Assessment of Serum Anti-mullerian Hormone Level, Antral Follicle Count and Age as Indicators of Ovarian Reserve Response in Women Diagnosed with Infertility in Abia State, South Eastern Nigeria

Ebirien-Agana S. Bartimaeus¹*, Chukwuma E. J. Obi², Felix O. Igwe¹ and Edna O. Nwachuku¹

¹Department of Medical Laboratory Science, Rivers State University, Nkpolu-Oworukwo, Port Harcourt, Nigeria.
²Department of Medical Laboratory Science, Federal Medical Center, Umuahia, Abia State, Nigeria.

Authors’ contributions

This work was carried out in collaboration among all authors. All authors were involved in the conception and design of the experiments. Author CEJO performed the experiments. Authors EASB and CEJO analyzed the data. Authors EASB and CEJO wrote the manuscript. Authors EASB, CEJO, FOI and EON proof read the manuscript. All authors read and approved the final manuscript.

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(1) Dr. Georgios A. Androutsopoulos, Assistant Professor, Department of Obstetrics - Gynecology, School of Medicine, University of Patras, Rion, Greece.

(2) Dr. Tchoffo Herve, University of Dschang, Cameroon.

(3) Dr. Juliano Augusto Brum Scheffer, Brazil.

(4) Dr. Victor Manuel Vargas-Hernandez, Mexico.

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ABSTRACT

Aim: This study aimed at assessing serum anti-mullerian hormone level, antral follicle count and age as indicators of ovarian reserve response in women diagnosed with infertility.

Methodology: Subjects comprised of 200 females: 150 subjects and 50 controls, aged < 20 and up to 49 years, stratified into age < 20 years (control), age 20-29 years (group 1), age 30-39 years (group 2) and age 40-49 years (group 3). About 5 ml of blood sample for AMH determination was collected on day 2-3 of spontaneous menstrual cycle from all groups and control and serum anti-mullerian hormone analyzed using enzyme linked immunosorbent assay. Baseline transvaginal

*Corresponding author: E-mail: ebbyagana@gmail.com;
ultrasound scanning was carried out on the subjects in experimental groups and control on day 2-3 of un-stimulated menstrual cycle for the measurement of antral follicle count, using the 2-dimensional plane.

**Results:** The means±SEM of serum anti-mullerian hormone by experimental groups was 1602.44 ± 54.42 pg/ml for control, 848.06±23.04 pg/ml for group 1, 26.74±1.28 pg/ml for group 2, while group 3 is 10.37±1.26 pg/ml. The means were significantly different (P<0.0001). The mean±SEM of AFC by experimental groups was control; 7.82±0.14, group 1; 5.46±0.18, 1.78±0.10 for group 2, and 0.70±0.08 for group 3. The means of antral follicle count by experimental groups showed significant difference (p<0.0001). Results showed that anti-mullerian hormone level and antral follicle count decreased significantly (p<0.05) as the age of the subjects increases. Subjects in the control and experimental group 1 showed 100% high anti-mullerian hormone level indicating 100% potential of good ovarian response. The antral follicle count result also indicate that 100% and 75% of the control group and experimental group 1 respectively show good ovarian reserve. The ovarian response and reserve in the subjects decreased substantially as the age of the subjects increased. Positive correlations were also observed between the AMH and AFC across the ages of the population studied.

**Conclusion:** The study reveals that good ovarian response and reserve in the population is related to the age of the subjects.

**Keywords:** Anti-mullerian hormone; antral follicle count; infertility; age; pregnancy.

1. INTRODUCTION

Infertility is the inability of couples to accomplish conception or pregnancy after regular sexual intercourse for a period between one and two years [1]. One of the main desires of couples in developing nations of the world is procreation [2]. Evidence shows that fertility is a major problem linked with reproductive health in sub-Saharan Africa [3]. As a result of poor documentation and lack of well designed studies, an accurate prevalence of inability to be pregnant in developing countries has not been ascertained [4]. Many reports show that the inability to achieve pregnancy is the main reason why couples visit gynecological clinics in Nigeria [5,6].

The effects of inability to be pregnant in sub-Saharan Africa are tremendous; it could result to marital problems, divorce, depression, isolation, physical violence, stigmatization and economic hardship among others [7,8]. Infertility can be primary or secondary; primary when the woman has never conceived and this has a prevalent range of 0.6 to 3.4%. while, secondary infertility is when the woman has achieved pregnancy before irrespective of the outcome, with prevalent range of 8.7 to 32.6%. About 37% of infertility is found in females [9].

