
Inverse Correspondence of AMH and FSH levels in Women Presenting for Infertility Treatment

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Ovarian reserve refers to the finite amount of follicles, responsible for the support of the oocytes, in the human ovary. From this pool, a dominant follicle is selected to enter into the menstrual cycle, where the oocyte can be fertilized. This process is dependent on the interrelationship of many hormones, such as Follicle Stimulating Hormone (FSH) and Anti-Mullerian Hormone (AMH). FSH is a major component in a feedback loop that exists between hormones secreted from the ovaries and pituitary gland. This inverse relationship allows the ovaries and brain to communicate. AMH is produced by granulosa cells that surround the oocytes. Abnormal levels of these hormones may indicate poor quality or quantity of the ovarian reserve. Preferred methods of determining the ovarian reserve consider the patient's chronological age, the level of Day 3 FSH, and the follicle count through transvaginal ultrasound. The measurement of serum AMH levels is a relatively new method that is being considered for determination of the ovarian reserve, giving a more direct and accurate measurement. A negative association between circulating AMH and FSH levels in reproductive aged women is examined. This study illustrates AMH levels presenting the same indication of the FSH levels to the ovarian reserve.

Introduction

At birth, the human ovary contains a finite amount of primordial follicles that are responsible for the support of the oocytes. This pool of available follicles is referred to as the ovarian reserve. Folliculogenesis occurs when the follicles leave the primordial pool and develop into preovulatory follicles in the growing follicle pool. From this cohort, one dominant follicle passes into the menstrual cycle, where the oocyte can be fertilized. Follicle development is dependent on the interrelationship of many hormones, such as Follicle Stimulating Hormone (FSH) and Anti-Mullerian Hormone (AMH), secreted from the anterior pituitary gland and the ovaries, respectively (1). Abnormal levels of these hormones may indicate a woman's diminished ability or inability of conception. 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. Approximately 10% of the reproductive aged female population, accounting for about 6.1 million women, in the United States is affected by infertility. There are many factors that contribute to infertility, including but not limited to the quantity and quality of the ovarian reserve. Couples struggling to become pregnant may consider fertility treatments, including drugs and/or surgery (2). Since treatments can be financially and emotionally draining, it is important to consider the patient's ability of becoming pregnant based on the determination of the ovarian reserve. The preferred methods consider the patient's chronological age, the level of Day 3 FSH, and the follicle count through transvaginal ultrasound. The measurement of serum AMH levels is a relatively new method that is being considered for

determination of the ovarian reserve, giving a more direct and accurate measurement. The aim of this study is to demonstrate the association between circulating AMH and FSH levels in women of different reproductive ages.

Methods Used to Determine Ovarian Reserve

Age

Recent trends show an increase in women who have started to plan childbearing later in life, during their 30s and 40s. Although studies show there is a high reduction of follicles after the age of 35, the diminishing rate of follicles available significantly varies among women of all ages. When the hormones stop signaling the production of follicles and the ovarian reserve is completely depleted, this is known as menopause, the final phase of a woman's reproductive life (3). This occurs around the average age of 51, but can occur earlier or even later (4). Therefore, chronological age can be a misleading factor for establishing the ovarian reserve.

FSH

FSH is needed to protect a portion of the follicles in the growing follicle pool from atresia, stimulate the follicles to grow, and to select the highest quality follicle from its cohort to begin ovulation. Low levels of FSH are found 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. As antral follicles develop in the ovaries, they excrete Estradiol (E2) and Inhibin B. An increase in these hormones

TABLE 1

Descriptive Statistics						
Column	Size	Range	Max	Min	Mean	Std. Error
Age	21	11.80	15.20	3.40	7.15	0.55
FSH	21	8.47	8.54	0.07	3.06	0.57
AMH	21	15.00	41.00	26.00	35.24	0.84

signal the gonatropins in the pituitary gland to discontinue the release of FSH. Once ovulation occurs there is a diminish in E2, which signals for an increase of FSH to be released. An increase in FSH helps to prepare for the next cohort of follicles in the growing pool (5). This inverse relationship is a way for the ovaries and brain to communicate. Poor communication can be an indicator of poor follicle quantity (5,6). The Day 3 FSH test measures serum FSH levels on the third day of the menstrual cycle to ensure the greatest accuracy possible. However, this is an indirect measurement considering FSH is dependent on other hormones of the feedback loop.

