International Journal of Intelligent Computing and Technology
ISSN: 2457-0249 (Online)
Website: https://www.ijict.com January (2019) - Vol. 3, Iss.2 Pages:47-69

Reliability of Ovarian Reserve Markers in Predicting IVF Outcomes

[1]K Amuthavalli, [2]Dr D Sudharsanam, [3]Dr C Geethaharipriya,
[4]Dr J Malasree, [5]Dr Siddhardha Solosan, [6]Dakshina Moorthy Janani, [7]Dr R Ravindran

[1,2]Department of Zoology and Biotechnology, Loyola College, Chennai
[3]Prashanth Fertility Research Center, Chetpet, Chennai, [4]Sri Ramachandra Medical College, Chennai,
[5]Department of Clinical Microbiology, MMC Rajiv Gandhi Government General Hospital, Chennai,
[6]SRM University, Chennai, [7]Department of Physiology, University of Madras, Chennai

Article History- Received: December 2019; Published: January 2019

Abstract

Fertility varies significantly even among the age of women depend upon the Oocyte number and quality decline with age. It has measures developed to predict response to ovarian stimulation and reproductive potential. To evaluate the ovarian reserve can identify the patients who may experience poor response or hyper – response to exogenous gonatropthins and can aid in the personalization of treatment to achieve good response and minimize risk. Both AMH and AFC have good predictive value. AMH level becoming the gold standard biomarker to evaluate the ovarian reserve and to predict the ovarian response to stimulation.

Keywords: Antimullerian Hormone, Antral follicle count, Follicle Stimulation Hormone, Luteini Menstrual cycle length

1. INTRODUCTION

The decline in the fertility levels of women of late can be because of various events associated with the increase in the age, which include changes in the quality of oocytes, efficiency and frequency of ovulation, diseases related to uterine, and the pregnancy complication risks like hypertension and gestational diabetes. The reason for diminished ovarian reserve in women is attributed to some factors that include genetic factors, infections, smoking and adnexal surgeries. Also, for achieving pregnancy in subfertile women, ovarian reserves play a very key role. As a treatment procedure in an attempt to predict the outcome and response in couples prior to InVitro fertilization, ovarian reserves are estimated routinely through various ovarian reserve tests (ORTs). Most widely used tests include basal FSH (follicle stimulating hormone), AMH (anti-mullerian hormone) and AFC (antral follicle count). Depending on the oocyte quality and the declining follicular pool, diminishing ovarian reserve in women during mid thirties or even earlier, can be estimated. The identification of women with a lower reproductive potential is a great challenge for reproductive medicine specialists. Sharara and Scott emphasized that an ideal ovarian reserve parameter should be easily measurable, minimally invasive, and inexpensive and should have good predictive values.Serum and ultrasonographic markers have been tested to infer the gonadal reserve of infertile women, but none of them has been proven to confidentially reflect the complex follicular dynamics or to be strongly correlated with the size and/or quality of primordial follicles remaining in the gonads after each wave of follicular growth. In other words, those tests do not ideally reflect the pool of unrecruited follicles, which may be responsible for the continuity of ovulatory cycles and, therefore, for the long-term reproductive potential. Recent studies demonstrated that these tests are not ideal for prediction of pregnancy and its outcome; rather they are effective in ovarian reserve prediction itself.

Antimullerian hormone (AMH) which is also referred as mullerian inhibiting substance (MIS) is a dimeric glycoprotein member of the transforming growth factor beta family. It is also recognized as a good indicator of fertility potential, thus reflecting the ovarian reproductive age. During the menstrual cycle, the AMH levels are more or less constant and also cycle independent (1). Its concentration has been shown to decline with the aging (2). This biomarker reflects that, with the increase in age in mormo-ovulatory women, there is a consistent gradual decrease in the reproductive capacity (3, 4)

In both human and animal studies (5), it was suggested that the concentration of serum AMH reflects the overall size of the primordial follicle pool and is considered to be a benchmark for ovarian reserve. Small antral follicles of the ovary and the granulosa cells of preantral secrete the AMH (6, 7). In general for newborn baby girls, the AMH is very rarely detectable whereas the its levels peak after puberty after which steadily decrease until menopause when serum concentration become undetectable there is a gradual and consistent decrease till menopause when the concentration of the serum becomes undetectable [Mulders et al]. In the ovary, AMH's role is to participate in the control or regulation of the ovarian function, prominently in the development and selection of follicle. AMH assist in the initiation of human primordial follicle growth and reduces the sensitivity of follicles to FSH (follicle simulating hormone) thereby preventing multiple selections of the dominant follicle (8).

Several works report that instead of traditional parameters like FSH, age, inhibin and estradiol, AMH is considered to be a better predictor of the responses to controlled ovarian hyperstimulation (9). This is largely attributed to the fact that AMH levels remain consistent and constant throughout the menstrual cycle and they are unaffected by GN RH – agonist pituitary down – regulation of pregnancy (6). Compared to the animals, human species are considered to be sub-fertile relatively. The average monthly fecundity rate of approx. 20% means that, human couples trying to conceive need more exposure months might to conceive, particularly if monthly fecundity has come down with increasing female age. The percentage of sub-fertile couple (who fail to achieve vital pregnancy within a year) will amount to 10-20% in the women of age group over 35 yrs, with respect to 4% women in their twenties. These sub fertility rates may rise to 30-35% for moderately fecund women of 35 years and above who have tried to conceive for long time. The regular menstrual cycle maintenance to an age where natural fecundity has already reduced to zero means that women are largely unaware that this process is ongoing. The decline in female fertility due to age has also been shown in numerous reports concerning ART (in vitro fertilization (IVF)) programs. After an average female age of 34 years, the chance of producing a live birth in ART programs comes down steadily and reduces below 10% per cycle in women of 40 years and above. This female age effect has also been shown for the chance of an IVF embryo to implant after IVF(10)

OVARIAN RESPONSE PREDICTION

In order to obtain adequate response and outcome from the assisted reproductive technologies (ART), prediction of ovarian reserve is very essential. It offers the chance of customizing COS protocols for every individual patient. The accurate prediction about ovarian response could pave way to provide information regarding the chances of conceiving as well as the particulars about options which include egg adoption or donation. In order to govern a safe and efficient treatment, ovarian response prediction before the initiation of hormonal stimulation, is the only possible way. Based on the insights and inputs, it implies that female age is the first choice predictor for ovarian testing prior to ART. With reference to the one's ovarian reserve of a woman within a specific age group, if there is a test available which can provide reliable inputs or information regarding the ovarian reserve of a woman within a specific age group, it will enable the clinicians to provide corresponding treatment course. For example, in case of older women a superior ovarian reserve observation may be resulted to allow ART treatment, whereas in case of younger women having exhausted ovarian reserve will subsequently result in early application or even refusal of ART. Ultimately, the response to maximal ovarian stimulation may provide further information on the reserve capacity of the ovaries. In the following two sections the biological rationale behind ovarian reserve testing and the accuracy and clinical value of several tests will be discussed.

