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Research Article

RELATIONSHIP BETWEEN SERUM ANTIMULLERIAN HORMONE, FOLLICULAR STIMULATING HORMONE, AND LUTEAL HORMONE LEVELS WITH OVARIAN FOLLICULAR STATUS ON DAY THREE OF MENSTRUAL CYCLE

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ABSTRACT

Background: An assessment of the follicular reserve is essential to define the follicular status of the ovary. Various methods have been utilized to measure how many follicles are in the ovaries, including FSH, LH, AMH and AFC by Transvaginal ultrasound. This study aims to identify the correlation that exists between the different ovarian function markers. Objectives: To compare the relationship between serum AMH, FSH, and LH levels with AFC on day 3 of a menstrual cycle. Patients and methods: 100 infertile women in the department of obstetrics and gynecology at Al-Emameen Al-Khadimen Medical City were studied on day 3 of their menstrual cycle from April 1, 2014 to April 1, 2015. Measurements were taken for serum AMH, FSH, LH levels and AFC. Results: Median (range) serum levels of AMH, FSH, and LH were 2.45 ng/ml (0.24- 6.5), 5.6 mIU/ml (2.1-19.3), 2.3 mIU/ml (1.1-10.0) respectively, and AFC was 15 (1-35). Serum AMH levels correlated more strongly with follicular count ($r = 0.965$ $p < 0.001$) than levels of FSH ($r = -0.446$ $p < 0.001$) and LH ($r = 0.049$ $p = 0.625$). Conclusion: Serum AMH levels showed a stronger correlation with the AFC on cycle day 3. This is an indication that AMH may demonstrate ovarian follicular status better than the hormone markers usually used.

Keywords: Antimullerian hormone, follicular stimulating hormone, ovarian follicular status

INTRODUCTION

During fetal life, the indifferent gonad is composed of germ cells and at 6 to 8 weeks begins the differentiation to oogonia with a rapid mitotic multiplication. When the oogonia enter the first meiotic division arrested in prophase, they are transformed into primary oocytes. At approximately 18 weeks, a primary oocyte is encircled by a layer of epithelial cells (pregranulosa cells), creating the primordial follicle. At puberty, only 300,000 to 500,000 primordial follicles are left and undergo follicle maturation [1]

The hypothalamus secretes the peptide hormone gonadotrophin–releasing hormone (GnRH) in a pulsatile fashion to stimulate the pituitary secretion of the luteinizing hormone (LH) and the follicle stimulating hormone (FSH).

The initial stages of follicular development (primordial, primary, secondary and pre-antral) are independent of gonadotropin stimulation. FSH levels rise in the first days of the menstrual cycle when oestrogen and progesterone levels are low, which stimulates a cohort of small antral follicles on the ovaries [2]

The ovarian follicles consist of an oocyte surrounded by two cell types that are involved in the ovarian steroidogenesis (oestrogen and progesterone). These are the theca and the granulosa cells, representing an ovary's functional unit [3], which respond to LH and FSH stimulation, respectively. LH stimulates the production of androgen from cholesterol within theca cells. These androgens are converted into oestrogens by the process of aromatization in the granulose cells, under the influence of FSH [4].

The Anti-Mullerian hormone (AMH), which is also referred to as the Mullerian inhibiting substance (MIS), belongs to the transforming growth factor beta (TGF-β) superfamily [5]. AMH has a molecular weight of 140 Kda [6] and is a homodimeric, disulfide–linked glycoprotein. AMH is expressed quite intensely in Sertoli cells from testicular differentiation and accounts for the regression of the mullerian duct by a fetal life's eighth week. AMH may not play a role in the development of females, but granulosa cells produce it later in a female's life, which hints at the possibility of autocrine and paracrine action in the development of follicles [6].

