Serum Anti- mullerian Hormone, Follicle Stimulating Hormone, Luteinizing Hormone, LH/FSH ratio, Estradiol and their correlations with age in Infertile Women in Nigeria

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Abstract

Infertility is a cause of concern because of its psychosocial effect. Globally, infertility is said to affect 8%-10% of couples. This study aimed at evaluating serum levels of Anti- mullerian Hormone (AMH), Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Estradiol, Luteinizing Hormone/Follicle Stimulating Hormone (LH/FSH) ratio and their correlation with age in women with infertility. treated in the University of Benin Teaching Hospital, Benin City, Nigeria.

This was a retrospective study. Data of 458 women seen in the ARRT Clinic of the Obstetrics and Gynaecology Department between May 2021 and June 2023 were analyzed. Subjects were stratified into four age groups. Serum levels of AMH, FSH, LH, Oestradiol and LH/FSH ratio were statistically analyzed and their correlation with age determined. Biochemical assays were done using the ELISA method. Statistical analysis was done using SPSS version 21. Students' t-test and Pearson's correlation determined. Statistical significance was established at $p \leq 0.05$.

Mean Serum AMH, FSH, LH, Oestradiol, LH/FSH ratio was 1.1±1.4ng/ml, 7.7±6.3mIU/ml, 4.4±3.9mIU/ml, 50.9±37.4pg/ml, 0.62±0.35 respectively. Serum AMH decreased significantly with age (from 3.09±2.4 to 0.63±0.86,p=0.003). Serum FSH and LH increased significantly with age (from 5.63±2.7 to 11.94±11.0 (p=0.001) (3.56±2.0 to 5.88±5.59 (p=0.001) respectively. Mean LH/FSH Ratio values decreased from 0.64 ± 0.36 to 0.55 ± 0.37 (p=0.201). Serum AMH showed a statistically significant negative correlation with age (r=-0.422, p=0.001). Serum FSH and LH showed a statistically positive correlation with age (r=0.268, P=0.001), (r=0.156, P=0.001) respectively. Oestradiol showed a weak negative correlation and LH/FSH ratio show weak positive correlation with age. Serum AMH showed a stronger level of negative correlation with age compared with the other hormones evaluated, therefore it may be a more reliable marker of ovarian reserve, hence, useful in the assessment of response to treatment protocols in Assisted Reproduction.

Key Words: Infertility, Anti- mullerian Hormone (AMH), Follicle Stimulating Hormone (FSH), luteinizing Hormone (LH), Estradiol (ES), LH/FSH Ratio.

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Introduction

Infertility, which is the inability of a couple to conceive despite regular, adequate and unprotected intercourse within one year is on the increase. While it is reported to affect 10%-30% of couples in Sub-Saharan Africa,[1] there seems to be paucity of information on prevalence rate in Nigeria. However, some studies have reported the prevalence rate of between 20-30% in Nigeria.[1] The problem of infertility places a psychosocial burden on the couples affected, especially in African culture; therefore, appropriate diagnosis and management especially with Assisted Reproduction, where applicable is important.

Assessment of the ovarian reserve is important both in infertility work up and treatment. Biochemical assays for Anti-mullerian Hormone, Follicle Stimulating Hormone, luteinizing Hormone, Estradiol are some of the tests that have been commonly used for the assessment of ovarian reserve;[2] these were done along with antral follicular count under ultrasonographic guidance. Assessment of the ovarian reserve helps in predicting reproductive age, and detecting early ovarian ageing, thereby predicting chances of conception in women desirous of pregnancy, and in counseling women desirous of delaying child bearing.[3]

Serum Anti-mullerian Hormone has recently emerged as a novel clinical marker of ovarian reserve.[2] It is expressed in the growing pre-antral or small antral follicles in the ovary and reflects ovarian follicular pool.[2] One of its advantages is the liitle inter and intracycle variability and gradually decreasing value with increasing age. Some authors[4,5,6,7] have reported that Serum Anti-mullerian Hormone levels are age-specific and a readily available clinical marker of ovarian reserve. Thus clinically, Serum Anti mullerian Hormone determination is utilized in assessing ovarian

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reserve in infertility diagnosis, premature ovarian failure and polycystic ovary syndrome.[8,9,10] Follicle Stimulating Hormone and luteinizing Hormone are determinants of ovarian activity[8]. Follicle Stimulating Hormone is also used as an indirect method of assessing ovarian reserve.[11,12]

This study is aimed at evaluating the serum levels of Anti-mullerian Hormone, Follicle Stimulating Hormone, Estrogen, luteinizing Hormone/Follicle Stimulating Hormone ratio, and their correlation with age, with the aim of determining their level of usefulness in assessing ovarian reserve, which is a very important factor in determining response to treatment in Assisted Reproduction Program.

