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Original Research Article

Comparative study between serum markers Follicular stimulating hormone, Anti Mullerian hormone and Antral Follicular Count on Transvaginal ultrasonography as a better predictor of ovarian reserve in patients of In Vitro Fertilization

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Abstract: The objective of study was to identify the correlations between the tests currently used in ovarian reserve assessment: anti-Mullerian hormone (AMH), follicle stimulating hormone (FSH) and antral follicle count (AFC) and to distinguish the most reliable markers for ovarian reserve in order to select an adequate strategy for the initial stages of infertility treatment. In this prospective study, 112 infertile women were assessed and AMH, FSH and AFC were determined on days 2 of their menstrual cycles. AMH and AFC positively correlates with oocytes retrieved. FSH negatively correlates with oocytes retrieved. Currently, AMH should be considered as the more reliable of the ovarian reserve assessments tests compared to FSH. There is a strong positive correlation between serum AMH level and AFC. The use of AMH combined with AFC may improve ovarian reserve evaluation.
Keywords: Anti-Mullerian hormone (AMH), follicle stimulating hormone (FSH) and antral follicle count (AFC).

INTRODUCTION

The term "ovarian reserve" refers to the quantity and quality of a woman's current reservoir of oocytes and is closely associated with reproductive potential [1]. It can be used as an indirect measure of a woman's reproductive age. Infertility refers to the inability of a woman to become pregnant after having unprotected intercourse for a year [2].

There are many factors that contribute to infertility, including, but not limited to the quantity and quality of the ovarian reserve. However, a woman's age is not the only factor determining her ovarian reserve; decreases can occur at younger ages and be partially or totally responsible for infertility. Couples struggling to become pregnant may consider fertility treatments, including multiple repeated tests, drugs and/or surgery [3]. Since treatments can be financially and emotionally draining, it is important to consider the patient's ability of becoming pregnant based on the limiting measures. Measurement of ovarian reserve is very important in predicting a woman's response to various fertility treatments and helps us decide on appropriate fertility medication dosage levels for that treatment. Follicle development is dependent on the interrelationship of many hormones, such as follicle stimulating hormone (FSH) and anti-Mûllerian hormone (AMH), secreted from the anterior pituitary gland and the ovaries respectively. Abnormal levels of these hormones may indicate a woman's diminished ability or inability of conception [4].

During fetal life, germ cells rapidly proliferate by mitosis to yield approximately 6 to 7 million oogonia by 16 to 20 weeks of pregnancy. From that point forward, the germ cell population begins an inexorable exponential decline via gene-regulated apoptosis. Transformed to oocytes after entering the first meiotic division, the number of germ cells present at birth is around 1 and 2 million in number and to about 300,000 to 500,000 by the onset of puberty. Between 35 to 40 years of reproductive life, only about 400 to 500 oocytes will ovulate; the rest are lost through atresia. During the reproductive years, the rate of follicular depletion is relatively constant and gradual until age 37 to 38 (when approximately 25,000 oocytes remain) and then accelerates over the 10 to 15 years preceding menopause. At the time of menopause, fewer than 1,000 follicles remain [5].

Menstrual characteristics in older women correlate with the number of follicles that are left. The ovaries of menstruating older women with regular periods contain 10-fold more follicles than those of perimenopausal women having irregular and infrequent menses; follicles are absent in the ovaries of postmenopausal women. Irrespective of age, the

interval from loss of menstrual regularity to menopause is approximately 5 years. Recently assessment of ovarian reserve to determine the protocol for treatment of female infertility has become necessary. Traditionally, age, follicle stimulating hormone (FSH), estradiol (E2) levels and antral follicle count (AFC) by transvaginal ultrasonography (TVS) at the early follicular phase have been used for evaluation of ovarian reserve. For years the levels of FSH and E2 were considered to be specific n sensitive biochemical markers for assessment of low ovarian reserve. However, it has been found that the FSH level is above the norm only in cases when the ovary function is largely decreased. Later stage identification of the AFC is considered to be more reliable in assessment of the ovarian reserve. Follicle count can be determined easily with the help of high resolution sonographic systems. Although, there are well-known difficulties in obtaining correct AFC such as high inter-observer differences and anatomical variations. It has been suggested that AFC predicts poor response much better than basal FSH. Thus, by some investigators AFC is considered as the first choice test. Of late, anti-Mullerian hormone (AMH) levels became very important in assessment of ovarian reserve.