AMH is expressed in granulose cells [7] from primordial follicles, with the highest expression in secondary, pre-antral and small follicles [8,9]. It continues to be expressed in an ovary's growing follicle until the antral stage, wherein the follicle is 4 – 6 mm [9,10]

Ovaries secrete AMH into circulation, so AMH can be measured in the serum and thereby reflect the ovarian pool. The decrease in the quantity of small antral follicles may precede a decrease in circulating AMH. AMH has recently been assessed as a possible clinical marker of ovarian reserve [12-16] Thus, peripheral AMH concentration can be used to reflect the quantity of oocytes that can be collected in ensuing ovarian stimulation cycles [17]

AMH is an excellent indicator for ovarian functional reserve because it is created by preantral follicles, which carry the potential of maturation, leading to an excellent correlation between the serum AMH level and the quantity of follicles holding the potential of maturation [6,18]

In past studies, the levels of serum AMH have been measured at various times throughout the menstrual cycle, resulting in an absence of fluctuation [1]. This is in line with the continuance of non–cyclic growth of small follicles during the cycle [19]

In the ovary, the major physiological role of AMH appears to be development inhibition of follicles during the early stages [20,21]

preventing a too early depletion of follicular reserves [22]. Furthermore, studies of the in vitro culture of mouse neonatal ovaries and human cortical strips have concluded that AMH plays an inhibitory role in primordial follicle recruitment [23]

According to other widely accepted theories, AMH might also act as a co-regulator of steroidogenesis in granulosa cells. This is because the estradiol level in follicular fluid from small antral follicles seems to be connected with AMH. It is likely that FSH induces a down regulation of AMH expression through rising follicle estradiol levels [24,25]

There are at a minimum three biological characteristics that AMH displays which are clinically worthy and not in alignment with the conventional hormonal predictors of ovarian reserve. First, AMH is exhibited in granulosa cells of small growing follicles [8,9] Second, follicles that grow past the early antral follicle stage begin to lose their ability to express AMH, largely avoiding AMH measurements from bias that are linked to early follicular development, which is especially common in ovarian-aged women. Third, the most recent studies have corroborated the theory that AMH production by ovarian follicles is likely FSH interdependent [25]

Therefore, peripheral AMH measurements could furnish useful information about follicle activity under little or no influence of the hormonal dynamics at luteal–follicular transition, which is the reason AMH does not show intra- and inter- cycle variation.

Transvaginal ultrasound assessment (TVS) of ovarian volume and antral follicle count (AFC) are methods that are both sensible and cost-effective for ovarian reserve determination, especially in women above 35 years, because they provide a correlation with chronological age that is stronger than day 3 FSH level when compared to other hormonal parameters [26]. AFC strongly correlates with serum AMH levels but has inter- and intra-cycle variations (27). AFC also does not reflect the pre-antral follicle pool. Thus, AMH could provide an assessment of the follicular pool that is more accurate [19, 28]. AMH levels should be considered in conjunction with a transvaginal scan of the ovaries to accurately assess ovarian reserve [29].

This study will compare the relationship between serum AMH, FSH, and LH levels with AFC on day 3 of the menstrual cycle of infertile women to discover which hormone is more strongly correlated to ovarian follicular status.

MATERIALS AND METHODS

This is a prospective study, conducted using 100 infertile women who were selected while attending an outpatient clinic in the department of obstetrics and gynecology at Al-Emameen Al-Khadimen Medical City from April 1, 2014 to April 1, 2015.This study was approved by the ethical committee of the Arab Board Scientific Council for Medical Specialties of Obstetrics and Gynecology (EC/2018/22). Verbal consent was obtained from all the women.

Patient inclusion criteria:

1. Aged 20 to 40 years.
2. Exhibiting a regular menstrual cycle of 25 to 35 days.
3. The presence of both ovaries.
4. No current or past diseases that could affect ovaries, gonadotrophin, or sex steroid secretion, clearance, or excretion.
5. Exhibiting a BMI of 18-29 kg/m^2.
6. Not currently participating in hormone therapy.
7. No history of chemotherapy or radiotherapy.
8. Adequate visualization of ovaries at transvaginal ultrasound scanning.

