Comparative advantage of anti-Mullerian hormone over other ovarian reserve metric (basal hormonal test) in prediction of fertility in women with varying menstrual cycle

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ABSTRACT

Objective: Anti-Mullerian hormone has been indicated as a novel biomarker for ovarian reserve assessment. This study aimed to determine the comparative advantage of serum levels of AMH, FSH, LH, E2, and LH/FSH ratio among women with varying menstrual cycles and duration of menstruation.

Material and Method: A total of 90 subjects, which consisted of sixty subjects and thirty healthy subjects as control, were recruited. Blood samples were collected on day 3 of the menstrual cycle and evaluated for ovarian markers using the ELISA technique. All data were analyzed using SPSS version 23.0.

Results: AMH and LH/FSH levels were lower in women with varying menstrual cycles than in the control group. FSH, LH, and E2 levels were significantly higher in women with varying menstrual cycles and flow duration than in the control group (P<0.05). AMH was negatively correlated with age (r= -0.72), BMI (r= -0.4), FSH (r= -0.3), LH (r = -0.2) (p<0.05) and E2(r= -0.2, p<0.05). Also age was positively correlated with FSH (r=0.5, p<0.05), E2(r=0.3, p<0.05) and BMI (r=0.4, p<0.05). The level of AMH was not significant with cycle length and days of flow (p>0.05). This implies that AMH can be measured independently of the cycle phase. This shows that AMH was lower in women with varying menstrual cycle with an increase in gonadotrophin and E2. The strong negative relationship between age and AMH implies that age is determining factor of ovarian reserve.

Conclusion: AMH combined with age and FSH may improve ovarian reserve evaluation, making AMH a better marker.

Keywords: Ovarian reserve, AMH, Fertility

INTRODUCTION

Most women are now postponing childbearing worldwide as a result of extensive use of contraception, desire for higher education, lack or disruption of employment, socioeconomic concerns, and the growing popularity of assisted reproductive technology (ART), which has given them the impression that female fertility may be manipulated at any stage of life (1). On the other hand, human fertility reduces with increasing age and more so in women as with increasing age, the quality and quantity of a woman's egg pool/ovarian reserve diminish. Many women currently seeking fertility treatments are in their advance reproductive age(s). More so, the treatment-seeking behaviour of sub-fertile couples in developing countries portends further delays.
A recent study conducted by Hidadz et al., 2019 (2) showed that many sub-fertile couples start seeking fertility treatment from herbal products and associated traditional services. Through religious leaders, the woman will eventually be advanced in age by the time they visit ART centers. Epidemiological data have consistently shown that fertility declines as early as the middle of the third decade (3), and female age remains the most crucial determinant of success (4) in an IVF program.

Age of women seemed to be a better predictor of success in IVF, but the emergence of the ability to measure AMH recently had added a great deal of spice into the daily practice in ART (5) and its ability to indicate ovarian reserve and predict ovarian response to stimulation has distinctly improved the planning of ovarian stimulation protocols, and so increased safety and efficiency as well as aiding in the counselling of patients. AMH is a far preferred biological marker that determines the ovarian reserve in women of all ages as compared to any other basal hormonal markers (5), and for that matter, IVF practitioners may prefer to have such a biological marker that can predict a patient's response to Controlled Ovarian hyperstimulation (COH).

Currently, a variety of tests (hormonal profiling) can be used to estimate a woman's ovarian reserve or the reproductive potential of the oocytes remaining within the ovary. Ovarian reserve testing modalities include antral follicle count (AFC), ovarian volume, early follicular phase follicle stimulation hormone (FSH) and oestradiol (E2) levels, inhibin B, the clomiphene citrate (CC) challenge test, and anti-Mullerian hormone (6). However, identifying which of these tests are most accurate in predicting the ovarian response to COH and the potential for predicting pregnancy is yet to be definitively established. As a result, practitioners currently rely on a variety of different tests as part of their infertility evaluation and to predict ovarian response to COH (7) (8). AMH is one of the most recent tests developed to measure ovarian reserve. AMH is a glycoprotein belonging to the beta-transforming growth factor (8-TGF) superfamily secreted from the granulosa cells of small ovarian follicles (9). In the developing female human embryo, the absence of AMH allows the Mullerian ducts to differentiate into the upper portion of the vagina, cervix, uterus, and fallopian tubes (10).