Materials and Methods

Study location/population

This was a retrospective study carried out in the department of Obstetrics and Gynaecology, University of Benin Teaching Hospital. Data of 458 patients seen in the Assisted Reproduction Clinic of the department between May 2021 and June 2023 were analyzed. The Subjects were diagnosed with either primary or secondary infertility. The Subjects were classified into three age groups, viz: 20-29 years, 30-39 years, 40-49 years.

Sample Collection/Analysis

Blood samples were collected from the women by venipuncture under aseptic conditions on Day 3 of their menstrual cycle and analyzed for Serum Anti-mullerian Hormone, Follicle Stimulating Hormone, luteinizing Hormone, Oestradiol and FSH/LH ratio calculated. Serum Anti-mullerian Hormone was assayed using the Enzyme-linked immunosorbent assay method. FSH, LH and Oestradiol were all analyzed using the enzyme-linked immunosorbent assay method. LH/FSH ratio was calculated.

Statistical Analysis

All Data Analysis were performed using the Statistical Package for Social Sciences software version 22. Variables were presented as mean ± SD. Student t test was used to compare between the means of the variables. Pearson's correlation was used to determine correlation between the variables. Levels of significance was established at $p \leq 0.05$.

Results

Data of a total of 458 subjects aged 18-49 years was analyzed. They were divided into three age groups, with 20.5% (94), 61.1% (303), 13.3% (61) in the (20-29 years), (30-39 years) and (40-49 years) age groups respectively. Mean age of the women was 33.9±5.3 years. (Table 1). Mean Serum AMH level was 1.1±1.4ng/ml. Serum AMH levels decreased across the age groups, from 3.09±2.1 (20-29 years) to 0.63±0.86, which was statistically significant. P=0.003 (Table 1). Mean Serum FSH was 7.7±6.3mIU/ml. Serum FSH levels increased with increasing age, from 5.63±2.7mIU/ml (20-29 years) to 11.94±11.0mIU/ml (40-49 years) which was statistically significant. P=0.001 (Table 1).

Mean Serum LH level was 4.4±3.9mIU/ml and it showed a statistically significant increase with age

Table 1: Serum AMH, FSH, LH, LH/FSH, ESTROGEN Levels according to age groups

Age group	N	Age	AMH (ng/ml)	FSH (mIU/ml)	LH	LH/FSH (mIU/ml)	Oestrogen (pg/ml)
ALL	458	33.9±5.3	1.1±1.4	7.7±6.3	4.4±3.9	0.62±0.35	50.9±37.4
20-29	94		3.09±2.4	5.63±2.70	3.56±2.0	0.64±0.36	48.68±26.76
30-39	303		1.22±1.44	7.43±5.36	4.30±3.79	0.63±0.38	52.11±40.42
40-49	61		0.63±0.86	11.94±11.05	5.88±5.59	0.55±0.37	48.31±35.87
P value			0.003*	0.001*	0.001*	0.201	0.635

*Statistically significant difference. P<0.05

TABLE 2: Correlation of SERUM AMH, FSH, LH, LH/FSH ratio with age

PARAMETERS	r	P value
Serum AMH (ng/ml)	-0.423	0.001*
Serum FSH (mIU/ml)	0.268	0.001*
Serum LH (mIU/ml)	0.156	0.001*
Serum Oestrogen (pg/ml)	-0.013	0.780
LH/FSH Ratio	0.105	0.302

