).

A popular recommendation is to measure serum progesterone on cycle day 21, that’s because in an ideal 28-days menstrual cycle ovulation usually occurs on or around day 14, and so day 21 lies in the midluteal phase just when serum progesterone levels reach their peak and approximately one week before the next cycle.

This test is used also to measure the quality of the luteal function based upon the fact that the amount and duration of progesterone production reflect the functional; capacity of the corpus luteum. However, an accurate judgment requires daily serum progesterone determinations that are both costly and impractical (Li TC et al., 1990).

Some have suggested that the sum of a few progesterone determinations may suffice, but recommendations have varied (Jordan et al., 1994).

The inevitable conclusion is that random serum progesterone concentrations defy confident interpretation and have little value beyond documenting ovulation.
**Determination of urinary LH excretion**

The midcyclic LH surge is relatively a brief event, typically lasting between 48 and 50 hours from the beginning to the end. LH has a short half life and is rapidly cleared in urine.

A wide variety of different commercial products are now available to allow women to determine not only whether they ovulate or not but also when ovulation occurs precisely, generally known as (ovulation prediction kits) or (LH kits).

These products are designed to detect the midcyclic LH surge in urine. Ovulation prediction kits turn positive when the urinary LH concentration exceeds a threshold level normally seen during the LH surge. In most cycles the test is positive only for a single day and occasionally for 2 consecutive days.

To detect LH surge reliably the test should be done on daily basis, generally beginning 2 or 3 days before the expected surge and based on the overall length of the cycle. The first positive test provides the needed information and no need for repetition of the test.

Twice daily testing might be recommended because it decreases the incidence of false negative results i.e failure to detect LH surge in an ovulatory cycle.

The morning sample is the ideal sample to be tested because it would be the most concentrated. However results correlate best with serum LH peak, when testing is performed in the late afternoon hours or early evening hours (4:00 – 10:00 pm). That’s because, LH surges start in the early morning hours and are not detected in urine except after several hours later. Ovulation usually follows 14 - 26 hours after detection of the urinary LH surge and almost always within 48 hours (Miller PB, Soules MR, 1996).
**Microscopic examination of endometrial biopsy**

Endometrial biopsy is another test of ovulation, based on the characteristic histological changes resulting from the action of progesterone on the endometrium.

During the follicular phase of the cycle, the endometrium exhibits a proliferative pattern, reflecting the growth stimulated by the rising levels of estrogen derived from the dominant ovarian follicle. During the luteal phase, progesterone secreted by the corpus luteum causes the secretory transformation of the endometrium. Anovulatory women are always with extended exposure to estrogen. In the absence of exogenous progesterone administration, a secretory endometrium is an evidence of recent ovulation.

**Indications for performing an endometrial biopsy**

- Women with chronic anovulation to exclude of associated endometrial hyperplasia.
- Women suspected to have chronic endometritis.

Microscopic examination of endometrial biopsy could be an effective method for evaluation of ovulation, however it is not the best method when compared with other techniques which are highly effective, simple and reliable.

**Serial transvaginal ultrasonographic examinations**

This method involves direct observation of a characteristic sequence of changes that occur prior to and immediately after ovulation occurs.

Serial transvaginal ultrasonographic examination offer detailed information about the size and the number of preovulatory follicles and provides an accurate estimate of when ovulation occurs.
In its final stages the preovulatory follicle grows at a predictable pace around 2mm/day (average 1 – 3 mm/day). After ovulation the follicle abruptly decreases in size, its margins become less distinct, the density of internal echoes increase, and the volume of fluid in the Douglas pouch increases (Echochard et al., 2000).

Abnormal patterns of follicular growth could be seen by ultrasonography for example; the follicle may grow at an abnormal pace, or collapse when still relatively small, or continue to grow but fail to rupture and persist as a cyst for days after the LH surge, the lutenized unruptured follicle (Petsos et al., 1985)

**Advantages**

Transvaginal ultrasonography can detect the presence of a lutenized unruptured follicle which is a rare condition predisposed to by treatment with prostaglandins (Smith et al., 1996)

Serial transvaginal ultrasonographic examination to monitor the size and number of developing ovulation is essential for the safety and effectiveness of the induction process especially with exogenous gonadotropins, however costs and logistical challenges might be difficult to justify. Therefore it should only be done in whom the safety and effectiveness of treatment truly depends on the information they offer.

**Examination of cervical mucus**

Examination of cervical mucus could be used as a method for evaluation of ovulation, before ovulation the cervical glands become exposed to the highest levels of estrogen.

Immediately before ovulation cervical mucus will have the following characteristics under the influence of estrogen being, stretchable when pulled with a forceps from the cervix up to 8-10cm
(positive Spinnbarkeit), demonstrates a highly ferning characteristic pattern similar to that on examination of amniotic fluid, clear, and watery.

After ovulation occurs and under the effect of progesterone cervical mucus becomes unstretchable (negative Spinnbarkeit), thick, opaque, and lacks ferning (Mark D Hornstein et al, 1996).

**Patient evaluation could be done through assessment of the ovarian reserve which could be done using one of the following various tests:**

1. Determination of basal serum FSH and serum estradiol.
2. Dynamic ovarian reserve tests (clomiphine citrate challenge test).
3. Determination of serum levels of anti-mullerian hormone.
4. Determination of serum basal levels of inhibin–B.

**Ovarian reserve tests**

These are tests that generally describe the size and the quality of the remaining follicular pool. Over the past several years, ovarian reserve tests have emerged as a new, important, and highly useful tool in evaluation of infertile women. Ovarian reserve tests are generally reliable but certainly not infallible. Rigid interpretation and application of test results risks inappropriate recommendations for treatment or for no treatment, and both must be avoided. An abnormal test result does not exclude the possibility of pregnancy. Except perhaps when grossly abnormal, test results should, therefore, not to be used to deny treatment but only to obtain prognostic information that can help to guide the choice of treatment and best use of available resources. Otherwise, although the
probability of pregnancy may be low, one can accurately who will be among those few with abnormal test results that succeed. Ultimately, regardless that the statistical prognosis may be, the success rate for any individual woman will be zero or 100%.

**Indications for the use of ovarian reserve tests**

Ovarian reserve tests should be done in females having any of the following characteristics:

- Age older than 35.
- Unexplained infertility regardless of age.
- Family history of early menopause.
- Previous ovarian surgery as ovarian drilling, ovarian cystectomy or unilateral oophorectomy.
- Previous chemotherapy or irradiation of the ovaries.
- Smoking females.
- Demonstrated poor response to exogenous gonadotropin stimulation.

**Basal FSH and Estradiol levels as measurements for ovarian reserve**

It is well known that fertility declines with increasing female age, it was also found that this is associated with rising serum FSH levels which made it logical that detection of serum FSH levels might have a prognostic value.
Numerous studies have investigated the relationship between cycle day 3 FSH concentrations or FSH/LH ratios and IVF cycle outcomes; all observed that these measures correlate closely with the ovarian response to exogenous gonadotropin stimulation and the likelihood for success. As values increase, peak Estradiol levels, the number of oocytes retrieved, and the probability of pregnancy or live births steadily decline (Barroso et al., 2001) currently, in most laboratories, cycle day 3 serum FSH levels above 10-15 IU/L are considered abnormal.

Early follicular phase Estradiol levels may provide additional useful information for evaluation of the ovarian reserve. Like FSH, a high cycle day 3 Estradiol concentration (greater than 80 pg/mL) also predicts low fecundability (Buyalos et al, 1997). Early elevations in serum Estradiol reflect the advanced follicular development and early selection of a dominant follicle observed in older cycling women that are driven by rising FSH levels. A premature elevation concentrations may also tend to suppress the FSH level, masking an elevation that might otherwise reveal a low ovarian reserve. Therefore, measurement of both FSH and Estradiol on cycle day 3 may help to decrease the incidence of false-negative tests based on measurement of FSH alone.

**Clomiphene citrate challenge test (dynamic test)**

It is a provocative and a sensitive test of ovarian reserve that probes the endocrinial dynamics of the cycle under both basal and stimulated conditions before (cycle day 3 FSH and Estradiol) and after (cycle day 10 FSH), treatment with clomiphene citrate a dose of 100mg/day cycle days 5 to 9 (Navot et al, 1987). when given to menstruating fertile women less than 35 years of age, clomiphene
typically stimulates transient increase in gonadotropin levels; LH generally rises more than FSH.

However, in women with low ovarian reserve, that pattern may be reversed; FSH may increase more than LH, sometimes to frankly elevated concentrations.

Although the mechanisms responsible are not entirely clear, evidence suggests that the smaller follicular cohorts in aging women produce less inhibin-B and Estradiol, resulting in less negative feedback inhibition on clomiphene-induced pituitary FSH release (Yong et al., 2003).

The clomiphene challenge test can identify women who might go unrecognized if evaluated by basal cycle day 3 FSH and Estradiol levels alone.

However, in women with a normal day 3 FSH level, a high day 10 value has the same poor prognosis as an elevated day 3 FSH concentration. An elevated cycle day 3 FSH level or abnormal clomiphene challenge test correlates consistently with a poor prognosis for IVF success (less than 10%), regardless of age (Yanushpolsky et al., 2003).

The stimulated day 10 Estradiol level has no prognostic value (Scott et al., 1993).

The prognosis for women with an abnormal ovarian reserve test is generally poor, even if they are young. In contrast, the prognosis for women with normal test results relates to their age; a normal test result does not improve the poorer, age-specific prognosis for older women.

Ovarian reserve tests are generally reliable, but they can also be misleading. Consider that interpretation generally hinges on whether
selected hormone levels are above or below critical threshold concentrations in a single test cycle and that both basal and stimulated FSH levels may vary between cycles, especially in older cycling women, thus, results and interpretation can also vary (Hannoun et al., 1998). Women with low cycle day 3 FSH levels generally exhibit less between cycle variability than those with higher levels. However, the response to gonadotropin stimulation does not vary with basal FSH levels in those in whom serial testing reveals large variations and yields both normal and abnormal results; such women generally respond poorly to stimulation, even in cycles in which day 3 FSH levels are comparatively low.

Therefore, whereas repeated testing in women with abnormal tests in efforts to identify an optimal cycle for treatment is unnecessary because the prognosis does not change (Scott et al., 1990), repeat testing in older women with normal test results may be prudent before complicated and costly treatments begin.

Numerous other methods for measuring the ovarian reserve have been investigated including

- Ovarian volume and early follicular phase antral follicle counts( Yong et al., 2003).
- Basal and clomiphene or exogenous FSH stimulated inhibin-B levels (Kwee et al., 2003).
- Response (FSH, Estradiol, inhibin-B) to stimulation with GnRH agonist (Ranieri et al., 2001) or human menopausal gonadotropins (Fabregues et al., 2000).
- Basal and GnRH agonist or gonadotropin stimulated antimullerian hormone levels ( Van Rooij et al., 2002)
• Transvaginal ultrasound examination at the outset of the menstrual cycle to observe the number of small antral follicles which reflects the size of the resting follicular pool (Scheffer et al., 1999) and correlates with the age and response to gonadotropin. stimulation; observation of 10 or fewer follicles might lead to cycle cancellation.

• Low basal or stimulated inhibin-B levels suggest a low ovarian reserve, but studies of their predictive value have yielded conflicting results (Kwee et al., 2003).

When the clomiphene challenge test is used as a screening test for infertile women of all ages, the prevalence of abnormal tests is approximately 10% overall, increases with age, and is disproportionately high in women with unexplained infertility i.e abnormal tests are associated with poor prognosis regardless of age, other identified causes of infertility, or type of treatment (Scott et al., 1993).

Careful studies in women whose infertility remains unexplained after rigorously thorough evaluation have provided direct evidence to suggest that a premature decline in ovarian reserve may be responsible (Leach et al., 1997).

Life table analysis of outcomes observed in infertile women after exclusion of tubal or peritoneal disease and male factor reveal that cumulative success rates decrease with advancing age in women with a normal screening clomiphene-challenge test and are dramatically lower in women with abnormal tests regardless of age (Scott et al., 1995).

These observations indicate that ovarian reserve tests have applications beyond prognostication for women considering IVF.
Determination of serum levels of anti-mullerian hormone

Antimullerian hormone (AMH), a member of the transforming growth factors (TGF) family, was identified as a factor that causes regression of the mullerian ducts during male fetal development.

In females, AMH, also known as mullerian inhibiting substance, is produced in the granulosa cells of ovarian follicles and is secreted into the circulation. A few early studies demonstrated that serum levels decrease with age earlier than other markers such as FSH and antral follicle count (Van Rooij et al, 2002).

Determination of serum basal levels of inhibin–B

Inhibin – B is a heterodimeric glycoprotein released by the granulosa cells of the follicle.

A recent study has shown that women with low day-3 inhibin-B levels (Less than 45pg/ml) had a poorer response to super ovulation for IVF and were less likely to conceive a clinical pregnancy. It has also shown that a decrease in inhibin-B probably precedes the increase in FSH levels (Seifer et al, 1997); however, the role of inhibin –B in predicting pregnancy outcome is unclear (Corson et al, 1999).

What is a poor responder?

Unfortunately, there is no universally accepted definition for the "Low", "poor", "bad" or "non"-responder, although these patients have definitely lower pregnancy rates compared with "normal" responders.

Numerous criteria have been used to characterize poor response. The most important criteria for defining poor responders are:
• Number of developed follicles and/or number of oocytes retrieved after a standard-dose ovarian stimulation protocol. The proposed number varies among different authors, and ranges from less than 3 to less than 5 dominant follicles on the day hCG administration. (Land et al., 1996; Fridstrom et al., 1997; Raga et al., 1999), and/or less than 3 to less than 5 retrieved oocytes (Chong et al., 1986; Rombauts et al., 1998; Surrey et al., 1998).

• Peak Estradiol level is another criterion used, as it correlates with the number of developing follicles. A peak Estradiol level of less than 300 to less than 500 pg/ml has been proposed as being crucial for defining poor response (Garcia et al., 1983; Brzyski et al., 1988; Raga et al., 1999), although a level than 100 pg/ml on day 5 of stimulation has also been suggested (Schoolcraft et al., 1997).

• An elevated day 3 FSH more than or equal 7 to more than or equal 15 mIU/ml has been proposed as an additional criterion to define poor ovarian response (Droesch et al., 1989; Feldberg et al., 1994; Karande et al., 1997; Faber et al., 1998).

• Likewise, an advanced age of the patient of more than or equal 40 years (Karande et al., 1997).

• Disappointing or no response to the clomiphene challenge test (Navot et al., 1987).

• Failed “leuprolide screening test” (Katayama et al., 1988), may be predictive of poor response.

