Review Article

Ovarian reserve

Pinkee Saxena, Surveen Ghumman[1], Shashi Prateek[2]

Department of Obstetrics and Gynaecology, Deen Dayal Upadhyay Hospital, Hari Nagar, New Delhi
[1]IVF & Reproductive Medicine, Max Multispeciality Hospitals, Panchsheel Saket, Patparganj, New Delhi
[2]Department of Obstetrics & Gyanaecology, Subharati Medical College, Meerut, Uttar Pradesh, India

Abstract

Nowadays more and more couples are delaying childbirth. As the age of the couples advances, their chances of achieving pregnancy decrease. This is due to the natural process of ageing of gonads. During counselling and treatment of infertile couples, clinicians are often faced with questions like what is the chance of conception. Hence there is need to test for functional ovarian reserve. An ideal ovarian reserve test is affordable, convenient, reproducible and sensitive. Various clinical and biochemical markers have been used to predict the same. These tests help to predict the poor response or hyper response to ovarian stimulation and help to formulate the treatment plans in infertile couple. They however cannot predict future fertility or occurrence of pregnancy.

Keywords: AFC, AMH, ovarian reserve

Address for correspondence: Dr. Pinkee Saxena, Deen Dayal Upadhyay Hospital, Hari Nagar, New Delhi, India.
E-mail: drpinkee@hotmail.com

INTRODUCTION

Changing lifestyle has led to delay in childbearing in most of the couples. The postponement of maternity has led to an increased demand of assisted reproduction technology. Hence, there is need for the functional evaluation of the ovarian reserve to counsel infertile couple regarding their reproductive chance.[1] It also guides the clinician to decide about the treatment protocols and individualize the treatment.

The ovary of a female fetus has 6 to 7 million oocytes at 20 weeks. Atresia of these oocytes starts *in utero*. At the time of birth, 1 to 2 million oocytes are available. The rate of follicular atresia then slows down, and at puberty, 300,000 to 400,000 oocytes remain. During each ovulatory cycle, a cohort of follicles gets recruited, of which, one grows to become the dominant follicle, and the rest undergo atresia. At the time of menopause, the number falls to below 1000.

Ovarian reserve is a complex clinical phenomenon that is greatly influenced by age, genetics and the environment variable. The decline in woman's ovarian reserve is an irreversible phenomenon. The rate at which a woman loses her primordial follicle varies from person to person. Ovarian reserve refers to the residual oocyte granulosa cell that is available for fertilization at any given age. The quantitative and qualitative decline of these cells is seen with advancing age. Ovarian reserve provides information regarding the follicles that can be stimulated and the oocyte that can be retrieved from the ovary.

Treating clinicians are often faced with the challenge to predict the fertility potential of women. The ovarian reserve tests add more prognostic information to the

Access this article online

Quick Response Code:

Website: www.fertilityscienceresearch.org

DOI: 10.4103/fsr.fsr_6_18

How to cite this article: Saxena P, Ghumman S, Prateek S. Ovarian reserve. Fertil Sci Res 2017;4:74-80.

counseling of infertile couple. It helps to identify patients who are likely to have poor response or hyperresponse to gonadotrophin stimulation, but it cannot predict clinically important outcome such as pregnancy. It helps to differentiate patients with normal ovarian reserve from those with diminished ovarian reserve and helps in deciding treatment protocols in women with diminished reserve. It also helps in avoiding aggressive treatment in women with normal reserve. It, however, should not be used to exclude an infertile couple from seeking assisted reproductive technology (ART).

Various clinical and biochemical markers have been studied to test for the ovarian reserve. Sharara and Scott emphasized that an ideal ovarian reserve test should be easy to measure, minimally invasive, inexpensive and should have good predictive value.[2]

CLINICAL MARKERS

Common clinical markers used to determine the ovarian reserve are age and menstrual cycle length (MCL).

