

Ovarian Reserve

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Introduction

Ovarian reserve is an estimate of supply of oocytes remaining in the ovary and is closely associated with reproductive potential¹.

The decline in fecundity with female age, long before menopause occurs, is a well-known phenomenon². The concentration of primordial follicles in the ovarian cortex undergoes steady depletion with age, starting already before birth. An embryo at the age of 8 weeks has storage of 600,000 oocytes and, at the age of 5 months storage of 7000,000 oocytes. At birth, 1000,000 oocytes are present and by puberty only 25% of the oocytes remain. At the age of about 37 years the loss of follicles accelerates with rapid loss of reproductive capacity, although menstrual periods might still be regular. This precedes the menopause, when only 1000 follicles remain, by many years. The timing of the menopause, caused by dysfunctional ovaries, is determined by the store of germ cells and the rate of depletion during life.

The evaluation of ovarian reserve has been and still the focus of substantial clinical research. The assessment of ovarian reserve is valuable for determining stimulation protocols and predicting ART outcome. The concept of diminished ovarian reserve has gained general acceptance in infertility medicine. In in-vitro fertilization (IVF), the association of poor ovarian response due to diminished ovarian reserve with cycle cancellation and a significant decline in success rates is well known.

Correct identification of patients who are at risk for poor response can help physicians to individualize counseling and permit the patients to decide whether to undergo a demanding infertility treatment. Accurate assessment of ovarian response potential before the patient enters an IVF program is of pivotal importance.

The limited predictive value of age alone in estimating fecundity rates and response to the exogenous stimulation led to evaluation of other parameters.

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Physiology of Ovarian Reserve

Unlike sperm, which men create throughout their adult lives, women are born with a finite supply of oocytes. Women make all the oocytes they will ever have before they are born, this supply begins to be depleted even before birth and continue until menopause with accelerated rate of loss at around age 37 years. On average, a female fetus at 20 Weeks gestation contains approximately 7 million oocytes, reduced at birth to 1-2 million oocytes and only 25% are left at puberty, about 400,000 of recruitable oocytes. By the beginning of every menstrual cycle a relatively fixed proportions of all the remaining oocytes (1000) (Cohort) becomes recruited i.e. sensitized to gonadotrophins from which one follicle or two will achieve dominance and progress to ovulation³.

Factors affecting the ovarian reserve

Female Age

The importance of age in fecundity is shown by many observations. A decline of fertility is observed at the age of 34 years, whilst the last pregnancy is seen at an average age of 41 years and in this way female age might serve as an ovarian reserve test⁴.

Although age is an important factor in subfertility, it is not very exact in predicting the reproductive potential. Some women will be unable to conceive in their thirties, while others become pregnant in their forties. Clearly, there is a wide range in the relationship between ovarian function and age, and ovarian reserve appears to be responsible for these differences⁴.

FSH and estradiol

Early studies revealed that women with normal ovulatory cycles begin having subtle elevations in FSH in their early 30s and those levels tend to increase with age⁵. In 1989, one group started screening IVF candidates with a basal FSH on day 3. Pregnancy rates were highest in women whose FSH levels were below 15 IU/L; the rate decreased to less than 5% in women with levels above 25 IU/L. The investigators attributed this decline to diminished ovarian reserve because these patients developed fewer follicles, produced

fewer oocytes, and had fewer embryos available for transfer⁶.

Another team examined basal FSH levels, age, and IVF success rates in 1,478 cycles and found that FSH level was a better predictor of ultimate pregnancies than age⁷.

Smoking and ovarian reserve

It was noted that smokers exhibit diminished ovarian reserve more frequently than age-matched nonsmokers. These findings suggest that cigarette smoking accelerates the diminished ovarian reserve process that is normally seen with aging. However, smokers with adequate ovarian reserve undergoing IVF had similar pregnancy (and miscarriage rates) to age-matched nonsmokers⁸.

