Original research article

Comparative Study Between Anti-Mullerian and Follicle-Stimulating Hormone in Workup of Infertile Female

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Abstract

Objective: The aim of our study is to compare the relationship between serum FSH and AMH in infertile women and to observe their relationship with antral follicle count ,so as to determine which is a better predictor of infertility

Materials and Methods: This study includes 75 infertile women in age group 25-40 years. Blood samples were taken at day three for serum AMH and FSH levels, and AFC was done. AMH was estimated again on day 14 of the menstrual cycle.

Results: Mean serum AMH and FSH were 1.18 ± 0.57 ng/ml and 9.09 ± 2.51 mIU/ml on day three of menstrual cycle. Mean AMH levels on day fourteen was 1.12 ± 0.53 ng/ml, which was not significantly different from day three AMH level. There was a significant inverse relationship between serum AMH and FSH concentration ($r = -0.488$, $P < 0.001$). Moreover, there was a positive correlation between AMH and AFC ($r = 0.641$, $P < 0.001$).

Conclusion: There was a significant inverse correlation between serum AMH and FSH levels in infertile women. AMH is considered by us as a better predictor of ovarian reserve because it is relatively stable throughout the cycle. Furthermore, there was positive correlation between AMH and AFC.It denotes reduction of AMH levels in serum is the first indication of a decline in the follicular reserve.

Introduction

Ovarian reserve refers to the size of the nongrowing ,or resting,primodial follicle of the ovary.This ovarian reserve,in turn,presumably determine the number of growing follicles and the "quality"or reproductive potential of oocytes.Ovarian reserve can be used as indirect measure of woman reproductive age.[1]

Infertility refers to the inability of a woman to become pregnant after having unprotected intercourse for a specified amount of time, usually, within a year. [2] There are many factors that contribute to infertility (i)decrease ovarian reserve(ii)ovulatory factors (iii)tubal causes (iv)uterine causes(v)systemic causes like infections or chronic disease such as obesity ,or chronic renal failure(vi)cervical and immunologic factors (vii) unexplained infertility .

Decrease fecundability and diminished ovarian reserve begins in the early 30sand accelerate during the late30s and early 40s,reflecting decline in oocytes quantity and quality, commonly refer to as ovarian reserve.[][] A woman's age is not the only factor determining her ovarian

reserve. It can decrease at younger ages which may be responsible for infertility. [3] . 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. [2]

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. [2]

The woman's age and assays of serum FSH in the early follicular phase were among the earliest and most useful parameters used for evaluation of ovarian reserve. [4],[5] Various ultra-sound parameters are also used for evaluation of ovarian reserve, including ovarian volume [6],[7] and the antral follicle count (AFC), with varying degrees of reliability. [8],[9]

Low levels of FSH are seen during follicle development and high levels during ovulation. The variation in levels of FSH is due to a feedback loop that exists between the hormones secreted from the ovaries and pituitary gland. [3]

Follicle stimulating hormone level also depends on the estradiol (E2) level. As antral follicles develop in the ovaries, they excrete E2 and inhibin B. An increase in these hormones signals the gonadotropins in the pituitary gland to discontinue the release of FSH. Once ovulation occurs there is a decrease in E2, which signals for an increase of serum FSH, which helps to prepare for the next cohort of follicles in the growing pool. This accounts for variability of FSH during the menstrual cycle. Day three FSH has been the most used test of ovarian reserve .It is also the standard way of determining ovarian reserve, providing greatest accuracy. [2]

The measurement of serum AMH level is a relatively new method. It is being considered for determination of the ovarian reserve, since it gives more direct and accurate measurement. In women, AMH is produced by the granulosa cells (GC) of follicles. It is produced from the stage of the primary follicle to the initial formation of the antrum. Thus it is distinct from ovulation and is a step closer to being able to assess the true ovarian reserve. [10] AMH appears to accurately measure the active follicle pool, as active follicles only produce AMH. This is the basis of using AMH for determining ovarian reserve. [3]Unlike other serum markers,AMH can be measured at any time in the menstrual cycle and levels are best interpreted using age specific references ranges.