In past works, besides the complete history of the patient, the use of ovarian reserve markers such as follicle stimulating hormone (FSH), anti-mullerian hormone (AMH) and antral follicle count (AFC) are evaluated for ovarian response prediction. For the ovarian response to hormonal stimulation, key predictors considered are age, morphological characteristics (antral follicular count (AFC)), biochemical parameters (serum antimullerian hormone (AMH), basal FSH levels in the early follicular phase), and ovarian volume. Even though the ovarian reserves reduce with the increase in age, it doesn't give any indication as a predictor of ovarian response (11)

AGE

To determine the quality as well as quantity of the ovarian reserve, the age of the individual is considered to be the most crucial factor. The increase in age is associated with a decrease in the pregnancy rates and natural fecundity. It is understood that both the quantity and the quality of ovarian follicles significantly decrease with the age ranging from being 6-7 million at 20 weeks of intrauterine life, and reducing to 2 million at the birth and 3-4 lacs persisting

at puberty. Starting early years of age 30 the fecund ability decreases significantly, after 35years of age the infertility prevalence increases significantly and after 45 years of age about 99% of the women are expected to be infertile. As the reproductive capacity decreases substantially with the age of the women, there is a definite need to find out women of relatively younger age with diminished ovarian reserves and also to find mean aged women at which natural fertility on an average is lost (41 years) but still having enough OR. By treatment scheme adjustments or stimulation dose, by indicating the necessity of early treatment initiation before reserve diminishes by very much or by counseling aging initiation of invitro fertilization (IVF) treatment, management could be individualized. In case of younger women, for ART outcome, age is considered to be a better predictor than basal FSH levels. Elevated levels of FSH also might have a favorable outcome of IVF for women younger than 35 years of age. Age of the woman is a very important factor for determining the pregnancy potential (regularly cycling women) although fertility doesn't decline uniformly. As the chronological alone has a limited value on the ovarian reserve prediction in woman, it leads to use and development of various biophysical and biochemical markers of ovarian reserve.

MENSTRUAL PATTERN

Menstrual cycle length (MCL) is generally determined by the rate of follicular growth and its quality, as well as the duration of follicular phase. In general, the MCL ranges from 21-35 days exhibiting an average interval of 28 days. In the late 30s, the MCL starts to shorten gradually along with the increased number of serum levels of follicular stimulating hormone. Recent works have examined in a standard stimulation, the history of menstrual cycles that got cancelled because of poor follicular response is a better predictor of than FSH and age for subsequent cycles of treatment. Shortened follicular phases are suspected to be one of the reasons behind shortening of MCL on age basis. It is described that diminished inhibin-B production by small number of antral follicles as well as the consequent increase in the secretion of FSH. Based on recent studies, it was explained that for women with MCL less than 30 days, the rate of conception was significantly reduced and the adjustment of other predictors of fertility like age had very less effect too. MCL has significant correlation with ovarian response to embryo quality and gonadotropin stimulus in IVF/ICSI cycles, and even if the age is excluded, the rates of pregnancy are almost doubled among the women having MCL > 34 days than those having MCL < 26 days.

BASAL SERUM FSH

In order to predict ovarian responses to stimulation in ICSI/IVF cycles, Basal Serum FSH has long been regarded as the most reliable and important bench marker. The concentration of Basal Serum FSH rises on average about 10 years or more prior to the menopause (12). This is generally caused due to the negative feedback of FSH-modulating proteins from the ovary, primarily inhibin A and B (13, 14, and 15). Another factor chosen is the reduction of antral follicles in cycle days 2/3/4 due to the low levels of estrogen which is also a critical feature. FSH level determination includes the estradiol level as it indicates that FSH level was determined when the estrogen levels are low which shows a glimpse of functioning of hypothalamic-pituitary-gonadal axis.

The combination of age as well as basal FSH were found to be better than considering age alone in the prediction of IVF outcome historically. Irrespective of limitations which include variable serum FSH levels between and within menstrual cycles, external factors like smoking and disparities between assays, the basal FSH measurements were being relied on by many IVF centers. With the increase in follicle age, the secreted FSH amount also increases which indicates a diminished ovarian reserve. So, it is of great importance to note that the fluctuations between cycles may be an indicator for the decline in the ovarian reserves. For predicting the IVF outcome of women, instead of using age alone as a predictor it is suggested that combination of age and early follicular FSH predicts better results.

Generally as the basal FSH are estimated to be lower than 10 mIU /ml (considered borderline), a single measurement might not be precise and accurate. Therefore, subsequent cycle basal FSH levels might be a better aspect for consideration. The cut points which have higher specificity in the range 80 to 100% have lower sensitivities ranging 10 to 30%. Other limitations include the variations in serum FSH levels between and during menstrual cycles, and disparities between assays. Hence, to exclude women from going forward with ART, high FSH cannot be used. It has been demonstrated recently that FSH is a good predictor only at high threshold levels of greater than 10 mIU /ml, thus predicts a compromised ovarian reserve (16)

BASAL (DAY 2/3/4) ESTRADIOL

For OR testing, basal estradiol has also been examined, however it isn’t relied upon extensively. Estradiol is product of granulose cells and it is often considered as a reflection of the follicular activity. Similar to FSH, the testing of estradiol is also available on various

automated platforms. Although the estradiol testing is relatively faster, reproducible and economical, it cannot be used alone as a biomarker for OR.

During the early stages of the menstrual cycle, if increased levels of estradiol is observed, it indicates the advanced follicular development and early selection of dominant follicles which is inappropriate for day 2/3/4. It is reported that as a result of rapid folliculogenesis also, the estradiol levels elevate. Alternatively, enhanced ovarian reserve results in increased levels of estradiol too. If a woman has polycystic ovarian syndrome (PCOS), only small amounts of estradiol are produced by a large number of antral follicles.