• Another parameter used by several authors is the total gonadotropin dose used and/or the daily stimulation dose (Hofmann et al., 1989; Hugues et al., 1991; Ibrahim et al., 1991; Salat-Baroux et al., 1993; Hughes et al., 1994; Karande et al., 1997).
• Prolonged duration of gonadotropins stimulation (Toth et al., 1996).
• More logically but not more accurately, criteria such as at least cancelled one cycle of IVF (Schachter et al., 2001)
• Increased number of HMG or FSH ampoules used ( >44) (Shaker et al., 1992)
• Increased (> 300 IU/day) gonadotropin dose used (Faber et al., 1998).

• **Recently,** Kailasam et al (2004) considered those patients who fail to develop more than 3 preovulatory follicles after using more than 300 IU FSH daily or when it required more than 3000 IU FSH to recruit less than 4 follicles as poor responders.

It should be noted that, in a variety of studies, these criteria have been used either or in combination, thereby highlighting the complexity, the lack of uniformity in definitions and also the major difficulties encountered when comparing the different strategies proposed.

**Other Suggested classifications of poor responders**

• According to Gorgy and Taramissi, (2001), there are 2 subgroups of poor responders.

  1. The young (aged less than or equal 37 years) and slim (body weight less than or equal 70 kg), who developed less than five follicles following 9 days of ovarian stimulation with 225 IU/day and did not reach oocyte retrieval or required more than 600IU of gonadotropin per retrieved oocytes if they did reach that stage.

  2. The second subgroup includes patients more than 37 years and weighing more than 70 kg who had their cycles cancelled due to
less than 5 developed follicles following 9 days of ovarian stimulation with 300 IU/day of gonadotropins.

- Barry et al., 1998, proposed that 3 subgroups of poor responders should be identified.
  1. Patients with a low response to previous IVF attempts but normal basal FSH levels.
  2. Young patients with persistently high FSH levels.
  3. Older patients with an abnormal endocrinological profile.

Clinical experience has not shown any real advantage in subcategorizing patients however, as no significant differences regarding response to ovarian hyperstimulation protocols have been observed. In fact, low ovarian response is confirmed only after the patient has failed ovarian stimulation following an accepted as normal or standard ovarian stimulation regimen.

**Aetiology of poor ovarian response**

Although several possible etiologies have been suggested, it seems that a diminished ovarian reserve is the principal factor in poor ovarian response (Pellicer et al., 1998).

Women have a finite number of germ cells reaching a peak of 6 – 7 million germ cells by gestation week 20. From mid gestation onward and throughout the reproductive life, an irreversible attrition progressively diminishes the germ cell pool of the gonad (Peters, 1976), mainly through follicular atresia. The rate of follicular attrition is not constant but rather follows a bi-exponential pattern, with the change in exponential rate being determined by the number of remaining oocytes rather than age (Faddy et al., 1992).
There is a marked increase in the rate of follicular disappearance from age 37 – 38 years onwards at which the total number of follicles reaches 25000 (Faddy et al., 1992). It then takes around 13 years to reach menopause (1000 remaining follicles), regardless the age of menopause (Faddy et al., 1992).

The rate of follicular disappearance and its relation to the important reproductive events in the female life has been extensively studied, suggesting that after a period of optimal fertility from age 18 – 31 years, the quality of oocyte decreases in parallel to decreased number of follicles becoming severely impaired after 37 to 38 years of age reaching around 25000 follicles (Faddy et al., 1992; Gougeon et al., 1996). Several theories have been formulated to explain declining the quality of oocytes with age in the so-called "production line" hypothesis. Oocyte quality is established during fetal life, and oocytes that are less susceptible to non-disjunction are ovulated first, leaving poor quality of oocytes to be ovulated later in life. (Henderson and Edward 1986; Polani and Crolla, 1991).

For this reason, the associated reproductive impairments may be more closely related to decline in the ovarian follicle number than to age or to the interval before the menopause rather than the age at which that occurs.

Therefore in young women, premature reduction of ovarian follicle numbers, whether by excessive atresia or accidental or iatrogenic damage, could lead to an exaggeration of all reproductive problems.

This aspect could be very important, as those women who will become menopausal earlier (40 to 45 years) will have an accelerated decline in fertility (25000 remaining follicles) at an unexpected young age.
It has been estimated that 10% of women in their early 30s with reduced fertility which is otherwise unexplained (Scott et al., 1993; Hofmann et al., 1996). However, besides the "production line" hypothesis supported by some experimental data in mice (Brook et al., 1984; Meredith and Butcher, 1985) and in women (Hardy and Kuh, 1999; Freeman et al., 2000), other data on related to female ovarian aging point to an increased frequency of meiotic non-disjunction as time passes which is the most important mechanism responsible for the majority of aneuploidies in early embryos (Lamb et al., 1997).

In fact oocyte quality seems to be less impaired in younger patients with elevated FSH concentrations and reduced ovarian reserve and the implantation rates between young patients (< 35 years) with high or low basal serum FSH appear to be comparable (Chuang et al., 2003). Similarly, Van Rooig et al., (2003) comparing women less than 40 years old within normal serum FSH with younger women who have higher basal serum FSH concentrations, reported higher cycle cancellation rates and lower number of oocytes retrieved in the latter group of patients; however, once the oocytes were retrieved the younger women had near normal implantation rates.

These results suggest that estimation of the ovarian reserve is a better predictor of oocyte production capacity than of quality, whereas age affects oocyte quality.

This phenomenon can be explained by an age-dependant accumulation of damage due to either compromised granulose cell function (Warburton, 1989) or progressively defective microcirculation around the leading follicle with reduced oxygen concentrations in the follicular fluid (Van Blerkom et al., 1997) or a gradual increase in the intracellular oxidative stress (Tarin, 1995).
This hypothesis is however, disputed and other authors reported a reduction in the implantation rates in young women with high basal serum FSH concentrations after IVF (Toner et al., 1991; Scott et al., 1995; Akande et al., 2002; El Toukhy et al., 2002), suggesting that aneuploidy before (Roberts and O'Neill, 1995) or after (Benadiva et al., 1996) fertilization due to disruption of meiotic spindle assembly (Battaglia et al., 1996) or mitochondrial DNA deletions (Keefe et al., 1995) are not simply due to duration of exposure to risk of damage, but were probably present since fetal life. More universally agreed is the very poor reproductive outcome in women approaching 40 years old with poor ovarian response. In this group of women, it is necessary to relate the effect of the reduced ovarian reserve to the diminished oocyte quality. The result is that there is a higher proportion of "poor quality" oocyte due to either the "production line" hypothesis (Henderson and Edwards, 1968; Polani and Crolla, 1991) versus time related exposure to risk of damage (Te Velde and Pearsons, 2002).

Although reduced ovarian reserve is the most important and frequent aetiological factor in reduced ovarian response to ovarian stimulation, in some patients the presence of polymorphic FSH receptor in which the asparagine of the receptor protein is replaced by serine at position 680 requiring higher FSH concentrations for normal function, and it is probably not related to reproductive aging (Perez Mayorga et al., 2000).

Moreover, low responses have also been associated with:

- The presence of ovarian antibodies (Meyer et al., 1990).
- Reduced aromatase activity (Hurst et al., 1992).
- Decreased blood flow measured by Doppler ultrasonography (Pellicer et al., 1994).
- Reduced circulating surge-attenuating gonadotropin factor (GnSAF) bioactivity (Martinez et al., 2002)
Other possible acquired factors include obesity (Dechaud et al., 1998), chemotherapy, radiotherapy, pelvic surgery (Tulandi et al., 2002), pelvic infections or tubal disease (Keay et al., 1998), severe endometriosis (Barnhart et al., 2002) and heavy smoking (El Nemr et al., 1998).

**Prediction of poor ovarian response**
Proper identification of those who are at risk of poor ovarian response prior to stimulation is useful in counseling patients, and may be helpful in tailoring the best stimulation protocol and dosage of gonadotropin to individual patients.

Before analyzing the tests that have been described as predictors of ovarian reserve, it is important to emphasize that no test is absolutely predictive and the best test is of course the ovarian response itself.

Measurement of serum FSH concentrations on day 3 of the cycle represents one of the most widely used prognostic tests in assessment of the ovarian reserve. Basal serum FSH concentrations begin to rise on average a decade or more before the menopause (Ebbiary et al., 1994; Klein et al., 1996), are inversely correlated with ovarian follicular responsiveness to maximal exogenous gonadotropin stimulation (Cahill et al., 1994). This is caused by the negative feed back of the FSH-modulating proteins from the ovary, mainly inhibin-A and inhibin-B (Groome et al., 1996). Since inhibin-B is predominantly secreted by the early antral follicles, a decreased serum concentration of inhibin –B reflects a reduction of the antral follicle pool (Burger et al., 1998; Welt et al., 1999), and it is clearly associated with the FSH rise in the early follicular phase.

Subtle serum increase of FSH represents an early signal of the decline of the ovarian response despite regular menses, and it is associated with unexplained infertility (Cameron et al., 1998; Muasher et al., 1998).
There is a wide variation in what can be considered "high" FSH values, and basal high FSH concentrations > 10 or > 12 or > 15 mIU/ml have been reported as predictive of poor response and poor clinical outcome (Cameron et al., 1988; Scott et al 1989; Toner et al., 1991; Faber et al., 1998).

Moreover, even a single elevated FSH value might denote a reduced ovarian reserve (Scott et al., 1990). However, when basal serum FSH concentrations are used to predict the ovarian reserve, it is necessary to consider the possible variability between cycles of day 3 FSH reported by some (Brown et al., 1995) but not all authors (Penarrubia et al., 2004), which suggests that basal hormone values should be samples within 3 months of the assisted reproduction cycle (Creus et al., 2000).

Another marker of reduced ovarian reserve is the increased basal Estradiol concentrations in the presence of "normal" FSH concentrations due to early follicular recruitment that occurs consequently to a premature luteal elevation of FSH. This early luteal recruitment is probably due to a diminished follicular cohort that produces less inhibin. Elevated Estradiol concentrations may be able to suppress FSH concentrations to the normal range in women who have substantially diminished ovarian reserve and thus may cause false – negative test results. Basal Estradiol concentrations > 30 or > 45 or >70 pg/ml have been associated with poor IVF outcome (Licciardi et al., 1995; Smotrich et al., 1995). Higher inhibin – B concentrations throughout stimulation were related to higher oocyte yield (Engel et al., 2001), whereas lower serum concentrations of inhibin-B on day 3 very likely reflect lower follicular numbers and may serve as a good predictor of clinical outcome (Burns et al., 1996; Seifer et al., 1997).

In patient with day 3 serum inhibin – B concentrations < 45 pg/ml, the number of oocytes retrieved is lower, the cycle cancellation rate is higher.
and the clinical outcome is poorer compared with patients with day 3 inhibin -B concentrations more than or equal 45 pg/ml( Seifer et al ., 1997 ).

Recently Martinez et al, (2002) suggested a role of GnSAF, an ovarian factor that has a specific biological effect of reducing pituitary responsiveness to GnRH, in the prediction of the ovarian reserve. Authors demonstrated that on the day of HCG administration GnSAF concentrations are lower in women with poor ovarian response to ovarian stimulation compared to normal responders. Moreover they observed lack of GnSAF activity during the follicular phase of spontaneous cycles and a much more reduced and slower increase in circulating GnSAF following FSH stimulation in women with poor ovarian response to ovarian stimulation compared to patients with normal ovarian response) Martinez et al .,2002).

A new promising biochemical marker that could be used as a predictor of the ovarian reserve is the anti-mullerian hormone. Because the anti-mullerian hormone is produced exclusively by the small growing follicles and secreted into the circulation. Serum concentrations of the anti-mullerian hormone were significantly decreased over time in young ovulatory women, whereas other markers associated with ovarian aging such as serum concentrations of FSH and inhibin-B did not change during the same interval (De Vet et al., 2002).

Ultimately the high heritability found fir age at menopause suggests genetic control by an unknown number of genes. With development in molecular genetics, it might be possible to construct DNA fingerprints that will be able to identify women with genetic predisposition to what is so called ‘early ovarian aging’ (Te Velde and Pearson, 2002). some authors have suggested dynamic tests of ovarian reserve such as clomiphine challenge test ( Navot et al ., 19987), the lupron screening test
(Padilla et al., 1991), change in Estradiol concentrations after exogenous FSH stimulation (Fanchin et al., 1994) and the GnRH stimulation test (Karande and Gleicher, 1999) as useful tools to predict the ovarian response. Recently, the ultrasonographic characteristics of the ovary have been suggested to be predictive of the ovarian potential during ovarian stimulation. The antral follicle count as well as ovarian volume appeared to be indicative of poor response in assisted reproduction. Pellicer et al. (1998) reported an intimate relation between the number of selectable follicles (2-5 mm) as measured by three dimensional transvaginal ultrasonography and the number of selectable follicles in histological slices. According to this observation, it is possible that the number of the antral follicles originating from the cohort of the growing follicles reflects the size of the pool of resting follicles, and thus the ovarian reserve.

Bancsi et al. (2002), analyzing the odds ratios, statistical significance and receiver operating curve (ROC) area under the curve (AUC) for six basal ovarian reserve markers, observed that the number of antral follicles as a single predictor was the most powerful prognosticator of reduced ovarian response expressed by the largest ROC AUC of 0.87. This discriminative potential for poor response could increase if the day 3 inhibin-B and FSH are also considered.

In a multivariate analysis with 3 variables, the antral follicle count was selected in the first step, followed by inhibin-B in a step two and finally FSH in step 3. The ROC AUC increased in a stepwise manner from 0.87 to 0.92 (Bancsi et al., 2002).

Similar results were recently reported in a prospective study where various markers of ovarian reserve (FSH, LH, Estradiol, inhibin-B< antral follicle count) were evaluated in the natural cycle preceding assisted reproductive therapy in 60 women as prognosticators of their ovarian response to ovarian stimulation (Loverro et al., 2003).
Popular Protocols Of Ovulation Induction

Could be done either medical or surgical

Medical methods

These methods include; administration of clomiphene citrate commercially known as clomid, exogenous gonadotropin administration, GnRH agonist administration, insulin sensitizing agents, and dopamine agonist administration.

Induction of ovulation using clomiphine citrate

History

Clomiphene was first synthesized in 1956, and first used in clinical trials in 1960, and approved by the USA in 1967.

Pharmacology:

It is a non-steroidal triphenylethelene with both estrogen agonist/antagonist properties.

Mainly acts as an antagonist, its estrogenic effects when circulating levels of estrogen are very low.