AMH, also known as Mullerian-inhibiting substance, is a dimeric glycoprotein that belongs to the transforming growth factor – β family. In reproductive-aged women, AMH is expressed by small antral follicles. It is manifested by granulosa cells of the ovary. In the ovary AMH inhibits initial primordial follicle selection and decreases the sensitivity of pre-antral and small antral follicles to FSH. In comparison with other ovarian reserve assessment tests, AMH is characterized by a number of benefits. AMH levels are constant throughout the menstrual cycle and therefore can be measured at any day of the cycle. AMH levels are not affected by other hormonal variations, including the use of oral contraceptives [6]. Recent studies have shown that follicular depletion doubles when the primordial follicle amount is approximately 25,000. Women reach this physiological condition at the ages of 37-38 years. This age is determined as critical, after which there is a sharp reduction in the ovarian reserve. This trend is individual and changes in ovarian reserve can be associated not just with age. Thus, only a woman's age is insufficient to determine ovarian reproductive potential. This suggests the need for practical implementation of individual biological age-specific ovarian reserve tests, which can be highly reliable in assessment of a woman's ovarian reserve and reproductive potential at the early stages of infertility.

MATERIALS AND METHODS

This prospective study was performed during period of Aug 15 to Sep 16 at ART centre, INHS Asvini Sample size- 112 infertile women who underwent infertility treatment. Women who visited infertility clinic, consent was taken from them for being worked up were first subjected to a detailed history followed by clinical examination. TVS was done on day 2 of spontaneous menstrual cycles by same investigator throughout study. The numbers of antral follicles that measured 2-10 mm in size counted in each ovary. The sum of both counts was the AFC of the patient. Blood sample collected on same day for FSH n AMH levels. Long luteal protocol of controlled ovarian stimulation was selected for all the patients. In which from day 21 of menstrual cycle GnRH agonist injection was started. Inj leuprolide 1 mg/1 ml is the GnRH agonist was given subcutaneously daily for approximately 10 days or until the onset of menses or gonadotropin stimulation. This dose is reduced to half that is 0.5 mg/ml from either Day 2 of menstrual cycle or after 10 days of inj leuprolide, until the hCG administration.

From day 2 of menstrual cycle (when leuprolide dose was reduced) ovarian stimulation was started with gonadotropin. Inj follitropin alpha is a gonadotropin was given as s/c daily till the 3 or more than 3 follicles attained size of > 18mm. Follicular monitoring was done at regular interval to observe growth of follicle. According to growth of follicle dose of follitropin alpha was moderated. When 3 or more than 3 follicles have attained size more than 18 mm, HCG trigger was given for ovulation. After 36 hours of HCG trigger (ovulation induction) ovum pick up was done and number of oocytes retrieved were noted.