La Marca *et al*. showed that serum AMH level, unlike other ovarian reserve tests, do not change significantly throughout the menstrual cycle. [11] Other studies have also confirmed that the intercycle and intracycle variability of serum AMH levels is not very significant.So, measurement of AMH can be done at any time during the menstrual cycle. Hence, it has been suggested that serum AMH values are more convenient and more effective than other serum ovarian reserve tests like FSH and inhibin B or E2. [12] It is said to be the best single indicator of response to fertility drugs. Several studies have shown that serum AMH is an excellent marker to determine ovarian responsiveness in an *in vitro* fertilization (IVF) program. AMH serum levels were also shown to correlate with the number of oocytes retrieved upon ovarian stimulation. [13]

Therefore, we have planned this study to determine the variability in the levels of serum AMH on two different days of menstrual cycle and compare the relationship between serum AMH and FSH (which is traditional marker of ovarian reserve) levels in infertile women and their relation with AFC as to determine which is a better predictor of infertility.

Materials and methods

The study was approved by the Ethics Committee of Patna Medical college. All patients were selected after obtaining informed consent. The study was a prospective study, conducted in the outpatient unit in Department of Obstetrics and Gynaecology, PMCH PATNA between June 2019 and June 2020. The study included 75 infertile females with poor ovarian reserve. The group was recruited by selective sampling. Information about body mass index (BMI), alcohol consumption, chronological age, age at menarche, menstrual cycle was recorded using a case report form.

Women attending obstetrics and gynaecology outpatient department with complaints of infertility were evaluated for poor ovarian reserve by transvaginal sonography (TVS). Infertility was defined as the inability of a woman to become pregnant after having unprotected intercourse for a specified amount of time, usually, within a year. [2] Poor ovarian reserve was defined as the number of early antral follicles 2-10 mm in diameter on TVS. [14] 75 infertile women positive for above findings and meeting the following criteria were included in the study:

INCLUSION CRITERIA:-

(i) Age group of 30-40 year age
(ii) regular, ovulatory menstrual cycles every 21-35 days;
(iii) both ovaries present;
(iv) no current or past diseases affecting ovaries or sex steroid secretion, clearance or excretion; (v) a BMI 19-25 kg/m ^{2} ;
(vi) no current hormone therapy; and
(vii) adequate visualization of ovaries at TVS.

EXCLUSION CRITERIA:-

(i) patient with PCOD
(ii) Abnormal uterine bleeding
(iii) Evidence of endocrine disorder (abnormal TSH ,Prolactin,Testosterone and LH)
(iv) patient already treated for infertility
(v) current and past disease affecting ovaries

Blood samples were taken on day three of the menstrual cycle in all women. Serum was assayed for FSH and AMH concentration. Sample was taken again on 14 days of the menstrual cycle in these women and analysed for AMH. Serum levels of AMH were determined by a the ultrasensitive AMH/Müllerian inhibiting substance enzyme linked immunosorbent assay [15] and FSH is estimated by ADVIA Centaur CP autoanalyzer by chemi-luminescence immunoassay. [16]

Statistical analysis

Analysis was performed using the latest available SPSS(statistical package for social sciences) version for Windows. The results are expressed as mean and standard deviation. The comparison between AMH level on day 3 and day 14 of the menstrual cycle was done

by *t*-test. Pearson correlation and linear regression analysis was conducted between serum AMH, serum FSH level and AFC. P <0.05 was considered as significant.

Results

The mean age of infertile women with diminished ovarian reserve was 35.39 ± 2.46 years. Mean FSH level in these 75 infertile women was 9.10 ± 2.51 mIU/ml, with a range of 4-15.6 mIU/ml. The mean AMH level on day 3 was 1.18 ± 0.58 ng/ml, with a range of 0.3-2.7 ng/ml and on day 14 was 1.12 ± 0.53 ng/ml. Other baseline characteristics of infertile women are shown in [Table 1]. Comparison of mean of AMH on day 3 and day 14 was not statistically different. Although AMH and FSH are not directly dependent on each other, there was a significant inverse relationship between serum AMH concentrations and serum FSH concentration ($r = -0.448$, $P < 0.001$) was taken on day 3 of menstrual cycle. This correlation is shown in [Figure 1].

