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# ANTIMULLERIAN HORMONE SERUM LEVELS: ASSESSING FERTILITY POTENTIAL OF NIGERIAN FEMALES

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## ABSTRACT

Antimullerian hormone (AMH) is one of the markers used to assess ovarian reserve. While this has been reported to be dependent on genetic and environmental factors, the treatment approach based on AMH level is individualized. This study was designed to compare the efficacy of antimullerian hormone with other markers of ovarian reserve. A longitudinal analytical study was carried out on one hundred and forty-two (142) females comprising hyperprolactinaemic (34), hypergonadotrohic hypogonadism (18), primary hypogonadism (18), polycystic ovarian syndrome (PCOS) (8), diminished ovarian reserve (18), and 46 normal hormonal profile volunteers based on diagnostic criteria. Blood samples were collected from the participants on day 3 for follicle stimulating hormone, luteinizing hormone, estradiol, and antimullerian hormone estimation, and day 21 of the menstrual cycle for prolactin and progesterone. Enzyme linked immuno-sorbent assay technique was used to determine the hormonal levels of the participants. Antimullerian hormone level was higher ($p < 0.05$) in PCOS when compared with volunteers with normal hormonal profile while no significant differences in antimullerian hormone was observed in all infertile groups ($p > 005$). An inverse association ($p < 0.05$) between antimullerian hormone and estradiol, antimullerian hormone and age as well as estradiol and age were observed in this study. There were no significant correlations between antimullerian hormone and luteinizing hormone as well as between antimullerian hormone and follicle stimulating hormone. In conclusion, antimullerian hormone level was found to be significantly associated with polycystic ovarian syndrome. This also proved to be a marker of age-related ovarian reserve.

**Keywords:** Infertility, Ovarian reserve, Antimullerian Hormone, Follicle stimulating hormone, Estradiol

## INTRODUCTION

Infertility remains a common medical problem globally, with female factors contributing almost equally to male factors (Romero, 2017, Makrigiannakis *et al*., 2011). In recent years, assessment of ovarian reserve to determine the strategy for treatment of female infertility has become essential. Traditionally; age (Faddy *et al*., 1992) follicle stimulating hormone (FSH), estradiol (E2) (WHO, 1991), antral follicle count (AFC) (Verhagen *et al.,* 2008) and until recently, antimullerian hormone (AMH) (Jirge, 2011) have been used for evaluation of ovarian reserve. Antimullerian hormone (AMH), belongs to the transforming growth factor – β family (VanDisseldrop *et al.,* 2008). In the ovary, it inhibits initial primordial follicle recruitment and decreases the sensitivity of pre-antral and small antral follicles to FSH (La Marca *et al.,* 2007). Basic research data obtained from adult ovary indicate that AMH is likely to be involved in the regulation of follicular steroidogenesis (Fanchini, 2003) and experiments conducted in animals suggest that AMH reduces aromatase activity and the number of leutinizing hormone (LH) receptors in FSH-stimulated granulosa cells (Josso *et al.,* 1998), and also influences testosterone production by theca cells (Ingraham *et al*., 2000).

The prediction of poor outcomes during ovarian stimulation is vital for the counseling and management of infertile women in clinical practice (Nelson *et al.,* 2007) on the basis of reduced levels of AMH (Fleming *et al.,* 2013). It is instructive to note from a previous study that AMH values have effectively predicted women at risk of menopause (Sangeeta *et al*., 2015) .While the success of assisted reproductive technology in infertile women depends on the levels of AMH, the individualization in the treatment approach based on AMH levels does not rely on generalized reference values. It is also well documented that

antimullerian hormone is strongly involved in the pathobiology of polycystic ovarian syndrome (PCOS) (Laven *et al.*,2004; Pigny *et al.*, 2006) where the increase in serum AMH reflected the increased number of small antral follicles. Clinical evidence reported by Seifer *et al*., (2007) showed that AMH is a valuable parameter in the monitoring of follicular exhaustion due to ovarian aging. A previous study conducted elsewhere by Riggs *et al*., (2008) reported a negative correlation between AMH and FSH; it is not fully established if serum AMH measurements reflect ovarian follicular status better than the conventional hormonal parameters, notably follicle stimulating hormone in our locale. Accumulating evidence suggests that fertility potential and function may be different across racial and ethnic groups and such racial differences have been demonstrated in pubertal timing, infertility, outcomes after assisted reproductive technology treatment and reproductive ageing (Dewailly *et al.*, 2014). The existence of such genetic differences in ovarian reserve and thus fertility potential may have important clinical implications. However, the mechanisms that underlie such are not clear. The aim of this report was to determine the plasma levels of antimuullerian hormones in Nigerian females with different reproductive pathologies. Another objective was to evaluate the fertility potential of the participants, via AMH levels.