Transvaginal Ultrasound

Ultrasonography used in conjunction with other tests, such as Day 3 FSH, may provide a more accurate reading (4). Transvaginal ultrasound measures the antral follicle count (AFC), when follicles are in an advanced stage of folliculogenesis (3). This method is a subjective evaluation, and does not always provide an accurate follicle count (7). It has not been proven that any method used in determining the ovarian reserve is completely reliable. The two most widely used methods are the ultrasonographics and FSH, both suggest the AFC (4). A method that measures the ovarian reserve directly would be useful.

AMH

A proposed test for ovarian reserve is the circulating levels of AMH. AMH is produced by the granulosa cells that surround the oocytes in the primordial follicles and suppresses follicle recruitment. As the granulosa cells enlarge, the level of AMH is diminished, and the follicles enter the growing pool and become regulated by FSH (8). Considering AMH is directly involved with the primordial follicles, it is no surprise that studies show a significant correlation with AFC (4). The measurement of AMH serum levels may be a more direct and accurate method of determining the ovarian reserve.

The aim of this study is to demonstrate the inverse relationship between AMH levels and, a commonly used method, FSH levels from a collection of blood samples in patients undergoing treatment for infertility.

Materials and Methods

Patients

The study population consisted of 21 infertile patients ranging in ages 26-41 with normal menstrual cycles. The blood samples were collected on the third day of the patient's

untreated cycle for AMH and FSH so an accurate comparison could be made regarding the hormones. Women presenting with polycystic ovary syndrome (PCOS) were excluded from the study.

Hormone Assay

The patients' blood were collected in serum separator tubes. The samples were allowed to clot, centrifuged, decanted, and stored at -20°C in cryotubes. The tubes were thawed to be analyzed. The serum levels were run in duplicate on a DSX™ Four-Plate Automated ELISA Processing System (Dynex Technologies; Chantilly, VA) per manufacturer's instructions (Beckham Coulter, Brea, CA). The limit of detection (LoD) was 0.08 ng/mL with a 95% probability and the limit of quantitation (LoQ) is 0.16 ng/mL at a total imprecision of 20%.

Statistical Analysis

The initial FSH tests were conducted to determine the outcome of hormonal treatments of IVF patients. The student's test was performed to examine relationships between FSH, AMH, and chronological age.

Statistical calculations were performed with SigmaPlot for Windows, version 11.00 (Jandel Scientific Corporation, San Rafael, CA) and MedCalc, version 11.4.20 (MedCalc Software, Belgium). Linear regression analysis was used to investigate the relationships between the measures of the study. All the linear regression graphs had 95% confidence intervals.

A receiver operator curve (ROC) used the mean levels of FSH to consider probable ranges of AMH that can be used for analysis.

Results

A statistical summary for each of the parameters considered in the study are illustrated in Table 1.

In Figure 1, a relationship between FSH and chronological age is demonstrated. There is a significant 33% increase in FSH levels from the the <30 age group to the 31-36 age group. Another 26% increase occurs at the age group >36. FSH levels increased at predictable rates with the increase of age.

A correlation is not found between AMH levels and chronological age due to the relatively low sample size. AMH levels decreased by 40% from the range of women classified in the range of <30 to the range of 31-36 years. The levels decreased only by 4% for the women >36 years. The results are illustrated in Figure 2.

The regression analysis is used to show any linear correlation. Figure 3 shows a correlation between serum AMH and FSH levels.