It is a racemic mixture of two stereoisomer, enclomiphine (cis clomiphine) and zuclomiphene (transclomiphene).

Enclomiphine is more potent and responsible for the ovulatory function of clomiphene, zuclomiphene is weaker but has a longer half-life. Serum levels remain detectable for weeks and may gradually accumulate for a series of cycles, but with no clinical significance or side effects (Dickey et al,1996).

Mechanism of action

It competes for and binds for estrogen nuclear receptors leading to their depletion as it also prevents their recycling (Clark et al,1982)
At the level of the hypothalamus circulating levels of estrogen are perceived lower than what they really are.

This leads to triggering of the compensatory mechanisms increasing gonadotropin releasing hormone secretion leading to increased levels of FSH and LH secretion.

If administered in normal ovulatory females this leads to increased frequency of GnRH pulses (Kerin et al., 1985)

But if administered in anovulatory females with PCO in whom GnRH pulse frequency is already abnormally high, clomiphine increases amplitude and not the frequency of the pulses (Kettel et al., 1993) leading to follicular maturation, together with increased estrogen levels leading to the occurrence of LH surge and ovulation.

Clomiphene citrate works by stimulating the normal endocrinological mechanisms that control hypothalamic-pituitary-ovarian feed back.

**Indications for clomiphine citrate therapy**

It is the drug of choice for ovulation induction in

1. **anovulatory infertile women with:**
   - Normal thyroid function.
   - Normal prolactin levels with no galactorrhea.
   - Having normal levels of endogenous estrogen (greater than approximately 40 pg/ml).

2. **it could also be used in cases having:**
   **Luteal phase deficiency;**
   It is both logical and effective to use clomiphine citrate in patients with abnormally short luteal phase, which is usually
associated with abnormally low levels of follicular phase FSH (Sherman et al, 1974).

The use of clomiphine increases serum progesterone levels than in normal spontaneous cycles, likely, because preovulatory follicular development is optimized and also because treatment may result in more than on corpus luteum (Guzick et al, 1990).

**Unexplained infertility**

In cases with unexplained infertility, Clomiphine citrate is used empirically and is mostly effective when combined with intrauterine insemination (IUI) in an effort to increase the number of ova and sperms (Guzick et al., 1998). The efficacy of treatment in these cases can be attributed to correction of the unrecognized luteal phase deficiency or to the superovulation of more than a single ovum (Meyer et al, 1995).

**Clomiphine treatment regimens**

Clomiphine is administered orally starting from the third day to the fifth day of a spontaneous or progestin induced cycle.

In females with amenorrhea, we can start clomiphine immediately once pregnancy has been excluded.

Regarding the dosage, we usually start by giving 50 mg tablets daily for a 5 day interval and increases by 50 mg increments in subsequent cycles until ovulation is achieved (most females will respond at a dose of 50 mg per day (52%) or a 100 mg per day(22%))(Gysler et al., 1982).
**Monitoring of clomiphine treatment**

Clomiphine citrate treatment could be monitored by a variety of methods among of which are the following methods; serial transvaginal, ultrasonography (folliculometry), determination of serum progesterone level; a level > 3ng/ml is regarded as evidence of ovulation.

Another method is monitoring of midcyclic LH surge which could be done easily using the available commercial kits (LH surge typically occurs 5 to 12 days after the end of treatment) (most often day 16 or 17 of the cycle when clomiphine is administered from day 5 to day 9 of the cycle) (Opsahl et al., 1996)

**Results of clomiphine therapy**

Clomiphine will successfully induce ovulation in approximately 80 % of properly selected candidates (Imani et al., 1998)

Among anovulatory infertile women who ovulate in response to clomiphine treatment, the overall cycle fecundability is approximately 15 %. Cumulative pregnancy rates of 70 to 75 % can be expected over 6 to 9 cycles of treatment (Imani et al., 2002)

**Risks of clomiphine treatment**

Risks of the use of clomiphine therapy include the following:

- Multiple pregnancy with an increased risk of 5 to 8 % and is mainly due to multifollicular development (Correy et al, 1982)
- Ovarian hyperstimulation syndrome (OHSS), which is a common complication, however, with clomiphine therapy it usually occurs in a mild form (transient abdominal discomfort, nausea, vomiting, and diarrhea).
• Congenital anomalies and miscarriage; however both are still controversial.

• Relation to ovarian cancer

Two epidemiologic studies suggested that there is an increased risk of ovarian cancers in females receiving induction of ovulation.

The first study was a case control study that concluded that ovarian cancer increased nearly 3 folds in women undergoing induction of ovulation, however, study methodology was widely criticized (Whittemore et al., 1992).

The main point of criticism was that the study compared infertile treated women to fertile untreated women (rather to fertile untreated women), and it is known that nulliparity and infertility themselves might be risk factors of infertility.

The second study was also a case control study concluding that the risk of ovarian rumors was increased in women treated with clomiphine for more than 12 cycles. The point of criticism in that study is that it included cancers of all types and tumors of low malignant potential despite their different pathophysiology (Rossing et al., 1994).

However, subsequent studies have been reassuring, and women with concerns should be counseled that there is no relationship between the use of clomiphine and ovarian cancers.

• Relation to breast cancer

Most of studies have found no evidence between the use of fertility drugs and increased incidence of breast cancer (Ricci et al., 1999)
**Treatment options after clomiphine citrate failure**

Options include the following:

- Adjuvant treatment with glucocorticoids or exogenous human chorionic gonadotropin (hCG).
- Preliminary suppressive therapy i.e the use of oral contraceptive pills.
- The use of insulin sensitizing agents (metformin).
- Aromatase inhibitors
- Surgical treatment via ovarian drilling.

**Extended course of clomiphine treatment**

The course might be extended from 5 days with a daily dose of 50 mg/day to (7 to 10) days with the same daily dose; this will result in 50% improvement in clomiphine resistant anovulatory women (Isaacs et al., 1997).

**Administration of clomiphine and glucocorticoids**

Numerous studies have found that combined treatment with clomiphine citrate and glucocorticoids can successfully induce ovulation in many who fail to respond to clomiphine alone (Parsanezhad et al., 2002) Whereas, some studies have suggested that combined treatment with clomiphine and glucocorticoids is most effective in women with elevated serum dehydroepiandrosterone sulfate (DHAS) (Daly et al., 1984).

The largest randomized trial alone included 200 clomiphine resistant, anovulatory, infertile females. A result of over 80% receiving combined treatment of clomiphine at a dose of 200 mg daily, cycle days from 5 to 9, and dexamethasone at a dose of 2 mg daily cycle days from 5 to 14, had
good ovulation compared to 20% of controls treated with clomiphine and placebo; the cumulative pregnancy rates increased 10 folds in females receiving combined treatment of clomiphine and glucocorticoids (40%), than in those receiving placebo (4%)(Parsanezhad et al., 2002).

The mechanism of action of glucocorticoids is unclear; however possibilities include direct action on the oocyte and indirect effects on the intrafollicular growth factors and cytokines that may act synergistically with FSH (Keay et al., 2003).

Regarding side effects, no evidence of any important side effects have been reported when used in proper doses.

**Administration of clomiphine citrate and hCG**

There is few data to demonstrate the value of administration of hCG and clomiphine.

The use of HCG with clomiphine could be considered as a surrogate LH-surge to trigger ovulation in clomiphine induced cycles. However, the use of HCG is of limited indications mainly for few candidates who require intrauterine insemination (IUI), but repeatedly fail to detect the LH surge using the commercial kits, despite other evidence of successful ovulation induction. In such circumstances HCG administration is best postponed until the preovulatory follicle reaches or exceeds 20 mm in mean diameter. Ovulation occurs 34 to 46 hours after HCG injection (Andersen et al., 1995), so IUI is usually performed approximately 36 hours later.

On the other hand, when LH surge can be detected, adjuvant hCG treatment has no value and only adds unnecessary expenses.

The question when to administer HCG is a dilemma, and serial transvaginal ultrasonography is required to demonstrate the phenomenon, and to ensure that the ovulatory stimulus is delivered at the appropriate time.
If HCG is administered blindly, this could result in follicular atresia and anovulation, which is a distinct disadvantage and a potential consequence.

**Preliminary suppressive therapy**

Thinking of anovulation as a hypothalamic-pituitary–ovarian axis dysfunction, it would be quiet logic to think that an interval of preliminary suppressive therapy might help to restore harmony and ovulatory function. The idea is consistent with clinical observations of a few normal menstrual cycles immediately following discontinuation of oral contraceptives in some women who previously exhibited their normal menstrual pattern. Studies have demonstrated that a 2-month interval of continuous oral contraception will effectively suppress serum LH and androgen levels and that ovulation rates exceed 70%, and cumulative pregnancy rates exceed 50% in previously clomiphine resistant anovulatory women. (Branigan et al., 2003).

A long acting GnRH agonist, alone or in combination with oral contraceptive, can be used for the same purpose. Combined suppressive therapy with a GnRH and oral contraceptives (6 months) achieves a greater and longer lasting reduction in serum LH and androgen levels than in treatment with oral contraceptives alone. It also prevents the otherwise inevitable estrogen deficiency symptoms that develop when a GnRH agonist is used alone, spontaneous resumption of the normal menstrual pattern usually follows (Genazzani et al., 2000), potentially obviating the need for clomiphene treatment.
Insulin sensitizing agents

Insulin resistance and hyperinsulinemia are now recognized as a common feature of polycystic ovary syndrome (PCO), and an important contributing cause of hyperandrogenism and chronic anovulation that characterize the disorder.

Anovulatory infertile women with PCO and hyperinsulinemia are also more resistant to clomiphine treatment.

Furthermore, Obesity contributes to insulin resistance. In obese anovulatory infertile women with PCO, weight loss alone (5% or more) decrease hyperinsulinemia and often restores ovulatory cycles (Hollmann et al, 1996) (Jakubowiez et al, 1997).

In one study, 90% of patients who lost an average of 10 kg/m^2 in a dietary and exercise program resumed spontaneous ovulation and 78 % ultimately achieved pregnancy, 27 % without other interventions (Clark et al 1998). It is important to emphasize that weight loss amounting to no more than 5 % can have significant beneficial effects (Muschelli et al 1997).

Almost all obese and many non obese anovulatory women with PCO are insulin resistant. The resulting compensatory hyperinsulinemia can contribute further to ovarian hyperandrogenism and also interfere directly with ovulation.

All obese anovulatory women with PCO should be screened for impaired glucose intolerance and diabetes anyway; a 2- hour oral glucose tolerance test is now the preferred method of assessment.

Recognition of the role of the role of insulin resistance in the pathophysiology of PCO stimulated intense interest in the use of insulin sensitizing agents for treatment of this condition. One of the most important agents is metformin.
**Metformin** is an oral hypoglycemic, biguanide class that acts mainly through reducing hepatic gluconeogenesis, and also decreases intestinal absorption of glucose and increases glucose uptake and utilization peripherally.

The role of metformin in management of an ovulation has been tested in various studies. Many of these studies had been randomized trials which compared metformin with placebo or no treatment (Vandermolen et al, 2001) (Fleming et al, 2002).

Others have compared treatment with both metformin and clomiphine to treatment with clomiphine alone in previously clomiphine resistant women. (Malkawi et al, 2002) (Sturrock et al, 2002), or in unselected women with polycystic ovarian syndrome, (El- Biely MM, Habba M, 2001).

Most studies, (Fleming et al., 2002) (Yarali et al., 2002) but not all, (Vandermolen et al, 2001) have observed that metformin treatment alone can induce ovulation in anovulatory females with PCO. Ovulation rates in metformin-treated women have varied widely among studies, ranging from a low of 8% to a high of 82%; overall, the observed ovulation rate is approximately 40%

A meta analysis including 7 studies involving a total of 310 women with PCO found that women treated with metformin were nearly 4 times more likely to ovulate than those receiving placebo or no treatment (Lord et al, 2003).

Combination treatment with metformin and clomiphine achieves ovulation approximately 4 – 9 times more often than clomiphine treatment alone and often induces ovulation when clomiphine treatment has failed. Overall, 70-90% of anovulatory women with PCO treated with metformin ovulate spontaneously or after addition of clomiphine (Lord et al, 2003).
Since most women with PCO are insulin resistant and metformin treatment can restore ovulatory cycles in many of them, the drug is now approved as a first line of treatment option for anovulatory infertile women suffering this disorder.

Possible side effects of metformin include:

- GIT upset as nausea, vomiting, abdominal cramps and diarrhea that can be severe enough to limit the dose or even discontinuation of treatment (Lord et al, 2003).
- Lactic acidosis can be a rare complication of metformin treatment, although recent systematic reviews have questioned whether there is a true casual relationship. (Salpeter et al., 2003) (Stades et al., 2004).

Other insulin sensitizing agents that could be used in induction of ovulation include:

- Thiazolidinediones
- Rosiglitazone
- Pioglitazone

**Thiazolidinediones**
Clinical experience with this drug is limited in cases with PCO. Troglitazone was used in the past, until withdrawn from the market in the year 2000, after repeated reports of hepatic damage and in some cases leading to death. (Graham et al., 2003).

**Rosiglitazone** and **Pioglitazone** are two newer medications in the class currently in use for treatment of type II diabetes mellitus. They are generally well tolerated; the main side effects are weight gain and fluid retention.
However, like Troglitazone, these agents may cause hepatic toxicity and monitoring of liver enzymes is recommended during the first year of treatment.

Whereas, metformin is a category B drug and appears safe for use during pregnancy, Rosiglitazone and Pioglitazone are class C drugs with evidence of teratogenicity in animals.

In one randomized controlled trial of Rosiglitazone in clomiphine resistant anovulatory women with PCO, for 14 of 25 subjects (56%) successfully ovulated, 4 of 12 (33%) in response to Rosiglitazone alone (4 mg twice daily) and 10 of 13 (77%) who also received clomiphine treatment (50–100 mg) (Ghazeeri et al., 2003).

Although, obviously preliminary, these data are consistent and promising with those from earlier studies with Troglitazone. Other studies have examined the effect of Pioglitazone treatment on menstrual cyclicity in women with PCO.

A pilot study involving 18 obese women with PCO found that Pioglitazone treatment restored menstrual cyclicity in 10 of 12 (83%) subjects with demonstrable hyperinsulinemia before treatment and in 2 of 6 (33%) women with normal pretreatment insulin levels. (Romualdi et al., 2003). Another small descriptive study observed that combined treatment with metformin and Pioglitazone restores regular cyclic menses in women who failed to respond to metformin alone. (Glueck et al., 2003).