## MATERIALS AND METHODS

This study was conducted among 142 females attending the infertility clinic of the Obstetrics and Gynaecology Department of the Lagos University Teaching Hospital (LUTH) Idi- Araba, Lagos; a tertiary health care institution. Ethical approval was obtained from the Health Research and Ethics Committee, College of Medicine of University of Lagos (CMUL/HREC /09/17/249) prior to the commencement of the study. Informed written consent was sought from each of the participants and a well-structured questionnaire was used to obtain information on bio - data of their reproductive histories. The inclusion criteria for the participants were women with a normal menstrual cycle ranging from 26–32 days, women who have not taken any contraceptive pills in the past one year and women between the ages of 18- 46 years. Those excluded include pregnant women and menopausal women, obese individuals, alcoholics, women who have had oophorectomy, tobacco smokers, and those aged 46 and above and below 17 years of age.

### Sample Collection

5ml of venous blood was collected from each participant into a plain bottle by venipuncture through the ante - cubital vein. Samples were collected from the participants (on day 3 for FSH, LH, prolactin, estradiol, and AMH estimation) and (day 21 for progesterone). The collected samples were centrifuged and the sera separated in an Eppendorf tube and kept frozen at -20 $^{\circ}$C till use.

### Biochemical Analyses

Enzyme-linked immunosorbent assay (ELISA) technique was used for the estimations of AMH, LH, FSH, prolactin, progesterone and estradiol levels in the participants. The principle was based on the assay system utilizing a high affinity specific monoclonal antibody directed against a distinct antigenic determinant. The antigen was sandwiched between the solid phase and enzyme linked antibodies after a simultaneous reaction. After incubation, and subsequent washing to remove unbound labeled antibody/antigen, reaction with TMB-substrate produced a blue color that changes to a yellow color after the addition of a stop solution. The intensity of color is directly proportional to the amount of hormone in the sample and the intensity was measured at 450 nm wavelength. The absorbance for each calibrator was plotted against their corresponding concentration and the concentrations of the hormones determined by extrapolation from the curve.

### Working Diagnosis

The participants were classified into different pathologies based on their hormonal profile results as follows:

Hypergonadotropic hypogonadism: Participants with LH, and FSH levels above 10 iu/L and 12 iu/L respectively accompanied with low progesterone levels less than 18 nmol/L.

Hyperprolactinaemia: Participants with prolactin levels above 550miu/L despite normal levels of LH, FSH, estradiol and progesterone (Roberts *et al*., 2008).

Polycystic Ovarian Syndrome: Participants with LH/FSH ratio greater than 2.5 were classified as having polycystic ovarian syndrome and primary hypogonadism include participants with progesterone levels of less than 18 nmol/L despite normal levels of LH, FSH, and prolactin (Chun, 2014).

Low Ovarian Reserve: Participants with FSH/LH ratio greater than 2.0 were classified as having low ovarian reserve (Toner and Seifer, 2013).

### Statistical Analysis of Data

The results generated from this study were subjected to statistical analysis using SPSS version 17. Quantitative data were expressed as mean ± SD. The difference between two means was assessed by student's t-test. Within group and between group analyses was done using one-way analysis of variance (ANOVA). Categorical variables were assessed by Chi square. Pearson's correlation coefficient was used to establish associations between groups.

## RESULTS AND DISCUSSION

The pattern of reproductive pathologies in our participants was such that the proportions documented with hypergonadotrophic hypogonadism, hyperprolactinaemia, polysctic ovarian syndrome, primary hypogonadism and low ovarian reserve were 12.7%, 23.9%, 5.6%, 12.7% and 12.7% respectively (see Table 1). The prevalence of PCOS in this study is fairly low compared with what was earlier reported for Caucasians (Asuncion *et al.,* 2013) where a prevalence rate of 6.5- 6.7% was documented. This could be attributed to the non- homogenous nature of our study population, which was entirely made up of participants with both normal and impaired hormonal status. The mean levels of antimullerian hormone in PCOS (48.29 ± 20.15 Au/ml) was significantly higher than the levels in participants with normal reproductive hormone profile (32.97 ± 5.11Au/ml, p = 0.050). These and other results are shown in table 2. Tables 3 and 4 show correlation studies of AMH with other reproductive hormones in various reproductive pathologies and age respectively. Table 5 depicts the levels (mean ± SD) of AMH in different age groups of the participants with different pathologies and subjects with normal reproductive hormonal profile. The Interrelationship between antimullerian hormone and reproductive hormones is illustrated in table 6.