Age

Age is a factor that has been used since long to predict the quality and quantity of the ovarian reserve. Studies have shown that the fecundability declines after the early 30s.[3] The prevalence of infertility increases significantly after the age of 35 years, and by the age of 45, around 99% of the patients are expected to be infertile.[4] Genetic factors, smoking, infections and adnexal surgeries are the other determinants of diminished ovarian reserve in older women.[5] Age alone has a limited value in predicting individual ovarian responses.[6] In a recent study, however, Scheffer *et al.* have stated that age is the best predictor of embryo quality.[7] Tehraninezhad *et al.* in their study[8] have also found age to be a superior variable to predict clinical pregnancy.

Menstrual cycle

MCL is usually determined by the rate and quality of growth of the follicle and the duration of the follicular phase. The pattern of menstrual cycle in a woman remains consistent until the late 40s, after which a gradual shortening in cycle length is seen. In addition, in the late 30s, a higher serum level of follicular stimulating hormone (FSH) and lower serum levels of inhibin are seen in women.[9]

Brodin *et al.*[10] in their study found a correlation among MCL and pregnancy and delivery rate, independent of age. They observed that in women with cycles >34 days, delivery rate was twice as compared with those having cycles <26 days. A significant association of MCL was also noted with the response of the ovary to gonadotropin drugs and the quality of the embryos obtained in IVF/ICSI cycles.

BIOCHEMICAL MARKERS

They can be either basal measurement or dynamic tests. Basal tests include FSH, estradiol (E2), inhibin B, anti-Müllerian hormone (AMH) and ultrasonography and dynamic tests include clomiphene citrate challenge test (CCCT) and other tests. Basal test measures are dependent on changes that occur in the ovarian milieu during the follicular growth, and it reflects the reserve of the ageing ovary. The dynamic test evaluates the response of the hypothalamic–pituitary–ovarian axis to stimulation.

Follicle stimulating hormone

Early follicular phase serum FSH is the commonly used endocrine test for determining the ovarian reserve. It is based on the feedback inhibition of FSH secretion by ovarian hormones and, hence, is an indirect marker of the ovarian reserve. At the beginning of the menstrual cycle, the E2 and inhibin B levels inhibit FSH secretion from the pituitary. In women with diminished ovarian reserve, the production of ovarian hormones is insufficient, and this leads to elevated pituitary FSH secretion.

Van der Steeg *et al.* studied the predictive value of basal FSH for achieving spontaneous pregnancy in ovulatory subfertile women younger than 40 years. He said that when FSH levels exceeded 8 IU/L, there was a reduced chance of achieving pregnancy.[11] Ashrafi *et al.* in their study observed that in women with serum FSH levels ≥15 IU/mL, fewer oocytes were aspirated, and they had a higher cycles cancellation rate than women with lower FSH levels, with no significant difference in gonadotropin doses administered.[12] Klinkert *et al.* observed that the pregnancy rate was less in women with FSH levels ≥15 IU/L as compared to those with lower levels; however, this was not statistically significant.[13] Luna *et al.* in their study observed that pregnancy rates in women aged <35 years with elevated basal FSH were higher than those of older women with normal levels of the hormone.[14] They recommended that young women with elevated basal FSH should be counseled differently from older women. Van Montfrans *et al.* in his study also said that basal FSH should not be the decisive factor to the initial management of infertile women with regular menstrual cycles.[15] A recent study has also stated that FSH is less reliable than other markers such as AMH and antral follicle count (AFC) for assessing the ovarian reserve.[16] Hence, high FSH levels

should not be used as the criterion alone to exclude women from ART.

Estradiol

E2 levels are a reflection of the ovarian response. Early elevations in basal serum E2 are due to the advanced follicular development and the early selection of a dominant follicle, as seen in older women, due to rising FSH levels.[9] It has been observed that women with E2 levels <20 pg/mL or ≥80 pg/mL have a higher artificial reproductive techniques (ART) cycle cancellation rate.[17] Combining E2 with FSH on cycle day 3 is shown to have reduced the incidence of false-negative tests obtained when FSH alone was used. The elevation of both indicates poor ovarian response.