Cancelled ART cycles are observed in large numbers for E2 levels less than 20 pg/ml or greater than or equal to 80 pg/mL. Measurements of both E2 and FSH on cycle day 2/3/4 is helpful to reduce the false to diminish the incidence of false levels, but need more study and validation. This could be attributed to a variation between assays used and there may be concerns about cycle – to – cycle variability

INHIBIN B

A glycoprotein hormone, Inhibin B, which is a member of β super family 18 (transforming growth factor), is produced from the tiny antral follicles and inhibits the FSH release 28 selectively. Stimulated by association of FSH itself with insulin like growth factor, inhibin-B regulates or inhibits the secretion of pituitary FSH and paracrine action on the follicles that were developing. For serum inhibin levels more than or equal to 45 pg/ml, large number of oocytes and greater response to estrogen were observed. Consequently for lower levels, cancellations are thrice more frequent comparatively. With the age of women the size of the follicle cohort decreases resulting in the decrease of inhibin B levels. Therefore it is to be noted that Inhibin B externally influences FSH ovarian reserve test. Recent investigations conducted on infertile women in the age group 24-40 years demonstrated a negative correlation between FSH and inhibin-B and positive correlation between FAC and inhibin-B.

AMH

Anti-Mullerian Hormone (AMH) is considered as a novel biomarker which has a crucial role in the development of follicles and testicles. Determination of AMH has been proposed in clinical practices for ovarian reserve prediction and reproductive potential as it can be considered as a marker which can predict and evaluate the activity and quantity of retrievable follicles in the initial stages of maturation. It is produced from antral follicles and preantral follicles in sizes up to 6 mm. AMH acts as an inhibitor for FSH mediated granulose cell

proliferation, aromatase activity and follicular growth (17). In male, AMH is secreted by Sertoli cells and its biological function in the fetal life, whilst in postnatal males it has a regulatory function in the gonad impacting on reproductive fertility. In women, AHM is secreted by granulose cells to inhibit the early stages of follicular development. AMH displays several unique characteristics that have led to its widespread adoption in the field of reproductive endocrinology and assisted conception. Primarily, as the bilateral oophorectomy associated with premenopausal women and the menopause is integrated with undeteactable levels of AMH, it appears to be completely derived from ovary. Secondly, it is the only hormone in the ovary which is produced by granulose cells (single-type cell) which permits it to be used as a biomarker of granulose cell function. Apart from that, it is synthesized and secreted by granulose cells from larger to smaller antral follicles (4 to 6 mm). When compared to that of FSH, Estradiol, inhibin-B or AFC, very minor variations in serum levels were observed between consecutive, conducted among infertile women in the age group 20-40 years. Also, AMH allowed authors to find out that cycles were able to produce significant amount of oocytes, thereby decreasing the gap between the conventional IVF and IVM. Besides, AMH has unique characteristics which include, independence of FSH circulation, better stability across and between the menstrual cycles, and its ability to be measured by enzyme-lined immunosorbent assary (ELISA) constitute its appeal stronger.

AMH FUNCTION AND CLINICAL UTILITY

» Fertility
- Determination of ovarian status
- Use in IVF for controlled ovarian stimulation (COS)
- Evaluate potential fertility prior to chemotherapy
- Male infertility

» Reproductive Aging
- Evaluation of menopause
- Granulosa cell tumors
- Polycystic Ovary Syndrome
- Patho-physiology
- Intersex disorders
- Ambiguous genitalia differentiation
- Precocious or delayed puberty

It is described that AMH has been used to quantify ovarian reserves primarily in order to estimate the response of an infertile woman to controlled ovarian stimulation. Besides it is proved that AMH can also be used to monitor and diagnose the women with PCOS

(polycystic ovary syndrome) and concentration of AMH levels are elevated more in normogonadotropic anovulatory women with PCOS.

CLINICAL MEASUREMENT OF AMH

Over the past decades there has been continuous evolution of the assays from solo laboratory versions to the most recent diagnostic system lab (DSL) and immunotech (IOT) assays. The research works that have been carried out till date possess both the assays DSL and IOT in equal numbers. The reported AMH values from previous works are not consistent as IOT assay gave higher values than those obtained with DSL assay. As a result of this, in the current work, a new assay (AMH generation II assay) which is a hybrid of both assays is developed. It is calibrated to IOT standards and is in the process of replacing both DSL and IOT assays.

Although potentially confusing, clinicians historically accustomed to interpreting that, as the hybrid AMH Gen II assay is calibrated similar to the old IOT assay, it is expected to give similar values and therefore IOT assay cannot be troubled. Consequently, those using DSL assay believe that the values of AMH from the new AMH Gen II assay are expected to be 40% higher than the earlier reported values. Although the studies reported that rapid reduction in the initial stages of luteal phase caused cyclical fluctuations significantly in AMH levels, the excursions from mean levels are relatively small (+3% to 19%). These fluctuations are in accordance with the inter-cycle variability reported for AMH. Consequently, during the menstrual cycle, the inter and intra cycle variability in the AMH levels in the clinical setting may be considered to be the low point in order to permit random timing to measure AMH level.

It is reported in one of the recent works that the initial primordial follicular recruitment rates increased gradually till the puberty age and then observed a decline progressively in the menopause. This indicates that the concentration of AMH levels in both childhood as well as adulthood at any specific age may mirror primordial follicular recruitment rates instead of simply the number of primordial follicle. Consequently across the life span of female, circulating AMH exhibits an increase initially followed by a gradual decline nonlinearly as it is well established for the pool of primordial follicles.

Fertility preservation measures including oocyte freezing can then be undertaken early to ensure that these young women have an opportunity to potentially avoid oocyte donation in the future. It is tempting to apply a similar approach to generic health screening for young women, regarding their potential reproductive life span. However, as age of the menopause

demonstrates strong heritability, AMH would need to do substantially better than just asking when their mother went through the menopause and extrapolating back.

Women with a very low or poor ovarian reserve (AMH <1.0pm01/1) although pregnancy is feasible, the likelihood of success is limited. Withholding treatment and pursuing alternatives like oocytes donation have been suggested as a cost effective approached, but for many moving on to donor gametes without first pursuing attempts at IVF and the inherently low success rates is not an option. Instead. Accurate counseling to ensure that their expectations regarding risk of cycle cancellation, reduced oocyte yield , low embryo number and reduced chance of success are set appropriately, will potentially minimize the associated disappointments of a couple.

The amount of follicles can be evaluated quantitatively by AMH but cannot be done by AFC. AMH level has very low inter and intracycle variability and remains largely stable during the menstrual cycles(17,18) However, factors like oral contraceptive pills and smoking can alter and quantify the variability(19). Recent studies has postulated that AMH is an excellent predictor of poor ovarian response to the ovarian stimulations even though the perfect test is the response of ovaries to ovarian stimulation itself (20,21)

ANTRAL FOLLICLE COUNT (AFC)

The antral follicle count (AFC) is termed as the total number of follicles that are smaller than the diameter of 10mm detected by transvaginal sonography (TVS) in both the ovaries during the initial stages of follicular phase. AFC is often regarded as a predictor of the number of oocytes that are retrieved in controlled ovarian hyperstimulation protocols, predicting pregnancy loss in IVF pregnancy, and cancellation rates in IVF. For a particular individual there isn’t any difference between right and left sided AFCs. Compared to the ovarian volume measurements and complex endocrine challenge tests, AFC is often considered to be a better predictor for poor IVF response or hyper IVF response. Similar to AFM, the AFC is also established to be equally beneficial in the determination of ovarian reserve status or responsiveness.