The ROC determined 0.88 as the AMH cut off for the range of levels. The range was rounded up to include the ranges of above one (>1) and below one (<1), as illustrated in Table 2.

TABLE 2

Receiver Operator Curve Results			
Group	Size	FSH Mean	SEM
<1 AMH	6	9.53	1.29
>1 AMH	15	6.20	0.38

Discussion

The results for the levels of FSH corresponding with age are as expected. Age affects the quality of antral follicles, and therefore the FSH levels show the decrease in quality (10). It has been found that even when FSH is measured on the third day of the cycle, the levels can vary significantly from month to month (3). In these types of cases it would be difficult to indicate a normal level of FSH for the patient, and the results may be misleading. During menopause, the antral follicle count (AFC) is not sufficient enough to produce enough Inhibin B to reduce the level of FSH. The high level of FSH can not be seen until the patient has reached menopause (12). Therefore, FSH may be used as an indicator of the ovarian reserve, but the response to the feedback loop is prolonged and does not reveal the future ovarian reserve lifespan. Although AMH and FSH are not directly dependent on each other, there is an inverse correlation. When these hormones are measured and used as indicators for fertility treatment outcomes, AMH predicts a greater percentage of successful pregnancies when AFC is considered (8). Considering the faults that are found in Day 3 FSH testing, and the correlation that is found between AMH and FSH, it only makes sense to use the more direct and reliable measurement of the two hormones.

Although a decrease in AMH levels are seen in older women, it is seen in women throughout all of the age ranges. AMH is directly related to the ovarian reserve because it is secreted from the follicles in the primordial pool. The ovarian reserve that a female is born with and the degree of decline of the primordial follicle pool throughout the lifespan varies significantly among every female. Yet, throughout the cycle, AMH levels remain consistent (7). This makes AMH a more reliable test that is not constrained to a time frame that it must be measured during the menstrual cycle. AMH is a direct measurement to the primordial follicles that are available and show a decrease in the available pool before any other method, providing a huge advantage. This diagnostic test can detect a decrease in AMH levels five years before a difference in the levels of FSH or Inhibin B are noticed (11). There is a correlation between AMH levels and the duration of prior menstrual cycles. One of the first signs of approaching menopause is the shortening of menstrual cycles. AMH shows a correlation with the (8). Better understanding of a patient's ovarian reserve would allow for physicians to create a better treatment plan for their patient. Therefore, AMH may be used in conjunction with ultrasonography to ensure the best possible indication of the ovarian reserve.

Compared with the indirect measurement of FSH, AMH also has a higher positive correlation of the oocyte count

investigated in a study of IVF patients (7). Many factors, such as age, play a role in a pregnancy's success that can effect the quantity and quality of the oocytes found in the follicles. There is a correlation found between the quality of the oocytes retrieved from an ovary after hyperstimulation (9). It is undecided if the levels of AMH are an indicator of the quantity of the follicles or the quality in terms of the follicle maturity (10). In any case, the measurement is significant.

Every lab has a different scale that considers a FSH cut off value normal for indication of a successful pregnancy. It is generally excepted that FSH under 10 mIU/mL (<10) is normal and above 10 mIU/mL (>10) is abnormal for a female during her reproductive years (7). It is critically important to set a range for AMH levels as well when determining the outcome for infertility patients. The ROC suggests 0.88 ng/ml as a cut off value for AMH levels determining normality. Rounded >1 ng/mL in AMH levels corresponds with the normal range for FSH levels, and <1 ng/ml in AMH levels with the abnormal range for FSH levels. This value is in the same range compared to other findings of the cut off value as 0.99 ng/ml and 1.05 ng/ml (9).

The first step in AMH being accepted as the most reliable indicator of ovarian reserve, and of infertility outcome is to prove that AMH is at least as good of an indicator as the commonly used tested FSH levels. This study shows the predicted and intended correlation.

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