Review Article

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Tests for Ovarian Reserve

Pratibha Singh[1], Meenakshi Gothwal[2], GarimaYadav[3]

Abstract

Ovarian reserve tests are used for determination of the functional potential of ovary. They reûect the number and quality of oocytes in it.

These tests are frequently used by the Gynecologist dealing with Infertility & IVFspecialists to predict the chances of success for ovarian stimulation and retrieval of follicles by knowing the reserves of ovary. Ovarian reserve is affected by many factors like biological age, chemotherapeutic drugs, radiation exposure, certain surgeries etc. It is well known that as the age of women increases fertility declines. So it becomes important for the IVF consultants to know the chances of success and the optimum dosages of ovarian stimulation protocols to be used; also the side effects can be predicted in some. These tests are useful in fertility assessment, as it provides a guide to ovarian reserve and identifies women that may need to consider either egg freezing or trying for a pregnancy sooner rather than later if their long-term future fertility is poor. Ovarian reserve can be measured by dynamic and static tests. Commonly used ovarian reserve tests are serum FSH, serum AMH and the Antral Follicle Count (AFC); these are static tests. Dynamic ovarian reserve tests are not commonly performed in clinical setting. Of these static tests AMH has certain advantages, due to its less inter and intra cycle variability. Many more uses of these tests have come up in recent years. These tests have some limitations so should be used with the knowledge of their limitations in clinical settings.

Keywords: Antral Follicle Count (AFC); Follicle Stimulating Hormone (FSH); Inhibin; Anti Mullerian Hormone (AMH).

Introduction

Women have finite number of eggs in their ovary, maximum number are in fetal life at around 20-24 weeks of intrauterine gestation. In humans approximately 85% of the oocytes are lost before birth, making ovary the only organ to lose such huge number of potentially functioning cells before it starts working [1,2]. There are about 2 million primordial follicle at birth, at menarche about 400,000 are left [2,11]. These eggs gradually continue to decline throughout a women's life and exhaustion of follicles lead to menopause. Ovary has follicles in various phases of development-primordial follicle, primary follicle, preantral and antral follicle, maturing follicle and also atretic follicles.Total reserve of ovary is constituted by growing (formed after recruitment) and nongrowing follicles (includes primordial follicles) was proposed by Gleicher (1). As women age number of follicular pool keeps declining as a large number of follicles undergo atresia. Ovarian aging is thus declining follicles and is directly proportional to women's chronological age. After thirties fecundity starts to decrease and is further speeded up as she approaches forties. With increasing age

[1]Professor [2,3]Assistant Professor, Department of Obstetrics & Gynecology, All India Institute of Medical Sciences, Jodhpur, Rajasthan 342005, India.

Corresponding Author:
Pratibha Singh,
Professor, Department of Obstetrics & Gynecology AIIMS Jodhpur, Rajasthan 342005, India.
E-mail: drpratibha69@hotmail.com

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follicular recruitment, folliculogenesis as well as quality of egg decreases; this causes infertility and abortions. Genetic factors also has significant influence on the ovarian aging, and FMR1 gene has been associated with it. Besides the age other factors implicated for the ovarian senescence include- prior cancer treatment in form of chemotherapy and radiotherapy, severe endometriosis, autoimmune and genetic factors etc. Prior ovarian surgery like ovarian drilling, cystectomy, fulguration etc also has effect on ovarian reserve as they may destroy or remove a large pool of these follicles. Tests of ovarian reserve are commonly used to predict reproductive aging and response to ovarian stimulation protocols. These tests are useful not only in fertility assessment, as it provides a guide to ovarian reserve and identifies women that may need to consider either egg freezing or trying for a pregnancy sooner rather than later if their long-term future fertility is poor