Inclusion criteria-

- Patients undergoing in vitro fertilization within age group of 35 year

Exclusion criteria-

- Age>35
- Inability to retrieve all oocyte
- Empty follicle syndrome

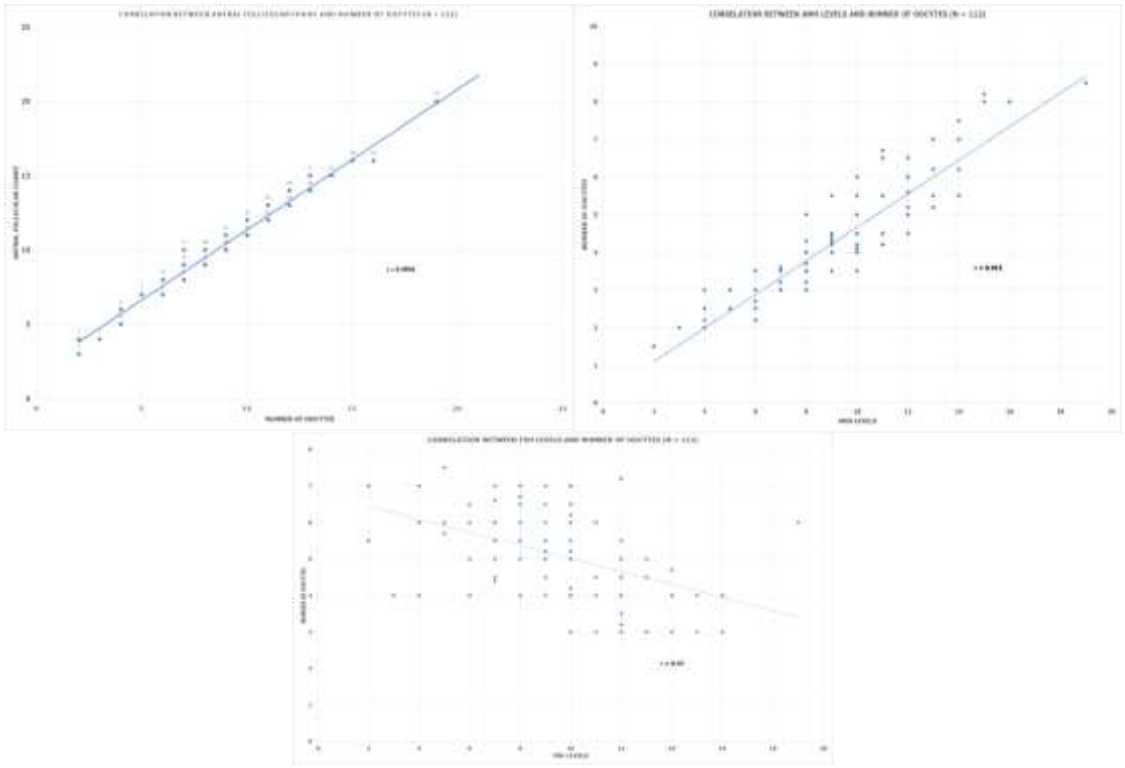
STATISTICAL ANALYSIS-

All the collected data then entered into the Microsoft excel sheet. It was then transferred to SPSS version 18 for statistical analysis. Correlation analysis performed with pearson's correlation.

RESULTS

Coefficients(a)						
Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	-1.371	0.377		-3.632	0
	Afc	0.901	0.038	0.884	23.522	0
	Fsh	-0.002	0.047	0	-0.041	0.967
	Amh	0.229	0.078	0.111	2.94	0.004
a. Dependent Variable: No oocytes retrieved						

- Oocyte retrieved shows positive correlation with AFC>>AMH
- Positive correlation with AFC (r=-0.71, p<0.0001).
- There was a significant negative correlation between FSH and oocyte retrieved



DISCUSSION

The presumed linkage in the relationship between baseline FSH and random AMH is that both hormones are indicators of ovarian reserve. The present study was meant to infer the variability of AMH during the menstrual cycle and that, if there is a direct feedback mechanism between these two hormones; rather, to find a relationship between their levels and with AFC and we believe that they are independent indicators of ovarian reserve. [7]. Baseline FSH level increases in infertility and our study also shows mean FSH level is 9.1 ± 2.51 mIU/ml, which is on the higher side. These Baseline FSH levels have for many years been used to predict a patient's response to ovulation induction and success with IVF [8]. Determinations of FSH, however, are characterized by many difficulties. One quite obvious problem is the inconvenience of a required blood draw on the day 2 or 3 of menses. The second issue of concern is the degree of cycle-to-cycle fluctuation in baseline FSH levels, at least partially caused by the dependency of FSH levels on the negative feedback from E2 level.

Anti-Müllerian hormone does not exhibit these difficulties. It is relatively stable throughout the cycle and therefore can be drawn at random. This is also evident by our study results which show AMH levels are not statistically different on day 3 and day 14 of the menstrual cycle. This makes serum AMH a more reliable test, as it is not constrained to a time frame for measurement. It is also said not to be affected by other hormonal variations, including the use of oral contraceptives [9]. In our study, we have noted a significant inverse correlation between serum AMH and subsequent baseline FSH on day 2 within the same menstrual cycle, which is similar to as previously noted in some other studies [10]. Serum FSH level on the third day of the menstrual cycle ensures the greatest

accuracy possible. Our study shows a positive correlation between AMH and AFC but not between FSH and AFC. It has been shown that compared with the indirect measurement of serum FSH, serum AMH also has a higher positive correlation of the oocyte count investigated in a study of IVF patient. A decrease in serum AMH level is detected 5 years before a difference in the serum levels of FSH or inhibin B is noticed. It has been reported that one of the advantages of serum AMH over other measures may be that it gives an earlier indication of a declining ovarian reserve. Although the present analysis does not address the utility of a random serum AMH value to predict cycle outcomes, but we can surely conclude AMH's role as a peripheral signal of the size of the growing follicle pool [12]. Thus, the possible explanation to our result can be that most of the decrease in AFC in the initial years, which correlated only with AMH, but not with FSH. Better understanding of a patient's AMH status would allow for physicians to create a better treatment plan for the infertile patient. Therefore, AMH may be used in conjunction with ultrasonography to ensure the best possible outcomes, though it is undecided if the levels of serum AMH are an indicator

CONCLUSION

The AFC levels and AMH should be considered as more reliable. Measuring AMH levels in combination with AFC may improve the assessment of ovarian reserve for evaluating fertility potential and monitoring infertility treatment

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