Demographic characteristics of each patient were assessed, including age, BMI, and day of menstrual cycle. Only those who were on day 3 of their menstrual cycle were included. A full history of each patient was taken, and a complete general examination was done, which included:

- Vital signs.
- A general examination.
- An abdominal examination to exclude any abnormalities.
- BMI, which was determined by dividing a patient's weight in Kilograms by the square of her height in meters.
- A transvaginal ultrasound was performed to assess AFC. The same technician then conducted an ovarian ultrasound with a 6.5 MHz transvaginal probe.
- A sample collection and preparation. For hormonal analysis, each patient was measured for serum AMH, FSH, and LH levels through blood sampling by venipuncture. Serum was collected and frozen at -2 degrees Celsius until it was needed for analysis. The AMH levels of the serum were identified using an enzyme-linked immunosorbent assay (ELISA), while the levels of serum FSH and LH were identified using mini-VIDAS.

Statistical analysis

Mean, standard deviation, range, and median was assessed. Pearson correlation was done between different parameters expressed as r (correlation coefficient) and p (level of significance). $p < 0.05$ was considered significant. The data were also expressed as box plot figures to demonstrate the distribution of data within the study group. Microsoft Excel 2013 and SPSS Version 17 (Statistical Package for Social Sciences) were utilized in the collection and analysis of results.

RESULTS

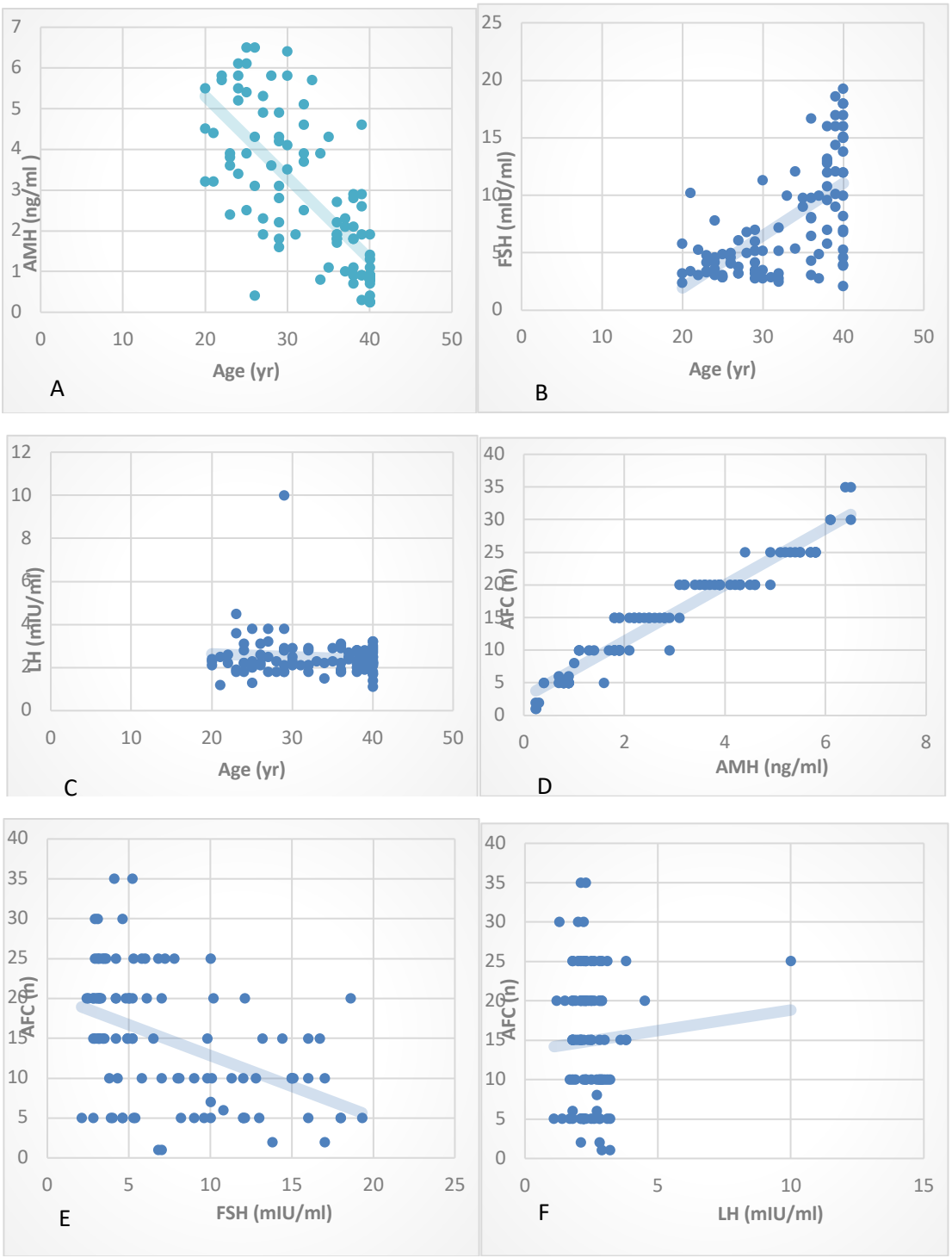
One hundred infertile women were included in the analysis. The demographic characteristics of the participants is shown in Table 1.