The number of preantral or small antral follicles (2 to 6 mm) declines with the age whereas the number of large follicles (7 to 10mm) remains mostly constant which is an indicative that small AFCs represent functional OR. Lower level of AFC (3-10 total antral follicles) is associated with poor response. Several works postulated that the AFC is one of the key predictors of ovarian response. During the investigation and testing in infertile women, as the

endovaginal ultrasound evolution is performed generally, AFC should be made a routine test of gonadal reserve. However, meta analysis states that AFC and AMH, as a combination or alone doesn't improve the ongoing pregnancy rate prediction, however the age of the women is considered as the most crucial factor related to live birth rate (21). The problems associated with AFC include the cycle-cycle differences, intraobserver differences and the biological variations.

2. MATERIALS AND METHODS

This comparative study was done on 383 infertile couples. The couples were introduced for ICSI program for either male or female infertility or both. The pituitary gland of the female was inactivated by administering gonadotrophin releasing hormone which caused the follicles to develop in the ovaries. Once the follicles reached a mean diameter of 16 mm to 22 mm, a booster injection of HCG (Human Chorionic Gonadotrophin) was injected in order to cause the eggs to mature inside the follicles and induce rupture. After 36 hours the oocytes were retrieved transvaginally under the guidance of ultrasound.

The collected eggs were incubated for 2hours in fertilization media at 37 ºC temperature in a 6% carbon dioxide atmosphere. After 2 hours the eggs were exposed to 7% hyaluronidase in order to facilitate removal of the surrounding cumulus cells and maturity of the eggs were checked. The semen samples were taken by masturbation. They were analysed (WHO 2010) and pure sperms were prepared by Gradient Separation Method by using 80% lower phase and 40% upper phase. The sediment sperm was washed in sperm media and final concentration of 0.6 to 0.8M/Ml was prepared for injection.

A 60mm petridish was marked with patient`s name and lab no. and 9 drops of 5µl equilibrated fertilization media was placed in the center of the dish and over layered with equilibrated oil . Mature eggs were placed, one in each drop with sperm and 7% polyvinylpyrrolidone(PVP) in the center drop. The conventional ICSI procedure was performed by micro injections. The patients had intracytoplasmic sperm injection (icsi) done to their eggs and incubated in CO2 incubator overnight. After 18 hrs of incubation the eggs were checked for pronucleus under a Stereomicroscope and the fertilized zygotes were changed to fresh equilibrated cleavage media and left undisturbed until second day. On the third day, the embryos were checked for number of blastomeres and fragments . They were scored as grade 1 for embryos with 7 to 8 blastomeres with nil or 25% fragmentation and

grade 2 for 50% fragmentation and grade 3 for 75% fragmentation or for blastomeres less than 5. After scoring, the embryos were changed to equilibrated Blastocyst medium and Embryo transfer was performed depending on quality or quantity of embryos. Otherwise the embryos were cultured till day 5 for transfer. On day 5 embryos were scored again as grade-1, 2, 3 according to the quality of inner cell mass and quantity of trophectoderm cells. The best embryos, at least two were chosen for transfer and shifted to fresh equilibrated blastocyst medium in a four well dish until the embryos were transferred to the uterus.

3. METHODOLOGY:

AMH GEN-II ELISA: (MANUAL METHOD)

AMH Gen II ELISA, a novel assay, is considered to be two-site enzymatically amplified immune assay. In this assay, samples, controls and calibrators are incubated in anti-AMH body coated microtitration wells. The anti-AMH antibody labeled with biotin is added to each microtitration well after the incubation and washing is completed. After second round of incubation and washing, streptavidin-horseradish peroxidase (HRP) is added to the microtitration wells. Following a third round of incubation and washing, the substrate tetramethylbenzidine (TMB) is then added to these wells. Finally, acidic stopping solution is added to these titration wells. The substrate's enzymatic turnover degree is evaluated by the measurement of dual wavelength absorbance at 450 nm, and another between 600 to 630 nm. The sample's absorbance that was measured is directly proportional to the AMH concentration levels. The calibration curve for absorbance vs AMH concentration is plotted using some set of AMH calibrators. From the curve, the concentration of AMH in the samples can be calculated.

ACCESS AMH ASSAY: (AUTOMATED CLIA METHOD)

The Access AMH assay is a one-step simultaneous immunoenzymatic ("sandwich") assay. A reaction vessel consisting of TRIS buffered saline with proteins, mouse monoclonal anti-AMH antibody which is conjugated to alkaline form of phosphatase in MES buffer, and paramagnetic particles that are coated with a mouse monoclonal anti-AMH antibody in TRIS buffer, is taken and the sample is added to it. The reaction vessel is incubated after which the bound solid phase materials are held in a magnetic field whereas the while unbound materials in the vessel are washed away. After this incubation process, the chemiluminescent substrate (Lumi-Phos* 530) is added to the reaction vessel and light that is generated by the reaction is measured using a luminometer. The light produced is quantified and is directly proportional

to the sample's AMH concentration. A multi-point calibrated curve is used to determine the amount of analyte present in the sample.

4. RESULTS

The table shows the relationships between AMH and study outcomes. The AMH was correlated with oocytes retrieved, eggs fertilized but not with outcome of pregnancy. Age and AMH level data are correlated and presented in Figure 1

Table A: Statistics of AMH data

	Age	Anti-mullerian Hormone	Right Ovary	Left ovary	Sum of right & left ovary	Number of eggs ret	Number of eggs fertilised
N Valid	383	383	383	383	383	378	378
Missig	0	0	0	1	0	5	5
Mean	33.40	3.68	6.33	6.27	12.59	9.80	5.47
Median	33.00	3.30	5.00	5.00	11.00	9.00	5.00
Std. Deviation	4.654	2.533	3.572	3.725	7.046	6.262	3.852
Minimum	21	0	0	0	1	0	0
Maximum	46	11	35	37	72	43	23