However, as early as 36 weeks gestation, female fetuses begin producing AMH, which steadily increases in production until follicles reach the antral phase (11). With increasing age, there is a steady decline in levels of AMH until it becomes undetectable, which correlates with the onset of menopause (12). This rise and fall of the AMH level correspond with the number of oocytes remaining in the ovary (13).

As a metric, AMH represents the pool of oocytes remaining in the ovary. It results in both consistent inter-and intra-menstrual cycle measurements (14). As a measure of ovarian reserve, AMH testing is recommended and routinely used in ART practice in Europe and North America. Although committee opinions from major organizations support the routine use of AMH, it is unclear what proportion of IVF centers in Nigeria are currently using AMH to guide IVF cycle management with other metrics of ovarian reserve (basal hormone tests). There are lots of researched and published works about the use of AMH in developed countries; however, no prior study has attempted to assess ART practitioners' perceptions and practice patterns about the use of AMH in Nigeria.

Infertility is a public health problem, and the prevalence in Nigeria appears to be increasing. Poor ovarian reserve, quality, and quantity of the primordial follicle in a female ovary are potential factors that may further increase the incidence of infertility in female subjects. It is not known whether varying menstrual cycles and changes in the duration of menstrual flow could affect AMH and reproductive hormones. The incidence of AMH and reproductive hormones abnormalities may be higher among women with varying menstrual cycles and duration of menstruation than in the general population.

Anti-Mullerian hormone (AMH) has been established to be a better biomarker for ovarian reserve assessment and more preferable by fertility specialists because it is not affected by the menstrual cycle even though it decreases with aging.

The evaluation of serum levels of the anti-Mullerian hormone is a novel biomarker in assessing ovarian reserve in females in Benin City.