E2, however, has low predictive accuracy and lacks high sensitivity and specificity cutoff levels.[18] It may be used as a guide for starting stimulation with gonadotropins; however, it should not be used to exclude couples from ART programs.

Inhibin B

Inhibins are glycoproteins secreted by the granulosa and theca cells. It plays a major role in the selection of the dominant follicle and has a regulatory effect on the secretion of FSH.[19] Women with inhibin B concentration levels ≥45 pg/mL have increased oocyte retrieval rate and lesser cycle cancellation rate, as observed by Seifer *et al.*[20] However, few studies have shown that inhibin B alone is not a very useful marker of the ovarian reserve.[21] The routine use of inhibin B is, hence, not recommended in infertile couples.

Anti-Müllerian hormone

AMH is a glycoprotein hormone that is expressed by the granulosa cells of the secondary, preantral and small antral follicles <4 mm in size. As the follicles grow, AMH expression decreases. Further follicular growth is then determined by FSH action.[22] AMH, thus, acts as a modulator of follicle recruitment and plays an important role in folliculogenesis. Compared to other markers, AMH levels do not vary much during the menstrual cycle and, hence, is reproducible.[22] Serum AMH has also been shown to be a reliable marker for ovarian ageing and in predicting reproductive status.[23]

Women with polycystic ovary syndrome have an increased number of antral follicles compared with normal women.[24]. Serum AMH levels are, hence, found to be two to three times higher in women with PCOS.[25] Women with low AMH levels prior to IVF may have either an increased risk of cycle cancellation or poor response to stimulation. Van Rooij *et al.* observed that serum AMH levels correlated well with preinduction AFC and the number of oocytes retrieved in ART cycles.[26] In a meta-analysis, it was seen that AMH levels have similar predictive value as AFC in identifying poor responder.[27]

Some have proposed a cutoff value range of 0.7-0.75 ng/mL for AMH for the identification of poor responders.[28,29] Although there seems to be a good sensitivity and specificity, it is seen that the prevalence of young women with AMH levels <0.7 ng/mL is low. Others have considered serum AMH levels lower than 0.1-0.35 ng/mL as cutoff to minimize false positives. It identifies patients who are at very high risk for cycle cancellation.[30,31]

High AMH levels before IVF are also useful in identifying women at risk for hyperresponse and ovarian hyperstimulation syndrome (OHSS). Vembu in his study has suggested a cutoff value of serum AMH to predict the hyperresponse in PCOS group as 6.85 ng/mL and in non-PCOS group as 4.85 ng/mL.[32] In these patients, starting a low dose of FSH followed by the use of GnRH antagonists or using GnRH antagonist for the triggering of ovulation instead of hCG[33] can be done to prevent the development of OHSS.

The measurement of AMH levels may be useful in the prediction of poor response and cycle cancellation as well as hyperresponse and ovarian hyperstimulation in ART. It also plays a role in the individualization of treatment strategies in patients undergoing in vitro fertilization (IVF) treatment. However, the AMH cannot predict the qualitative ovarian response in ART or pregnancy.

OVARIAN ULTRASONOGRAPHY

Antral follicle count

AFC is the total number of follicles observed in both the ovaries in the early follicular phase using transvaginal ultrasonography. It is a very reliable marker of the ovarian reserve.[30,34] A count of 8-10 is considered as a predictor of a normal response. The diameters of the follicle used to define antral follicles ranges from 2 to 10 mm. Haadsma *et al.* in their study observed that the number of small antral follicles, 2-6 mm in size, declines with age, but that of 7-10 mm in size remains constant. They said that the small antral follicle correlates well with the ovarian reserve tests and appears to represent functional ovarian reserve better.[35]

Compared to other tests, AFC has the best discriminating potential for a poor ovarian response. It, however, lacks the sensitivity and specificity to predict the nonoccurrence of pregnancy.[36] The presence of more than 14 antral follicles is considered to be a predictor of hyperresponse.[37]