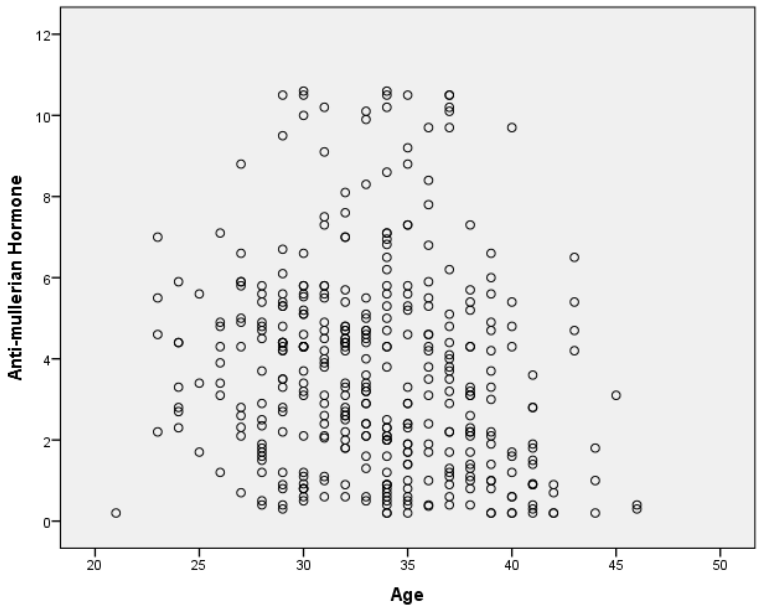

Figure 1: AMH levels for corresponding age group

Table 1: Correlations – Age and AMH levels

			Age	Anti-mullerian Hormone
Spearman's rho	Age	Correlation Coefficient	1.000	-.208**
		Sig. (2-tailed)	.	.000
		N	383	383
	Anti-mullerian Hormone	Correlation Coefficient	-.208**	1.000
		Sig. (2-tailed)	.000	.
		N	383	383

**. Correlation is significant at the 0.01 level (2-tailed).

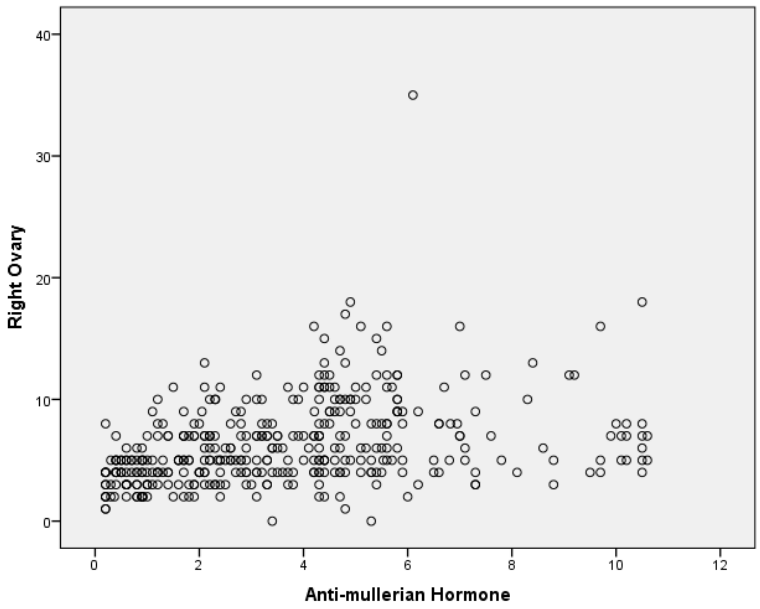

Figure 2: Right ovary and AMH level data

Table 2: Correlations – AMH and Right ovary

			Anti-mullerian Hormone	Right Ovary
Spearman's rho	Anti-mullerian Hormone	Correlation Coefficient	1.000	.426**
		Sig. (2-tailed)	.	.000
		N	383	383
	Right Ovary	Correlation Coefficient	.426**	1.000
		Sig. (2-tailed)	.000	.
		N	383	383

**. Correlation is significant at the 0.01 level (2-tailed).

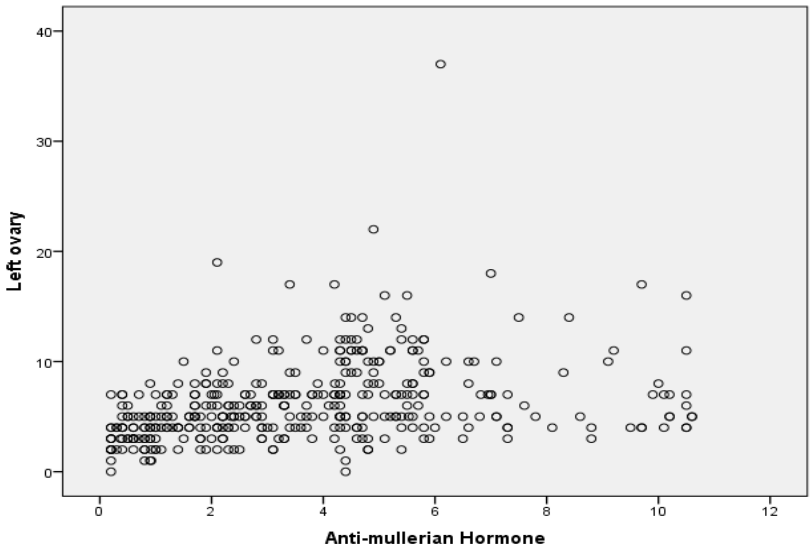

Figure 3: Left ovary and AMH level data

Table 3: Correlations – AMH and Left ovary

			Anti-mullerian Hormone	Left ovary
Spearman's rho	Anti-mullerian Hormone	Correlation Coefficient	1.000	.412**
		Sig. (2-tailed)	.	.000
		N	383	382
	Left ovary	Correlation Coefficient	.412**	1.000
		Sig. (2-tailed)	.000	.
		N	382	382

**. Correlation is significant at the 0.01 level (2-tailed).

The sum of left and right ovaries data and AMH level data are correlated and presented in Figure4 and tabulated in Table4.

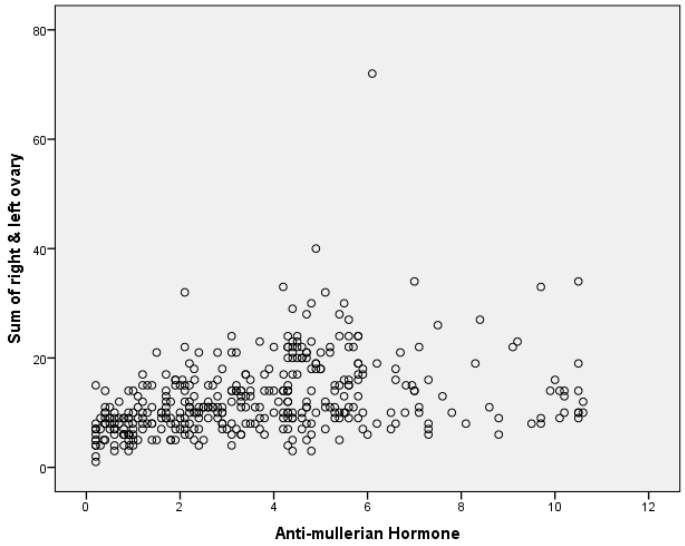

Figure 4: Sum of Right and Left ovaries and AMH level data

Table 4: Correlations – Sum of right and left ovaries and AMH

			Anti-mullerian Hormone	Sum of right & left ovary
Spearman's rho	Anti-mullerian Hormone	Correlation Coefficient	1.000	.442**
		Sig. (2-tailed)	.	.000
		N	383	383
	Sum of right & left ovary	Correlation Coefficient	.442**	1.000
		Sig. (2-tailed)	.000	.
		N	383	383

**. Correlation is significant at the 0.01 level (2-tailed).

Number of eggs retrieved and AMH level data are correlated and presented in Figure 5 and tabulated in Table 5.