Tests for Ovarian Reserve

Ovarian reserve started to emerge with rise in ART and IVF in 1980s. Ovarian reserve can be tested by many methods, both dynamic and static tests are used for this purpose. Dynamic tests tells us the response of ovaries to different drugs, hence better response can be predicted with different stimulation protocols. But these tests requires testing at multiple times and a runs a theoretical risk of further declining the compromised ovarian pool. So in clinical practice static tests serve a more useful purpose. An ideal test should be reproducible, with no or minimum inter-cycle and intra-cycle variation, easy to use and observer independent, non-invasive, rapidly interpretable and can be performed anytime of the menstrual cycle; with good sensitivities and specificities. It would be better if decline in ovarian reserve can be detected at an early stage so as to enable timely treatment

Static Ovarian Reserve Tests- These are the tests commonly used in clinical practice, due to ease of testing. They comprise of-

a. Hormonal tests- FSH, AMH, Inhibin B, Estradiol (E2) levels
b. Biophysical Tests - USG & Doppler
c. Ovarian tissue Biopsy–used in research settings only.

A. Hormonal tests

1. Serum Follicular Stimulating Hormone (FSH)

This was one of the first test identified for testing ovarian reserve almost 30 years back [12,13]. FSH is a glycoprotein hormone having alfa and beta units, and is secreted from pituitary in response to hypothalamic gonadrophins. Menstrual cycle causes significant variation in the values; so is generally measured on Day 2 or 3 of menstrual cycle. This test is one of the simplest and commonly being used test, but requires a functional hypothalus-pituitary-ovarian axis. A high FSH on day 2/3 predicts poor response. Accuracy is dependent on the cut-off values used, also methods used for detection of FSH in different laboratories can have different cut-off values. Basal FSH more than 10-20 IU/L have specificity of 83-100% [3]. Specificity further increases if higher cut-off value are used [4]. This test is dependent on day of menstrual cycle and differences in different cycles can be a limiting factor. It is of value only if elevated. A normal FSH may still have a poor response to stimulation as it is a late marker; when combined with estradiol it gives better prediction of ovarian functional reserve. Example- in the perimenopausal woman normal FSH and high Estradiol predicts a declining follicular pool, if only FSH is measured which might be normal, may fail to predict the reserve and hence the response. A single abnormal FSH level in women less than 40 years of age may not predict a poor response to ovarian stimulation or failure to achieve pregnancy [14] and a repeat testing is desired. FSH cannot predict OHSS (Ovarian hyper stimulation syndrome).

2. Estradiol (E2)

It is not measured in isolation and is generally combined with measurement of FSH on Day 2/3 and gives a basal values. Increased levels on cycle day3 suggests early follicular recruitment & development, and predicts a poor response to stimulation [5] though FSH may be within normal range.

3. Inhibin B

It is also secreted by small antral follicular granulosa cells, and is a peptide hormone. Low Inhibin B levels (<45pg/ml) predicts poor ovarian reserve. It is also dependent on day of the menstrual cycle with inter cycle and intracyclevarations. If measured alone, may not give meaningful insight in ovarian response.

4. Anti Mullerian Hormone (AMH)

AMH is a glycoprotein, secreted from the granulosa cells of preantral and small antral follicles, and is a peptide growth factor. It belongs to TGF (Transforming Growth Factor) beta super

family. Levels of AMH are not dependent on hypothalamic gonadotrophinsand remains stable throughout the cycle. There is no inter-cycle variation in normal ovulating woman [15], and values are not affected by recent hormonal intake or drugs. Independence from the day of menstrual cycle makes this test a choice of test for the clinicians. Recruitment of follicles from the primordial pool is inhibited by this hormone by modifying the sensitivities of FSH in follicles. Low AMH cut-offs of 0.2-0.7ng/ml have a sensitivity of 40-97% and specificity of 78-92% in predicting poor response to ovarian stimulation [3]. Polycystic Ovarian disease have a high AMH value as predicted due to large number of preantral follicles. A value>3.5 ng/ml predicts a high likelihood of ovarian hyper-stimulation syndrome (OHSS) [6].