Maseelall *et al.* in their study observed that women with AFC ≥11 (follicles measuring between 2 and 10 mm present on both the ovaries) were more likely to have a live birth.[38] In a meta-analysis, it was seen that women with AFC ≤4 were 8.7 times [95% confidence interval (95% CI 2.4 to 31.7) more likely not to be pregnant and 37 times (95% CI 13.68–100.45) more likely to have their cycle cancelled than women with a AFC of four or more.[39] The sensitivity and specificity of AFC to predict cycle cancellation was 67 and 95%, respectively. However, due to 12% sensitivity to predict no pregnancy and 67% sensitivity to predict cycle cancellation,[39] AFC must not be used as a criterion for ART exclusion. It is a useful tool for counseling on the low probability of achieving pregnancy and determining individualized treatment protocols in IVF cycles.

Ovarian volume

The routine use of ovarian volume as a predictor of ovarian reserve is controversial. The study conducted by Gibreel *et al.* observed 93% (95% CI 87 to 97) and 92% (95% CI 89 to 94) specificities for the prediction of nonpregnancy and cycle cancellation, respectively, with a 3.0 mL cutoff.[39] In a meta-analysis performed by Hendriks *et al.*, the predictive value of ovarian volume for poor response to stimulation was inferior,[36] as noted by Broekmans *et al.* in a previous study.[18]

Ovarian blood flow

Ovarian blood flow has been studied in natural and stimulated reproductive cycles.[40] Shrestha *et al.* in their study showed that high-grade ovarian perifollicular blood flow in the early follicular phase during ovarian stimulation was associated with a higher clinical pregnancy rate.[41] However, the value of ovarian blood flow is still indeterminate.[39]

OTHERS

Clomiphene citrate challenge test

CCCT was first described by Navot *et al.* In this test, 100 mg clomiphene citrate was given from day 5 for 5 days. Serum FSH, LH and E2 levels were determined on day 3 and day 10. Diminished ovarian reserve was determined by day 3 FSH levels >14.9 mIU/mL or day 10 FSH levels > day 3 FSH level.[42] However, a meta-analysis has shown that CCCT is no better than basal FSH in predicting a clinical pregnancy.[43]

Exogenous FSH ovarian reserve test

The exogenous FSH ovarian reserve test (EFORT) measures the increase in E2 and inhibin B 24 h after the administration of 300 IU of recombinant FSH on cycle day 3.[44] It tests the functional response of the ovary. Increased levels of E2 and inhibin B after EFORT has a good predictive value for the number of ovarian dominant follicles that can be obtained after stimulation. However, this test lacks uniformity.

Gonadotrophin releasing hormone agonist stimulation test

In this test, serum estradiol is measured on day 2 of the cycle followed by the subcutaneous administration of gonadotropin analogue (triptorelin 100 μg). E2 levels are measured 24 h later and on day 3, and values are compared. A rise in E2 level is considered indicative of good ovarian reserve. It is found to have a good ability for the prediction of poor ovarian reserve, but is not superior to inhibin B or AFC in this regard.[45]

WHICH TEST TO CHOOSE?

Ovarian reserve testing should be performed for women older than 35 years who have not conceived after 6 months of attempting pregnancy and women at a high risk of diminished ovarian reserve.[46]

According to the NICE guidelines,[47] a women is said to have sufficient ovarian reserve if she either has an AFC of >4 or serum AMH level >5.4 pmol/L or serum FSH level <8.9 IU/I.

As already stated, an ideal ovarian reserve test should be convenient, reproducible with little intracycle or intercycle variability and demonstrate high specificity to minimize the risk of wrong diagnosis of diminished ovarian reserve. It should also have the ability to identify those at risk of developing ovarian hyperstimulation. Tests such as FSH are an indirect measure of the ovarian reserve and have substantial intracycle or intercycle variability. The provocative tests such as CCCT have not been commonly performed due to the expense and inconvenience, because they require more than one visit.

