Chapter

Ovarian Reserve

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Abstract

The human ovary is a complex structure that is controlled by endocrine, paracrine, and autocrine mechanisms. The number of eggs retrieved after controlled ovarian stimulation in in vitro fertilization depends on the physiological follicular reserve pool of ovaries. Ovarian reserve is decided genetically and decreases with advancing age and gets affected by ovarian surgery, chemotherapy, radiotherapy, and autoimmune disorders. Environmental influences like chronic smoking, hyperglycemia, and conditions interfering ovarian vascularity also reduce the ovarian reserve. This chapter summarizes the methods to assess the ovarian reserve. This helps in deciding the initiating dose of gonadotropins for controlled ovarian hyper stimulation for optimal follicular response.

Keywords: ovary, controlled ovarian hyper stimulation, in vitro fertilization, ovarian reserve, ovulation

1. Introduction

Each natural ovulatory menstrual cycle has a 25% probability of spontaneous conception. Infertility is investigated when there is failure to conceive naturally following 1 year of unprotected intercourse in cases where the female is ≤35 years of age or following 6 months of unprotected intercourse for women >35 years of age. In 40% of the cases it can result due to female factor, in another 20% of cases it's the male factor, which is the cause, and combined factors can cause infertility in about 20% of cases. The causative female factors can be further classified into tubal factors (40%), ovulatory factors (40%), uterine factors (10%) and cervical factors (10%). The commonest etiology of female infertility is ovulation dysfunctions and fallopian tube anatomical and physiological obstruction. The cornerstone first line examinations and investigations for the subfertile couple should detect of ovulation and pituitary and ovarian secretion of hormones by hormonal assay (early follicular FSH and LH levels, mid-luteal progesterone) to assess the endogenous hypothalamo-pituitary-ovarian endometrial axis, and evaluation of tubal patency and function by diagnostic hysterolaparoscopy. GnRH hormone in hypothalamo-pituitary portal circulation cannot be detected in peripheral blood samples.

2. Physiology of folliculogenesis

Ovaries are almond-shaped organs located in the pelvis on either side of the uterus. In addition to production of ova the ovaries are also a distinct endocrine organ producing hormones primarily estrogen and progesterone that are very important for normal reproductive function. The adult ovary can be divided into

3 main regions superficial to deep. These are the a. Cortex that consists of tunica albuginea ovarian follicles (primordial, primary, secondary, small medium, large Graafian follicles, and corpus luteum, atretic follicles. The B. medulla consists of blood vessels and nerves in connective tissue. The innermost hilum contains large spiral arteries and Leydig cells.

Normally the ovary produces a single dominant follicle in each menstrual cycle that undergoes maturation and results in ovulation (**Figure 1**). The granulosa cells of the growing follicles produce estradiol during the first half of the menstrual cycle or the follicular phase. The folliculogenesis begins with recruitment of a primordial follicle into a pool of growing follicles and it is a long process almost a year for a primordial follicle ultimately resulting in ovulation of the follicle or death by atresia. The process of folliculogenesis can be divided into 2 phases. First phase is "preantral" or "gonadotrophin independent" phase, where there is growth and differentiation of oocyte. The preantral phase mainly controlled by local factors growths factors by autocrine and paracrine processes. The second phase is the "gonadotrophin dependent" phase or the "antral phase" when there is a fast growth in the size of the follicle itself and it is under the control of gonadotrophin. The Antral phase is regulated by FSH and LH and also by paracrine growth factors that cause intracellular signaling and multiplication of cells.

The process of this folliculogenesis goes through multiple sub phases too. These include: 1. primordial follicle recruitment; 2. preantral follicle development; 3. Selection and growth of antral follicle; and 4. follicle atresia. The primordial follicle is the reproductive unit of ovary. It gives rise to dominant follicle. In human female fetus, primordial follicles are formed 6 and 9 months of gestation and many of the primordial follicles undergo apoptosis. The number of primordial follicles decreases progressively due to recruitment and apoptosis and very few of them are present after menopause. Primordial follicle recruitment is completely dependent on paracrine mechanism largely controlled by growth factors especially TGF-beta superfamily.