To conclude, these data suggest that Rosiglitazone and Pioglitazone may well emerge as useful additions to the range of therapeutic options for ovulation induction in women with PCO.
Aromatase inhibitors

Letrozole is an aromatase inhibitor that is considered as a potential option for clomiphine resistant anovulatory females. Surprisingly, the drug has been advocated as a possible first line alternative to clomiphine treatment for ovulation induction. Whereas, clomiphine stimulates secretion of endogenous FSH by decreasing central negative feedback for estrogen via estrogen receptor antagonism, Letrozole does so by inhibiting peripheral estrogen production. Theoretically, Letrozole might offer advantages over clomiphine, primarily because it has no direct peripheral anti-estrogenic effects like those exerted by clomiphine in some females.

In the only randomized controlled trial, comparing Letrozole (2.5 mg daily, cycle days 3 to 7) and clomiphine treatment (50 mg daily, cycle days 3 to 7), conducted in normal ovulatory women, peak endometrial thickness was similar despite markedly lower estrogen levels in women receiving Letrozole. (Fisher et al, 2002).

In a small descriptive study involving 4 women who failed to ovulate and 8 who ovulated but exhibited poor endometrial proliferation during clomiphine treatment (50 – 100 mg), Letrozole treatment (2.5 mg daily, cycle days 3 – 7) successfully induced ovulation in 9 of the 12 women overall when exogenous hCG was administered to augment an endogenous LH surge or to trigger ovulation once a preovulatory follicle reached or exceeded 20 mm in mean diameter (Mitwally, Casper, 2001).

Aromatase inhibitors deserve to receive additional; studies but, at the present time, their use in clomiphine resistant anovulatory women and their efficacy as a first line e alternative to clomiphine treatment remain uncertain.
**Induction of ovulation with exogenous gonadotropins**

Exogenous gonadotropins should be used only by clinicians having the training and experience necessary to provide safe and effective treatment.

**Gonadotropin preparations**

Preparations of exogenous gonadotropins used for ovulation induction come in three different varieties; urinary, purified urinary, and recombinant formulations.

For almost 30 years, the only exogenous gonadotropins available were human menopausal gonadotropins (hMG, menotropins), an extract prepared from urine of postmenopausal women containing equivalent amounts (75 IU) of FSH and LH per ampoule or vial and requiring i.m. injection. Urinary menotropins also contain small but measurable and varying amounts of hCG, most of it added intentionally during the manufacturing process to provide the appropriate amount of LH activity and some derived from other sources (Stokman et al., 1993). Relatively crude gonadotropin extracts like traditional hMG, also contained significant amounts of uncharacterized urinary protein that can be antigenic (Van de Weijer et al, 2003).

Contemporary, hMG preparations are more highly purified than in the past and can be administered subcutaneously (Gocial et al, 2000).

Twenty years ago, more purified urinary FSH preparations were developed by removing LH from urinary extracts using immunoaffinity columns containing anti-hCG antibodies. Early preparations of purified urinary FSH (75 IU) contained less than 1 IU LH but a considerable amount of urinary protein, and still required i.m. administration. The more highly purified products now in use contain less
than 0.001 IU LH and much lower levels of urinary protein and can be administered subcutaneously.

Ten years ago, the invitro production of recombinant human FSH was achieved through genetic engineering. Advantages include the absence of urinary protein, more constant supply, and less batch to batch variation in biologic activity.

A recombinant form of human LH having physiochemical, immunologic, and biologic activities comparable to those of human pituitary LH has been developed (Le Cottonec et al., 1998) and was approved for use in Europe in the year 2000.

Clinical experience with recombinant LH is still quiet limited, but seems likely to expand in the near future.

**Indications for exogenous gonadotropin treatment**

- **Hypogonadotropic hypogonadism**

  Women with Hypogonadotropic hypogonadism (hypothalamic amenorrhea, WHO group I) are the most obvious candidates for ovulation induction with exogenous gonadotropins.

  Gonadotropin therapy in women with this disorder can be viewed as hormone therapy intended to stimulate normal cyclic ovulation once fertility becomes a priority.

  In women with hypogonadotropic hypogonadism, the drug of choice is menotropins because it contains both FSH and LH.

  Although not always required (Keenan, Moghissi, 1992), luteal-phase support with supplemental hCG (2000 – 2500 IU every 3- 4 days) (Messinis et al, 1988) or progesterone (Hamilton et al, 1993) may be needed to compensate for low levels of endogenous LH secretion that prove to be insufficient to support normal luteal function. Premenstrual spotting or a grossly short luteal phase suggests the possibility.
Some have observed that supplemental hCG treatment can improve cycle fecundity (Messinis IE, Bergh T, Wide L, 1988), however its value has not been conclusively demonstrated, probably because endogenous LH levels vary in women with hypogonadotropic hypogonadism and only those with profoundly low LH concentrations (Less than approximately 3 IU/L) can benefit from luteal phase support (The European Recombinant Human LH Study Group, 1998).

Since, supplementary hCG also increases the risk for ovarian hyperstimulation syndrome, treatment is best reserved for women who exhibit evidence of poor luteal function after ovulation induction. Some women with secondary hypogonadotropic hypogonadism related to hyperprolactinemia become candidates for treatment with exogenous gonadotropins because they can not tolerate dopamine agonist therapy. Consequently, it is important to know that hyperprolactinemia has no apparent adverse effect on the response to exogenous gonadotropins (Farine et al., 1985)

- **Clomiphine resistant anovulation**

  When clomiphine treatment fails to achieve ovulation, exogenous gonadotropins are an obvious option. Serum gonadotropin concentrations in clomiphine resistant anovulatory women with PCO (WHO group II) are normal; in many, LH levels are relatively high. In this population of women, treatment superimposes boluses of exogenous gonadotropins on basis of erratic endogenous FSH and LH secretion. Purified FSH preparations offer a theoretical advantage over conventional menotropins because they avoid the risk of amplifying endogenous LH hyper secretion. However, in practice, there is no evidence that purified FSH has greater efficacy than hMG, and either may be used.
Clomiphene resistant anovulatory women with PCO generally respond to relatively low doses of gonadotropin stimulation. In many who are very sensitive, the therapeutic range is extremely narrow and higher doses can cause ovarian hyperstimulation. Luteal support is rarely required after induction of ovulation by gonadotropins in women with PCO, due to already present high levels of endogenous LH sufficient to support normal luteal function.

- **Unexplained infertility**
  
  In this context, higher doses of exogenous gonadotropins are used because these females ovulate normally with no endocrinial dysfunction (Dodson, Haney, 1991). Luteal support is not required because the combined contributions of 2 or more corpora lutea may be reliably expected to yield supraphysiologic luteal phase serum progesterone concentration.

**Exogenous gonadotropin treatment regimens**

Early retrospective studies established that daily treatment, frequently adjusted according to the clinical response is the mostly effective treatment regimen (Olive DL, 1995). The dose and duration of gonadotropin treatment required to successfully induce ovulation vary among women, some times even among cycles within women, and must be determined empirically. Whereas, many women are extremely sensitive to low doses of gonadotropins (75 – 225 IU daily), others require greater stimulation (300 – 450 IU daily). Although there is a direct relationship between body weight and dose requirement, the response threshold can not be predicted even in obese women (Chong et al, 1986). It can’t be overemphasized that safe and effective ovulation induction with exogenous gonadotropins depends heavily on the experience and the clinical judgment of the clinician.
Step-up regimen

In women with hypogonadotropic hypogonadism (WHO group I) and clomiphene resistant anovulation (WHO group II), initial attempts to initiate ovulation should begin with a low daily dose (75 IU daily). In a step-up treatment regimen designed to define the effective threshold of response. After 4-7 days of stimulation, a serum Estradiol level, with or without transvaginal ultrasonography, provides the first measure of response. Once the serum Estradiol begins to rise, ovarian ultrasonography should be done every 1-2 days. When the mean diameter of the dominant follicle reaches 15-18 mm, hCG is administered to trigger ovulation, which is expected 36-48 hours later.

Early and frequent monitoring is a must in patients with PCO undergoing induction with gonadotropins because they are sensitive to low doses of FSH, having a larger number of recruitable follicles (Van der Meer, et al 1998).

Insulin resistant women may be less sensitive to gonadotropin stimulation than those who are not (Dale, et al 1998).
Metformin treatment before and during gonadotropin stimulation improve the response, limit the number of smaller developing ovarian follicles (De Leo, et al 1999), reduce the likelihood of cycle cancellation for excessive stimulation, but not in all women(Yarali et al., 2002).

Step-down regimen

The alternative step-down approach to gonadotropin stimulation is designed to approximate the pattern of serum FSH concentrations observed in spontaneous ovulatory cycles. Treatment begins with a higher dose (150–225 IU daily) and decreases gradually to promote growth of the only more sensitive dominant follicle, while withdrawing the support from the less sensitive follicles in the cohort. Since many anovulatory women are
extremely sensitive to low dose of exogenous gonadotropin, therefore, the step-down method is best applied only after determination of the response threshold in one or more previous stimulation cycle.

**Sequential treatment with clomiphine and gonadotropins**

Clomiphine-resistant anovulatory women and those with unexplained infertility can benefit from sequential clomiphine and gonadotropin therapy. The typical cycle involves a standard course of clomiphine treatment (50 – 100 mg daily), followed by low dose FSH or hMG (75 IU daily) beginning on the last day of clomiphine therapy or the next day; treatment is monitored and individualized thereafter as in standard gonadotropin –stimulated cycles. In most studies cycle’s fecundity in sequential treatment cycles has approached that achieved with gonadotropin alone (Lu PY et al., 1996). In all studies, the dose and duration of gonadotropin therapy and the associated costs of monitoring were significantly decreased by 50 % or more. Logically, the sequential therapy is generally useful in women who respond to clomiphine, otherwise, treatment does not effectively begin until gonadotropin therapy starts.

**Adjuvant treatment with GnRH agonists**

The elevated endogenous LH levels in many clomiphine resistant anovulatory women with PCO predispose to premature follicular lutenization during exogenous gonadotropin stimulation, and can be prevented by using long acting GnRH agonist before and continued during exogenous gonadotropin stimulation(Manzi et al., 1995). Also elevated endogenous LH levels have been implicated as a contributing factor in the higher incidence of spontaneous miscarriage observed in those who conceive (Tulppaia, et al., 1993).
On the other hand, adjuvant GnRH agonist therapy has no proven benefits for unselected subfertile women receiving gonadotropins to induce superovulation (Dodson et al., 1991), and may increase the amount and duration of gonadotropin stimulation required.

Although combined treatment with GnRH agonist and exogenous gonadotropins, is the established standard for controlled ovarian hyperstimulation in IVF cycles, it has no prove advantage over gonadotropin stimulation alone for ovulation induction.

**Monitoring of gonadotropin therapy**

It is achieved by serial serum Estradiol measurements and ovarian ultrasonography

Its aim is achieving ovulation; avoid ovarian hyperstimulation and minimizing the risk of multiple pregnancies.

**Serum Estradiol levels**

It properly reflects the ovarian response to stimulation. Gonadotropins are administered in the evening generally between 5 and 8 P.M., and serum Estradiol measurements are obtained in the early morning.

Results are usually available by midday and new instructions regarding the dose and duration of treatment and the next schedule evaluation are determined before the evening dose. Estradiol levels usually double after 2 to 3 days over the days before peak follicular development is achieved. Best results are generally obtained when Estradiol concentrations peak are between 500-1500 pg/ml with existing gonadotropin stimulation regimens. Pregnancy is low at levels below 200 pg/ml (Reuter et al., 1996)

**Ultrasonography**
It defines the size and number of follicles contributing to the measured Estradiol level. Normally, in spontaneous ovulatory cycles the dominant follicle emerges by day 8 to 12 and grows at a rate of 1-3 mm/day and grows most rapidly one to two days immediately preceding ovulation. When the LH surge occurs the follicle measures about 20 – 24 mm in mean diameter. In exogenous gonadotropin stimulated cycles, dominant follicle exhibit a similar linear growth pattern, but reach maturity at a smaller mean diameter and over a wider range of sizes. The likely hood of ovulation increases with increased mean follicular diameter. Ultrasonography is of great value in exogenous gonadotropin stimulated cycles because it plays a role in measuring the endometrial thickness which correlates with serum Estradiol concentrations (Shoham et al., 1991). Few pregnancies result from cycles in which endometrial thickness is approximately less than 7 mm on the day of hCG induced ovulation (Reuter et al., 1996).

**Risks of exogenous gonadotropin therapy**

In addition to the obviously big cost, exogenous gonadotropin treatment also possesses significant risks. The chief risks are mainly, multiple pregnancy and ovarian hyperstimulation syndrome. They can never be completely avoided, but their risk could be minimized through careful management.

In fact, gonadotropins should be reserved for ovulation induction in infertile women with hypogonadotropic hypogonadism and clomiphine-resistant anovulation and for intentional controlled ovarian hyperstimulation for older
subfertile women and those with unexplained infertility, including women who ovulate in response to treatment but ultimately fail to conceive.

**Multiple pregnancy**

Multiple pregnancies are high risk pregnancies at any age because they are frequently complicated by preterm delivery, low birth weight, gestational diabetes and preclampsia and are associated with both, high infant mortality and morbidity (American College Of Obstetricians and Gynaecologists, 1998).

Almost all high order multifetal pregnancies result from the use of exogenous gonadotropins for ovulation induction, superovulation, and assisted reproductive technologies (ARTs) (Centers For Disease Control and Prevention, 2000).

Several factors contribute to the risks of multiple pregnancies associated with exogenous gonadotropin therapy. Although in recent years the attention has been focused upon embryo transfer practices in ART centers, less than half of treatment related multiple pregnancies results from invitro fertilization.

In IVF cycles, the risk of multiple pregnancy relates to the numbers of embryos transferred and increases with serum Estradiol concentrations, the total number of developing ovarian follicles, and with decreasing maternal age, but does not correlate well with the number of larger preovulatory follicles (Dickey RP, Pyrzak R, 2002)

The risk could be reduced if ovulation was simply not triggered when the Estradiol level or number of the maturing follicles was excessive.

Some have suggested varying cycle cancellation criteria that might be used to guide treatment and limit risks of multiple pregnancies (Dickey et al., 2001).

Management options other than cycle cancellation include conversion to IVF and transvaginal aspiration of excess follicles.
Women who conceive a high order multiple pregnancy despite all efforts to avoid the complication, must choose from among three difficult options:

- Termination of the entire pregnancy.
- Continuing the pregnancy bearing the previously mentioned risks.
- Multifetal pregnancy reduction which sacrifices a portion of the pregnancy in an effort to save the whole, but for many it is inconvenient due to moral, religious, ethical and personal attitudes.