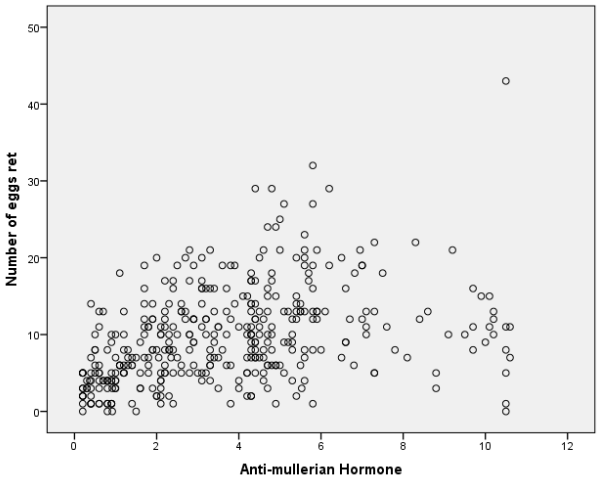

Figure 5: Number of eggs retrieved vs AMH

Table 5: Correlations – AMH and No. of eggs retrieved data

			Anti-mullerian Hormone	Number of eggs ret
Spearman's rho	Anti-mullerian Hormone	Correlation Coefficient	1.000	.457**
		Sig. (2-tailed)	.	.000
		N	383	378
	Number of eggs ret	Correlation Coefficient	.457**	1.000
		Sig. (2-tailed)	.000	.
		N	378	378

**. Correlation is significant at the 0.01 level (2-tailed).

Number of eggs fertilized and AMH level data are correlated and presented in Figure 6 and tabulated in Table 6

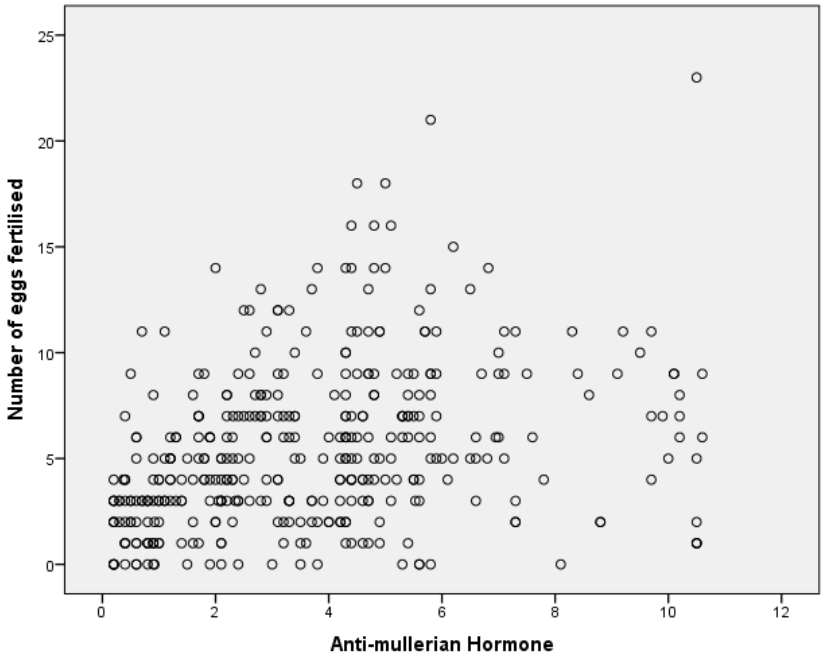

Figure 6: Number of eggs fertilized vs AMH

Table 6: Correlations – AMH and No. of eggs fertilized

			Anti-mullerian Hormone	Number of eggs fertilised
Spearman's rho	Anti-mullerian Hormone	Correlation Coefficient	1.000	.389**
		Sig. (2-tailed)	.	.000
		N	383	378
	Number of eggs fertilized	Correlation Coefficient	.389**	1.000
		Sig. (2-tailed)	.000	.
		N	378	378

**. Correlation is significant at the 0.01 level (2-tailed).

Number of eggs retrieved and Age data are correlated and presented in Figure 7 and tabulated in Table 7

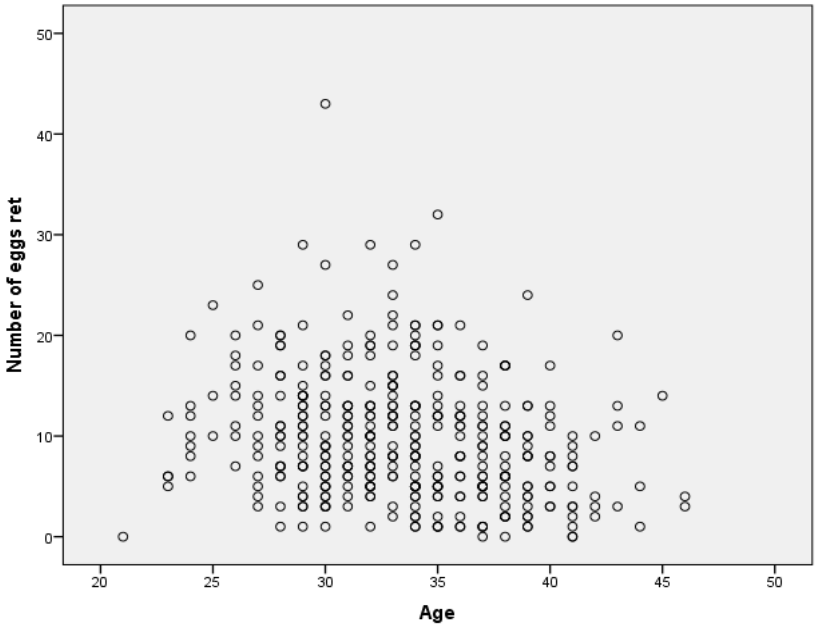

Figure 7: Number of eggs retrieved vs Age

Table 7: Correlations – No. of eggs retrieved vs Age

			Age	Number of eggs ret
Spearman's rho	Age	Correlation Coefficient	1.000	-.245**
		Sig. (2-tailed)	.	.000
		N	383	378
	Number of eggs ret	Correlation Coefficient	-.245**	1.000
		Sig. (2-tailed)	.000	.
		N	378	378