A primary follicle is in the next sequence that has the presence of one or more cuboidal granulosa cells arranged in a single layer around the oocyte. The most important event at this stage is the FSH receptor expression and oocyte growth and

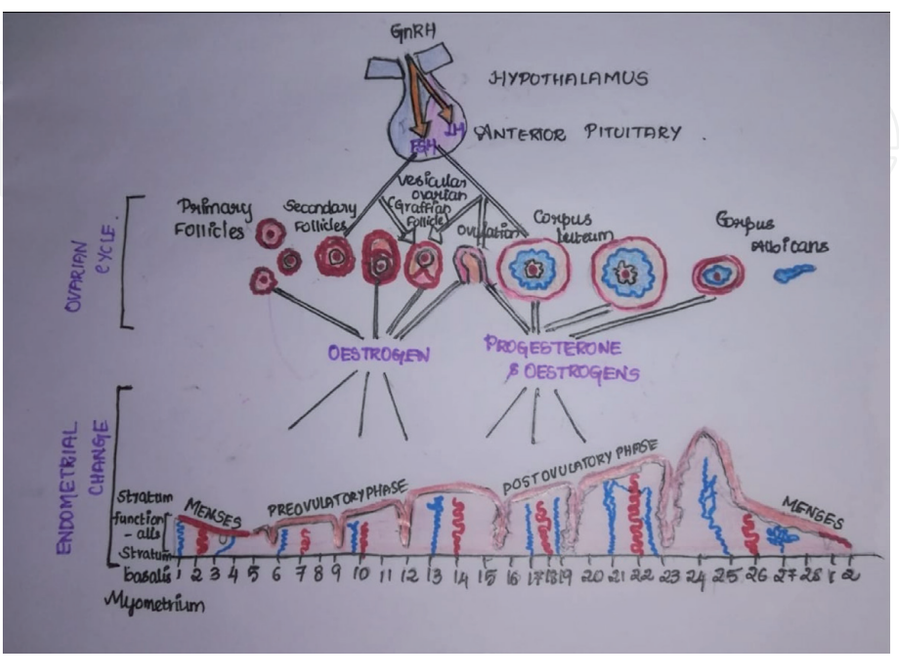

Figure 1.
Folliculogenesis, ovulation and corpus luteal formation.

maturation. Granulosa cells express receptors for FSH at this stage and the stimulus for this is FSH itself. Further development of primary follicle into preantral follicle is FSH dependent. The development of primary follicle is associated with striking change in oocyte too. Even there is a development of intimate connections between oocyte and granulose cells through the transzonal oocyte processes and gap junctions (cumulus oophorus complex). FSH is an important factor in supporting follicle growth after antrum formation and in also preventing apoptosis thus it is a survival factor for antral follicles. Thus FSH plays a very important role in selection and dominant follicle development. The proliferation of granulosa cells is a very important feature for development of dominant follicles. FSH is the most important factor for granulosa cell proliferation.

As the granulosa cells proliferate and a dominant follicle develops it acquires the capacity to produce estradiol. FSH mediates the granulosa cells to acquire the above potential. The progressive increase in estradiol production by the dominant follicle from Day 7-Day 12 of menstrual cycle is only possible due to increased levels in P450AROM gene expression by FSH in granulosa cells.

The physiologic mechanism by which dominant follicle produces estradiol is the two cells two-gonadotrophin concept. When FSH recruits follicles to preovulatory development, their granulosa cells develop LH receptors, start undertaking aromatization and also inhibin production. Inhibin has the capability to increase LH stimulated thecal androgen production. LH receptors are present on theca cells throughout the menstrual cycle. LH acts on theca cells to produce primarily androstenedione and to a lesser extent testosterone. Theca cell synthesized androstenedione is transported by paracrine circulation into the granulosa cells. In granulosa cells androstenedione and testosterone are aromatized to estrone and finally into estradiol by 17-β-hydroxysteroid dehydrogenase type I. This is known as the two-cell, i.e., theca cell and follicular cell, two-gonadotropin, i.e., FSH and LH and two hormone, i.e., estradiol and progesterone theory of regulation of estrogen synthesis in the human ovary as shown in **Figure 2**. Granulosa cell derived inhibin takes part in a paracrine mechanism communicating with theca cells and thus amplifying androgen synthesis. This theca cell derived androgen is converted to estradiol in granulosa cells of preovulatory follicles. Inhibin B has an early follicular phase elevation and lower values after ovulation. Thus it suggests Inhibin B is a granulosa cell product and can be a marker for follicular function, oocyte number and thus plays a role in follicular development in early part of the cycle.

2.1 Follicular phase

The follicular phase starts from the first day of menstrual cycle D1 until ovulation. Basal body temperature chart normally shows lower values during this phase. Development growth trajectories of the ovarian follicles characterize this first phase of the cycle. Folliculogenesis starts during the last few days of the preceding menstrual cycle and continues till the release of the mature follicle at the time of ovulation. The primary development of the follicle to the preantral follicle is not gonadotropin dependent, and further follicular growth beyond this point requires gonadotropin action.