Another study did not show a negative effect of smoking on pregnancy rates in IVF patients. However, these investigators evaluated a younger population and no information on ovarian reserve was provided (baseline FSH, estradiol, or CCC test). Because the toxic effect of cigarette smoking is exposure-related, it is more apparent in the older reproductive group (over age 35)⁹.

Ovarian Reserve Tests

Ovarian reserve screening is one mechanism by which fertility specialist can partially predict the reproductive potential of a specific patient as well as the potential of her oocytes to result in a healthy pregnancy.

Measurement of ovarian reserve can only be approximated as precise tests are not currently available and it gives couples the advantage of a more realistic estimate of the likelihood of fertility treatment.

The methods for assessing ovarian reserve are classified into two groups:

- Passive tests
- Dynamic tests.

Passive Tests

Female Age

In an ART population, age was found by different authors to be a poorer predictor for pregnancy than basal serum FSH concentration.

However the age was found by others to be a predictor for pregnancy rate in ART programs^{10,11}.

It was concluded in one study¹² that maternal age in combination with basal FSH concentration is an accurate predictor for pregnancy rate in women entering an ART program.

Cycle day 3 serum FSH concentration

Cycle day 3 FSH concentration is an indirect estimate of ovarian reserve, being a measure of the amount of inhibin B/ estradiol that a cohort of follicles is producing, and the feedback effects at the level of the pituitary gland¹³.

Patients with low basal FSH concentrations (threshold values vary between 10.8 and 25 m IU/ml) responded better to ovulation induction, as demonstrated by the number of mature oocytes. Some studies showed FSH to be a better predictor than age, although an age-related decline in fecundity remained.

Besides the finding that normo-ovulatory women under the age of 35 years have a large inter-individual variation in follicular phase FSH concentrations¹⁴, the inter-cycle variability and changes across the cycle and inter-laboratory differences must also be discussed.

The validity of FSH screening depends on the time in the cycle the sample is collected. Timing is considered optimal when circulating E2 levels are at their nadir, which is typically around cycle day 3. Some patients will have inappropriately high E2 levels on day 3, which suggests that they may be farther into their follicular phase than is clinically apparent. In these circumstances it is possible that the higher circulating E2 level might be able to suppress FSH levels back into the normal range even if the patient has diminished ovarian reserve⁴.

Basal FSH/LH ratios

Although extensive data are available regarding the high level of specificity of basal FSH levels, the fact remains that the test may have limited sensitivity. Stated otherwise, patients with high ratios are typically low responders with very poor pregnancy rates (highly specific), but a substantial group of patients will have normal levels and yet still respond poorly to stimulation with associated poor pregnancy rates. Some investigators have suggested that an increased FSH/LH ratio may predict an elevated FSH level.

Cycle day 3 serum estradiol concentration

The follicular phase length changes from a mean of 16.9 days in patients aged 18-20 years, to a mean of 10.4 days in regularly menstruating women aged 40-45 years¹⁵. A more well-advanced follicular recruitment by cycle day 3 might be responsible for the condensed follicular phases in older women. This early dominant follicle selection is expressed by relatively high serum estradiol concentrations.

FSH and estradiol

It is important to correlate the basal FSH value with the estradiol level. Basal estradiol values above 50 pg/mL decrease FSH levels through negative feedback on the pituitary. Basal estradiol readings above 50 pg/ml have been associated with a poor response to ovulation induction in assisted reproduction and with fewer pregnancies¹⁶.

When ovarian cysts are not actively producing estradiol, a premature estradiol elevation signifies early recruitment and is commonly seen in perimenopausal women. Therefore, to interpret basal FSH values correctly, it's important that estradiol not to be elevated¹⁶.

Cycle day 3 serum inhibin B concentration

The inhibins are dimeric peptides composed of an α -subunit, a β_A -subunit (inhibin A) and a β_B -subunit (inhibin B)¹¹.