Table 1: General characteristics of the study group.

	Mean	Standard deviation	Range	Median
Age (year)	32.43	6.57	20-40	33.5
BMI (Kg/m2)	22.36	3.31	18-29	22
FSH (mIU/L)	7.6	4.74	2.1-19.3	5.6
LH (mIU/L)	2.47	0.96	1.1-10.0	2.3
AMH (ng/ml)	2.82	1.83	0.24-6.5	2.45
AFC (n)	14.91	8.19	1-35	15

The median age was 33.5 years (with a range of 20-40), the median BMI was 22 Kg/m^2 (with a range of 18-29), and the median length of menstrual cycle was 28 days (with a range of 25-35). Median serum levels of AMH, FSH, and LH were 2.45 ng/ml (with a range of 0.24-6.5), 5.6 mIU/ml (with a range of 2.1-19.3), and 2.3 mIU/ml (with a range of 1.1-10.0) respectively. The ultrasound scans that were conducted showed the median number of AFC to be 15 (with a range of 1-35).

The serum levels of AMH were negatively correlated with age ($r = -0.718$ and $p < 0.001$), as shown in Figure 1A and Table 2. This reveals a decrease of AMH serum levels with age.



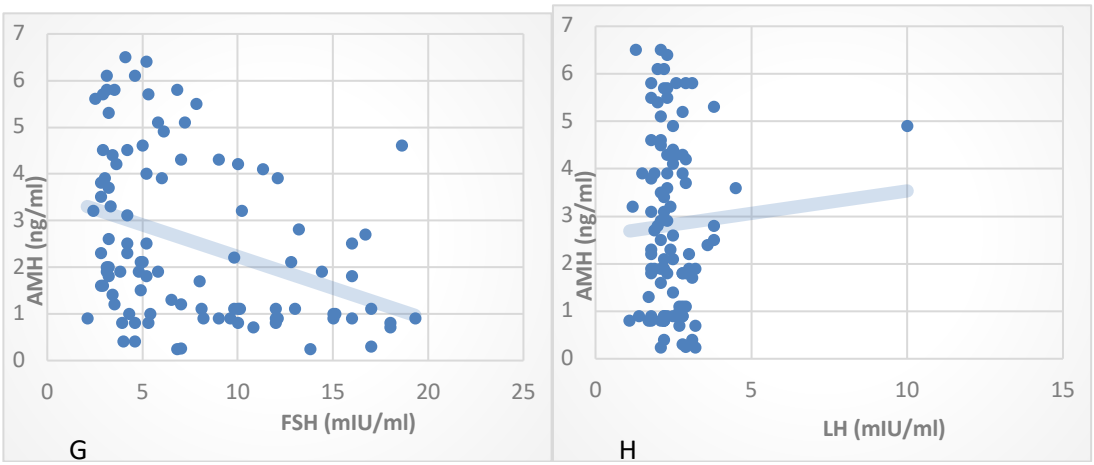


Figure 1: Correlation between AMH (A), FSH (B) and LH (C) with age. Correlation of AFC with AMH (D), FSH (E) and LH (F). Correlation of AMH with FSH (G) and LH (H).

Table 2: Correlation between AMH, FSH and LH with age. Correlation of AFC with AMH, FSH and LH. Correlation of AMH with FSH and LH.

	Age		AFC		AMH	
Measure	**r**	**p**	**r**	**p**	**r**	**p**
AMH	-0.718	< 0.001	0.965	< 0.001		
FSH	0.631	< 0.001	-0.446	< 0.001	-0.359	< 0.001
LH	-0.093	0.358	0.061	0.547	0.049	0.625

Serum levels of FSH show an increase with age ($r = 0.631$ and $p < 0.001$), as seen in Figure 1B and Table 2. There did not exist a correlation between age and LH levels ($r = -0.093$ and $p = 0.358$), as seen in Figure 1C and Table 2. The correlation between serum AMH levels and AFC ($r = 0.965$ and $p < 0.001$) was stronger than with serum levels of FSH and AFC ($r = -0.446$ and $p < 0.001$), as displayed in Figure 1D, Figure 1E, and Table 2). Serum levels of AMH and FSH were significantly correlated with AFC, but serum LH levels were not correlated ($r = 0.061$ and $p = 0.547$), as shown in Figure 1F and Table 2.