A large body of evidence has demonstrated a greater clinical value of AMH and AFC compared to FSH.[16,48] AMH has been shown to have better reliability than other

markers to predict ovarian reserve and response to stimulation.[49,50] It is a highly sensitive and superior real-time biomarker, which tells us about the pool of primordial follicle.[51] Other tests are based on the ovarian response to the hormones produced by the follicle during folliculogenesis. AFC measures only antral follicles, and results are operator dependent.

At times, discordance is seen in these ovarian reserve tests. In a large study of 5354 women[52] tested, one in five had discordant AMH and FSH values defined as AMH <0.8 (concerning) with FSH <10 (reassuring) or AMH ≥0.8 (reassuring) with FSH ≥10 (concerning). Of the women with reassuring FSH values ($n = 4469$), the concerning AMH values were found in one in five women in a highly age-dependent fashion, ranging from one in 11 women under 35 years of age to one in three women above 40 years of age. This can be explained by the fact that AMH is more sensitive, and its levels decline much earlier than the rise in FSH levels. This is due to normal ovarian ageing. On the other hand, of the women with reassuring AMH values ($n = 3742$), one in 18 had concerning FSH values, a frequency that did not vary in a statistically significant fashion by age.A total of 366 infertile patients undergoing IVF were studied by Gleicher *et al.*[53] They observed that the oocyte yields significantly decreased from patients having normal AMH and FSH to normal AMH/abnormal FSH or normal FSH/abnormal AMH and both abnormal. Except at very young and very old ages, normal AMH level is a better predictor of higher oocyte yields than normal FSH level. Women with normal AMH level but abnormal FSH level do not follow far behind until age 42. Beyond 42 years, normal FSH level predicts good oocyte yield even with abnormally low AMH level.

An evaluation of ovarian response in women undergoing IVF with discordant baseline serum AMH level and AFC was performed on 1046 women.[54] Among them, 32.2% had discordant AMH and AFC results. When AMH and AFC were discordant, it was seen that the ovarian responsiveness was intermediate between that when both were concordant on either end. Women having higher AMH within the same AFC quartile had a higher number of retrieved oocytes and cumulative live-birth rate.

CONCLUSION

Ovarian reserve test is an indirect marker of woman's follicular pool, which tells us about her sensitivity to ovarian stimulation and success with these procedures. They, however, cannot predict future fertility or the timing of decline or the cessation of future fertility. Currently available ovarian reserve tests do not provide sufficient evidence to be solely considered ideal, but they occupy an important place in initial counseling, predicting unsatisfactory results that could be improved by individualized induction schemes and reducing the excessive psychological and financial burden of the couple.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Leridon H. 30 years of contraception in France. Contracept Fertil Sex 1998;26:435-8.
2. Sharara FI, Scott RT Jr. Assessment of ovarian reserve and treatment of low responders. Infertil Reprod Med Clin N Am 1997;8:501-22.
3. Faddy MJ, Gosden RG, Gougeon A, Richardson SJ, Nelson JF. Accelerated disappearance of ovarian follicles in mid-life: Implications for forecasting menopause. Hum Reprod 1992;7:1342-6.
4. Menken J, Trussell J, Larsen U. Age and infertility. Science 1986;233:1389-94.
5. Rowe T. Fertility and a woman's age. J Reprod Med 2006;51:157-63.
6. Sharif K, Elgendy M, Lashen H, Afnan M. Age and basal follicle stimulating hormone as predictors of in vitro fertilization outcome. Br J Obstet Gynaecol 1998;105:107-12.
7. Scheffer JB, Scheffer BB, de Carvalho RF, Rodrigues J, Grynberg M, Mendez Lozano DH. Age as a predictor of embryo quality regardless of the quantitative ovarian response. Int J Fertil Steril 2017;11:40-6.
8. Tehraninezhad ES, Mehrabi F, Taati R, Kalantar V, Aziminekoo E, Tarafdari A. Analysis of ovarian reserve markers (AMH, FSH, AFC) in different age strata in IVF/ICSI patients. Int J Reprod Biomed (Yazd) 2016;14:501-6.
9. Speroff L, Fritz MA. Female infertility. In: Speroff L, Fritz MA, editors. Clinical Gynecologic Endocrinology and Infertility. 7th ed. Philadelphia, PA, USA: Lippincott Williams & Wilkins; 2005. p. 1015-22.
10. Brodin T, Bergh T, Berglund L, Hadziosmanovic N, Holte J. Menstrual cycle length is an age-independent marker of female fertility: Results from 6271 treatment cycles of *in vitro* fertilization. Fertil Steril 2008;90:1656-61.
11. van der Steeg JW, Steures P, Eijkemans MJ, Habbema JD, Hompes PG, Broekmans FJ, *et al.* Predictive value and clinical impact of basal follicle-stimulating hormone in subfertile, ovulatory women. J Clin Endocrinol Metabol 2007;92:2163-8.
12. Ashrafi M, Madani T, Seirafi Tehranian A, Malekzadeh F. Follicle stimulating hormone as a predictor of ovarian response in women undergoing controlled ovarian hyperstimulation for IVF. Int J Gynecol Obstet 2005;91:53-7.