**Ovarian hyperstimulation syndrome**

It is an iatrogenic complication of ovulation induction with exogenous gonadotropins. It may be occasionally observed in clomiphine induced cycles and rarely in spontaneous pregnancies associated with conditions characterized by supraphysiologic concentrations of hCG (multiple gestations, molar pregnancy).

Risk factors for this disorder include young age, low body weight, PCO higher doses of gonadotropins, and previous history of hyperstimulation (Byalos RP, Lee CT, 1996). The risk increases with serum Estradiol levels and the number of developing ovarian follicles and when supplemental doses of hCG are administered after ovulation for luteal phase support (Enskog et al., 1999)

Ovarian hyperstimulation syndrome is normally self-limited and resolves spontaneously within days.

For patients who appear to develop high-risk signs such as rapidly increasing estradiol levels; concentrations over 2500 pg/ml, or massive follicular recruitment, we may decrease medication dosages, change the ratio of individual medications in the regimen or stop medications altogether.

Withholding medications at midcycle or delaying the administration of hCG is a maneuver some times referred to as coasting.
Coasting without further gonadotropin stimulation and delaying administration of hCG for one to three days until Estradiol levels plateau or decline can reduce the risk of hyperstimulation (Ulug et al., 2002). A lower dose of hCG (5000 IU) also may help to reduce the risk (Whelan JG, 3rd, Vlahos NF, 2000). Alternatively, a GnRH agonist (leuprolide 0.5-1mg) can be used to trigger an endogenous LH surge and induce ovulation, thereby avoiding the longer duration of action and further stimulation of hCG (Sagle et al., 1988). When luteal support is judged necessary, exogenous progesterone administered by injection (50mg daily) or vagianlly (suppositories 100mg or 8% gel, daily) are preferable to supplemental doses of hCG (Forman et al., 1990).

Rizk,1993 suggested the use of the “ten commandments” for the prevention of ovarian hyperstimulation syndrome

1. **Withholding hCG**
   This is the most commonly used method in preventing OHSS in patients predicted to be at high risk of developing this syndrome. The serum estradiol levels above which hCG should be withheld is variable (Rizk and Aboulghar, 1991).

2. **Delaying hCG administration**
   Several authors have reported successful reduction in severe OHSS by delaying hCG or coasting. It was suggested that prolonged coasting in GnRH-agonists/FSH/hCG cycles could prevent life endangering complications of OHSS (Sher et al, 1993).

3. **The use of GnRH-agonist to trigger ovulation**
   This eliminates the risk of clinically significant OHSS (Kol et al, 1996).

4. **Follicular aspiration**
It was found that follicular aspiration has no protective effect on
the occurrence of OHSS (Aboulghar et al, 1992).

5. Intravenous albumin administration
   Experience in subjects with different forms of third space fluid
   accumulation have shown that albumin is efficient is preventing and
correcting haemodynamic instability.

6. Glucocorticoids
   Effective in prevention of OHSS (Rizk, 1993).

7. Luteal phase support with progesterone
   Should be used in patients at high risk of developing OHSS (Rizk and

8. Cryopreservation of embryos and subsequent replacement
   It was reported that Cryopreservation of all prezygotes in patient at
   risk of severe hyperstimulation did not eliminate the syndrome, but the
   chances of pregnancy were excellent with subsequent frozen-thawed
   transfers ( Queenan and co-workers, 1997).

9. Selective oocyte retrieval in spontaneous conception cycles
   This is done through puncturing of most of the ovarian follicles 35 hours
   after hCG administration as in IVF programmes.

10. Prevention of OHSS in patients with PCO
    The low dose of rFSH and hMG protocols have been compared in
    the treatment of patients with history of severe OHSS. The low dose of rFSH
    proved to be as effective as low dose of hMG protocol in producing
    reasonable ovulation and pregnancies in patients with PCO and giving a
    history of severe OHSS; the protocol was safe regarding the risk of
    developing OHSS (Aboulghar and colleagues, 1996).
Laparoscopic ovarian drilling has been used successfully for prevention of OHSS in patients with PCO. Recently, Almedia and Rizk reported the first case of micro laparoscopic ovarian drilling under local anesthesia in patients with PCO (Almedia and Rizk, 1998).

Management of OHSS

Since ovarian hyperstimulation is a self limiting disease, its treatment should be symptomatic and conservative. Although the severity of its symptoms may demand radical, intensive care. Treatment is generally medical, with laparotomy reserved for cases with abdominal catastrophes (Joseph et al, 2000).

OHSS has got a broad pathophysiological spectrum ranging from mild illness to severe disease. It is classified into mild, moderate and severe.

Mild illness is characterized by ovarian enlargement, lower abdominal discomfort, mild nausea and vomiting, diarrhea and abdominal distention and could be managed by patient reassurance and close observation.

Persistence or worsening of symptoms or ascites indicate a progressing or moderate illness, which requires treatment with antiemetics and potent analgesics, oral fluid intake that should be maintained at not less than 1 liter/day to maintain electrolyte balance.

Serious illness is uncommon with an incidence of 1%, including severe lower abdominal pain, rapid weight gain, tense ascites, homodynamic instability, respiratory distress, progressive oliguria and impairment in liver and kidney functions.

Other more serious complications include renal failure, adult respiratory distress syndrome, ovarian rupture and intraperitoneal haemorrhage, haemoconcentration and thromboembolic complications (Abramov et al, 1999).
This type of cases will require immediate hospitalization for close monitoring and performing of:

- Leucocytic count.
- Hemoglobin concentration and hematocrite.
- Prothrombin time and partial prothrombin time.
- Full electrolyte panel should be done on daily basis.
- Liver function tests.
- Measurement of daily weight and abdominal girth.
- Chest X-ray and pulse oximetry are mandatory for patients with dyspnea or other signs of chest compromise.
- Repeated abdominal ultrasonography should be reserved when paracentesis is considered a line of management for ascites.
- Fluid administration with 1 liter of normal saline over one hour as a bolus together with maintenance of a fluid protocol to counter the effects of hypovolaemia, third space fluid retention, vomiting and diarrhea.
- Subcutaneous heparin 5000 IU twice daily through out the hospital stay to prevent thromboembolic complications.

Knowledge and prompt recognition of risk factors for ovarian hyperstimulation are essential for its prevention. Rapidly rising serum Estradiol levels and observation of a large number of small and intermediate sized ovarian follicles are high risk indicators and signals to proceed with great caution.

**Breast and ovarian cancer**; in this context, no casual relationship between exogenous gonadotropin treatment and breast or ovarian cancer
has been established, although long term studies are certainly warranted, prolonged treatment is best avoided when there is little hope of success.

**Induction of ovulation using exogenous GnRH therapy**

**Mechanism of action**

Exogenous pulsatile GnRH therapy generally stimulates only normal physiologic levels of pituitary gonadotropin secretion and allows normal feedback modulation of the pituitary response by ovarian steroids and peptides to operate. Consequently, follicular recruitment, selection, growth and development in women using the GnRH pump progress as they do in the normal menstrual cycle (Filigoi et al, 1991)

**Indications for exogenous gonadotropin therapy**

The best candidates are women with hypogonadotropic hypogonadism, because treatment is specific, physiologic and highly effective as the GnRH pumps provide proper instructional signals that pituitary gonadotropes properly receive. The GnRH pump also is effective in women with hyperprolactinemia and offers an alternative to exogenous gonadotropins when dopamine agonist treatment fails or can’t be tolerated.

**Exogenous GnRH agonist regimens**

Exogenous gonadotropin agonist therapy is most effective when administered intravenously, and in low doses (2.5-5.0 ug/pulse) at a constant interval (every 60-90 minute) (Filigori et al, 1994). Higher doses might be needed in those who fail to ovulate (Timmerman-Van Kessel et al., 2000).
In women with primary hypogonadotropic hypogonadism, low doses as (2.5ug/pulse) can effectively induce ovulation, but follicular phase concentrations of LH may remain lower than normal and luteal phase concentrations of progesterone are often reduced. Longer durations of treatment are required because available stores of endogenous gonadotropins are markedly reduced due to historically low levels of endogenous GnRH secretion.

In women with secondary idiopathic hypogonadotropic hypogonadism treatment should begin at a low dose of GnRH (2.5 ug/pulse); the higher the dose (5.0 ug /pulse) is associated with high follicular phase LH and Estradiol levels, a short follicular phase, multiple folliculogenesis, and a higher risk of multiple pregnancy, possibly because previous pituitary or ovarian priming confers a greater sensitivity to GnRH therapy.

The endocrinal response of women with PCO to pulsatile exogenous GnRH (5.0ug/pulse) is markedly abnormal, but can be normalized by pretreatment with a long acting GnRH agonist (daily subcutaneous administration) for 6 to 8 weeks immediately before starting exogenous pulsatile GnRH treatment (Filicori et al, 1994).

After ovulation has been achieved, GnRH therapy can continue at the same or a slower pulse frequency (every 120 -140 minutes) which is simpler, much less costly, and just as effective to discontinue the pump once ovulation has occurred and to support the luteal phase with small doses of hCG (2000 IU every 3 days) (Filicori et al, 1994) or exogenous progesterone.

**Advantages and Drawbacks**

GnRH treatment has numerous advantages.

The method is simple to use and, requires no extensive and costly monitoring in contrary to exogenous gonadotropin therapy.
More important is that it is associated with lowered risk of multiple pregnancy and ovarian hyperstimulation. However, because GnRH therapy requires maintenance of an indwelling intravenous catheter for an interval of 2 to 3 weeks or even longer, many women fear needle displacement or other technical problems and are reluctant to use the method or reject it altogether.

**Induction of ovulation using dopamine agonists**

**Mechanism of action**

Dopamine agonists inhibit pituitary lactotrope prolactin secretion directly.
This allows the hypothalamic-pituitary-ovarian axis to escape from the suppressive influence that hyperprolactinemia has on pulsatile GnRH secretion and to resume normal operation, thereby restoring the ovulatory function.

**Indications for dopamine-agonist therapy**

Dopamine agonists are the treatment of choice for hyperprolactinemic infertile women with ovulatory dysfunction.
It can be highly effective in women with galactorrhea and normal serum prolactin levels. (Pallida et al., 1985)

**Dopamine agonist treatment regimens**

Treatment should begin with small doses and increase gradually until the dose required to maintain and restore euprolactinemia is established.
Bromocriptine treatment usually begins with a dose of 1.25-2.5mg administered at bed time, most women will respond at a dose of 2.5-5.0 mg daily but some may require as much as 10mg daily. Cabergoline treatment usually begins with a dose of 0.25mg twice weekly, increasing thereafter every 4 weeks until the therapeutic dose is established. Most women achieve normal prolactin levels with 0.5-1mg weekly. Cabergoline has proven effective in 70 – 85 % of hyperprolactinemic women who are resistant to or can not tolerate bromocriptine therapy (Webester et al., 1994).

**Possible side effects of dopamine agonist therapy**

They are common, but generally well tolerated including nausea, vomiting, dizziness, nasal stuffiness and orthostatic hypotension (Factor SA, 1999).

These side effects could be minimized by starting with small doses that could be gradually increased to larger ones.

**Risks of dopamine agonist therapy**

No evidence available about the role of dopamine agonist therapy and increased risk of spontaneous miscarriage or congenital anomalies of the newly born (Webester et al., 1994).

**Surgical management**

- **ovarian wedge resection**

In 1939, ovarian wedge resection was the first established treatment for anovulatory women with PCO. It is now obsolete when medical treatment
and laparoscopic procedures became available alternatives for management of the problem.

- **Laparoscopic ovarian drilling**

It is considered as a contemporary version of the classical surgical procedure and another treatment option in clomiphine resistant, hyperandrogenic, anovulatory women. The technique involves multifocal ovarian cautery, diathermy, or laser vaporization (approximately 10 to 20 sites per ovary) aiming to decrease both intraovarian and systemic androgen concentrations by ablating some of the hypertrophic stroma in polycystic ovaries. Indeed, postoperative serum testosterone levels typically are reduced, at least for a time; (Armar et al., 1990), inhibin concentrations are also lowered (Kovacs et al., 1991), and both changes likely to contribute to an associated increase in FSH levels.

Laparoscopic ovarian drilling is a reasonable therapeutic option for clomiphine resistant anovulatory infertile women, but the temporary effects of treatment, the risk of postoperative adhesions, and the theoretical risk of adverse effects on ovarian reserve necessitate careful consideration and discussion.

The procedure is best reserved for women who are unable or unwilling to accept the costs and risks associated with gonadotropin therapy (Yildiz et al, 2003).
**Introduction**

Poor response is not a rare occurrence in controlled ovarian hyperstimulation. Although not fully accepted, the most dominant criteria for poor ovarian response are:

- Small number of follicles developed or oocytes retrieved.
- Low Estradiol levels after the use of standard stimulation protocols.

What's really disappointing is that there is no ideal predictive test as the poor responder is revealed only during ovulation induction however increased levels day 3 FSH and Estradiol as well as decreased levels of inhibin-b can be used as an assessment of the ovarian reserve.

Special protocols have been proposed for management of low ovarian response in IVF and in induction of controlled ovarian hyperstimulation in this group of responders.

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. The few available relevant studies do not indicate that recombinant FSH improves the outcome.

Flare up GnRH agonist protocols (including all dosage varieties) produce better results than standard long luteal 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.

The few available data obtain with GnRH antagonists have not shown any benefits.
Adjuvant therapy with growth hormone (GH) or GH-releasing factors is no significant improvement. 
The limited data with nitric oxide donors are promising.  
Pretreatment with combined oral contraceptives prior to stimulation may help ovarian response. 
Well designed, large scale randomized controlled trials are needed to assess the efficacy of the different management strategies and protocols. 
That will be discussed later in this chapter. 

Correct controlled ovarian stimulation is of great importance in assisted reproductive technologies. Therefore analysis of the ovarian reserve of the patient is mandatory to tailor the best ovarian stimulation regimen. When the ovarian reserve is reduced, the induction of a multifollicular growth remains a challenge. In patients with reduced ovarian response to stimulation regimens, even more attention is required to achieve acceptable reproductive outcome. Although it is difficult to standardize the characteristics that categorize patients as poor responders, it has been estimated that among patients undergoing IVF treatment the prevalence of poor ovarian response is 9 – 24 % (keay et al.1997). 
Several factors could be associated with reduce ovarian response. However, reduced ovarian reserve either in older patients (Akande et al.,) or in young patients with early ovarian aging ( Nikolau and Templeton 2003 ) represents the most frequent aetiologial factor, also patients having history of previous ovarian surgery ( Nargund et al.,1995), and pelvic adhesions ( keay et al., 1998). Whatever the aetiology, one of the main problems is how to predict reduced ovarian response, and although several tests have been suggested, no very
accurate predictive test is available. A variety of different stimulation protocols have been suggested but the lack of any large scale, randomized, controlled trials of any of the different management strategies and the lack of a uniform definition of the population may result in comparing heterogeneous groups of patients, making it difficult to draw any definitive conclusions.