Ovarian reserve refers to the number and quality of remaining primordial follicles, which measure women's reproductive potential or fecundity. Clinically, diminished ovarian reserve (DOR) has been defined as a reduced response to ovarian stimulation in women of reproductive age with regular menses and without any other anatomical or other non-hormonal reason preventing the ability to reproduce (15). It indicates a low number and impaired development of primordial follicles. Women with markedly DOR have low chances of conception with their gametes, even with assisted reproductive technique (16). Diminishing ovarian reserve is normal peri-menopausal and happens in women during their mid to late thirties and at times earlier (15). In 2002, 7.4 % of married women in the US experienced infertility (17). Specifically, 10.9% of US women aged 18 to 44 years, which is within the age range of the thesis study participants, experience impaired fecundity based on data from 2006 to 2010 (18). Premature ovarian failure (POF) affects 1% of the general population (19) and refers to women who reach menopause before the age of 40 by means other than medical intervention (20). The women (with intact, functioning ovaries and no other known reason for delayed conception) who do not meet the criteria for premature ovarian failure are classified as having diminishing ovarian reserve (15). An ideal ovarian reserve test should predict both ability and inability to have a live-born baby with or without treatment. In an ideal world, it should also predict the preservation of current levels of ovarian activity. This is particularly relevant in the present social climate when increasing numbers of women defer childbearing. However, the availability of a wide range of tests of the ovarian reserve at present suggests that there is no definitive test. There are two types of tests of ovarian reserve: static and dynamic. Static tests assess specific parameters relating to ovarian reserve at a single point in time and involve both ultrasound and biochemical parameters. Dynamic tests assess ovarian response to exogenous stimulation. Usually, this involves measurement of hormonal concentrations in a serum sample before and after stimulating the ovaries using FSH, clomiphene citrate (CC), or a gonadotrophin-releasing hormone (GnRH) agonist. There are several existing reviews on the tests of ovarian reserve.
Ovarian aging will have begun before women notice any clinical changes to their menstrual cycles; therefore, they are often unaware that they may be at greater risk of infertility. Ovarian reserve testing has been explored as a means to determine a woman's fertility potential and provide an assessment of ovarian aging. Although chronological age alone serves as a good marker of ovarian reserve, some women will experience a decline in their natural fertility sooner than average, while some older women may maintain above-average ovarian function. Identification of these two groups, in which ovarian reserve is inconsistent with chronological age, may be useful for counselling and planning treatment (23). Many tests of the ovarian reserve have been tried. However, testing has mainly been performed on infertile populations, with little data on the distribution in the normal fertile population. Ovarian reserve testing cannot be used to predict infertility or time to infertility; therefore, its application to the general population as a screening tool is untested. Most studies have used these tests to try to predict a woman's ovarian response and prognosis with fertility treatment and IVF. Overall, markers of the ovarian reserve have been shown to correlate with egg quantity and response to ovarian stimulation but not with egg quality. The most commonly used test of ovarian reserve is the cycle day three or basal FSH level. An elevated basal FSH level (≥ 14 IU/L) is the first sign of ovarian aging that can be detected in women and usually occurs in women aged 35 to 40 (24). Physiologically, the follicular pool is reduced to approximately 10% of the levels present at puberty (25). The rise in basal FSH is due to a loss in ovarian feedback (inhibin-A and B) as the available follicular cohort diminishes. Basal FSH levels are easy to obtain, and no special skills are required to perform the test or interpret the results; therefore, it is easily accessible. However, basal FSH levels are predictive for poor response to ovarian stimulation and non-pregnancy only when the levels are extremely elevated (23). Although a high threshold may improve the usefulness of the test in predicting a poorer prognosis, only a small number of women will have abnormal tests at this threshold. In addition, it has been associated with a false positive rate of 5% (23). Elevated basal FSH levels are also less predictive of pregnancy for women < age 35 (26, 27). An ovarian antral follicle count can be performed early in the menstrual cycle. Antral follicles between 2 mm and 10 mm can be identified by transvaginal ultrasound performed by an experienced sonographer using a vaginal transducer with a minimum frequency of 7 MHz (28). Antral follicles are sensitive to FSH and are considered to be representative of the available follicle pool. The number of antral follicles seems to correlate with the number of primordial follicles in the ovary, with a decline in primordial follicles being reflected in a lower number of antral follicles (29). In later reproductive years, the proportion of antral follicles to total follicles may increase as the ovary allows a higher proportion of follicles to be selected. This may reflect a loosening of the selection process (30). The decline in AFC may not be as steep as the decline in fertility. Although the decline in AFC is correlated with both the menopause transition and ovarian response to stimulation, it is not a good predictor of pregnancy (23). Anti-Müllerian hormone is produced by the granulosa cells of pre-antral and small antral follicles but not dominant follicles (31). AMH levels decrease with decreasing AFC, which, in turn, is a marker of the primordial follicle count. Levels remain consistent throughout the menstrual cycle (32) and become undetectable in women after menopause (33). Although AMH provides moderate value in the prediction of ovarian response in IVF, it is a poor predictor of pregnancy (23). The clomiphene challenge test is performed by administering 100 mg of clomiphene daily from day 5 to day 9 of the cycle. FSH is measured on day three and day 10. If an adequate response to clomiphene is generated, the rise in FSH will be suppressed by the release of estradiol and inhibin-B by developing follicles. Systematic reviews have not shown a benefit to the clomiphene challenge test over basal FSH or AFC (Broekmans et al., 2006). Inhibin-B and basal estradiol are not more useful predictors of poor response or pregnancy than basal FSH (23). However, basal estradiol levels are often screened in conjunction with FSH and can confirm correct timing in the menstrual cycle. An elevated estradiol level may also falsely suppress FSH levels.

Ovarian reserve tests performed before ART treatment may be useful for counselling, but they have a poor predictive power for pregnancy (31). AMH is useful for the prediction of poor ovarian response with IVF (31). Although significantly abnormal results are associated with lower pregnancy rates (< 5%), only about 3% of women on ART treatment will have results in this category (23). In general, ovarian reserve testing is useful for predicting egg quantity and ovarian response to the stimulation but has little value for the prediction of egg quality. Therefore, although these tests may be useful for counselling before ART treatment, testing should not be used to exclude women from ART treatment, and abnormal tests do not preclude the possibility of pregnancy. These test results can be used to obtain individual prognostic information to help to guide the choice of treatment and best use of resources.