The secretion of gonadotropins from anterior pituitary is regulated by gonadotropin releasing hormone (GnRH), steroid hormones, and various peptides released by the dominant follicle. The growth of follicular size and number of granulosa cells in each follicle leads to an increase in estradiol serum concentrations in the early follicular phase. FSH receptors exist only on the granulosa cell membrane. There is increase in the number of FSH receptors with the gradual rise in serum FSH levels during the late follicular phase. The rise in serum FSH along with the rise in FSH

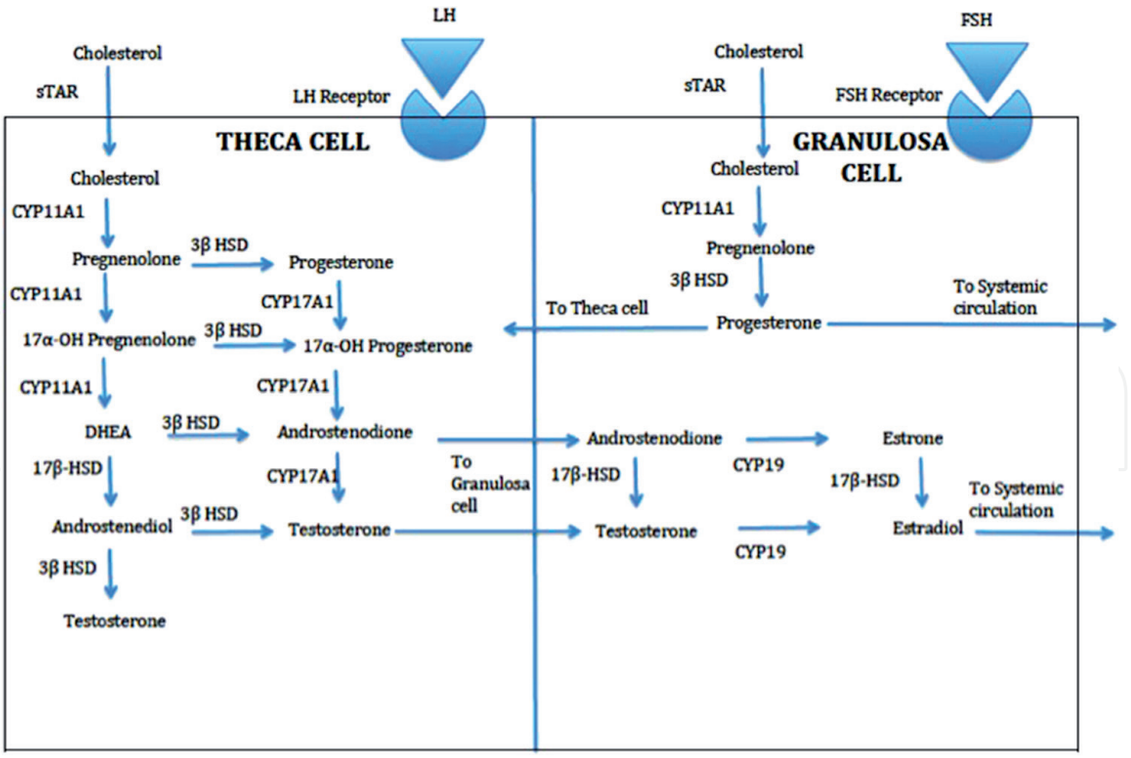

Figure 2.
Theca and granulosa cells (two cells) respond to luteinizing hormone and follicle stimulating hormone (two gonadotropins) to produce 17-OH progesterone and estradiol (two hormones).

receptors leads to an increase in estradiol secretion by granulosa cells. It has to be noted at this point clearly that the increase in FSH receptor numbers is due to an increase in the population of granulosa cells and not an increase in the concentration of FSH receptors per granulosa cell. FSH has a positive stimulating effect for the formation of LH receptors on granulosa cells thus allowing the production of small quantities of progesterone and 17-hydroxyprogesterone (17-OHP) that exert a positive feedback on the estrogen-primed pituitary to augment luteinizing hormone (LH) release. Without LH, the increased progesterone synthesis from granulosa cells under the influence of FSH advances the endometrium and the resulting asynchronous development reduces the chances of embryo implantation (**Figure 2**). This phenomenon is also called as ENLOP or Elevated Non Luteinised Origin of Progesterone leading to displaced window of implantation. The addition of LH in follicular phase reduces premature progesterone increase and improves the likelihood of implantation and clinical pregnancy.

FSH is elevated during the early follicular phase, declines as estradiol secretion increases from the granulosa cells, has a second rise before ovulation and then begins to decline until ovulation. FSH also stimulates several steroidogenic enzymes including aromatase, and 3β-hydroxysteroid dehydrogenase (3β-HSD). The secondary rise of FSH is important for inducing LH receptors on theca cells and granulosa cells and thereby preparing for the action of LH surge.