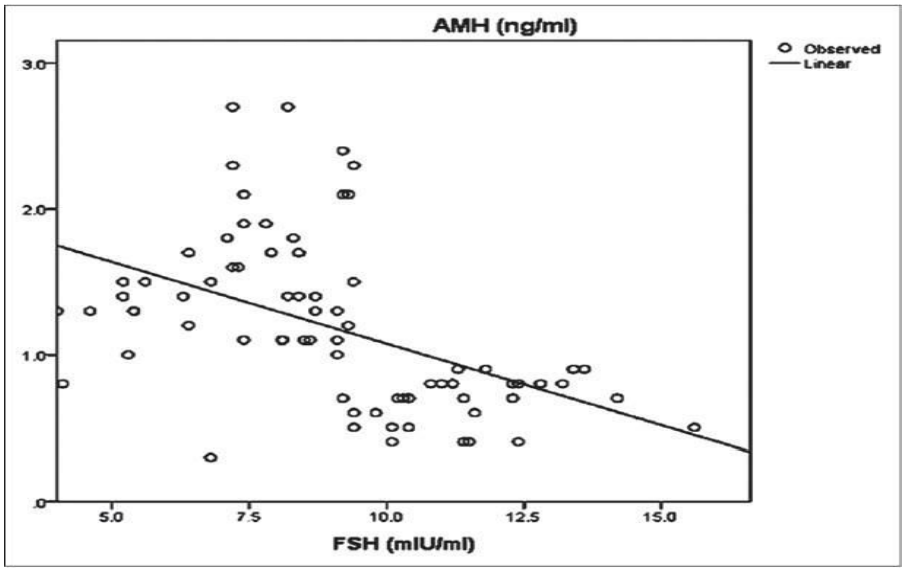

Fig 1: relationship between serum AMH and FSH

Table 1: Baseline characterstic of study group

Parameter	Mean±SD	Range
Age (years)	35.39±2.46	30-40
BMI (kg/m^2)	23.26±1.47	18.6-25
Days of menstrual cycle	28.50±1.37	26-33
AFC (mm)	6.4±2.5	2-10
AMH on day 3 of menstrual cycle (ng/ml)	1.18±0.58	0.3-2.7
AMH on day 14 of menstrual cycle (ng/ml)	1.12±0.53	0.3-2.5
FSH on day 3 of menstrual cycle (mIU/ml)	9.10±2.51	4-15.6

SD: Standard deviation, BMI: Body mass index, AFC: Antral follicle count, AMH: Anti-mullerian hormone, FSH: Follicle stimulating hormone