Ovarian reserve testing may be considered in women > age 35 to screen for age-related infertility, although its results may be useful only for counselling and to aid women in their decision-making process. Testing in women < 35 years may be considered if they have risk factors for decreased ovarian reserve, such as a single ovary, previous ovarian surgery, poor response to FSH, previous exposure to chemotherapy or radiation, or unexplained infertility. Although markers of ovarian reserve are not good predictors for pregnancy rate with ART for women < 35, (27) identification of these women may prompt shorter delay to infertility investigations and treatment.
MATERIAL and METHODS

This is a cross-sectional study of female participants with varying menstrual cycles attending fertility clinic in Central Hospital, Benin – City, Edo State, Nigeria, on day 3 for the measurement of AMH, FSH, LH, and E2 in Central Hospital, Benin-City, Edo State, South-South, Nigeria. The study was carried out in Benin-city, an urban area, the capital of Edo state, with a population of 1147188 according to the 2006 Nigeria census. It is located at latitude 6.340 N and longitude 5.600E with an altitude of 87.88m. Participants are educated, aged between 18-45 years, dark in complexion, occupationally engaged, with normal, overweight, obese, married, and unmarried. A total of ninety (90) subjects were recruited in this study; sixty (60) subjects recruited were within the age of 26-45years, while thirty (30) healthy women within the age of 18-25 years served as control. The sample size will be determined using the formula (34) with a 4% prevalence of AMH as an ovarian marker (35).

Known women of ages 18-45 with the varying menstrual cycle (cycle length-25-35days; duration of menstruation 2-7days), presence of both ovaries and lack of morphologic abnormalities, No evidence of endocrine disorders (normal level of TSH, FT4, protection, and testosterone). Not on any hormonal treatment for at least 3months, and a BMI ranging from 18-27 kg/m² all attending fertility clinic in Central Hospital, Benin City. Healthy females served as control. Women with a history of ovarian endometriosis, ovulatory factors such as polycystic ovary syndrome (PCOS), women with ages >45years were excluded from the study.

Blood samples were collected aseptically from the antecubital vein twice weekly from each female participant on day 3 of the menstrual cycle for the measurement of AMH, FSH, LH, and E2. Blood samples were centrifuged at 3,500rpm for 10 minutes, and all sera were stored at -200C until the time of analysis.

The research was designed to evaluate the comparative advantage of AMH over another ovarian reserve metric (Basal hormonal tests) in the prediction of fertility. The study was carried out within 12 months (May 2017- May 2018). Both qualitative and quantitative data were collected using a semi-structured self-questionnaire. The questionnaire has two (3) sections. Section A (Socio-Demographic characteristics), Section B. (Medical/family history). The questionnaire was distributed among female participants on day 3 of the spontaneous menstrual cycle.

Serum levels of AMH, FSH, LH, and E2 were measured using the ELISA method (Immunotech Beckman Coulter Laboratories, 16507, CA, USA).

Ethical clearance was obtained from the Edo state ministry of Health Benin City, Edo State, Nigeria. Informed consent was obtained from the individual subject before the commencement of the study.

Data from the study were analyzed using SPSS version 23.0 software. Results obtained are presented as mean±standard deviation (SD), the comparison between female participants on day 3 of menstrual cycle and control will be performed using student's unpaired t-test, chi-square, and correlation. The statistical significance will be set at p<0.05.