**. Correlation is significant at the 0.01 level (2-tailed).

Number of eggs fertilized and Age data are correlated and presented in Figure 8 and tabulated in Table 8

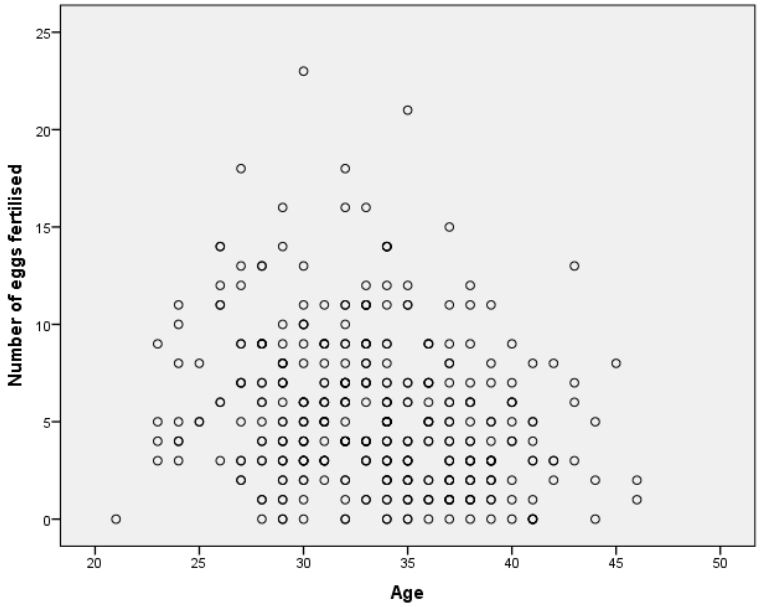

Figure 8: Number of eggs retrieved vs Age

Table 8: Correlations – No. of eggs fertized vs Age

			Age	Number of eggs fertilised
Spearman's rho	Age	Correlation Coefficient	1.000	-.260**
		Sig. (2-tailed)	.	.000
		N	383	378
	Number of eggs fertilised	Correlation Coefficient	-.260**	1.000
		Sig. (2-tailed)	.000	.
		N	378	378

The summary of all the data which includes statistical parameters (Table 9), correlation with age (Table 10) and correlation with AMH (Table 11) are tabulated accordingly.

Table 9: Summary of statistics

	Mean	Median	Standard Deviation	Min Value	Maximum Value
Age	33.4	33	4.65	21	46
Anti-Mullerian Hormone	3.68	3.3	2.53	0	11
Right Ovary	6.33	5	3.57	0	35
Left Ovary	6.27	5	3.72	0	37
Sum RO+LO	12.59	11	7.04	1	72
No. of eggs retrieved	9.8	9	6.26	0	43
No. of eggs fertilized	5.47	5	3.85	0	23

Table 10: Correlation with Age

	Correlation coefficient-r value	P value
Anti-Mullerian Hormone	-0.208	<0.05*
No. of eggs retrieved	-0.245	<0.05*
No. of eggs fertilized	-0.26	<0.05*

*p <0.05 is statistically significant at 95% CI.

Table 11: Correlation with AMH

	Correlation coefficient-r value	P value
Right Ovary	0.426	<0.05*
Left Ovary	0.412	<0.05*
Sum RO+LO	0.442	<0.05*
No. of eggs retrieved	0.457	<0.05*
No. of eggs fertilized	0.389	<0.05*

*p <0.05 is statistically significant at 95% CI.

S.No	**Characteristics**	**Frequency (n)**	**Percentage (%)**
1.	**AGE (n=383)**		
	20-25	15	3.9
	26-30	97	2.5
	31-35	148	3.9
	36-40	96	2.5
	41-45	25	6.5
	More than 45	2	0.52
2.	**AHM(n=383)**		
	<0.3ng/ml	12	3.1
	0.3-0.6 ng/ml	27	7.0
	0.7-0.9 ng/ml	23	6.0
	1-3 ng/ml	110	28.7
	>3 ng/ml	211	5.5
3.	**Right Ovary (n=383)**		
	0-5	200	5.2
	6-10	138	3.6
	11-15	36	9.4
	16-20	8	2.1
	>20	1	0.2
4.	**Left Ovary (n=382)**		
	0-5	200	52.3
	6-10	134	35.0
	11-15	38	9.9
	16-20	8	2.1

	>20	2	0.5
5.	**No. of eggs Retrieved (n=378)**		
	0-10	224	56.3
	11-20	133	35.1
	21-30	19	5.0
	31-40	1	0.3
	41-50	1	0.3
6.	**No. of eggs fertilized (n=378)**		
	0-5	214	56.6
	6-10	121	32.0
	11-15	36	9.5
	16-20	5	1.3
	>20	2	0.5

5. DISCUSSION

The parameters including FSH, E2, are not reliable to predict the outcome of pregnancy but AMH provide an approach to predict the number of oocytes retrieved and embryo quality rather than biochemical and clinical outcome of pregnancy. The previous studies have also described the AMH concentration values in predicting embryo quality, oocyte quality, and ICSI outcome. Lower concentration of AMH may be attributed to poor oocyte retreived. The results of the present study also indicate that there is no significant correlation between basal serum AMH level and the outcome of pregnancy.

The AMH value is correlated with good quality embryo which is turn is coorelated with implantation and pregnancy rates. Even though AMH is considered as a useful biomarker for prediction of ovarian reserve, ovarian path physiology like PCOS and menopause, its role in predicting pregnancy remains unclear. Although embryo quality and oocyte quality diminishes with the increase in age, it is tough to correlate pregnancy rate with age. Both age and AMH have been proved to be independent predictors of live birth.

6. CONCLUSION

Ovarian reserve evaluation helps identify patients who have poor response or poor hyper response to stimulation of ovaries for assisted reproduction techniques. This helps in personalization of treatment and hence to achieve good response and minimizes safety risks. The ideal ovarian reserve test should be reproducible, have limited inter- & intra-cycle variability, and can demonstrate high specificity to minimize the risk for not correctly categorizing women as having reduced ovarian reserve. It can be said that there is no perfect measure of ovarian reserve but, both AMH and AFC level have good predictive value(22)

Hence this study aims to Composite measures that use both methods which can potentially be used to provide a complete assessment of ovarian reserve, although AMH has been proved as a better predictor of oocyte yield in patients having discordant AFC and AMH results. The convenience of untimed sampling, objectivity, and potential standardization of AMH level makes this a trusted method for evaluation of ovarian reserve in women.