2.2 Luteal phase

The positive feedback from the rising estrogen levels during the follicular phase results in gradual increase in LH level by mid follicular phase that is low to start with during the early follicular phase. Estradiol levels must be greater than 200 pg/mL for approximately 50 hours in duration for the positive feedback effect of LH release to occur. During the early follicular phase, LH secretion occurs at a pulse frequency of

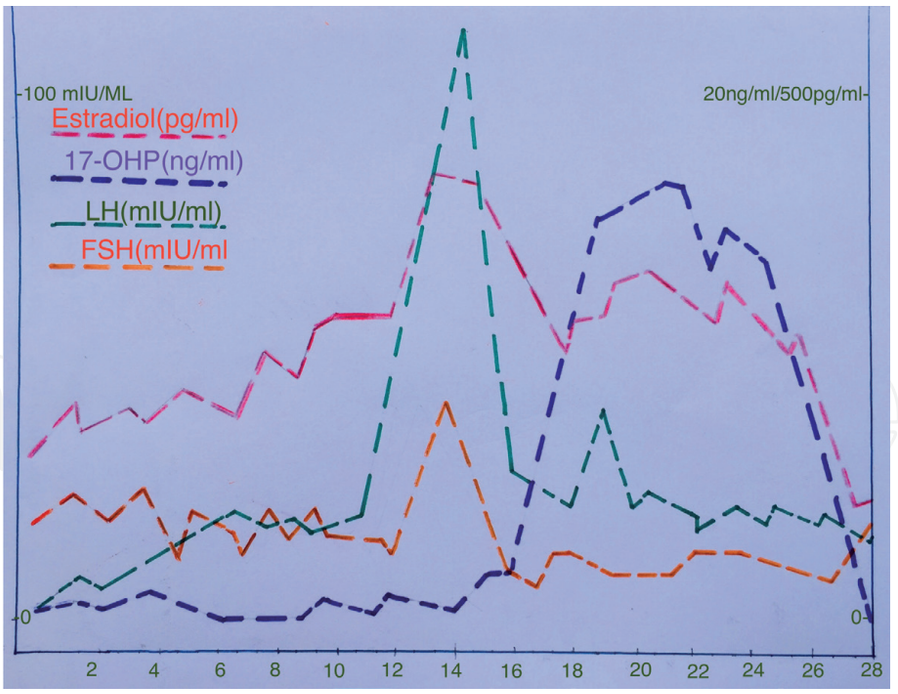

Figure 3.
The cyclical harmonious balance of gonadotropin secretion from pituitary and estrogen and progesterone secretion from ovary results in LH surge resulting in final maturation of oocyte and ovulation.

60 to 90 minutes with relatively constant pulse amplitude. During the late follicular phase prior to ovulation, the pulse frequency and may be amplitude of LH secretion increases. LH pulse amplitude increase results in ovulation [1, 2]. After ovulation corpus luteum is formed and theca cells continue to secrete progesterone.

The reduction of steroid production by the corpus luteum and the sudden fall of inhibin A allow the follicle stimulating hormone (FSH) to increase during the last few days of the menstrual cycle. In the late luteal phase, as corpus luteum lso degenerates there is no the estrogen and progesterone secretion from the ovary resulting in FSH rise because of increased GnRH pulsatile secretion. This elevation in FSH is very crucial for the recruitment of a cohort of ovarian follicles in each ovary out of that one follicle will be destined to ovulate during the next menstrual cycle. As menstruation starts the FSH levels begin to decline due to the negative feedback of estrogen and the negative effects of inhibin B produced by the developing follicle [1, 2]. The cyclical hormone changes are depicted in **Figure 3**.

3. Ovarian reserve

Ovarian reserve plays an important role in achieving pregnancy following any treatment in infertile and sub fertile women. The main function of the ovary in a woman is the production of a mature and viable oocyte that is capable of fertilization and subsequently leads to an embryo development and implantation. At birth, each ovary has a fixed number of oocytes available for folliculogenesis during the later life. This fixed number of available oocytes is termed as "the ovarian reserve" of the woman. Delayed childbearing, voluntary or involuntary, is a common feature in couples visiting fertility clinics nowadays as they are career oriented and mostly working. The estimation of ovarian reserve is routinely performed prior to interventions through various ovarian reserve tests (ORTs) in an effort to predict the response and outcome in couples in vitro fertilization techniques and to even counsel them. The ovarian reserve estimation has to be routinely performed prior

to any interventions for infertility through various ovarian reserve tests (ORTs) in an effort to predict the response and outcome in couples seeking help for infertility treatment such. The widely used tests are basal follicle stimulating hormone, Anti-Mullerian Hormone (AMH) and Antral Follicle Count (AFC). Ovarian reserve reduction is a physiological phenomenon characterized by declining follicular pool and oocyte quality. The reduction of ovarian reserve starts at about 30 years in south Asian population and at 35 years in Caucasians [3].This rate of age-related reduction of follicle count in the human ovary is more than doubles when numbers fall below a critical figure of 25,000 at ~37.5 years of age [4].