Although serum inhibin -B concentrations are lower in older women, it remains a matter of debate as to whether inhibin B is a good marker for ovarian response^{17,11}.

Inhibin A seems to be secreted by the dominant follicle, since it increases just after the rise in estradiol concentration during the late follicular phase^{11,18}. In this way, inhibin A may mark follicle maturity. The dominant follicle in older women has been shown to contain fewer granulosa cells than the dominant follicle in younger women¹⁹, and this might be the reason why inhibin A concentrations are lower in older women¹⁸.

Inhibin B is possibly secreted by the developing cohort of follicles in a cycle, since its concentration rises across the luteal-follicular transition and peaks in the mid-follicular phase²⁰. In this way, inhibin B may indicate the number or quality of developing follicles^{11,18}. Decreased inhibin B concentrations were also found in women with diminished ovarian reserve, despite having non-elevated FSH concentrations, suggesting that a fall in inhibin B concentration might be an earlier marker for limited ovarian reserve than was elevated FSH concentration²¹.

No data are available on serum inhibin B concentrations in relation to pregnancy within the general subfertile population⁴.

Serum Anti -müllerian hormone level (AMH)

Also known as Müllerian-inhibiting substance (MIS), is produced by granulosa cells of preantral and small antral follicles. Since the number of ovarian follicles declines with age; (AMH) might be used as a marker for ovarian reserve.

Ovarian volume

During a woman's life, ovarian volume changes from 0.7 cm³ at the age of 10 years, to 5.8 cm³ at the age of 18 years²².

However, at the age of 40 years the ovaries tend to decrease in size, and they decrease even further after menopause²³.

The ovarian volume, measured by vaginal ultrasound, has been described in different studies, and was shown to correlate with response to ovulation induction in ART cycles in several investigations²⁴. In women with small ovaries (<3 cm³) the cancellation rate of IVF is higher (likelihood ratio 3.8-6.8). However, the likelihood ratio of a positive test (ovarian volume <3 cm³) related to pregnancy²⁴ is 1.0, which indicates that this is not a good test to predict pregnancy.

Others²⁵ also found a correlation (albeit less strong) between ovarian volume and reproductive success in ART cycles, and concluded that ovarian volume might be an important predictor of ovarian reserve. The likelihood ratio of a positive test in relation to pregnancy in this study was 1.4, and in relation to cancellation was 2.0, which means that the test has a limited value. Small ovarian volume (smallest <5 ml) was also shown to be predictive of achieving a clinical pregnancy in a later study by these authors²⁶.

In an additional study²⁷, ovarian volume was found to be a predictor for the number of growing follicles, but not of the number of recovered oocytes. Likelihood ratios in these studies could not be calculated.

Antral follicle count

The number of antral follicles as counted early in the follicular phase (follicles smaller than 10 mm, in absence of a larger follicles or cysts) is found to be a predictor of the number of oocytes collected in an IVF program, as well as the cancellation rate²⁷.

This test might be representative of ovarian reserve, and indeed an age-related decrease in antral follicle count has been observed²⁸.

The number of selectable follicles and the total number of follicles within an antrum was found to be significantly reduced in women with a low response to ovarian stimulation in the prior cycle, as compared with normal responders (all aged <35 years)²⁹.

Others³⁰ found the number of antral follicles to be related with reproductive age in women with proven fertility, and this might reflect the ovarian reserve, though this was tested in a fertile population. The importance of the number of antral follicles in a general subfertile population has not been reported.

Ovarian Biopsies

Increasing age shows a decline in follicular density, as found in ovarian biopsies of subfertile women³¹. A significantly lower number of follicles in ovarian biopsies were found in a group of women with premature ovarian failure, as compared to women with chronic anovulation or low ovarian reserve³².

A significant correlation between follicular density and subfertility has not yet been found, however. In future, although ovarian biopsies may form part of the evaluation of ovarian reserve, the performance of this test clearly has excessive clinical implications, and further investigations are required before definitive conclusions can be drawn.