A negative relationship between AMH and FSH levels was discovered ($r = -0.359$ and $p < 0.001$), as shown in Figure 1G and Table 2. There was no correlation between AMH and LH levels ($r = 0.049$ and $p = 0.625$), displayed in both Figure 1H and Table 2.

DISCUSSION

In this study, the relationship between AMH, FSH and LH levels with AFC on day 3 of a menstrual cycle was evaluated, and the strength of the correlations were also compared. The levels of serum AMH were found to be correlated more strongly with AFC than with FSH and LH levels ($r = 0.965$ and $p < 0.001$).

In a study by Silva–Vera Marisol et al., there was a strong correlation between AMH levels and the quantity of antral follicles ($r = 0.303$) [30]. Dehghani et al. found an association between serum AMH levels in the early follicular phase and the ovarian reserve, while also discovering that heightened levels of serum AMH on day 3 were associated with chemical pregnancy success [31]. Ficicioglu et al. discovered a relationship between AMH levels and the quantity of oocytes collected [14]. In a study by Goksedef et al., the levels of serum AMH displayed a strong correlation with antral follicle count ($r = 0.400$), and this correlation was stronger than other ovarian reserve parameters [32]. Similar results were found by de Vet A et al. in a study on the relationship between AMH and antral follicle count, with a correlation of 0.71 [18]

A study by Barbakadze et al. concluded that AMH should be deemed a more reliable ovarian reserve assessment test than FSH due to its significant positive correlation with AFC ($r = 0.57$, $p < 0.001$). Thus, ovarian reserve evaluation could be enhanced if AMH combined with AFC was used [33]

Bala J et al. also found a positive correlation between AMH and AFC ($r = 0.641$ and $p < 0.001$). This then meant that a reduction of AMH levels in serum was the first indication of a declining follicular reserve [34]