3.1 Ovarian reserve testing

Ovarian reserve tests (ORT) serve as an indirect measures of a woman's remaining follicular pool when she presents herself for infertility treatment. ORT should be easy to perform, should be sensitive, specific, valid, and help to individualize the starting dose of gonadotropins for multifollicular development. The ovarian reserve testing helps to differentiate normoresponders, hyporesponders, and hyperresponders. Ovarian reserve is deciphered through a number of markers. These markers also help to prognosticate poor responders. Ovarian reserve is predicted clinically using a combination of clinical, biochemical and biophysical tests.

The tests are being used all over the world but the sensitivity and specificity of these test to detect the oocyte number, quality, and fecundity has to be still ascertained with further research [5]. More recently, their value in predicting hyperresponse and hypo response and thus using safe stimulation regimes to prevent OHSS is also explored [2]. The interpretation of the results of the ovarian reserve test is complicated by the lack of uniform definitions for hypo or hyper-responders and uniform threshold values to identify abnormal results. Several static and dynamic ovarian functional markers like biological (age), biochemical, biophysical, and histological tests have been used to identify ovarian reserve [1, 2].

In most cases of decreased ovarian reserve, the cause remains undetected. In specific cases like exposure to chemotherapy, pelvic irradiation, and genetic abnormalities there is a premature decrease in ovarian pool of oocytes. Cigarette smoking has been associated with a decrease in ovarian reserve. With diminished ovarian reserve a reproductive age woman has regular periods with normal or shortened duration of menstrual cycles but there is a decrease in response to ovarian stimulation and fecundity. Thus women of same age can differ in their response to ovarian stimulation and thus the fecundity can vary.

3.1.1 Age

It is long established that ovarian reserve reduces progressively with age. This is due to a combination of two factors the body 'spending' the eggs through routine ovulation and the ovaries aging and preparing for menopause. Individual variation of the ovarian reserve can be explained by the two instances given as- a young woman with certain reproductive health problems may start out with smaller than the normal reserve of healthy eggs, and some women's reserves decrease more quickly than others with age. Fecundity in both natural and stimulated ovarian cycles declines with maternal age, beginning in the late 20s and becoming more abrupt in the late 30s. The fall in ovarian reserve with age in is a universal phenomenon in all ethinic groups. The initiation and rate of this decline varies considerably with ethinicity. Calender Age *per se* cannot determine ovarian responses. Ovarian reserve can also be traced indirectly by other biochemical and biophysical markers of ovarian function [6–11].

3.1.2 Basal follicle stimulating hormone

One of the most classically used biochemical levels to measure ovarian reserve is the Basal follicle stimulating hormone (FSH) levels measured on day 3 of the menstrual cycle. An increase in FSH levels occurs due to follicle depletion as the age of the woman progresses [9, 10]. The measurement of FSH is easy, and inexpensive reproducible and its specific. FSH levels are known to have diurnal, intra- and intercycle variations that have to be kept in mind. There is definite precise parameter value to detect a woman with poor ovarian reserve. A vague demarking values more than 25 IU/L was used arbitrarily in some studies to detect high basal FSH. Several subsequent reviews did not identify values to satisfy the specificity and sensitivity for basal FSH as a test for poor ovarian response to stimulation or prediction of non-pregnancy. In women with regular menstrual cycles, FSH can predict a poor response adequately only at very high levels, and hence will be helpful only to a small number of women as a screening test for ovarian reserve testing and further counseling. It is thus clear that the ovarian aging begins several years prior to any elevation in FSH levels is noted and thus a normal test cannot rule out a poor ovarian response in some women. When FSH level is combined with other markers it can be used to counsel couples and planning treatment option regarding a poor response but it should not be used to exclude regularly cycling women from ART. The specificity of basal FSH testing in a general sub-fertile population or elevated levels in young, regularly cycling women is thus unclear and needs further studies [11–13]. Additionally the reliability of FSH is challengeable because of its pulsatile and circadian release and its isoforms. There are no cut off values available to predict poor responders.

3.1.3 Anti-Mullerian hormone

Anti-Mullerian hormone (AMH) is a dimeric glycoprotein exclusively produced by granulosa cells of preantral (primary and secondary) and very small and small antral follicles (2–6 mm) in the ovary. The serum levels of AMH reflect the number of follicles that have made the transition from primordial pool into the growing pool but still it is not under gonadotrophin control. The secretion of AMH starts once there is a follicular transition from the primordial to the primary stage, and it continues until the follicles reach till the follicles attain antral stages of diameters 2–6 mm. The number of the small Antral Follicles indirectly reflects size of the primordial follicle pool. With the decrease in the number of the antral follicles with age, AMH production seems to reduce and become undetectable at and after menopause. The physiological function of AMH is to modulate primordial follicle recruitment. It inhibits the action of FSH on follicular growth and selection. AMH is considered to be reflective of FSH independent follicular growth, so it is a direct measure of ovarian reserve. AMH reflects qualitative and quantitative assessment of ovarian reserve. AMH levels also strongly correlate with basal antral follicle count (AFC) measured by transvaginal ultrasonography. Serum AMH levels correlate inversely with age from 25 years onwards and reaches undetectable levels after menopause, thus AMH levels is an important ovarian reserve marker. Serum AMH levels can be measured on any day of the cycle and does not exhibit inter-cycle variability unlike other biochemical markers. Threshold values of 0.2–1.26 ng/ml, have been used to identify poor responders with 80–87% sensitivity and 64–93% specificity. Thus by understanding of its clinical implications, AMH too has the potential to predict a hyper-response during treatment as well. The nomograms of the values of AMH can predict and identify the age-related physiological decline in the AMH levels and thus ovarian reserve, and abnormal deviation in the levels of AMH can be