This study is limited by the study was conducted in a monodominant follicle cycle. Despite these limitations the current study demonstrated that AMH was correlated with oocytes retrieval and embryo quality but not with pregnancy outcome.

REFERENCES

1. Cook CL, Siow Y, Taylor S, Fallet M.E. Serum mullerian-inhibiting substance levels during normal menstrual cycles. Fertil Steril. 2000; 73: 859-861.
2. Fanchin R, Taieb J, Lozano DH, Ducot B, Frydman R,et al. High reproducibility of serum anti-Mullerian hormone measurements suggests a multi-staged follicular secretion and strengthens its role in the assessment of ovarian follicular status. Human Reproduction. 2005; 20: 923-927.
3. Van Rooji IA, Broekmans FJ, Scheffer GJ, Looman CWN, Habbema JDF, et al: Serum antimullerian hormone levels best react the reproductive decline with age in normal women with proven fertility: a longitudinal study. Fertil Steril. 2005; 83: 979-87.
4. Tehrani FR, Solaymani DM, Azizi F. A single test of anti-Mullerian hormone in late reproductive-aged women is a good predictor of menopause. Menopause. 2009; 16:797-802.
5. Kevenaar ME, Meerasahib MF, Kramer P, Van de Lang-Born BM,De Jong FH, Groome NP, Themmen AP, Visser JA. Serum anti-mullerian hormone levels reflect the size of the primordial follicle pool in mice. Endocrinology. 2006; 147:3228-3234.
6. La Marca A, Broekmans FJ, Volpe A, Fauser BC, Macklon NS: ESHRE Special Interest Group for Reproduction Endocrinology-AMH Round Table Anti-Mullerian hormone (AMH): What do we still need to know? Hum Reprod. 2009; 24:2264-2275.
7. Weenen C, Laven JS, Von Bergh AR, Cranfield M, Groome, NP, Visser JA, Kramer P, Fauser BC, Themmen AP. Anti-Mullerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. Mol Hum Reprod. 2004; 10:77-83.
8. Arabzadeh S, Hossein G, Rashidi BH, Hosseini MA, Zeraati H. Comparing serum basal and follicular fluid levels of anti-Mullerian hormone as a predictor of in vitro fertilization outcomes in patients with and without polycystic ovary syndrome. Ann Saudi Med. 2010;30(6):442-7. Doi:10.4103/0256-4947.71063.
9. Fanchin R, T Taieb J, Lozano DH, Ducot B, Frydmman r. Bouyer j. high reproductibility of serum anti- mullerian hormone measurement suggests a multi staged follicular secretion and strengthens its role in the assessment of ovarian follicular status > hum reprod. @005; 20(4) ;923-927 .
10. La Marca A, Nelson SM, Sighinolfi G, Manno M, Baraldi E, Roli L et al antimullerian based prediction model for a live birth in assisted reproduction. Reprod Biomed online 2011; 22 (4) ;341-9.

11. M.Al-Azemi, S.R.Killick, S.Duffy et al., "Multi – marker assessment of ovarian reserve predicts oocyte yield after ovulation induction,"Human Reproduction,vol.26,no.2,pp.414-422,2011.
12. N.A.A Ebbiary, E.A Lenton, and I.D.Cooke,"Hypothalamic pituitary ageing: progressive increase in FSH and LH concentrations throughout the reproductive life in regularly menstruating women, "Clinical Endocrinology, vol41,no.2,pp.199-206,1994.
13. N.P.Groome, P.J Illingworth, M.O'Brien et al, "Measurement of dimeric inhibin B throughout the human menstrual cycle, "Journal of Clinical Endocrinology and metabolism,vol.81,no.4,pp.1401-1405,1996
14. H.G.Burger, N.Cahir, D.M Robertson et al, " Serum inhibins A and B fall differentially as FSH rises in perimenopausal women,'' Clinical Endocrinology, vol.48,no.6,pp.809-813,1998.
15. C.K.Welt, D.J.J.McNicholl, A.E.Taylor, and J.E.Hall, "Female reproductive aging is marked by decreased secretion of dimeric inhibin," The Journal of Clinical Endocrinology and Metabolism, vol.84, no.1, pp.105-111, 1999.
16. F.J.Broekmans, J. Kwee, D.J.Hendriks, B.W.Mol, and C.B.Lambalk, " A systematic review of tests predicting ovarian reserves and IVF outcomes." Human Reproduction Update,vol.12,no.6,pp.685-718,2006.
17. A.La Marca, G.Stabile, A.C.Artenisio, and A.Volpe, " Serum antiMullerian hormone throughout the human menstrual cycle,'' Human Reproduction , vol.21,no12,pp. 3103 – 3107,2006.
18. A.la Marca, S.Giuline, A.Tirelli et al, " Anti – Mullerian hormone measurement on any day of the menstrual cycle strongly predicts ovarian response in assisted reproductive technology.'' Human Reproduction, vol. 22,no.3,pp.766-771,2007.
19. M.Dolleman, M.J.Faddy, J.van Disseldorp et al., The relationship between anti – mullerian hormone in women receiving fertility assessments and age at menopause in subfertile women: evidence from large population studies," Journal of Clinical Endocrinology and Metabolism,vol.98,no.5,pp.1946-1953,2013.
20. N.P.Polyzos, S.M.Nelson, D.Stoop et al, "Does the time interval between antimullerian hormone serum sampling and initiation of ovarian stimulation affect its predictive ability in vitro fertilization – intracytoplasmic sperm injection cycles with a gonadotropin – releasing hormone antagonist? A retrospective single – center study,'' Fertility and Sterility, vol.100, no.2, pp.438 – 444.2013.
21. S.L.Broer, M.Dolleman, J.van Disseldrop et al., " Prediction of an excessive response in invitro fertilization from patient characteristics and ovarian reserve tests and comparison in subgroups: an individual patient data metal – analysis," Fertility and Sterility, vol.100,no.2,pp.420.e7-429.e7,2013.
22. Li,H.W.,Lee,V.C.,Lau,E.Y.,Yeung,W.S.,Ho,P.C.,Ng,E.H.,2014.Ovarian response and cumulative live birth rate of women undergoing invitro fertilization who had discordant anti –Mullerian hormone and antral follicle count measurements: a retrospective study. PLoS one9,e108493

How to cite this articles :
K.Amuthavalli, "Reliability of Ovarian Reserve Markers in Predicting IVF Outcomes", International Journal of Intelligent Computing and Technology (IJICT), Vol.2, Iss.2, pp.47-69, 2019