**Therefore, to sum up.**

- Before starting ovarian stimulation, a prospective analysis of the ovarian reserve together with definition of the goals and selection of the proper stimulation protocols are mandatory. (Penzlas 2004).
- It is of crucial importance to predict the poor ovarian response to ovarian stimulation in order to tailor the correct stimulation regimens.
- Although several tests have been suggested no very accurate predictive test is available to asses the ovarian response to stimulation protocols.
- A variety of different stimulation protocols have been suggested but the lack of large scale, prospective, randomized, controlled trials of the different management strategies does not allow any definitive conclusion to be drawn.

**Aetiology of poor ovarian response**

Although several possible aetiologies have been suggested, it seems that a diminished ovarian reserve is the principal factor in poor ovarian response (Pellicer et al., 1998).

Women have a finite number of germ cells reaching a peak of 6 – 7 million germ cells by gestation week 20. From mid gestation onward and throughout the reproductive life, an irreversible attrition progressively diminishes the germ cell pool of the gonad (Peters, 1976),
mainly through follicular atresia. The rate of follicular attrition is not constant but rather follows a bi-exponential pattern, with the change in exponential rate being determined by the number of remaining oocytes rather than age (Faddy et al., 1992).

There is a marked increase in the rate of follicular disappearance from age 37 – 38 years onwards at which the total number of follicles reaches 25000(Faddy et al., 1992). It then takes around 13 years to reach menopause (1000 remaining follicles), regardless the age of menopause (Faddy et al., 1992).

The rate of follicular disappearance and its relation to the important reproductive events in the female life has been extensively studied, suggesting that after a period of optimal fertility from age 18 – 31 years, the quality of oocyte decreases in parallel to decreased number of follicles becoming severely impaired after 37 to 38 years of age reaching around 25000 follicles (Faddy et al., 1992; Gougeon et al., 1996).

Several theories have been formulated to explain declining the quality of oocytes with age in the so-called "production line" hypothesis. Oocyte quality is established during fetal life, and oocytes that are less susceptible to non-disjunction are ovulated first, leaving poor quality of oocytes to be ovulated later in life.( Henderson and Edward 1986; Polani and Crolla, 1991).

For this reason, the associated reproductive impairments may be more closely related to decline in the ovarian follicle number than to age or to the interval before the menopause rather than the age at which that occurs.

Therefore in young women, premature reduction of ovarian follicle numbers, whether by excessive atresia or accidental or iatrogenic damage, could lead to an exaggeration of all reproductive problems.
This aspect could be very important, as those women who will become menopausal earlier (40 to 45 years) will have an accelerated decline in fertility (25000 remaining follicles) at an unexpected young age. It has been estimated that 10% of women in their early 30s with reduced fertility which is otherwise unexplained (Scott et al., 1993; Hofmann et al., 1996).

However, besides the "production line" hypothesis supported by some experimental data in mice (Brook et al.,1984; Meredith and Butcher,1985) and in women (Hardy and Kuh,1999; Freeman et al., 2000), other data on related to female ovarian aging point to an increased frequency of meiotic non-disjunction as time passes which is the most important mechanism responsible for the majority of aneuploidies in early embryos (Lamb et al.,1997).

In fact oocyte quality seems to be less impaired in younger patients with elevated FSH concentrations and reduced ovarian reserve and the implantation rates between young patients (<35 years) with high or low basal serum FSH appear to be comparable (Chuang et al., 2003).

Similarly, Van Rooig et al., (2003) comparing women less than 40 years old within normal serum FSH with younger women who have higher basal serum FSH concentrations, reported higher cycle cancellation rates and lower number of oocytes retrieved in the latter group of patients; however, once the oocytes were retrieved the younger women had near normal implantation rates.

**These results suggest that estimation of the ovarian reserve is a better predictor of oocyte production capacity than of quality, whereas age affects oocyte quality.**

This phenomenon can be explained by an age-dependant accumulation of damage due to either compromised granulose cell function (Warburton, 1989) or progressively defective microcirculation around the leading
follicle with reduced oxygen concentrations in the follicular fluid (Van Blerkom et al., 1997) or a gradual increase in the intracellular oxidative stress (Tarin, 1995).

This hypothesis is however, disputed and other authors reported a reduction in the implantation rates in young women with high basal serum FSH concentrations after IVF (Toner et al., 1991; Scott et al., 1995; Akande et al., 2002; El Toukhy et al., 2002), suggesting that aneuploidy before (Roberts and O'Neill, 1995) or after (Benadiva et al., 1996) fertilization due to disruption of meiotic spindle assembly (Battaglia et al., 1996) or mitochondrial DNA deletions (Keefe et al., 1995) are not simply due to duration of exposure to risk of damage, but were probably present since fetal life. More universally agreed is the very poor reproductive outcome in women approaching 40 years old with poor ovarian response. In this group of women, it is necessary to relate the effect of the reduced ovarian reserve to the diminished oocyte quality.

The result is that there is a higher proportion of "poor quality" oocyte due to either the "production line" hypothesis (Henderson and Edwards, 1968; Polani and Crolla, 1991) versus time related exposure to risk of damage (Te Velde and Pearsons, 2002).

Although reduced ovarian reserve is the most important and frequent aetiological factor in reduced ovarian response to ovarian stimulation, in some patients the presence of polymorphic FSH receptor in which the asparagine of the receptor protein is replaced by serine at position 680 requiring higher FSH concentrations for normal function, and it is probably not related to reproductive aging (Perez Mayorga et al., 2000).

Moreover, low responses have also been associated with:

- The presence of ovarian antibodies (Meyer et al., 1990).
- Reduced aromatase activity (Hurst et al., 1992).
- Decreased blood flow measured by Doppler ultrasonography (Pellicer et al., 1994).
- Reduced circulating surge- attenuating gonadotropin factor (GnSAF) bioactivity (Martinez et al., 2002)
- Other possible acquired factors include obesity (Dechaud et al., 1998), chemotherapy, radiotherapy, pelvic surgery (Tulandi et al., 2002), pelvic infections or tubal disease (Keay et al., 1998), severe endometriosis (Barnhart et al., 2002) and heavy smoking (El Nemr et al., 1998).

**Prediction of poor ovarian response**

Proper identification of those who are at risk of poor ovarian response prior to stimulation is useful in counseling patients, and may be helpful in tailoring the best stimulation protocol and dosage of gonadotropin to individual patients.

Before analyzing the tests that have been described as predictors of ovarian reserve, it is important to emphasize that no test is absolutely predictive and the best test is of course the ovarian response itself.

Measurement of serum FSH concentrations on day 3 of the cycle represents one of the most widely used prognostic tests in assessment of the ovarian reserve. Basal serum FSH concentrations begin to rise on average a decade or more before the menopause (Ebbiary et al., 1994; Klein et al., 1996), are inversely correlated with ovarian follicular responsiveness to maximal exogenous gonadotropin stimulation (Cahill et al., 1994). This is caused by the negative feed back of the FSH-modulating proteins from the ovary, mainly inhibin-A and inhibin-B (Groome et al., 1996). Since inhibin-B is predominantly secreted by the early antral follicles, a decreased serum concentration of inhibin –B reflects a reduction of the antral follicle pool (Burger et al., 1998; Welt et
al., 1999), and it is clearly associated with the FSH rise in the early follicular phase.

Subtle serum increase of FSH represents an early signal of the decline of the ovarian response despite regular menses, and it is associated with unexplained infertility (Cameron et al., 1998; Muasher et al., 1998). There is a wide variation in what can be considered "high" FSH values, and basal high FSH concentrations > 10 or > 12 or > 15 mIU/ml have been reported as predictive of poor response and poor clinical outcome (Cameron et al., 1988; Scott et al. 1989; Toner et al., 1991; Faber et al., 1998).

Moreover, even a single elevated FSH value might denote a reduced ovarian reserve (Scott et al., 1990). However, when basal serum FSH concentrations are used to predict the ovarian reserve, it is necessary to consider the possible variability between cycles of day 3 FSH reported by some (Brown et al., 1995) but not all authors (Penarrubia et al., 2004), which suggests that basal hormone values should be samples within 3 months of the assisted reproduction cycle (Creus et al., 2000).

Another marker of reduced ovarian reserve is the increased basal Estradiol concentrations in the presence of "normal" FSH concentrations due to early follicular recruitment that occurs consequently to a premature luteal elevation of FSH. This early luteal recruitment is probably due to a diminished follicular cohort that produces less inhibin. Elevated Estradiol concentrations may be able to suppress FSH concentrations to the normal range in women who have substantially diminished ovarian reserve and thus may cause false – negative test results. Basal Estradiol concentrations > 30 or > 45 or > 70 pg/ml have been associated with poor IVF outcome (Licciardi et al., 1995; Smotrich et al., 1995). Higher inhibin – B concentrations throughout stimulation were related to higher oocyte yield (Engel et al., 2001), whereas lower serum
concentrations of inhibin-B on day 3 very likely reflect lower follicular numbers and may serve as a good predictor of clinical outcome (Burns et al., 1996; Seifer et al., 1997).

In patient with day 3 serum inhibin – B concentrations < 45 pg/ml, the number of oocytes retrieved is lower, the cycle cancellation rate is higher and the clinical outcome is poorer compared with patients with day 3 inhibin –B concentrations more than or equal 45 pg/ml (Seifer et al., 1997).

Recently Martinez et al, (2002) suggested a role of GnSAF, an ovarian factor that has a specific biological effect of reducing pituitary responsiveness to GnRH, in the prediction of the ovarian reserve. Authors demonstrated that on the day of HCG administration GnSAF concentrations are lower in women with poor ovarian response to ovarian stimulation compared to normal responders. Moreover they observed lack of GnSAF activity during the follicular phase of spontaneous cycles and a much more reduced and slower increase in circulating GnSAF following FSH stimulation in women with poor ovarian response to ovarian stimulation compared to patients with normal ovarian response) Martinez et al.,2002).

A new promising biochemical marker that could be used as a predictor of the ovarian reserve is the anti-mullerian hormone. Because the anti-mullerian hormone is produced exclusively by the small growing follicles and secreted into the circulation. Serum concentrations of the anti-mullerian hormone were significantly decreased over time in young ovulatory women, whereas other markers associated with ovarian aging such as serum concentrations of FSH and inhibin-B did not change during the same interval (De Vet et al., 2002).

Ultimately the high heritability found fir age at menopause suggests genetic control by an unknown number of genes. With development in
molecular genetics, it might be possible to construct DNA fingerprints that will be able to identify women with genetic predisposition to what is so called ‘early ovarian aging’ (Te Velde and Pearson, 2002). Some authors have suggested dynamic tests of ovarian reserve such as clomiphene challenge test (Navot et al., 1998), the lupron screening test (Padilla et al., 1991), change in estradiol concentrations after exogenous FSH stimulation (Fanchin et al., 1994) and the GnRH stimulation test (Karande and Gleicher, 1999) as useful tools to predict the ovarian response.

Recently, the ultrasonographic characteristics of the ovary have been suggested to be predictive of the ovarian potential during ovarian stimulation. The antral follicle count as well as ovarian volume appeared to be indicative of poor response in assisted reproduction. Pellicer et al.(1998) reported an intimate relation between the number of selectable follicles (2-5 mm) as measured by three dimensional transvaginal ultrasonography and the number of selectable follicles in histological slices. According to this observation, it is possible that the number of the antral follicles originating from the cohort of the growing follicles reflects the size of the pool of resting follicles, and thus the ovarian reserve. Bancsi et al.(2002), analyzing the odds ratios, statistical significance and receiver operating curve (ROC) area under the curve (AUC) for six basal ovarian reserve markers, observed that the number of antral follicles as a single predictor was the most powerful prognosticator of reduced ovarian response expressed by the largest ROC AUC of 0.87.

This discriminative potential for poor response could increase if the day 3 inhibin-B and FSH are also considered.

In a multivariate analysis with 3 variables, the antral follicle count was selected in the first step, followed by inhibin-B in a step two and finally
FSH in step 3. The ROC AUC increased in a stepwise manner from 0.87 to 0.92 (Bancsi et al., 2002).

Similar results were recently reported in a prospective study where various markers of ovarian reserve (FSH, LH, Estradiol, inhibin-B, antral follicle count) were evaluated in the natural cycle preceding assisted reproductive therapy in 60 women as prognosticators of their ovarian response to ovarian stimulation (Loverro et al., 2003).

**Induction protocols for poor responders**

**High doses of gonadotropins**

The initial unresponsiveness to gonadotropin stimulation unavoidably leads the clinician to increase the dosage of medication for controlled ovarian hyperstimulation, and indeed this action forms part of the definition of a poor responder.

According to most authors, the common initial dose is at least 300 IU/day. Few studies have been conducted on this issue however, as high doses of gonadotropins have been used in the majority of regimens employed (See table 1).

One group (Cedrin-Durnerin et al., 2000), in a prospective randomized study, compared a high fixed versus a step- down of gonadotropins on a minidose flare- up GnRH agonist regimen. Patients were pretreated for 14 days with 10 mg/day norethisterone, ceasing at cycle day 0, followed by
100 ug triptolerin s.c. From day 1, reduced to 25 ug S.C. from day 3 of the cycle.
Purified FSH at a dose of 450 IU/day i.m. was administered from day 3 either in a fixed manner or decreasing to 300 IU/day, and finally to 150 IU/day.
Thus, these authors showed by applying this decremental flare-up GnRH agonist protocol, that there were increased cancellation rates and similar pregnancy rates but, as expected, a significantly reduced number of ampoules of GnRH were used.
Doubling of the starting hMG dose, from 225 IU to 450 IU/day from day 5 of ovarian stimulation onwards, has been evaluated in a prospective randomized study (Van Hoff et al., 1993).
These authors concluded that such an approach was ineffective in enhancing the ovarian response in poor responders, this being in accordance with the hypothesis that follicular recruitment occurs only during the late luteal and early follicular phases of the menstrual cycle. Similar doses (450 IU/day) were also used in a retrospective study (Karande et al., 1990), where in 34 poor responders the mean number of retrieved oocytes (2.67) was still low, as was the pregnancy rate (12%). The latter group concluded that, there was no benefit from the incremental increase in FSH dose.
The same conclusions were obtained in another retrospective study (Land et al., 1996), where 126 previously poor responders were given 450 IU/day hMG. The women showed an increased number of oocytes retrieved but the pregnancy rate remained low (3.2%).
Conversely, in a prospective study with historical controls, the effects of the increased gonadotropin doses in poor responders were evaluated as improved (Hofmann et al., 1989). The authors showed increased pregnancy rates (33.3 versus 6.7%) and lower cancellation rates (9 versus
35 %), by using 450 IU/day of purified FSH (in step-down fashion), rather than 300 IU/day. The benefits of using a very high dose of purified FSH were also reported in a retrospective study (Crosignani et al., 1989) which included 116 poor responders and resulted in satisfactory follicular growth in 70 % of cases.