Few recent studies have shown that this hormone levels may fluctuate significantly in one menstrual cycle, specially in younger women [11]. The main limitation of this test depends on assay variability and lack of standardised international assay [11], European and US assay methods are different. Automated assay platforms can offer precision and is faster to do with better sensitivities as compared to ELISA [16]. Variations has also been observed with Oral contraceptive pills, or GnRH agonist which temporarily decrease the levels of AMH, and returns to normal 3-4 months after stopping these drugs [11, 17]. Women with smoking, obesity and low vitamin D values may lower the levels of AMH.

B. Biophysical Tests

1. USG- AFC

Measurement of number of antral follicle of size 2-10mm in each ovary is used as a marker of ovarian reserve. It is measured by transvaginalsonography on day 2 or 3 of the menstrual cycle. It is a simple test with less inter-observer varaiblityin trained hands and the results are available immediately. AFC <5-6 predicts poor ovarian reserve. If AFC count of 3-4 follicle are taken as cut off specificity increases to 73-97% [18]. This test is dependent on observer experience and machine resolution, also obesity may compromise its reliability. This test must be done in the early follicular phase due to its inter cycle variations; different training of sonographers in measuring AFC, methodology used and equipment resolution may also lead to variations in measurement. It has the advantage of immediate results and can predict OHSS, like AMH. High AFC count is associated with high likelihood of development of OHSS after stimulation protocols.

2. Size of Ovary (volume)

As the follicular pool of ovary decline the ovarian volume also declines, but if used alone this test does has much sensitivity and specificity, it is also significantly influenced by inter-observer variability. This makes it a poor test with limited clinical utility if used alone, however, if combined with other sonographic parameters it may have better clinical value.

3. Ovarian Blood Flow Doppler

Measurement of stromal blood flow has been used to predict ovarian reserve. Poor stromal blood flow has been linked to poor ovarian reserve. This is generally combined with the above sonography parameters to predict a better response.

Dynamic Ovarian Reserve tests are-

1. Clomiphene Challenge tests (CCT)
2. GnRH Agonist stimulation test (GAST)
3. Exogenous FSH ovarian reserve test.

Clomiphene Challenge Test

This test measures response of ovaries after administering Clomiphene Citrate orally and is given from Day 5th to 9th of the menstrual cycle for 5 days. Serum FSH is measured on day 3 and day 10. Good ovarian reserve is predicted by increase in follicular recruitment with rise in Estradiol levels and a fall in FSH levels on Day10. If the 10th day FSH is 10-22 IU/L over the baseline, it has a sensitivity and specificity of 35-98% and 65-98% respectively in ovarian reserve predicting [7].

GonadotrophinAgonist Stimulation Test

In this test InjGnRH is given subcutaneously and flare response of E2 is measured. Significant increase or flare of Estradiol (80-180pmol/L) in response to stimulation is indicative of a good reserve of ovaries.

Exogenous FSH Ovarian Reserve Test

In this test menstrual cycle Day 2 serum Estradiol & Inhibin B are measured. Recombinant FSH 300IU injection is given subcutaneously next day. On 10th day serum Estradiol and Inhibin B are measured again. Increase in Estradiol of 110-150 mmol/L and Inhibin B of 70-100ng/ml is suggestive of a good ovarian response and reserve.

When is testing for Ovarian Reserveindicated?

These tests are not generally needed in all patients coming for infertility treatment; it is indicated in women age >35 years or family history of early menopause, Unexplained infertility, young patients with poor response to Gonadotrophins stimulation and cycle cancellation or less than 4 ovum retrieved in IVF programs. Some people also recommend this test in women going for oocyte donation programs. This test is also done in women with history of prior ovarian surgery (endometriosis, ovarian drilling, fulguration etc) or cancer treatment with radiation therapy or chemotherapy. These tests do not predict a woman's chances of natural conception.