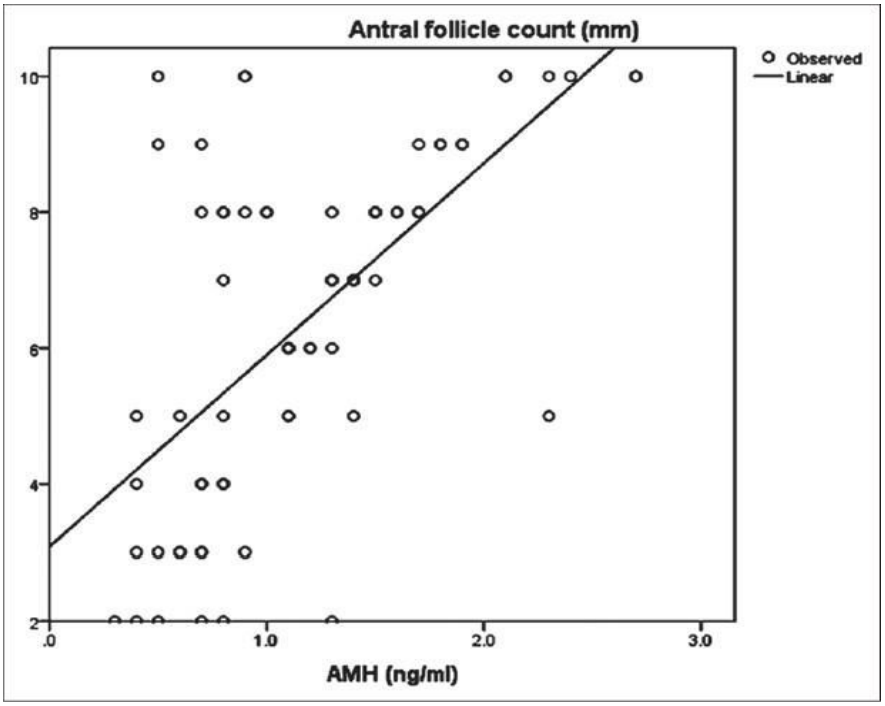

Fig 2: Relationship between serum Anti-mullerian hormone and Antral follicle count(r^2=0.411, p<0.001)

Discussion

The presumed linkage in the relationship between baseline FSH and random AMH is that both hormones are indicators of ovarian reserve. This present study is meant to derive the variability of AMH during the menstrual cycle ,and if there is a direct feedback mechanism between these two hormones

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. [17] Determinations of FSH are characterized by many difficulties. Firstly and the most ovious problem is inconvenience of a required blood draw on the day 3 of menses. Secondly it is the cycle-to-cycle fluctuation in baseline FSH levels, because FSH levels are partially dependent on the negative feedback from E2 levels. [2]

Anti-Müllerian hormone does not exhibit these difficulties. It is relatively stable throughout the cycle [11],[18],[19],[20] 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 restricted to a time frame for measurement. It is also not affected by other hormonal variations, including the use of oral contraceptives. [21],[22]

In our study, we have noted a significant inverse correlation between serum AMH and subsequent baseline FSH on day 3 within the same menstrual cycle ($r = -0.488$, $P < 0.001$), which is similar to as previously noted in some other studies. [23] Serum FSH level on the third day of the menstrual cycle ensures the greatest accuracy possible. [2] AMH null mice with low FSH levels was seen to present with a large number of growing follicles. This fact led to the hypothesis that, in the absence of AMH, follicles show a tendency to become more sensitive to FSH action. [24] Further, it has been reported that FSH and E2 down-regulate AMH gene expression in GC of rat follicles. [25] Thus, serum AMH level can, therefore, be used as a reliable biomarker of ovarian reserve (similar to serum FSH levels). [26] Another

study similar to our study also reported that serum AMH concentration shows a negative linear correlation with basal FSH levels in women who have a poor response to controlled ovarian stimulation with human gonadotropins. [27]

Our study shows a positive correlation between AMH and AFC ($r = 0.641$, $P < 0.001$) 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 patients. [28] Shebl *et al*. showed a correlation between serum AMH level and the quality of the oocytes retrieved from the ovary after hyperstimlation. [29] AMH knock-out mice showed three times more small non-atretic growing follicles, and a reduced number of primordial follicles compared with wild mice. [24] AMH in serum is said to be a direct measure of the primordial follicles that are available and show a decrease in the available pool before any other method, providing a huge advantage. A decrease in serum AMH level is detected 5 years before a difference in the serum levels of FSH or inhibin B is noticed. [2] 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. 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 of the quantity of the follicles or the quality in terms of the follicle maturity. [30]

Conclusion

Mean serum FSH levels were 9.10 ± 2.51 mIU/mL, which is in the higher range, as occurs in infertility. There was a significant inverse correlation between serum AMH and FSH levels in infertile women and we consider serum AMH, a better predictor of ovarian reserve as it is relatively stable throughout the cycle and can be assayed at different time during the cycle. Our study shows a positive correlation between AMH and AFC ($r = 0.641$, $P < 0.001$), but not between FSH and AFC denoting reduction of AMH levels in serum is the first indication of a decline in the follicular reserve of the ovaries.

The results reported here may not apply to other female populations as in this study females selected had diminished ovarian reserve. Future studies of the correlation between serum FSH and AMH should be conducted using normally fertile women.

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