RESULTS

Table 1: Shown the socio-demographic variables of the ninety (90) subjects in the study revealed that 33.3% were between the ages of 18-25 years and 66.3% were between 26-45 years. Of the total subjects, 100.0% were females. In addition, 44.4% were singles, 44.4% were married, and 11.1% had divorced. Of the total subjects, 44.4% were traders, 11.1% were nurses, 22.2% were students, and 22.2% were medical laboratory scientists. Of the total subjects, 11.1% had a primary level of education, 33.3% had a secondary level of education, and 55.6% had a tertiary level of education. In addition, 50.0% were normal (BMI of 23.6kg/m²), 47.8% were overweight (BMI of 28.6 kg/m²) and 2.2% were obese (BMI of 33.5 kg/m²).

Table 2: Shown level of AMH, FSH, LH, E2 among female participants and its control group. It was observed that AMH was significantly lower (p<0.05) in the participants than the control group while FSH, LH, E2 were significantly higher in participants than the control group (P<0.05) except LH/FSH ratio, which was not statistically significant (p>0.05).

Table 3: Shows the correlation analysis between serum levels of anti-Mullerian hormones, age, body mass index, and sex hormones. It was observed that there was a significant negative relationship between AMH and FSH, LH, age, and BMI (p<0.05) except with E2 and LH/FSH (p>0.05). It was also observed that there was a significant negative relationship between age and AMH and a significant positive relationship with FSH, E2, and BMI (p<0.05) except with LH (p>0.05). In addition, it was observed that there was a significant negative relationship between BMI and AMH and a significant positive relationship with age (p<0.05) except with FSH, LH, and E2 (p>0.05).

Table 4: Shows a post-hoc (Bonferroni) multiple comparisons of AMH, FSH, LH, and E2 with days of menstrual flow. It was observed that levels of FSH and LH in subjects with 2-3days flow were statistically significantly higher than subjects with longer days of flow (p<0.05), while AMH and E2 were non-significant across the group.

Table 5: Shows a post-hoc (Bonferroni) multiple comparisons of AMH, FSH, LH, and E2 with the length of the cycle. It was observed that the levels of FSH and LH at 28–31 days and 24–27 days of the menstrual cycle, respectively, in subjects, were statistically significantly higher than subjects with a menstrual cycle of 32-35days (p<0.05), while AMH and E2 were non-significant across the group (p>0.05).
Table 1: Distribution of demographic factors of respondent

| Demographic Factors | Total N = 90 | Test N=60 | Control N=30 | X² | P-Value |
|---------------------|-------------|----------|-------------|----|---------|
| Age (Years)         |             |          |             |    |         |
| 18-25               | 30(33.3%)   | 0(0.0%)  | 30(100.0%)  | 12.84 | P=0.005** |
| 26-50               | 60(66.3%)   | 60(100.0%) | 0(0.0%)     |    |         |
| Marital Status      |             |          |             |    |         |
| Married             | 40(44.4%)   | 22(55.0%) | 18(45.0%)   | 20.00 | P=0.001** |
| Single              | 40(44.4%)   | 20(50.0%) | 20(50.0%)   |    |         |
| Divorce             | 10(11.1%)   | 6(15.0%)  | 4(10.0%)    |    |         |
| BMI (Kg/M²)         |             |          |             |    |         |
| Normal              | 45(50.0%)   | 25(55.5%) | 20(44.4%)   | 39.27 | P=0.001** |
| Overweight          | 43(47.8%)   | 23(53.5%) | 20(46.5%)   |    |         |
| Obese               | 2(2.2%)     | 2(100.0%) | 0(0.0%)     |    |         |
| Occupation Status   |             |          |             |    |         |
| Trader              | 40(44.4%)   | 20(50.0%) | 20(50.0%)   | 21.11 | P=0.001** |
| Nurse               | 10(11.1%)   | 7(70.0%)  | 3(30.0%)    |    |         |
| Student             | 20(22.2%)   | 12(60.0%) | 8(40.0%)    |    |         |
| Med.Lab.Scientist   | 20(22.2%)   | 14(70.0%) | 6(30.0%)    |    |         |
| Education Status    |             |          |             |    |         |
| Primary             | 10(11.1%)   | 8(80.0%)  | 2(20.0%)    | 26.67 | P=0.001** |
| Secondary           | 30(33.3%)   | 17(56.6%) | 13(43.4%)   |    |         |
| Tertiary            | 50(55.6%)   | 40(80.0%) | 10(20.0%)   |    |         |