used for counseling couples wishing to delay childbirth. Still the available evidence is not sufficient enough to suggest that serum AMH can be used as a single marker to predict pregnancy. Furthermore, studies of the levels of follicular fluid AMH has shown that oocytes obtained from follicles with higher levels of AMH have a better fertility potential compared to those with lower AMH levels [14–16]. Serum AMH estimations have also been useful to diagnose "Transitional Ovarian Failure" and "Insipient Ovarian Failure."

Studies conducted longitudinally in fertile women have clearly shown a decline in serum AMH levels with progressing age. AMH is one of the earliest markers to show a decline progressively in young women with aging thus offering the probability of a screening test for women to counsel against delay in childbirth. Levels of 0.5–1.26 ng/ml of AMH suggests impending menopause in next 3–5 years [17, 18]. AMH is also a most promising marker for predicting age of natural menopause too. The serum levels of AMH are not controlled by hypothalamus pituitary axis that makes it important marker in diagnosing conditions such as PCOS and premature ovarian failure.

3.1.4 Inhibin B

Inhibin B is a glycoprotein hormone produced by small ovarian follicular granulosa cells and thereby it is an indirect indicator of the follicular pool. Inhibin B is not a reliable parameter for measuring ovarian reserve though serum levels <45 pg/ml have been associated with poor response to controlled ovarian stimulation since it is not a reliable predictor of pregnancy. Inhibin B levels are lower in poor responders than in women with normal ovarian reserve. Inhibin B levels if exaggerated in stimulated cycle is an indicator of hyper response thus it can be used to monitor the response to exogenous FSH. Use of Inhibin B as a sole predictor of ovarian response is not recommended [19].

3.1.5 Basal estradiol

Estradiol is a steroid hormone secreted by the granulosa cells of the growing ovarian follicles. Day 2 or Day 3 basal estradiol is commonly assessed for observing the early oocyte development. Estradiol also exerts a negative feedback on the secretion of FSH from the pituitary thus high basal estradiol can reduce the FSH levels. Thus it is a helpful parameter in combination with FSH to establish the baseline ovarian reserve. Elevated basal estradiol has been associated with a poor response to ovarian stimulation. An early rise in serum estradiol is a characteristic sign of reproductive aging and can lower the elevated basal FSH into normal range thus resulting in misinterpretation of the test.

4. Clomiphene citrate challenge test

This is a dynamic test of ovarian function. This basically involves the Day 3 testing of basal FSH levels and serum estradiol. Administering 100 mg of Clomiphene citrate tablets per day for 5 days from Day 5 to Day 9 of the cycle follows this. The FSH level is measured on Day 10 of the cycle. In cases of low reserve FSH is elevated on Day 10. FSH is the primary stimulus for final follicular maturation. It is under negative feedback from estradiol and inhibin B. Basal FSH levels are elevated it indicates a diminished follicular pool. Clomiphene citrate challenge test is a good predictor of ovarian reserve but not an absolute indicator of ovarian hypofunction. Thus the clinical value of CCCT is not clearly better than basal FSH and AFC in

combination. The Day 3 or Day10 FSH levels more than 10 mIU/ml is considered abnormal by most studies. Though there are different opinions still CCCT remains a gold standard in testing ovarian reserve. The justification of this test is that as the ovarian follicle develops in patients with normal ovarian function and will produce levels of inhibin and estradiol sufficient enough to suppress FSH production by Day 10 of the cycle concerned [20].Other dynamic tests used are GAST (GnRH Analogue Stimulation Test) and EFFORT (Exogenous FSH Ovarian Reserve Test).