**Table 1: The Frequency of Reproductive Pathologies in the Study Population of Infertile Females**

| Reproductive Pathologies | Frequency (n) | Percentage (%) |
|---|---|---|
| Hypergonadotrophic hypogonadism | 18 | 12.7 |
| Hyperprolactinaemia | 34 | 23.9 |
| Primary hypogonadism | 18 | 12.7 |
| Polycystic ovarian syndrome | 8 | 5.6 |
| Diminished ovarian syndrome | 18 | 12.7 |
| Normal hormonal profile | 46 | 32.4 |
| Total number of subjects | 142 | 100 |

**Table 2: Serum Levels (Mean ± SEM) of Antimullerian Hormones in Infertile Female Subjects with Reproductive Pathologies in Comparison with Normal Hormonal Profile Subjects**

| Reproductive Pathologies | Antimullerian Hormone | p values |
|---|---|---|
| Polycystic ovarian syndrome | 48.29 ± 20.15 | 0.050* |
| Normal hormonal profile | 32.97 ± 5.11 | |
| Hyperprolactinaemia | 32.85 ± 4.78 | 0.987 |
| Normal hormonal profile | 32.97 ± 5.11 | |
| Hypergonadotrophic hypogonadism | 19.44 ± 6.66 | 0.151 |
| Normal hormonal profile | 32.97 ± 5.11 | |
| Primary hypogonadism | 20.64 ± 6.59 | 0.189 |
| Normal | 32.97 ± 5.11 | |
| Diminished Ovarian reserve | 35.77 ± 7.33 | 0.767 |
| Normal | 32.98 ± 5.11 | |

*significance. Serum levels of Antimullerian Hormones in subjects with polyscystic ovarian syndrome were significantly higher when compared with subjects with normal reproductive hormone levels.

**Table 3: Pearson Correlation Coefficient of AMH with Other Reproductive Hormones in Various Reproductive Pathologies**

| Infertile Subjects | Reproductive Hormones | AMH r (p) |
|---|---|---|
| Polycystic ovarian syndrome | LH | -0.206 (0.794) |
| | FSH | 0.159(0.841) |
| | PROL | -0.010 (0.990) |
| | PROG | 0.457 (0.543) |
| | E2 | -0133 (0.867) |
| Hyperprolactinaemia | LH | 0.099 (0.707) |
| | FSH | 0.074 (0.778) |
| | PROL | 0.087 (0.741) |
| | PROG | -0.-067 (0.797) |
| | E2 | -0.304 (0.236) |
| Hypergonadotrophic hypogonadism | LH | 0.057 (0.884) |
| | FSH | 0.158(0.684) |
| | PROL | 0.164 (0.674) |
| | PROG | -0.105 (0.788) |
| | E2 | -0.408 (0.276) |
| Primary hypogonadism | LH | -0.023 (0.953) |
| | FSH | 0.102 (0.793) |
| | PROL | 0.151 (0.699) |
| | PROG | -0.116 (0.767) |
| | E2 | -0.493 (0.178) |
| Diminished ovarian reserve | LH | 0.316 (0.407) |
| | FSH | 0.495 (0.175) |
| | PROL | 0.267 (0.487) |
| | PROG | 0.365 (0.334) |
| | E2 | -0.275 (0.474) |
| Normal | LH | 0.252 (0.245) |
| | FSH | 0.122 (0.580) |
| | PROL | 0.113 (0.607) |
| | PROG | -0.153 (0.486) |
| | E2 | -0.260 (0.231) |

## Table 4: Correlation Studies of Age and Reproductive Hormones

| Reproductive Hormones | Age r (p) |
|---|---|
| Antimullerian hormone | -0.237 (0.049) * |
| FSH | 0.030 (0.806) |
| LH | 0.205 (0.094) |
| E2 | -0.382 (0.001) * |

*significant. Antimullerian hormone showed a significant inverse correlation with age.