Values in parenthesis are expressed in percentage, * Non-significant - p>0.05, **Significant – p<0.05

Table 2: Level of ovarian marker reserve(amh) and others reproductive hormones in women with varying menstrual cycle and duration of menstruation

| Parameters                        | Subjects | Control | p-value |
|-----------------------------------|----------|---------|---------|
| AMH (ng/ml)                       | 0.65 ± 0.76 | 3.31±1.49 | P<0.05  |
| FSH (mIU/ml)                      | 8.94±7.32  | 3.82±1.53 | P<0.05  |
| LH (mIU/ml)                       | 7.17±4.88  | 5.15±2.97 | P<0.05  |
| E2 (pg/ml)                        | 76.25±33.70| 63.20±15.68| P<0.05  |
| LH/FSH ratio                      | 1.14±1.01  | 1.49±0.91 | p>0.05  |

Table 3: Correlation of anti-mullerian hormone with other ovarian reserve metrics

| Parameters         | R-value | P-value |
|--------------------|---------|---------|
| AMH (age)          | -0.724  | P<0.05  |
| AMH/BMI            | -0.377  | P<0.05  |
| AMH/FSH            | -0.329  | P<0.05  |
| AMH/LH             | -0.225  | P<0.05  |
| AMH/E2             | -0.161  | P<0.05  |
| AMH/LH: FSH        | 0.100   | P>0.05  |

Table 4: Multiple comparison of ovarian reserve marker(amh), fsh, lh, and estradiol with duration of menstruation

| BONFERRONI DEPENDENT VARIABLE | (I) FLOW DAY (DAYS) | (J) FLOW DAY (DAYS) | Mean Difference (I-J) | Std. Error | P-value |
|--------------------------------|---------------------|---------------------|-----------------------|------------|---------|
| AMH                            | 2-3 DAYS            | 4-5 DAYS            | .95                   | 1.23       | 1.000   |
|                                | 6-7 DAYS            | -1.15               | 1.38                  | 1.000      |
|                                | 6-7 DAYS            | -2.11               | 1.28                  | 1.000      |
|                                | 4-5 DAYS            | 2.11                | 1.28                  | .310       |
| FSH(MIU/ML)                    | 2-3 DAYS            | 4-5 DAYS            | 2.35                  | 1.53       | .381    |
|                                | 6-7 DAYS            | 6.67                | 1.71                  | .001       |
|                                | 6-7 DAYS            | -2.35               | 1.53                  | .381       |
|                                | 4-5 DAYS            | 4.31                | 1.58                  | .024       |
|                                | 4-5 DAYS            | -4.31               | 1.58                  | .024       |
| LH(MIU/ML)                     | 2-3 DAYS            | 4-5 DAYS            | 3.97                  | .98        | .000    |
|                                | 6-7 DAYS            | 5.24                | 1.10                  | .000       |
|                                | 6-7 DAYS            | -3.97               | .98                   | .000       |
|                                | 4-5 DAYS            | 1.26                | 1.02                  | .656       |
|                                | 4-5 DAYS            | -5.24               | 1.10                  | .000       |
|                                | 4-5 DAYS            | -1.26               | 1.02                  | .656       |
| E2(PG/ML)                      | 2-3 DAYS            | 4-5 DAYS            | 31.44                 | 24.66      | .617    |
|                                | 4-5 DAYS            | 41.40               | 27.63                 | .413       |
|                                | 6-7 DAYS            | -31.44              | 24.66                 | .617       |
|                                | 6-7 DAYS            | 9.95                | 25.55                 | 1.000      |