In general, high doses of gonadotropins have been used by the vast majority of authors and form a clear part of all protocols for ovarian stimulation in poor responders. Never the less, the results of studies evaluating the use of gonadotropins in these patients remain controversial, though the true prospective randomized studies have shown either minimal or no benefit.

**Use of recombinant FSH versus purified urinary FSH**

The use of recombinant FSH (rFSH) appears to be associated with better assisted reproduction results than do either purified urinary FSH or hMG (Out et al., 1996; Daya and Gunby, 2002). As patients include in these studies were not poor responders, these results stimulated the concept than rFSH might also improve oocyte and embryo quality in poor responders.

The use of rFSH versus purified FSH in poor responders was evaluated in a small (15 versus 15 patients), prospective randomized study (Raga et al., 1999).

The authors found an increased mean number of oocytes collected (7.2 versus 5.6), improved pregnancy rates (33 versus 6 %) and lower cancellation rates (13 versus 40 %).

Similarly, another prospective study, albeit with historical controls (De Placido et al., 2000), assessed the efficacy of 300 IU rFSH versus the same dose of purified FSH in the flare-up protocol involving 28 cycles of
poor responders in each group. These authors suggested that rFSH was associated a significantly larger number of oocytes retrieved (2.4 versus 1.7) and significantly increased pregnancy rates (14.3 versus 0%).

Therefore, it seems that there is no evidence that rFSH produces better results in poor responders, though larger prospective randomized trials are needed to elucidate this issue further.

**Luteal initiation of FSH**
This interesting approach was first suggested in the late 1980s (Rombauts et al., 1998). Healthy, small, antral follicles are present in late follicular phase, and initiation of their further development occurs under action of the premenstrual FSH rise (Gougeon, 1996).

The hypothesis was that earlier administration of FSH might increase the number of recruited follicles by opening the recruitment window earlier, in the late luteal phase of the preceding cycle.

In a prospective randomized controlled study in 2 patient groups (n=20 each), these authors administered leuprolide according to a standard short or long follicular protocol.

The control group received 150 IU/day of rFSH from cycle day 3, while the study group received the same dose from day 25 of the previous cycle.

Unfortunately, the results were not encouraging with increased cancellation rates (33 versus 25%), decreased number of oocytes collected (4.5 versus 6) and lower pregnancy rates (0 versus 5%); in addition, increased FSH doses (1950 versus 1500 IU) were needed for longer stimulation period (15 versus 11 days) (see table 1).
**Novel gonadotropin treatment regimens**

The critical role that LH plays in follicular development has only come to light in recent years.

In the normal ovulatory cycle, the latter stages of preovulatory follicular development are completed while FSH levels continue a steady decline. The traditional explanation was that; by virtue of its greater size, granulosa cell mass, FSH receptor content and microvascular development, the dominant follicle is more highly sensitive to FSH and thus able to continue development while smaller, less FSH –sensitive follicles in the cohort are not. Although still true, we now know that follicle selection and the final stages of follicular maturation are equally if not even more dependant on low circulating levels of LH (Levy DP, Navarro JM, Schattman GL et al., 2000).

In addition to stimulating production of thecal androgens as substrate for estrogen synthesis, LH stimulates granulosa cells via LH receptors induced by FSH and estrogen in larger but not in smaller follicles (Shima K, Kitayama S, Nakano R, 1987). LH then becomes the principal stimulus for the final stages of follicular maturation while, at the same time, declining concentrations of FSH starve the smaller, more FSH-dependant follicles into atresia.

The new paradigm, has suggested novel approaches to ovulation induction that may have particular value for anovulatory infertile women within PCO in whom existing treatment regimens still too often result in multifollicular development and ovarian hyperstimulation.
Low doses of hCG (Filicori M, Cognigni GE, Taraborrelli S et al., 1999) or recombinant LH (The European Recombinant Human LH Study Group, 1998) can selectively promote larger follicular growth, while, simultaneously hastening the regression of smaller follicles. To a limited extent, step-down gonadotropin treatment regimens in which the amounts of FSH stimulation are gradually reduced have exploited this phenomenon.

The practice of **coasting**, in which FSH is withdrawn altogether during the latter stages of follicular development does so even more. In the latter instance, the largest follicles generally continue to function, most likely because their LH receptor expression renders them receptive to circulating low concentrations of endogenous LH (Hillier SG, Whitelaw PF, Smyth CD, 1994), whereas, estrogen levels plateau or decline and smaller follicles arrest or begin to regress (Egbase PE, Al Sharhan M, Berlingieri P., et al 2000).

Continuing stimulation with low doses of hCG or recombinant LH after decreasing or discontinuing FSH treatment takes the whole advantage of the differential actions of LH on larger and smaller follicles by supporting continued development of the former. (Filicori M, 1999), and selectively excluding the latter (Loumaye E, Engrand P, Shoham Z et al., 2003), both by withdrawing FSH and by directly stimulating increased intrafollicular androgen concentrations (Filicori M, Flamigni C, Cognigni GE, et al., 1996).

Although, experience with sequential FSH and low dose hCG or recombinant LH remains quite limited, the results of preliminary investigations are quite promising. In women with hypogonadotrophic hypogonadism or PCO, recombinant LH treatment (225 – 450 IU) daily
during the latter stages of follicular development can decrease the number of developing follicles (Loumaye E, Engrand P, Shoham Z et al., 2003). In GnRH agonist- suppressed normal ovulatory women treated with 150 IU FSH daily for 7 days, a variety of treatment regimens involving combination of decreasing FSH (50, 25, 0 IU) and increasing hCG (50, 100, 200 IU) have been observed to support the development of larger follicles and to speed the regression of smaller follicles. Whereas, either hCG recombinant LH might be used, the longer half life of hCG may help to provide a more stable level LH activity between daily injections (Filicori M, Cognigni GE, Pocognoli P., et al 2002). Interestingly, low-dose hCG treatment during the late stages of follicular development appears to have a little effect on circulating progesterone or testosterone concentrations, at least in normal women, suggesting that the risk of causing premature lutenization or other adverse effects is low. By inducing the regression of smaller follicles, much treatment also may help to reduce the risk of ovarian hyperstimulation associated with exogenous gonadotropin therapy (Navot D, Relou A, Birkenfeld A et al., 1988).

The optimum sequence and relative amounts of FSH and LH/hCG to administer have not yet been defined and likely will vary with the goals of treatment and the endocrinology of individual women (Loumaye E, Engrand P, Shoham Z et al., 2003), but it seems very likely that new and more effective gonadotropin treatment regimens are likely to emerge in the near future.
Flare-up GnRH agonist regimens: short and ultra-short protocols

The flare-up regimens involve early follicular phase commencement of the GnRH agonist, with minimal delay before the onset gonadotropin administration (Howles et al., 1987; Marcus et al., 1993).

There are two theoretical advantages with this approach

- First, the ovarian suppression is not excessive
- Second, the initial stimulation of the GnRH receptors and consequent secretion of endogenous gonadotropins enhances the effects of the exogenously administered gonadotropins.

By contrast, it has been proposed that by decreasing the GnRH agonist dose, a lighter ovarian suppression could be obtained and, hence a better response to gonadotropin stimulation could be achieved.

Furthermore, several micro doses of GnRH agonists in the flare-up protocols have been tested, the aim being to achieve gonadotropin release while eliminating the phenomena of increased LH, androgen and progesterone noted in the classic flare-up protocols (Scott et al., 1993; Deaton et al., 1996). Thus, at least in theory, these regimens would be suited to patients with low ovarian response.

Although widely used, there are no prospective randomized controlled trials of flare-up protocols which can be used to assess their efficacy compared with standard protocols. The relevant studies are summarized in (table 2).

**Standard-dose flare-up regimens:**
In a prospective study with historical controls and using an ultra-short protocol (Howles et al., 1987), according to which 7 patients were administered 0.5 mg/day buserelin during only the first 3 days of the cycle a 0 % cancellation rate and 42.9 % pregnancy rate were found. Similarly, in a prospective study with no controls, the same flare-up regimen was used in 3 categories of various responders after a lupron screening test (Padilla et al., 1996). The group of 53 poor responders had a low cancellation rate (11.3 %) and a good pregnancy rate (29 %) despite the low number of oocytes retrieved. In accordance with these data, others (Toth et al., 1996) compared retrospectively the flare-up versus the luteal GnRH agonist regimen, and observed higher pregnancy rates (20.4 % versus 11.7 %) and lower cancellation rates with the flare-up protocol.

On the contrary, other authors failed to confirm any substantial benefit of using a classic flare-up protocol.

In a prospective study with historical controls (Karande et al., 1997), 80 poor responders were treated using a classic flare-up GnRH agonist regimen with leuprolide 0.5 mg/day from cycle day 2, and 450-600 IU/day hMG from cycle day 3. The authors found an increased number of retrieved oocytes (10 / cycle), but otherwise no improvement, with a high cancellation rate (23.4 %) and a low pregnancy rate (13.4 %). Hence, most studies conducted to assess the standard dose flare-up protocols (that are non-randomized) demonstrated a degree of improvement.

**Reduced-dose GnRH agonist flare-up regimens:**

The idea of minimizing the dose of GnRH agonist created the so-called “mini” and “micro” dose flare-up GnRH agonist regimens. Improved
outcome was observed in a prospective, non randomized trial with historical controls (Surrey et al., 1998), who treated 34 patients having poor ovarian response and no pregnancies in a previous IVF attempt with the long luteal regimen, with a micro dose flare-up GnRH agonist regimen (leuprolide 80 ug/day s.c. from day 3).

The cancellation rate was significantly decreased in the flare-up micro dose protocol, and the clinical pregnancy rate was increased. Impressively, results using the same micro dose leuprolide were also reported in a prospective study with historical controls (Schoolcraft et al., 1997). Here, 32 poor responders were pretreated for 21 days with a combined oral contraceptive (COC).

On day 3 post- COC, each patient received leuprolide 40 ug b.i.d. and GH 4 IU/day i.m., followed on day 5 post- COC by a high dose of gonadotropins (450 IU purified FSH). The cancellation rate was 12.5 %. A mean of 10.9 oocytes per patient was retrieved, and a very optimistic pregnancy rate of 50 % was obtained.

Even lower doses were used in a prospective study with historical controls (Scott and Navot, 1994), where in leuprolide was given to 32 women at a dose of 20 ug b.i.d. from cycle day 3, followed by high doses of FSH from cycle day 5. The authors found a higher peak of Estradiol levels and a higher number of retrieved oocytes.

Despite these findings, the initial optimism for the micro dose flare- up regimen was not supported the results of other studies.

Hence, in a retrospective analysis one group (Leondirs et al., 1999) compared a micro dose flare- up regimen with a long luteal with decreasing dose of GnRH agonist, and observed significantly higher cancellation rates (22.5 versus 8.2 %, $P = 0.032$), lower clinical pregnancy rates (47.3 versus 60 %, $P=NS$) and decreased number of
oocytes retrieved per cycle (13.3 versus 16.5, P=NS) with the micro
dose flare – up regimen.

The results derived from the use of reduced- dose GnRH agonist flare –
up regimens are controversial. There is a trend towards improvement, but
larger controlled and randomized studies are needed to support this issue.

**Luteal initiation of GnRH agonist regimens**

These regimens are characterized by the use of relatively low doses
GnRH agonists commencing in the mid-luteal phase of the cycle and
usually ending at the time of menses or shortly thereafter, in combination
with high doses of gonadotropins.

The possible mechanism of action is the reduced effect of GnRH
agonists on the ovarian receptors (Latouche et al., 1989; Kowalik et al.,
1998), that results in reduced ovarian suppression and consequently, in
increased ovarian response.

Despite the early discontinuation of the agonist, the incidence of
premature LH surge is low but the results are quite contradictory.

Only 2 published prospective randomized controlled trials showed no
statistically significant increase in pregnancy rates, whereas 7
prospective trials with historical controls and one retrospective study
demonstrated improved outcome these studies are summarized in table 3

In a prospective randomized controlled trial involving 78 cycles, a “stop
agonist “regimen was compared with a standard long luteal protocol
(Dirnfeld et al., 1999).
Thus, the use of GnRH agonist (buserelin 1 mg/day intranasally or triptolerin 0.1 mg/day S.C.) was initiated on day 21 of the pre-stimulation cycle and ceased on the day of confirmed pituitary suppression (Estradiol level < 140 pmol/L). Ovarian stimulation was induced with the use of 225 to 375 IU/day hMG or purified FSH (i.m.), commencing on the day of down regulation. No improvement was found, as the mean number of the retrieved oocytes remains unchanged and no significant increase in the pregnancy rate was noted.

Similar results were observed in another well designed, prospective randomized, controlled (Garcia-Velasco et al., 2000), where the “stop” versus “non-stop” protocol of the GnRH agonist, in addition to high doses of gonadotropins were compared. The authors used leuprolide (1 mg, s.c.) from day 21 of the cycle, ceasing on the day of menses, followed by 375 – 450 IU hMG and/or purified FSH daily (i.m.). A significantly higher number of aspirated follicles was found (8.7 versus 5.3 / cycle, P = 0.027), but there was no significant difference in either cancellation rate (5.7 versus 2.8 %) or pregnancy rate (18.7 versus 14.3 %).

These results are not supported by other studies which, although prospective, had historical controls. Thus in a prospective analysis involving 224 cycles (Faber et al., 1998), a low-dose mid-luteal GnRH agonist (leuprolide 0.5 mg, S.C.) was administered but then discontinued with the onset of menses. All of the patients had received GnRH agonist for at least 7 days, after which 450-600 IU of purified FSH or hMG were administered (i.m.) daily. The dose of gonadotropins was decreased 2
days prior to hCG administration, and this was referred to as the “stop-lupron protocol”.
The authors reported a low cancellation rate of 12.5 %, a high number of oocytes aspirated per cycle (11.1), and an impressive clinical pregnancy rate per transfer (32 %).
Interestingly, among the poor responders the authors did not find any statistical significant difference when comparing the use of hMG / purified FSH with purified FSH alone.
In accordance with this approach, another group (Pu-Tsui et al., 2002), in a 52 cycle, non-randomized prospective study, administered 0.5 mg leuprolide s.c. from day 21 to the next cycles’ day 2. After this, 300 – 450 IU / day hMG and purified FSH were used for controlled ovarian hyperstimulation. The patients showed a good response to stimulation (mean 7.5 oocytes /cycle) and encouraging pregnancy rates (20.5 % per embryo transfer).
The same protocol was also assessed by another group in an 82 – cycle prospective analysis with historical controls; both high pregnancy rates (33.3 %) and cancellation rates (31.6 %) were observed (Karande et al., 1997).