In our study, AMH correlated more intensely with AFC than did the other hormonal markers of follicular status because AMH is expressed most strongly in the stage of pre-antral and small antral follicle (4 – 6 mm), while vanishing when follicles are bigger than 8 mm (11). Because the number of follicles decreases with age while the levels of AMH also decrease, we found a negative correlation ($r = -0.718$ and $p < 0.001$). This is similar to the study by Nardo et al., where AMH correlated positively with AFC ($r = 0.52$, $p < 0.0001$), and AMH and AFC correlated negatively with age ($r = -0.30$ and -0.27 $p < 0.001$) [35]. In the study by Barbakadze et al., there also existed a strong negative correlation between age and AMH levels ($r = -0.67$ and $p < 0.0001$) [33]. Thus, we conclude that the reduction in ovarian reserve occurs as a physiological process in a woman's late reproductive period and is routinely connected to a decrease in the levels of AMH [13]

Serum FSH levels were negatively correlated with AFC in the current study ($r = -0.446$ and $p < 0.001$), with similar results being reported by Fanchin et al. ($r = -0.29$ and $p < 0.001$) [36] and Goksedef et al. ($r = -0.292$ and $p < 0.001$) [32] These data support the theory that FSH plays a stimulating role in granulosa cells of the antral follicle. This occurs because FSH levels are dependent on the negative feedback from E2, as well as the possibility of a complex relationship with AMH when compared to other hormonal parameters [32]

The current study shows no correlation between LH levels and AFC ($r = 0.061$ and $p = 0.547$), a result that agrees with Fanchin R. et al. ($r = 0.05$) [36] and Goksedef et al. ($r = 0.067$ and $p = 0.42$) [32]. According to Jeppesen JV et al., LH receptor expression was

maximal in granulosa cells from the antral follicle, so LH seems to affect the development of human follicles largely during the follicular phase [37]. Still, the role of LH on pre-antral follicle development is limited, in that they require LH in low concentrations to proceed through antral development.

AMH is also created by follicles that are hardly sensitive to FSH, which includes pre-antral follicles. Thus, AMH might be a more independent and reliable marker of early antral follicle activity than FSH on day 3 of a menstrual cycle [36]

A negative relationship was observed between FSH and AMH in the current study ($r = -0.359$ and $p < 0.001$). These data dispute the stimulating role of FSH on granulosa cells producing AMH [32] observed in the study by Yu Yet al., where FSH mainly enhanced the development of medium sized follicle (3 – 5 mm) in goats by increasing the production of Insulin Growth Factor I and steroids as a way to inhibit the apoptosis of granulosa cells [38]. But they do not dismiss its possible inhibitory action on AMH production. AMH is able to inhibit FSH responsiveness, so AMH reduction is an important requirement of follicle dominance selection. A study by Panidis D et al. discovered an inverse correlation between serum AMH and FSH levels in older women who had abnormal or exhausted follicular development. This led them to use AMH as a marker for ovarian reserve [39]

In our study, we did not find a correlation between LH and AMH levels ($r = 0.049$ and $p = 0.625$), which is aligns with the findings of Fanchin R et al. (36). We found that cells from normal ovaries usually produce a very little response to LH, while LH stimulates the production of AMH fourfold in cells from PCOS [40]

CONCLUSION

The results of the present study show that the levels of serum AMH are significantly correlated with ovarian reserve during the early follicular phase. This correlation is more significant than the correlations existing between other hormonal markers of follicular function. We can conclude that serum AMH measurement on day 3 of a menstrual cycle does a better job at predicting the number of antral follicles than most other hormone measurements that are currently in use.

Abbreviations

Abbreviation	Complete name
AFC	Antral Follicle Count
AMH	Antimullerian Hormone
ART	Artificial Reproductive Technology
E2	oestradiol
FSH	Follicular Stimulating Hormone
GnRH	Gonadotrophin Releasing Hormone
LH	Luteal Hormone
MIS	Mullerian inhibitory substance
TGF-β	Transforming growth factor beta
TVS	Transvaginal ultrasound
U/S	Ultrasound

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