*. The mean difference is significant at the 0.05 level.
Table 5: Multiple comparison of ovarian reserve marker (amh), fsh, lh, and estradiol with length of cycle

| BONFERRONI DEPENDENT VARIABLE | (I) CYCLE LENGTH (DAYS) | (J) CYCLE LENGTH (DAYS) | Mean Difference (I-J) | Std. Error | P-value |
|-------------------------------|-------------------------|-------------------------|-----------------------|------------|---------|
| AMH                          | 24-27 DAYS              | 28-31 DAYS              | 2.61                  | 1.46       | .233    |
|                              | 32-35DAYS               | 28-31 DAYS              | -3.4                  | 1.63       | 1.000   |
|                              | 24-27 DAYS              | 28-31 DAYS              | -2.61                 | 1.46       | .233    |
|                              | 32-35DAYS               | 28-31 DAYS              | -2.27                 | 1.20       | .185    |
|                              | 24-27 DAYS              | 28-31 DAYS              | -34                   | 1.63       | 1.000   |
|                              | 28-31 DAYS              | 28-31 DAYS              | -2.72                 | 1.20       | .185    |
|                              | 32-35DAYS               | 28-31 DAYS              | -4.35                 | 2.07       | .118    |
|                              | 32-35DAYS               | 28-31 DAYS              | -3.52                 | 1.52       | .001    |
|                              | 24-27 DAYS              | 28-31 DAYS              | -1.37                 | 1.86       | 1.000   |
|                              | 28-31 DAYS              | 24-27 DAYS              | 1.17                  | 1.86       | 1.000   |
|                              | 32-35DAYS               | 24-27 DAYS              | 5.52                  | 1.52       | .001    |
|                              | 24-27 DAYS              | 28-31 DAYS              | -4.35                 | 2.07       | .118    |
|                              | 28-31 DAYS              | 24-27 DAYS              | 1.17                  | 1.86       | 1.000   |
|                              | 32-35DAYS               | 24-27 DAYS              | -4.35                 | 2.07       | .118    |
|                              | 32-35DAYS               | 28-31 DAYS              | -3.52                 | 1.52       | .001    |
|                              | 24-27 DAYS              | 28-31 DAYS              | 2.62                  | 1.26       | .121    |
|                              | 28-31 DAYS              | 24-27 DAYS              | 4.96                  | 1.41       | .002    |
|                              | 32-35DAYS               | 24-27 DAYS              | -2.62                 | 1.26       | .121    |
|                              | 28-31 DAYS              | 32-35DAYS               | 2.33                  | 1.03       | .079    |
|                              | 32-35DAYS               | 24-27 DAYS              | -4.96                 | 1.41       | .002    |
|                              | 28-31 DAYS              | 32-35DAYS               | -2.33                 | 1.03       | .079    |
|                              | 24-27 DAYS              | 28-31 DAYS              | 68.74                 | 29.01      | .060    |
|                              | 28-31 DAYS              | 24-27 DAYS              | 76.98                 | 32.41      | .059    |
|                              | 32-35DAYS               | 24-27 DAYS              | -68.74                | 29.01      | .060    |
|                              | 28-31 DAYS              | 32-35DAYS               | 8.24                  | 23.78      | 1.000   |
|                              | 32-35DAYS               | 24-27 DAYS              | -76.98                | 32.41      | .059    |
|                              | 28-31 DAYS              | 32-35DAYS               | -8.24                 | 23.78      | 1.000   |

* The mean difference is significant at the 0.05 level. AMH—Anti-Mullerian hormone, FSH—a follicle-stimulating hormone, LH—Luteinizing hormone.

Figure 1: The correlation analysis between the serum AMH and FSH. The serum AMH levels are inversely correlated with FSH (r=-0.329**, p<0.05).

Figure 2: Correlation analysis between serum AMH and LH. The serum AMH levels are inversely correlated with LH (r=-0.225**, p<0.05).

Figure 3: The correlation analysis between serum AMH and E2. The serum AMH levels are inversely correlated with E2 (r=-0.161*, p>0.05).

Figure 4: Graph showing relationship of AMH and LH/FSH ratio.
Figure 5: Correlation analysis between serum AMH and BMI. The serum AMH levels are inversely correlated with BMI ($r = -0.377^{**}$, $p < 0.05$).

Figure 6: Correlation analysis between serum AMH and AGE. The serum AMH levels are inversely correlated with AGE ($r = -0.724^{**}$, $p < 0.05$).