5. Ultrasound parameters

5.1 Antral follicle count

After menarche, gradually a regular bi-fortnight ovulation is established. The immature oocyte covered with granulosa and theca cells rests in a small fluid filled cavity called as the antral follicle. These small fluid filled cavities are visualized sonologically in early follicular phase. These reflect follicles that were selected from the primordial follicle in this wave (wave theory of folliculogenensis) and so the antral follicle counts may vary in various mentstral cycles. There is no clear-cut consensus on the criteria to identify antral follicles. Various litreture reviews suggest that the follicles with a diameter of 2 to 10 mm can be considered as AFa [5, 21]. Thus, the antral follicle count (AFC) is the number of follicles with cavity less than 10 mm in diameter with Transvaginal Ultrasound (TVUS) imaging in the early follicular phase of the cycle (**Figure 4a–c**). Antral follicle count is a quantitative aspect of ovarian aging. As a direct marker of the cohort of growing follicles

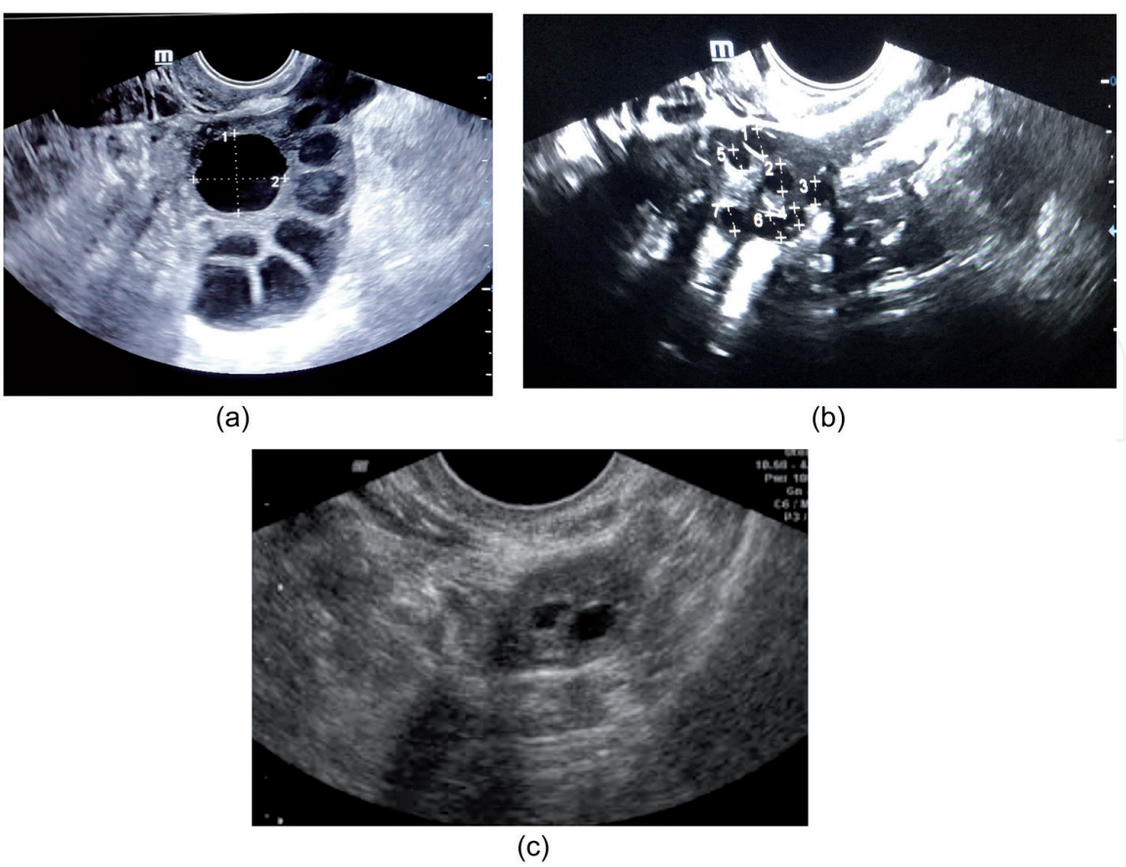

Figure 4.
(a) Ovarian antral follicular count in hypo responders; (b) ovarian antral follicular count in normal responders; and (c) ovarian antral follicular count in hyper responders.

in the early menstrual cycle, the AFC is believed to correlate strongly with the number of primordial follicles present in the ovary and, thus, the ovarian reserve. Antral Follicles are routinely measured by 2 D transvaginal ultrasonography in the early follicular phase, by taking the mean of two perpendicular measurements. Inversion made is useful for counting multiple follicles. The numbers of follicles in both ovaries are added for the total Antral Follicle count. (AFC). AFC has been predominantly used as a marker of ovarian reserve over a period of time. A count of 8–10 is taken as a normal response of ovaries. Different diameters are used to define antral follicles of varying sizes as those measuring 2–6 and 7–10 mm. There is no clear consensus regarding the size of antral follicles, which truly represent ovarian reserve. The number of small antral follicles (2–6 mm) is significantly related to age and also to all endocrine ORTs tested, suggesting the number of small antral follicles represents the functional ovarian reserve. It is seen that the number of antral follicles of 2–6 mm in size decreases with age and correlates with other markers such as serum basal FSH and CCCT whereas follicles of size 7–10 mm remains constant and thus, the former appears to be a more reliable marker of ovarian reserve. Measurements taken repeatedly of the antral follicles have shown that there is only a limited intercycle variability. 3D ultrasound imaging also does not carry any better advantage in comparison to 2D ultrasound for the detection of functional ovarian reserve [22–25]. Meta-analyses showed that women with AFC less than four were 8.7 times more likely not to get pregnant after IVF (two studies; 95% CI,) than women with AFC four or more. The sensitivity and specificity of AFC to predict cycle cancelation was 66.7 and 94.7%, respectively [21].