13. Klinkert ER, Broekmans FJ, Looman CW, Habbema JD, te Velde ER. The antral follicle count is a better marker than basal follicle-stimulating hormone for the selection of older patients with acceptable pregnancy prospects after *in vitro* fertilization. Fertil Steril 2005;83:811-4.
14. Luna M, Grunfeld L, Mukherjee T, Sandler B, Copperman AB. Moderately elevated levels of basal follicle-stimulating hormone in young patients predict low ovarian response, but should not be used to disqualify patients from attempting *in vitro* fertilization. Fertil Steril 2007;87:782-7.
15. van Montfrans JM, Hoek A, van Hooff MH, de Koning CH, Tonch N, Lambalk CB. Predictive value of basal follicle-stimulating hormone concentrations in a general subfertility population. Fertil Steril 2000;74:97-103.
16. Barbakadze L, Kristesashvili J, Khonelidze N, Tsagareishvili G. The correlations of anti-mullerian hormone, follicle-stimulating hormone and antral follicle count in different age groups of infertile women. Int J Fertil Steril 2015;8:393-8.
17. Frattarelli JL, Bergh PA, Drews MR, Sharara FI, Scott RT Jr. Evaluation of basal estradiol levels in assisted reproductive technology cycles. Fertil Steril 2000;74:518-24.
18. Broekmans FJ, Kwee J, Hendriks DJ, Mol BW, Lambalk CB. A systematic review of tests predicting ovarian reserve and IVF outcome. Hum Reprod Update 2006;12:685-718.
19. Klein NA, Illingworth PJ, Groome NP, McNeilly AS, Battaglia DE, Soules MR. Decreased inhibin B secretion is associated with the monotropic FSH rise in older, ovulatory women: A study of serum and follicular fluid levels of dimeric inhibin A and B in spontaneous menstrual cycles. J Clin Endocrinol Metabol 1996;81:2742-5.
20. Seifer DB, Lambert-Messerlian G, Hogan JW, Gardiner AC, Blazar AS, Berk CA. Day 3 serum inhibin-B is predictive of assisted reproductive technologies outcome. Fertil Steril 1997;67:110-4.
21. Corson SL, Gutmann J, Batzer FR, Wallace H, Klein N, Soules MR. Inhibin-B as a test of ovarian reserve for infertile women. Hum Reprod 1999;14:2818-21.
22. Visser JA, Themmen AP. Anti-Müllerian hormone and folliculogenesis. Mol Cell Endocrinol 2005;234:81-6.
23. Van Rooij IA, Tonkelaar ID, Broekmans FJ, Looman CW, Scheffer GJ, de Jong FH, *et al.* Anti-Müllerian hormone is a promising predictor for the occurrence of the menopausal transition. Menopause 2004;11:601-6.
24. Pigny P, Jonard S, Robert Y, Dewailly D. Serum anti-Mullerian hormone as a surrogate for antral follicle count for definition of the polycystic ovary syndrome. J Clin Endocrinol Metab 2006;91: 941-5.
25. Wang JG, Nakhuda GS, Guarnaccia MM, Sauer MV, Lobo RA. Müllerian inhibiting substance and disrupted folliculogenesis in polycystic ovary syndrome. Am J Obstet Gynecol 2007;196: 77e1-5.
26. Van Rooij IA, Broekmans FJ, te Velde ER, Fauser BC, Bancsi LF, de Jong FH, *et al.* Serum anti-Müllerian hormone levels: A novel measure of ovarian reserve. Hum Reprod 2002; 17: 3065-71.
27. Nardo LG, Gelbaya TA, Wilkinson H, Roberts SA, Yates A, Pemberton P, *et al.* Circulating basal anti-Müllerian hormone levels as predictor of ovarian response in women undergoing ovarian stimulation for *in vitro* fertilization. Fertil Steril 2009; 92:1586-93.
28. La Marca A, Giulini S, Tirelli A, Bertucci E, Marsella T, Xella S, *et al.* Anti-Mullerian hormone measurement on any day of the menstrual cycle strongly predicts ovarian response in assisted reproductive technology. Hum Reprod 2007;22:766-71.