Utility of Ovarian Reserve Testing in Clinical Practice

Chronological age of women is the 1st step for assessing the ovarian pool reserve. Poor responder to stimulation should also be assessed for ovarian reserve even if she is young. In older women success of IVF can be influenced by knowing the ovarian reserve. Damage to ovary because of prior ovarian trauma of surgery (fulgeration, drilling, cystectomy, compromised blood supply to ovary due to torsion or use of energy source etc) may also warrant testing for residual reserve.

Treatment failures as well as high or low Oocyte yield can be predicted by these tests. Non responder & hyper responders can be predicted by these tests so can help in tailoring treatment protocols according to the individual need with optimum outcomes. A poor reserve test indicates need for higher dosages for longer time to achieve optimum results. A high ovarian reserve indicates hyper responders, these patients may develop life threatening "Ovarian Hyper Stimulation Syndrome", indicating need for a lower dosages for shorter time

A low ovarian reserve test does not signify absolute inability to conceive; so it should not be the only criteria to refute IVF treatment. Ovarian reserve testing can possibly help couples to know the benefits and disadvantages of treatment and to decide the most suitable management.

As better diagnostic and treatment modalities have become available for early diagnosis and management of young cancer survivors, focus is shifting towards quality of long life in these patients These tests are also used for assessing the toxicities of various chemotherapeutic drugs and radiations on ovarian tissues. Surgeries known to cause damage to follicular pool can be detected early giving a chance of instituting some treatment to improve pregnancy outcome. These tests are said to be useful in predicting onset of menopause.

Which is the Best Test?

As there are many tests available for measuring ovarian reserve, clinicians often want a test which is easy to perform, can be done any time of menstrual cycle and not observer dependent, with not being influenced by exogenous hormones or drugs. No single test can adequately identify all women with diminished ovarian reserve, so a combinations of tests should be used. AMH and AFC have definite advantages over other tests. However, AMH is close to ideal expectation of a clinician as it detects the declining pool at a much earlier stage so treatment can be started early if desired.

Women identified with low reserve are a challenge to the IVF consultants, they require careful tailoring of the protocols used for controlled ovarian hyperstimulation; so is the case with if ovarian reserve is very high as it predicts risk of significant side effects and complications.

Dihydroepiandrosterone (DHEA) has been reported to improve pregnancy outcomes in women with diminished ovarian reserve, it probably works by increasing IGF-1 and androgen levels in ovary, which then enhances ovarian function [8]. It increases oocyte and embryo yield and pregnancy rate. A meta analysis on DHEAS, which included 9 studies suggested in favour of this drug for improving pregnancy rate, however they also suggested more studies on this drug to confirm these findings [9]. Reaearch is also going on to find drugs which might have an effect on ovarian reserve and pregnancy outcome, one such drug is Melatonin which might be useful in these women [10].

Though there are many tests available none of these tests have sensitivity & specificity of 100%. The population tested, cut-offs used, method of detection/ testing have a significant role in interpretation of these results. Often a combination of tests are used to have a better assessment of ovarian reserve as sometimes these tests are not in concordance with each other.

Conclusion

Tests of Ovarian reserve gives information of quantity and quality of oocyte present in it and hence the functional capacity of ovary. Besides age, familial and genetic factors, surgery on ovary, prior

cancer treatment may compromise the ovarian reserve. Ovarian reserve are commonly measured by FSH, AFC, AMH in clinical practice and are static tests. Dynamic tests are not commonly used. Serum AMH over the last few years has emerged as reliable test which can be measured any time of menstrual cycle, and is an early marker of diminishing ovarian reserve. The aim of testing ovarian reserve is to identify women with low reserve, prognosticate them realistically, and counsel them to modify and optimally plan their treatment. These test do not predict chances of pregnancy in a young healthy women; they should not be used to deny treatment to infertile couples. None of these tests have 100% sensitivity or specificity, hence the results must be interpreted in light of the population tested and the inherent flaws of the tests. Combination of tests may give a better prediction for ovarian reserve.

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