5.2 Ovarian volume

The volume of the ovaries is calculated by imaging and callipering the ovary in three perpendicular planes with using the formula of ellipsoid volume as (L1 × L2 × L3 × π/6). An alternative automatic method is calculation of Ovarian volume through "virtual organ computer-aided analysis" or VOCAL. The predictive performance of ovarian volume toward poor response is clearly inferior compared with that of AFC. Therefore, the AFC may be considered the test of first choice when estimating quantitative ovarian reserve before IVF. Total Basal Ovarian Volume (BOV) is obtained by adding the volumes of both. The ovarian volume is constant till the perimenopausal period and the measurement does not increase the

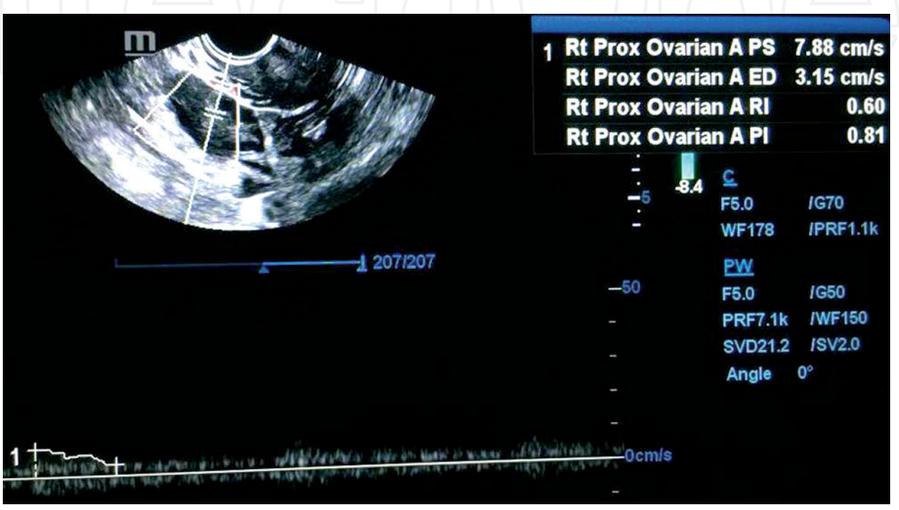

Figure 5.
Doppler ovarian stromal vascularity measurements with 2D doppler calculation of pulsatility index and resistivity index.

positive predictive value of AFC. Furthermore, the decrease in the Basal Ovarian Volume is a very late phenomenon women >40 years [25, 26]. Ovarian volume measurement, at a cut off value of 3 cm^3, showed specificity for prediction of cycle cancelation and non-pregnancy of 92% (three studies, 95% CI, 89–94) and 93% (three studies, 95% CI), respectively [21].

5.3 Ovarian stromal vascularity

The observation of the ovarian stromal Doppler flow during ovarian stimulation has been studied in IVF cycles. Poor ovarian stromal vascularization impairs the access of gonadotropins to the ovarian follicles. Power Doppler US in combination with 3D VOCAL is an appropriate approach for correlating the ovarian vascular network with the ovarian response to ART. The gradual increase in the Doppler flow noted during stimulation may provide additional information to AFC (**Figure 5**) [27–36].

6. Conclusion

Ovarian pathophysiology is complex. Ovarian folliculogenesis follicular rupture and luteal transition should be studied elaborately. Endometrial evaluation should be also done in a nonstimulated cycle. Serum hormone values should be measured in normal non-induced menstrual cycle to study the ovarian reserve and detect any undiagnosed synchronizing defects in embryo invasion and endometrial implantation window. Sonoendocrinology is a new imaging science deciphers the hormonal action on target organs. Antral follicle count, at a cut off value of less than four, had high specificity for the prediction of cycle cancelation in assisted reproduction. Ovarian volume, at a cut-off value 3 mL^3, had high specificity for the prediction of non-pregnancy and cycle cancelation in assisted reproduction. Doppler studies of ovarian stromal blood flow are promising, but more research is needed. AFC and ovarian volume provide direct measurements of ovarian response, while AMH, Inhibin B and estradiol are released from the growing follicles and so they reflect the follicular cohort that has been selected from the follicular pool.

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