29. Nelson SM, Yates RW, Fleming R. Serum anti-Müllerian hormone and FSH: Prediction of live birth and extremes of response in stimulated cycles − Implications for individualization of therapy. Hum Reprod 2007;22:2414-21.
30. Muttukrishna S, Suharjono H, McGarrigle H, Sathanandan M. Inhibin B and anti-Mullerian hormone: Markers of ovarian response in IVF/ICSI patients? BJOG 2004;111:1248-53.
31. Lekamge DN, Barry M, Kolo M, Lane M, Gilchrist RB, Tremellen KP. Anti-Mullerian hormone as a predictor of IVF outcome. Reprod Biomed Online 2007;14:602-10.
32. Vembu R, Reddy NS. Serum AMH level to predict the hyper response in women with PCOS and non-PCOS undergoing controlled ovarian stimulation in ART. J Hum Reprod Sci 2017;10:91-4. doi:10.4103/jhrs.JHRS_15_16
33. Griesinger G, Diedrich K, Devroey P, Kolibianakis EM. GnRH agonist for triggering final oocyte maturation in the GnRH antagonist ovarian hyperstimulation protocol: Asystematic review and meta-analysis. Hum Reprod Update 2006;12:159-68.
34. van Rooij IA, Broekmans FJ, Scheffer GJ, Looman CW, Habbema JD, de Jong FH, *et al.* Serum anti-Müllerian hormone levels best reflect the reproductive decline with age in normal women with proven fertility: A longitudinal study. Fertil Steril 2005;83:979-87.
35. Haadsma MA, Bukman A, Groen H, Roeloffzen EM, Groenewoud ER, Heineman MJ, *et al.* The number of small antral follicles (2-6 mm) determines the outcome of endocrine ovarian reserve tests in a subfertile population. Hum Reprod 2007;22:1932-41.
36. Hendriks DJ, Kwee J, Mol BW, te Velde ER, Broekmans FJ. Ultrasonography as a tool for the prediction of outcome in IVF patients: A comparative meta-analysis of ovarian volume and antral follicle count. Fertil Steril 2007;87:764-75.
37. Kwee J, Elting ME, Schats R, McDonnell J, Lambalk CB. Ovarian volume and antral follicle count for the prediction of low and hyper responders with in vitro fertilization. Reprod Biol Endocrinol 2007;5:9.
38. Maseelall PB, Hernandez-Rey AE, Oh C, Maagdenberg T, McCulloh DH, McGovern PG. Antral follicle count is a significant predictor of livebirth in *in vitro* fertilization cycles. Fertil Steril 2009;91(4 Suppl): 1595-7.
39. Gibreel A, Maheshwari A, Bhattacharya S, Johnson NP. Ultrasound tests of ovarian reserve: A systematic review of accuracy in predicting fertility outcomes. Hum Fertil 2009;12:95-106.
40. Chui DK, Pugh ND, Walker SM, Gregory L, Shaw RW. Follicular vascularity-the predictive value of transvaginal power Doppler ultrasonography in an *in-vitro* fertilization programme: A preliminary study. Hum Reprod 1997;12:191-6.
41. Shrestha SM, Costello MF, Sjoblom P, McNally G, Bennett M, Steigrad SJ, *et al.* Power Doppler ultrasound assessment of follicular vascularity in the early follicular phase and its relationship with outcome of *in vitro* fertilization. J Assist Reprod Genet 2006;23:161-9.
42. Navot D, Rosenwaks Z, Margalioth EJ. Prognostic assessment of female fecundity. Lancet 1987;2:645-7.
43. Jain T, Soules MR, Collins JA. Comparison of basal follicle-stimulating hormone versus the clomiphene citrate challenge test for ovarian reserve screening. Fertil Steril 2004; 82:180-5.
44. Kwee J, Schats R, McDonnell J, Schoemaker J, Lambalk CB. The clomiphene citrate challenge test versus the exogenous follicle-stimulating hormone ovarian reserve test as a single test for identification of low responders and hyper responders to *in vitro* fertilization. Fertil Steril 2006;85:1714-22.
45. Hendriks DJ, Broekmans FJ, Bancsi LF, Looman CW, de Jong FH, te Velde ER. Single and repeated GnRH agonist stimulation tests compared with basal markers of ovarian reserve in the