Figure 7: Correlation analysis between serum FSH and AGE. The serum AMH levels are positively correlated with AGE ($r = 0.453^{**}$, $p < 0.05$).

Figure 8: Correlation analysis between serum LH and AGE. The serum AMH levels are positive weakly correlated with AGE ($r = 0.155^{**}$, $p > 0.05$).

Figure 9: Correlation analysis between serum E2 and AGE. The serum AMH levels are positively correlated with AGE ($r = 0.259^{**}$, $p < 0.05$).
DISCUSSION

Infertility is a problem of public health importance in Nigeria and many other developing nations. This is due to its high prevalence and its serious social implications on affected couples and families (36). This research was aimed at evaluating the comparative advantage of anti-Mullerian hormone (AMH) over other ovarian reserve metrics (basal hormonal tests) in the prediction of fertility in women with varying menstrual cycles and duration of flow. A total of ninety (90) subjects were recruited in this study; sixty (60) subjects were recruited within the age of 26-45 years, while thirty (30) healthy females within the age of 18-25 years served as control. Measurement of these hormones served as ovarian markers to assess ovarian reserve and its integrity in females in the area of study.

This study showed that the serum level of AMH was significantly lower (p<0.05), LH/FSH ratio was lower than control (p>0.05), while serum levels of FSH, LH, and E2 were significantly higher than control (p<0.05). The significantly lower serum level of AMH in this study could be attributed to ovarian aging since AMH level is closely related to the early atrial follicle count, which is dependent on reproductive aging, which is in line with the findings of (37). In the present study, it was found that serum AMH levels in women with varying menstrual cycles were reduced with advancing age showing a strong negative correlation (p<0.05) before changes in other markers (e.g., FSH, LH, and E2) were apparent. These results are in line with those of previous studies and suggest that AMH could be used as a novel marker of ovarian aging. A previous study had reported that the number of antral follicles seems to correlate with the number of primordial follicles in the ovary, in which a decline in primordial follicles result to non-ovulating, this is in agreement with the previous study (38). In this study, the serum level of FSH was significantly higher than control (p<0.05), which could be attributed to age, that women with advancing reproductive aging, the serum FSH levels begin to rise, which reflect a reduction in the number of early antral follicles present that can be recruited to ovulate, this is in agreement with (39), which was also supported by (32, 33) that AMH levels decrease with decreasing AFC, which in turn is a marker of the primordial follicle count, as well remain consistent throughout the menstrual cycle and become undetectable in women after menopause. This shows that AMH, age, and atrial follicle counts are better markers in ovarian reserve assessment.

Serum FSH levels could not be used only to predict the decrease in ovarian reserve, but as LH/FSH decreases, ovarian reserve begins to fall off because FSH level is known to increase more significantly than LH as ovarian reserve declines which were observed in this study. Therefore, LH/FSH ratio levels should serve as a good predictor of ovarian reserve and could be applied to the clinical evaluation with AMH.

This study examined body mass index and ovarian reserve markers to assess the association between BMI and ovarian reserve in reproductive-age women. It was observed that obese (BMI > 30) women had a significantly lower level of AMH (p<0.05) than normal (BMI<25) weight, age-matched women, respectively. FSH and E2 were not found to be associated with BMI, and the AMH differences appeared to result from physiological processes other than decreased ovarian reserve. This agrees with a previous study by (42).

In this study (43), the level of AMH was non-statistically significant (P>0.05) using post hoc multiple comparisons across the length of cycle and days of flow, this implies that AMH can be measured independently of the cycle phase which is in agreement with the previous study (43).

Recent years have shown how AMH is an invaluable tool offering new insights into ovarian function through the reproductive years. It is already clear that AMH is crucial in maintaining the right tempo of folliculogenesis in the ovary, making it one of the most important ovarian hormones and one of the most crucial factors underpinning female fertility. Whether its action is exclusively intra-ovarian, within, or between follicles is challenging for future research.

CONCLUSION

In conclusion, this study provides strong evidence that the serum AMH level is an important marker of reproductive aging in women. Further research of large-scale and longitudinal design is necessary to confirm our results.

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