Diminished ovarian reserve is one of the major causes of inability to be pregnant. As a woman advance in age her number of good quality gametocytes in the ovaries decreases. Recently, appraising ‘ovarian reserve’ became a scheme in the treatment and management of infertility in women. Exact measurement of good quality pre ovulatory oocytes in the ovaries has long been a search or an alternative in reproductive medicine [10].

The main aim of assessing the ovarian reserve in women attending fertility clinics is to evaluate the amount and quality of the left over oocytes that can anticipate the possibility of pregnancy. The availability of a test with the ability of allowing useful information as it concerns personalizing the ovarian reserve of woman within a known age bracket would enable medical experts in reproductive medicine to make available personalized and concise treatment and management scheme [10].

Anti-Mullerian Hormone (AMH) is an ovarian hormone expressed in growing follicles that have undergone recruitment from the primordial follicle pool but have not yet been selected for dominance, it is considered an accurate marker of ovarian reserve, able to reflect the size of the ovarian follicular pool of a woman of reproductive age [11] and age has been reported as one of the established predictors of reproductive potential during infertility treatment [12,13].

In most laboratories in Nigeria, assessment of infertility in women is done mainly by assessing fertility hormones such as follicle stimulating hormone (FSH), luteinizing hormone (LH), progesterone, estradiol and prolactin as a result of limited laboratory resources. In South East Nigeria, literatures on the relationship between
AMH level, AFC and age as predictors of ovarian response is scarce. Therefore, this study was designed to assess serum AMH level, antral follicle count and age as indicators of ovarian reserve response in women diagnosed with infertility in Abia State, South Eastern Nigeria.

2. MATERIALS AND METHODS

2.1 Description of Study Area and Study Population

The participants in this study were patients attending the Fertility Clinic and Assisted Reproductive Technology Unit (ART) of Federal Medical Centre, Umuahia. At first visit, general data were obtained for criteria eligibility including full history as contained in the questionnaire issued. Subjects (infertile) and controls (fertile) that fulfilled the inclusion and exclusion criteria were enrolled in the study. The nature of the study was clearly explained to the recruited participants (subjects and controls). They were all assured that the information gathered through the study would be kept confidential, being collected anonymously.

The study consists of 200 females: 150 subjects and 50 controls, aged less than 20 and up to 49 years were considered and recruited for the study. The population of women studied was stratified into age <20 years which was used as the control, subjects in this group consists of relatively fertile women, age 20–29 years as group 1, age 30–39 years as group 2 and age 40–49 years as group 4 of the studied subjects.

2.2 Inclusive Criteria

The studied population were aged between 17 and 49 years of age, having unprotected sexual intercourse for a period of 1 year and above. While females used as the control subjects were within the age range range <20 years and regarded as relatively fertile women. Women diagnosed with primary infertility, oligoamenorrhea or amenorrhea and unexplained infertility within 20 to 49 years of age were used as the study participants.

2.3 Exclusive Criteria

Exclusive criteria included women below the age of 15 years and those diagnosed with primary infertility with pure polycystic ovarian syndrome (PCOS), pelvic inflammatory diseases, endometriosis, other pelvic pathologies and those already on IVF treatment with donor’s eggs were excluded from the study.

2.4 Experimental Design

This is a case control-study, carried out among women the fertility clinics and the assisted reproductive technology unit (ART) of Federal Medical Centre, Umuahia. The subjects were categorized based on age classification into control (<20 years), experimental group 1 (20–29 years), experimental group 2 (30–39 years) and experimental group 3 (40–49 years).

2.5 Sample Size Calculation

The sample size was calculated using the formula developed by Umeora, et al. [14]. In Nigeria, institution based incidence of infertility reported that the South-Eastern Nigeria has a prevalent rate of 11.2% [15]. From the calculation a total of 200 samples were used in this study.

2.6 Specimen Collection

Blood samples for AMH determination were collected on day 2–3 of spontaneous menstrual cycle from both subjects and control. About 5ml of the venous blood samples was withdrawn from the selected subjects and dispense into plain sample containers properly labeled with patient’s name and age. The blood samples were left to coagulate spontaneously, centrifuged at 1000 x g for 15-20 minutes and the serum separated immediately into plain sterile sample bottles with Pasteur pipette and the test carried out immediately. Samples not assayed immediately were stored frozen in the refrigerator at 2–8ºC for 7 days.

2.7 Measurement of Antral Follicle Count

A baseline transvaginal ultrasound scanning (TV/US) was carried out on both the subjects and controls, on day 2-3 of un-stimulated menstrual cycle to enable the measurements of antral follicle count (AFC), using the greatest 2-dimensional (2-D) plane. Probe 5-9 MHZ (Mindray DP 8800 plus) was used for the transvaginal ultrasound scanning.