Switching between various GnRH agonists did not seem to make an y difference. Thus, in a prospective study with historical controls and involving 36 poor responders, the use of nafarelin (0.6 mg / day commenced in the mid-luteal phase and discontinued on day 5 of ovarian stimulation) resulted in an enhanced efficacy of the gonadotropin treatment (Schachter et al., 2001). The number of retrieved oocytes was increased by 28 %, cancellation rates were decreased to 8.3 % and pregnancy rates increased to 19.4 %.
In another prospective study with historical controls, and using the same GnRH agonist at the same dosage but discontinuing it on day 1 of the next cycle, 39 poor responders were treated and showed a positive effect on the number of retrieved oocytes and the pregnancy rates (10.7 versus 2.8%) (Pinkas et al., 2000). (See table 1).

Subsequently, reducing by a factor of 2 and already low dose of the GnRH agonist had encouraging results (increased number of oocytes collected, decreased total gonadotropins used), as shown in another prospective study with historical controls (Olivennes et al., 1996). These authors used 0.1 mg / day leuprolide s.c. from cycle day 21, reducing it to 0.05 mg / day on down-regulation day in 98 cycles. However, the cancellation rate remained high (24%) and the pregnancy rate relatively low (16.3%).

The above approach was evaluated retrospectively in another study of 106 cycles (Feldberg et al., 1994). Triptolerin was used in the same step-down fashion (from 0.1 to 0.05 mg/day), where upon a higher number of oocytes and improved pregnancy rates (28.1% were observed, compared with the higher dose step-down triptolerin (from 0.5 to 0.1 mg / day) or single dose depot triptolerin (3.75 mg). Administration of a single dose depot GnRH agonist preparation (leuprolide 3.75 mg) on day 21 of the pre-stimulated cycle was also assessed in a prospective study with historical controls including 27 cycles (Serafini et al., 1988).

These authors observed a significantly increased pregnancy rate and number of oocytes retrieved, while the cancellation rate was decreased.

**GnRH antagonist regimens**
The relatively new GnRH antagonist regimens aim to avoid premature LH surge and, at the same time, to utilize the maximum of the ovarian oocyte cohort by minimizing the suppressing effects of the GnRH analogues on the ovarian receptors, thus avoiding ovarian suppression at the stage of the follicle recruitment (Kenigsberg et al., 1984).

Only three published studies have addressed the issue. One prospective randomized controlled study comparing the antagonist versus the flare–up agonist protocol, reported better results with the flare-up regimen. The other two studies (one randomized and one with historical controls) with the use of GnRH antagonists. These studies are summarized in table 4.

One group (Craft et al., 1999) used clomiphene citrate (100 mg/day, from cycled days 2 to 5) combined with the appropriate dose of gonadotropins (mean 375 IU/day), in 24 cycles of poor responding patients. The GnRH antagonist cetorelix was started on cycle day 6 at a dose of 0.25 mg/day. Compared to previous results with GnRH antagonists in the same patients, fewer abandoned cycles (29.2 versus 56.5 %), increased number of retrieved oocytes per cycles (6.4 versus 4.7) and increased pregnancy rates per transfer (23.5 versus 10 %) were observed. There was also a reduction in the amount of gonadotropin injections used. Never the less, the previously mentioned results did not reach statistical significance.

Another prospective randomized study (Akman et al., 2000) reported that the use of GnRH antagonists, together with high doses of gonadotropins (300 IU/day hMG + 300 IU/day purified FSH from cycled day 2) in previous poor responders, was associated with lower
cancellation rates (20 versus 25 %) and increased pregnancy rates (20 versus 6.25 %), as compared with gonadotropins alone. However, these differences were not statistically significant and no change in the number of the oocytes retrieved was observed.

The same authors (Akman et al., 2001) in a subsequent prospective, randomized controlled trial compared the multi dose GnRH antagonist protocol with the flare – up GnRH agonist regimen in poor responders (24 cycles in each group). These authors observed significantly less oocytes retrieved per cycle (4.5 versus 5.5) in the antagonist group (P = 0.032), but no significant difference was seen in either the cancellation or pregnancy rates (25 versus 20.83 % and 22.3 versus 26.3 %, respectively).

The available limited data, derived from small or preliminary studies, do not show any advantage from the use of GnRH antagonists. Therefore, larger, controlled, prospective randomized trials using GnRH antagonists are necessary to investigate this issue.

**Adjunctive use of GH or GH-releasing factor or pyridosigmine** The hypothesis that GH stimulated ovarian steroidogenesis, follicular development and enhances the ovarian response to FSH was proposed in 1986 (Jia el al., 1986). This action of GH is believed to be mediated via IGF-1 that acts in synergy with FSH, amplifying its effects on granulosa cells (Adashi and Rohan, 1993). This was the theoretical basis for the introduction of GH or GH-releasing factor (GH-RF) in the IVF protocols of poor
**responders.** Usually 4 to 12 IU GH are administered S.C., commencing on the day of controlled ovarian hyperstimulation with gonadotropins.

A total of nine prospective trials have been reported; four of these were non-randomized but had historical controls. Seven of the studies revealed essentially no change or no significant improvement in the clinical results. The studies are summarized in table 5.

In one large multicenter prospective randomized, double blind, placebo-controlled trial (Howles et al., 1999), GH-RF was administered and caused an increase in endogenous levels of GH. However, the final cancellation and pregnancy rates were similar to those found for the protocol without GH-releasing hormone (12.5 versus 16 % and 8.3 versus 8 % respectively).

No statistically significant improvements in cancellation and pregnancy rates were reported in another double-blind, placebo-controlled trial (Suikkari et al., 1996) in which 4 IU /day of GH was used as adjuvant therapy.

Increasing the GH dose to 12 IU /day in along luteal GnRH agonist regimen led to similar results in a prospective study with historical controls (Shaker et al., 1992).

The above discouraging findings were also reported by others (Hughes et al., 1994), who treated 21 previous poor responders with 12 IU/day GH in a prospective double-blind, placebo- controlled study and found no significant differences in serum Estradiol levels, duration of the follicular phase, total hMG dose and number of oocytes between the placebo or GH cycles.
Likewise, in a small, prospective, randomized, double-blind, placebo-controlled study, another group administered 18 IU unit GH on alternating days in a classic flare-up triptorelin protocol with 300 IU day of hMG, in 14 cycles of poor responders (Dor et al., 1995). Unfortunately, the results were also disappointing. The same conclusion was also reached in 2 additional small studies (Hugues et al., 1991; Levy et al., 1993).

Optimistic results were reached in a small prospective study with historical controls (Ibrahim et al., 1991), where 10 patients had higher number of oocytes collected (7.5 versus 3.5, P<0.001), and improved pregnancy rates (60 %).

Growth hormone secretion can also be increased by acetylcholine Which inhibits somatostatin secretion at the hypothalamic level (Delitala et al., 1988). Pyridostigmine is an acetylcholinesterase inhibitor which, by enhancing the action of acetylcholine, can increase GH secretion. This approach was evaluated in a randomized, double-blind, placebo-controlled study (Chung-Hoon et al., 1999), which included 70 poor responders who were given 120 mg/day Pyridostigmine orally, from the day of down –regulation until the day of hCG, together with a long luteal GnRH agonist regimen (triptorelin 0.1mg on day 21, hMG / FSH 300 IU/day, i.m.). Compared with placebo, Pyridostigmine was associated a significantly lower number of ampoules used (38.4 versus 48.3), a higher number of oocytes collected (5.9 versus 3.7) and improved ( but not significantly so) pregnancy rates (25.7 versus 11.4 %).
In a recent Cochrane review a meta-analysis was conducted of the trials assessing the effectiveness of GH adjuvant therapy in women undergoing ovulation induction (Kotarba et al., 2002).

In previous poor responders, the common odds ratio for pregnancy / cycle instituted was 2.55 (95% CI 0.64-10.12).

No significant difference was noted in either the number of follicles and oocytes, or gonadotropin usage.

Therefore, these published data do not support any benefit from the use of GH as adjuvant therapy in poor responders.

**Adjunctive use of glucocorticosteroids (dexamethasone)**

It has been suggested that dexamethasone may directly influence follicular development and oocyte maturation via its isoform (11-BHSD) in the granulosa cells (Smith et al., 2000) or indirectly, by increasing serum GH and consequently intrafollicular IGF-1 (Miell et al., 1993).

In addition, it may provoke immunosuppression within the endometrial microenvironmment (Polak de Fried et al., 1993).

To date, no studies have been reported involving poor responders. In one double-blind, placebo-controlled prospective randomized study in 290 cycles of normal responders (aged < 41 years), dexamethasone was administered at 1 mg / day in the long luteal protocol until the day prior to oocyte retrieval (Keay et al., 2001), and the authors found a significantly lower cancellation rate (2.8 versus 12.4 %, P=0.001) (see table 1).
These findings provided great encouragement, as they reveal a very low incidence of poor response with the use of corticosteroids; however, the data are limited and can only be considered as preliminary. Consequently, further trials are needed to support the role of corticosteroids in patients confirmed to be poor responders.

**Adjunctive use of nitric oxide (NO)-donors (L-Arginine)**

In humans, increased vascularization appears to play a critical role in the selection, growth and maturation of follicles in both natural and IVF cycles (Weiner et al., 1993).

L-Arginine is a potential vasodilator as a NO-donor; in fact, NO is derived invivo from L-Arginine by a NO-synthase enzyme that is either cytokinine-inducible or calcium-dependant (Moncada et al., 1991). It is also thought that NO is involved in follicular maturation and selection (Anteby et al., 1996), possibly due to its participation in periovulatory vasodilatory modulation, as proven in a rat model (Ben Shlomo et al., 1994).

Promising results were presented by one group (Battaglia et al., 1999), in a prospective randomized study in which 2 groups of 17 poor responders were compared, each of which was treated with the GnRH agonist flare-up regimen, high doses of purified FSH and orally administered L-Arginine.

Significantly higher numbers of collected oocytes were found (4.1 versus 1.6, P=0.049), as well as, a higher though not significantly so, pregnancy rate (17 versus 0 %, P = NS), and a lower cancellation rate (11 versus 76 %, P = 0.001) in the L-Arginine group (see table 1).
Clearly, these preliminary results require further verification, and additional studies are needed to investigate the role of these agents in controlled ovarian hyperstimulation, particularly in poor responders.

**Pretreatment with COC or progestogens**

COC administration aims to suppress endogenous gonadotropins and, at the same time (through its estrogen component), generate and sensitize more estrogen receptors.

Unfortunately, the administration of COC acts as a type of pituitary suppression by itself.

A few prospective and randomized studies have shown that COC pretreatment may be beneficial with regard to ovarian response and clinical pregnancy rates (Gonen et al., 1990; Biljan et al., 1998). This suggestion was not confirmed by pretreatment with progestins alone (Shaller et al., 1995), although the data were obtained from a patient cohort which excluded poor responders.

Although several investigators have used COC pretreatment in other experimental protocols for poor responders, only one retrospective study has been reported on this topic (Lindheim et al., 1996). These authors showed that COC administration prior to the GnRH-agonist protocol was associated with higher pregnancy rates and lower cancellation rates.

In conclusion, although there us a general feeling that COC pretreatment might be of assistance in the ovarian response in poor responders, only a minimal amount of published data exist which document this hypothesis.
Conclusions

Despite the plethora of predictive tests for poor ovarian response, the poor responder is relieved definitely only during ovarian stimulation. Furthermore, there is no uniformity in the definition of the “poor responder”, thereby, rendering many of the clinical trials incomparable. On the other hand, an accurate prediction of poor responders would help the clinicians to choose the most suitable alternative to classic IVF treatment, for example oocyte donation.

A second limitation of the published studies is the selection of the control group, with most of the prospective studies using historical controls and comparing patients with their prior “failed” IVF cycles.

A third limitation is that, at present, there have been no reports on any large scale, prospective, randomized, controlled trials of the different management strategies. At present, the results from only a few prospective and even fewer prospective, randomized, double-blind, placebo-controlled trials have been reported, and all have been small in terms of patient numbers.

The use of very high doses of gonadotropins to stimulate the ovaries is clearly unavoidable due to the lack of any initial ovarian responsiveness. Nevertheless, the results have been controversial with the prospective randomized trials showing either minimal or no benefit. Additionally, the few available relevant studies have suggested that the use of recombinant FSH might improve the outcome.
Although not derived from authentically prospective trials, optimistic data have been presented which suggest the beneficial use of flare-up GnRH agonist protocols (standard or micro dose), along with high doses of gonadotropins with or without GH. These regimens seem to have better results compared with those of the standard long luteal protocols.

A significant improvement was demonstrated with the use of the use low-dose, mid-luteal onset, GnRH agonist regimens, that discontinue with the initiation of ovarian stimulation, followed by high doses of gonadotropins (GnRH agonist “stop” protocols), according to the prospective studies with historical controls. However, the well designed trials fail to confirm this, and showed no significant improvement.

The few data available from the use of the GnRH antagonist do not show any benefits at present, though it is possibly too early to comment at this time. Certainly, further evaluation in this area is necessary.

Pretreatment with COC may help the ovarian response and, therefore appears to be beneficial.
Likewise adjuvant therapy with GH or GH-releasing factors causes in general either no change or a trend towards non-significant improvement.
The use of corticosteroids appears to reduce the incidence of poor ovarian response in the population undergoing IVF treatment.
Moreover, the encouraging limited data related to the use of NO –donors such as L-Arginine requires further evaluation.
In conclusion, there is a clear need to standardize the definitions of low ovarian response. Well designed, large scale, randomized controlled trials are needed to assess the efficacy of the different management strategies. The current results which are available are perhaps somewhat disappointing, but this should not be too surprising as most poor responders appear to have occult ovarian failure. Thus, the exhausted ovarian apparatus is unable to react to any stimulation, no matter how powerful this might be. The ideal stimulation for poor responders still remains a challenge, as the hypothesized diminished oocytes cohort and poor oocyte quality can not be reversed within the limits of our present capabilities.