**Table 5: Serum Levels (Mean ± SD) of Antimullerian Hormone in Infertile Subjects with Different Reproductive Pathologies and Normal Hormonal Profile Subjects**

| Reproductive Pathologies | Age Group | Antimullerian Hormone (Mean ± SD) |
|---|---|---|
| Normal Hormone Profile | 21-30 | 39.16 ± 21.43 |
| | 31-40 | 30.95 ± 17.85 |
| | >40 | 21.18 ± 35.65 |
| Hyperprolactinaemia | 21-30 | 34.57 ± 20.64 |
| | 31-40 | 32.69 ± 20.11 |
| | >40 | 22.14 ± 8.59 |
| Hypergonadotropic Hypognadism | 21-30 | 59.48 |
| | 31-40 | 14.14 ± 15.1.9 |
| | >40 | 16.49 |
| Polycystic Ovarian Syndrome | 21-30 | - |
| | 31-40 | 28.56 ± 28.78 |
| | >40 | 46.02 ± 61.14 |
| Primary Hypogonadism | 21-30 | 40.98 ± 26.15 |
| | 31-40 | 14.58 ± 16.59 |
| | >40 | 16.49 |
| Diminished Ovarian Reserve | 21-30 | 45.22 ± 17.15 |
| | 31-40 | 31.01 ± 22.92 |
| | >40 | 2.78 |

**Table 6: Interrelationship between AMH and Reproductive Hormones**

| Reproductive hormones | r (p) |
|---|---|
| FSH | -0.089 (0.458) |
| LH | -0.112 (0.354) |
| E2 | - 0.307 (0.009) * |

*significance. Antimullerian hormone showed a significant inverse correlation with estradiol (E2)

This study revealed a significantly higher antimullerian hormone levels in PCOS when compared with subjects with normal hormonal profile. This is in agreement what other reported studies elsewhere (Bungum *et al*., 2013; Neoklis, *et al.*, 2013). In conditions with high LH and normal or low FSH as in PCOS, AMH concentrations in this study showed no correlation with FSH and this observation is in tandem with the study of Neoklis *et al*., (2013). By implication, increased levels of AMH in PCOS are thus a reflection of growing follicles (Velvekar *et al*., 2016). A link with follicular growth implies the strength of AMH as a marker of severity of ovarian dysfunction and hyperandrogenism in women with anovulatory PCOS. However, AMH may not be a useful guide in the exact characterization of reproductive pathologies. This is evident from the Pearson correlation coefficient studies conducted between reproductive hormones and AMH in different reproductive pathologies. We have also shown in this study that both endocrine (FSH, LH and E2) and clinical marker (age) of ovarian reserve independently correlated with plasma AMH values, where an inverse association of AMH with age ($r = -0.237$, $p = 0.049$) and E2 ($r = -0.382$, $p = 0.001$) were observed. The level of AMH thus serves as a useful marker of age – dependent fall in the follicular potential of the ovary of the study participants. This observation was corroborated in our participants with normal reproductive hormone profile, where a gradual decrease in AMH was observed with aging and as well in those with hyperprolactinaemia. This underscores the importance of age as an important factor in determining quality and quantity of ovarian reserve. Interestingly, FSH did not show any association with age in our study participants. This is at variance with a positive correlation earlier reported by Velvekar *et al*, (2016). The reliability of FSH alone in assessing ovarian reserve in our study population is limited, in view of the lack of correlation with age observed. This is against the backdrop of the

World Health Organization classification of ovarian dysfunction which was hitherto based on serum FSH and estradiol levels. Consequently, the interpretation and use of follicle stimulating hormone should be done with caution. This may be due to the fact that FSH exhibits both inter and intra cyclic fluctuations, thus single day 3 FSH measurement may fail to be an accurate marker, suggesting evaluation of subsequent cycle's day 3 FSH (Perloe, *et al.*, 2000). Further examination of the interrelationship of AMH with other reproductive hormones showed a negative correlation with estradiol ($r = -0.307$, $p = 0.009$), while no significant correlations were observed with LH and FSH. The data on FSH is in agreement with a previous study (Omabe *et al*., 2013). The reported correlation between AMH and E2 could be attributed to the fact that AMH is not affected by the hypothalamic pituitary axis, oral contraceptives and other ovarian factors.

## CONCLUSION

From the data generated, AMH level could be a useful marker of age-related ovarian reserve in our study participants. However, no significant correlation was observed with other hormones in different reproductive pathologies. This study has provided baseline information on the usefulness of AMH in assessing fertility potential of Nigerian females and calls for further studies in this field.

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**Conflict of Interest:** None declared.

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