Ovarian stromal Doppler

Recently, there has been much interest regarding the potential role of transvaginal Doppler ultrasound in the measurement of intraovarian blood flow in the early follicular phase and its relation to subsequent ovarian responsiveness in IVF treatment. Several studies have shown that ovarian stromal blood flow at the baseline ultrasound scan is correlated with subsequent follicular response and may be a new indicator for predicting ovarian responsiveness in IVF treatment.

Dynamic Tests*Gonadotrophin agonist stimulation test (GAST)*

The magnitude of the increase in estradiol correlated strongly with IVF success. This test has not been validated outside of assisted reproduction, and how it can be applied to infertile couples is unknown. However, the cost of the test may preclude its use for generalized screening.

This test evaluates the estradiol serum concentration change from cycle day 2 to day 3 after administration of a GnRH agonist, the latter causing a temporary increase in pituitary secretion of FSH and LH³³. In response, the ovaries will produce estradiol. The test is dependent on the pituitary production of gonadotrophins and the response of the ovary to stimulation (i.e. the follicle reserve)^{34,13} and the number of oocytes appeared to be not significantly higher in the group with a normal test result.

According to these data, the ability of this test to differentiate between normal and diminished ovarian reserve appears limited. Also, the GAST has not been evaluated outside of ART systems.

Clomiphene citrate challenge test (CCCT)

The clomiphene citrate challenge test was described in 1987 by³⁵ as a mean of assessing ovarian reserve in women 35 years of age and older with unexplained infertility³⁵.

It is simple to perform and involves measuring day 3 FSH and estradiol, administration of clomiphene citrate from days 5 to 9, followed by repeat FSH on day 10. The test result is considered abnormal if either basal or day 10 FSH levels are above 10 IU/L.

The test relies on the ability of the pituitary gland to respond to decreased estrogen feedback by increasing FSH levels.

Clomiphene Citrate is a mixed estrogen agonist-antagonist with antagonistic effects at the pituitary gland, where it blocks the action of the circulating estrogen. In the early follicular phase, inhibin B and estradiol levels are increasing as a result of growth of small follicles, and restrain the FSH increase after clomiphene citrate.

The rationale behind the clomiphene citrate challenge test (CCCT) is that women with adequate ovarian reserve would have a developing cohort of follicles producing adequate estradiol and inhibin to overcome the effect of clomiphene citrate on the hypothalamic-pituitary axis. The CCCT can help identify patients with diminished ovarian reserve that was not detected by basal FSH measurement³⁵⁻³⁷.

The clomiphene citrate challenge test appears to be more sensitive than day 3 FSH alone.

An abnormal clomiphene citrate challenge test has excellent predictive values for diminished ovarian reserve and poor long-term pregnancy rates in natural cycles, during ovulation induction, and in IVF^{8,37}.

Although the test is quite specific, it has limited sensitivity, with a significant age-related diminution in reproductive potential occurring even among women with normal test results³⁷. The test may be superior to basal FSH screening because it is 2 to 3 times more sensitive than basal FSH screening alone. Although abnormal day 3 FSH values appear to be accompanied by abnormal day 10 values in most cases, the current literature does not contain enough data to recommend omission of the day 3 sample, and the authors continue to screen patients with both day 3 and 10 FSH levels.

The exogenous FSH ovarian reserve test (EFORT)

This test was introduced as a screening test for good and poor responders in IVF cycles³⁸. Day 3

FSH and estradiol serum concentrations are determined, as well as the estradiol response following a 300 IU FSH injection on day 3.

Others³⁹ performed the test in a different way, and found the estradiol concentration after 5 days of 300 IU FSH injections not to be an independent variable associated with pregnancy. The likelihood ratio of this study cannot be calculated, however. The EFORT has not yet been studied in the general subfertile population, nor has it been evaluated by others¹¹.

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