2.8 Determination of Anti Mullerian Hormone

Serum AMH was analysed using Enzyme linked immunosorbent assay (ELISA) kit by Elabscience Biotechnology Co. Ltd, China.
2.9 Statistical Analysis

The statistical software used for the analysis and graphics presentation is the Statistical Analysis System (SAS), STAT 15.1, developed by SAS Institute, North Carolina State University, USA. Data are presented as Means±SEM, comparison of means of groups that are more than two was done using analysis of variance (ANOVA), and the Tukey test of multiple comparison was used to test for variance within and across groups. Variation between two groups was done using the Student t-test analysis while Chi square analysis was used to compare percentages. The Pearsons correlation was used to determine the correlations between parameters. Variation in means of parameters was considered statistically significant at p < 0.05.

3. RESULTS

3.1 Distribution of Age (years) of Study Subjects

The distribution of the age of the subjects in the study population is shown in Fig. 1. The figure shows that 15% of the studied population were found in the control group (<20 years) of age. As shown in the figure 35% of the subjects were in group 1 (20—29 years) of age. Also 25% of the subjects were in group 2 (30-39 years). While 25% of the subjects were in age range of (30-49 years).

The means±SEM of serum AMH by experimental groups was 1602.44 ± 54.42 pg/ml for control (<20 years), 848.06±23.04 pg/ml for group 1 (20-29 years), 26.74±1.28 pg/ml for group 2 (30-39 years), while group 3 (40-49 years) is 10.37±1.26 pg/ml. The means of AMH by experimental groups showed that they were significantly different (P<0.0001). The mean of serum AMH for control subjects was significantly higher (P =0.05) than the AMH means in groups 1-3. Similarly, the comparison of the mean of AMH for experimental group 1 with the other experimental groups showed significant increase (p<0.05) in AMH level. No significant increase (p>0.05) in mean of serum AMH was seen between experimental groups 2 and 3. The box plot analysis showing the trend of decrease of serum AMH concentrations as the age of the subjects increase from control to experimental groups 3 is shown in Fig. 2.

![Fig. 1. Distribution of age (years) of study subjects](image-url)
The mean ± SEM of AFC by experimental groups is 7.82±0.14 for control, 5.46±0.18 for group 1, 1.78±0.10 for group 2, and 0.70±0.08 for group 3 and were significantly different (p<0.0001). The comparison of the means of AFC within the experimental showed significant decrease (p<0.05) between the groups as the age increases. Below is the box plot analysis showing the decrease in basal AFC as age of subjects increase from control to experimental group 3 (Fig. 2).

3.2 Classification of AMH as Indicator of Ovarian Response by Experimental Groups

In fertility profiles, serum AMH is classified into high level (>46.88 pg/mL) indicating good ovarian response and low level serum AMH (<46.88 pg/mL) indicating poor ovarian response [15]. Using this criterion, 25% of the subjects within the control group and experimental group 1 have high AMH level indicating 100% potential of good ovarian response with 0% low AMH level. Similarly, 25% of the study population within the experimental group 2 have low AMH level indicating 100% potential of poor ovarian response with no high AMH level while 1.0% of the study population within the experimental group 3 (4%) have high AMH level 24% have low AMH level indicating 96% potential of poor ovarian response. The high population of subjects with high AMH levels and low AMH levels were significantly different at (p<0.0001, \( \chi^2=192.32 \)). The classification of serum AMH in the experimental groups as indicators of ovarian response is shown in Table 1 and Fig. 3 respectively.

The association between serum AMH and age is further buttressed through the Receiver’s Operator Curve (ROC) Fig. 5. The curve showed an area under the courve (AUC) of 0.99320 as true positive which signifies a strong association between serum AMH and age.