prediction of outcome in IVF. J Assist Reprod Genet 2005;22:65-73.
46. ACOG Practice Committee. Committee opinion No. 618: Ovarian reserve testing. Obstet Gynecol 2015; 125: 268-73.
47. Fertility problems: assessment and treatment. Clinical guideline [CG156]. (February 2013). https://www.nice.org.uk/guidance/cg156/chapter/recommendations. [Last accessed on 2017 Sep].
48. Iliodromiti S, Anderson RA, Nelson SM. Technical and performance characteristics of anti-Müllerian hormone and antral follicle count as biomarkers of ovarian response. Hum Reprod Update 2015;21:698-710. doi:10.1093/humupd/dmu062.
49. Nelson SM, Fleming R, Gaudoin M, Choi B, Santo-Domingo K, Yao M. Antimüllerian hormone levels and antral follicle count as prognostic indicators in a personalized prediction model of live birth. Fertil Steril 2015;104:325-32. doi:10.1016/j.fertnstert.2015.04.032.
50. Fleming R, Seifer DB, Frattarelli JL, Ruman J. Assessing ovarian response: Antral follicle count versus anti-Müllerian hormone. Reprod Biomed Online 2015;31:486-96. doi:10.1016/j.rbmo.2015.06.015.
51. Jamil Z, Fatima SS, Cheema Z, Baig S, Choudhary RA. Assessment of ovarian reserve: Anti-Mullerian hormone versus follicle stimulating hormone. J Res Med Sci 2016;21:100.
52. Leader B, Hedge A, Baca Q, Stone K, Lannon B, Seifer DB, *et al.* High frequency of discordance between antimüllerian hormone and follicle-stimulating hormone levels in serum from estradiol-confirmed days 2 to 4 of the menstrual cycle from 5,354 women in U.S. fertility centers. Fertil Steril 2012;98:1037-42.
53. Gleicher N, Weghofer A, Barad DH. Discordances between follicle stimulating hormone (FSH) and anti-Müllerian hormone (AMH) in female infertility. Reprod Biol Endocrinol 2010;8:64.
54. Li HW, Lee VC, Lau EY, Yeung WS, Ho PC, Ng EH. Ovarian response and cumulative live birth rate of women undergoing *in-vitro* fertilisation who had discordant anti-Mullerian hormone and antral follicle count measurements: A retrospective study. PLoS One 2014;9:e108493.