3.3 Ovarian Reserve Status by Experimental Groups of the Studied Population

The mean±SEM of AFC for the control and experimental groups was control: 7.82±0.14, group 1; 5.46±0.18, 1.78±0.10 for group 2 and 0.70±0.08 for group 3. The means of basal AFC by experimental groups showed a significant difference (p<0.0001, F=621.83) for each of the groups. Ovarian reserve assessment from the present study may be classified as either positive which implies a good ovarian reserve status, when AFC >5, or as negative which implies a poor ovarian reserve status, AFC <4 [16]. Based on this criterion, the ovarian reserve status in the
The classification of AMH as an indicator of ovarian reserve by experimental groups is shown in Table 2 and Fig. 5. The table shows that in the studied population, 25% have AFC >5 (control group), with none being negative implying good ovarian status. This population lies within the age range < 20 years indicating 100% potential of good ovarian reserve for subjects within this age in the studied population. Similarly, 18% of the study population within the age range 20-29 years have AFC >5, which represents positive or good ovarian reserve status while 7% of the same age of the population are negative which indicate poor ovarian reserve status and this represents 75% and 25% respectively (experimental group 1). However, 50% of the studied population with AFC <4 indicating negative ovarian reserve status or poor ovarian reserve (experimental groups 2 and 3 with age range 30-39 years and 40-49 years respectively show 100% poor ovarian reserve status. The study showed that the high population of subjects with negative result or poor ovarian reserve status and positive results or good ovarian reserve status were significantly different (P <0.0001, \(X^2=158.87\)).

The Pearson's correlation of AFC by AMH weighted by age was positive (r=0.9418, p<0.0001). Pearson correlations between AMH and AFC of < 20 years old subjects (Control Group) was also positive (r=0.6412, P<0.0001). Positive correlations were also observed between AFC and AMH of 20-29 years old subjects (Group 1) (r = 0.8223, P <0.0001), of 30-39 years old subjects (r = 0.4230, P= 0.0022) and of 40-49 years subjects (group 3) (r = 0.5186, P < 0.0001) respectively.

![Boxplot of AFC by experimental groups](image)

**Table 1. Classification of AMH as indicator of ovarian reserve by experimental groups**

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Anti-Millerian Hormone (AMH)</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>High AMH</td>
<td>Low AMH</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Control</td>
<td>50</td>
<td>25.0</td>
<td>0</td>
</tr>
<tr>
<td>Group 1</td>
<td>50</td>
<td>25.0</td>
<td>0</td>
</tr>
<tr>
<td>Group 2</td>
<td>0</td>
<td>0.0</td>
<td>50</td>
</tr>
<tr>
<td>Group 3</td>
<td>2</td>
<td>1.0</td>
<td>48</td>
</tr>
<tr>
<td>(X^2)-value</td>
<td>192.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Experimental Group:** Control Group [<20 years, (Mean= 18.96±0.14 years)]; Group 1 [20-29 years, (Mean=25.46±0.41)]; Group 2 [30-39 years, (Mean=36.78±0.33)]; Group 3 [40-49 years, Mean= 46.40±0.34]

**AMH Classification:** Low AMH =≤46.88 pg/mL. High ≤46.88pg/mL. Significance Level P <0.0001
Fig. 4. Mosaic plot of contingency analysis of AMH level by experimental group

![Mosaic plot](image)

Fig. 5. Receiver Operator Curve (ROC) for ovarian reserve in association with AMH and age

AUC: 0.99320, using ovarian reserve = Positive to be the positive level

Table 2. Ovarian reserve status by experimental groups

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Ovarian reserve status</th>
<th>N</th>
<th>%</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Negative</td>
<td>0</td>
<td>0.0</td>
<td>50</td>
<td>25.0</td>
</tr>
<tr>
<td>Group 1</td>
<td>14</td>
<td>7.0</td>
<td>36</td>
<td>0</td>
<td>18.0</td>
</tr>
<tr>
<td>Group 2</td>
<td>50</td>
<td>25.0</td>
<td>0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Group 3</td>
<td>50</td>
<td>25.0</td>
<td>0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>X²-value</td>
<td>158.87</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.0001****</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Experimental Group: Control Group [<20 years, Mean= 18.96±0.14 years]; Group 1 [20-29 years, Mean=25.46±0.41]; Group 2 [30-39 years, Mean=36.78±0.33]; Group 3 [40-49 years, Mean= 46.40±0.34];

Ovarian Reserve Status: AFC <= 4: Negative or Poor Ovarian Reserve Status, AFC=>5: Positive or good ovarian Reserve Status. Significance Level ****=p<0.0001
4. DISCUSSION

Ovarian reserve indicates the woman’s reproductive potential, in terms of follicular and oocyte number and quality. It is well established that ovarian reserve generally declines with age [17]. In this study, it was observed that the serum AMH levels of control group (<20 years) has high serum AMH levels. Subjects in this group will naturally achieve pregnancy without ovulation induction or stimulation. A high serum AMH is associated with oocyte number and quality. A significant decrease in AMH values with increasing age across the four (4) experimental groups was also observed which shows that the observed changes are specific to age. This trend correlates with the studies of van Disseldorp, et al. [18] and Nelson, et al. [19]. de Vet, et al. [20] had earlier reported that early follicular phase hormonal evaluation carried out separately for a period of 3 years showed that serum AMH levels decreased significantly with age. This showed that AMH is a biomarker that possesses the ability to exhibit noticeable variations even when the woman’s menstrual cycle is supposedly normal, and would better recognize women that has difficulty in actualizing pregnancy. This suggests that serum AMH in the present studied population could be regarded as a good biochemical indicator of ovarian aging. This position is supported by the works of Barbakadze, et al. [21] and Fleming, et al. [22]. The AMH levels of the control group (<20 years) and group 1 (20-29 years) was observed to be significantly high (>46.88pg/mL), when compared with group 2 (30-39 years) and group 3 (40-49 years) which showed low AMH levels (<46.88 pg/mL). This observation is in consonance with the finding of van Disseldorp, et al. [18]. They reported a positive relationship of AMH concentration with age.

Our study also showed a declining trend in AFC as the age of subjects in the study increases. The control group (<20 years) of age and group 1 (20-29 years) have high AFC, indicating that participants in this age groups may not require ovarian stimulation or ovarian induction to actualize pregnancy and thus may demonstrate good oocytes retrieval and stimulation response should there be delayed conception and IVF intervention opted for. This observation is in line with the reports of Bansci, et al. [23] and ASRM, [24] that an AFC is recommended for the forecast of poor response during ovarian stimulation. A sharp and drastic decrease in basal AFC in group 2 (30-39) years and in group 3 (40-49) years of age was observed. This observation reflects failure in the number of oocytes in the ovaries due to increased age of the participants in these groups. This observation is in consonance with the position of Jamil, et al. [25] in their assessment of ovarian reserve using AMH versus FSH. Subjects in groups 3 and 4 of the study based on their AFC which is <4 will experience higher occurrence of cycle cancellation, they will also require higher ampoule of menopausal gonadotropin (HMG) when compared with the control group and group 1, with AFC >4. The observation agrees with the
The work of Chang et al. [16] that patients with AFC ≤3 had significantly higher cancellation rate and high dosage of HMG, unlike those with basal AFC of 4-10.

The AFC of the control group was significantly higher and statistically different (p<0.0001), when compared with group 1, group 2, and group 3. The AFC of group 1, was also significantly higher and statistically different (p<0.0001) compared with groups 2 and 3. While group 2 was only statistically different (p<0.0001) compared with group 3. This corresponds with the report of Jamil, et al. [25]. The present study also considered the association of ovarian reserve status of the experimental groups using AFC, which is classified as either positive or good ovarian reserve status, when the AFC is >5, or negative or poor ovarian reserve status, when the AFC is <4. The control group (<20 years) and group 1 (20-29 years) was observed to have very good or positive ovarian reserve status. While group 2 (30-39 years) and group 3 (40-49 years) as observed in this study had very poor or negative ovarian reserve status. Results of the present study agree with results of the study by Islam, et al. [26] that AFC and AMH are effective in predicting ovarian reserve and response to induction and that both are accurate for the estimation of ovarian reserve.

The positive correlations between serum AMH and basal AFC was observed across the four (4) experimental groups. Both experimental parameters also showed a strong association, which implies that high AMH levels will result to high AFC, and low AMH level will also lead to low AFC. This is in agreement with the studies of Nardo, et al. [27]. They observed a strong positive association between serum AMH levels and AFC. This observation also correlates with the work of Valvekar, et al. [10] where they reported that serum AMH levels are strongly correlated with basal AFC, in the midst of other ovarian reserve parameters used in recent reproductive practice. The strong correlations that exits between serum AMH and basal AFC was further buttressed in the present study through the Receiver Operator Curve (ROC) where the area under curve (AUC) was 0.99320. This also implies that there is no significant difference between the two (2) superior markers of ovarian reserve and ovarian response in terms of sensitivity and specificity. This finding corresponds with the studies of Broer, et al. [28] where in a meta-analysis study they identify no significant difference between serum AMH and basal AFC in forecasting ovarian response using the receiver's operational characteristics (ROC).

5. CONCLUSION

The present study reveals that AMH and AFC alongside with age are important diagnostic indicators of ovarian reserve and response in the population studied. Emphasis on the inclusion AMH measurement and AFC in the traditional reproductive hormonal profiles in relation to the age of the women in the assessment of infertility in the population before recommendation of assisted reproduction technology is thus imperative. This would make it possible for patients to entertain more realistic expectations from treatment options and minimize the psychological stress the patients go through in attempts to resolve the crisis of infertility.

CONSENT AND ETHICAL APPROVAL

The study was approved by the Research and Ethical Committee of the Federal Medical Centre, Umuahia, Abia State. In addition each of the recruited participants gave informed consent to participate in the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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