*Statistically significant difference. P < 0.05

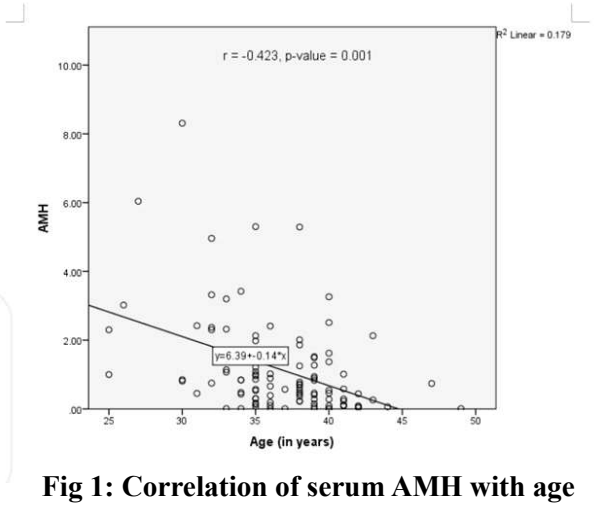

Fig 1: Correlation of serum AMH with age

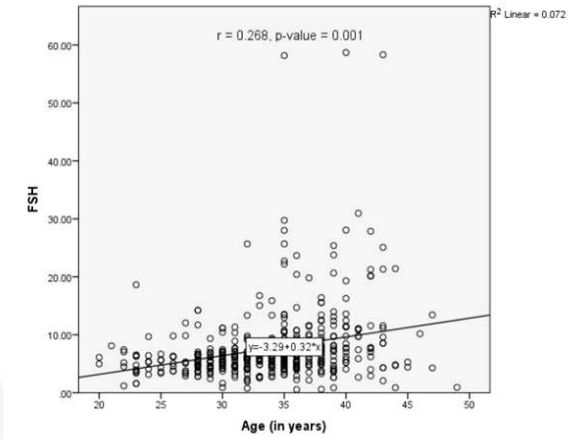

Fig 2: Correlation of serum FSH with age

from 3.56±2.0mIU/ml (20-29 years) to 5.85±5.59 (40-49 years) P=0.001 (Table 1). Mean Estrogen level was 50.9±37.4pg/ml and did not show any clear pattern or trend with increasing age (Table 1). Mean LH/FSH ratio was 0.62±0.35. Mean levels showed a non-statistically significant decline from 0.64±0.2 (20-29 years) to 0.55±0.37 (40-49 years) P=0.201 (Table 1). Serum AMH demonstrated a strong and statistically significant negative correlation with age (r=-0.422, P=0.001) (Table 2, figure 1). Follicle Stimulating Hormone demonstrated a statistically significant correlation with age (r=0.156, P=0.001) (Table 2, figure 2). Luteinizing Hormone also showed a statistically significant correlation with age (r=0.156, P=0.001) (Table 2). LH/FSH ratio showed a weak positive correlation with age, which was not statistically significant (r=0.089, P=0.06) (Table 2).

Discussion

Prevalence of infertility appears to be on the increase in Sub-Saharan Africa, Nigeria not left out. This poses a huge psychosocial burden on the couple. Proper management and positive treatment outcomes are of great importance, which brings succour to the affected individuals. The hormones assayed in our study are commonly used by Gynaecologists to evaluate infertile women and also assess ovarian reserve in preparation for Assisted Reproduction.

In this study, the largest number of women seen were in the age group 30-39 years; this is 61.1% of the study population. Similar findings were reported by Pande B[13] and co-authors in their study on infertile women in Eastern India. This finding seems to suggest an increasing prevalence of infertility in women of age group 30-39 years, which may not be limited to sub-Saharan Africa.

Mean Serum AMH level in our study was similar to that reported by Okunola O.T[3] and co-authors in their study in Ile-Ife, Nigeria and Jyoti B et al[14] in their study on infertile women in India. Serum AMH levels decreased significantly with age in this study. Several authors[13,15,16,17] have reported similar findings and this cuts across the globe. This is in keeping with what is already known that Serum AMH level decreases with increasing age. Seifer D.B et al.[15] in their study reported the rate of decline in mean AMH value, was 0.2 mg/ml/year through age 40 and then diminishes to 0.1mg/ml/year thereafter.

Mean FSH levels increased across the age groups; which was statistically significant. Mean LH levels also increased across age groups. Increase was statistically significant. Pande B.[13] , Prasad B.[18] and Okunola O.T[3] all reported similar findings in their studies. Follicle Stimulating Hormone and Leutinizing Hormone play an important role in Follicle development and oestrogen production. The elevated levels of these hormones have an impact on ovulation and menstruation thereby highlighting the role their imbalance play in infertility. LH/FSH ratio level decreased with age in this study, which was not statistically significant. Pande B.[13] in their own study reported a significant decrease.

Serum oestrogen showed a slight decrease with age in this study. Serum AMH levels showed a fairly strong and statistically significant negative correlation with age, serum levels decreased with increasing age. This corroborates findings by several authors[13,19,20,21,22] worldwide. Nelson S.M. et al[16] in the U.S. developed a normogram to demonstrate the decline in Serum AMH with age in infertility patients, to enable counseling of infertility patients regarding reproductive performance; while Mawusi D and Co-authors[19] in Ghana also studied age specific differences in Serum AMH in infertile women, to assist as a guide in planning of ovarian stimulation protocols. These findings are suggestive that AMH is an important biomarker in evaluating ovarian reserve and is clinically useful in predicting ovarian response to stimulation in assisted reproduction protocols. It is considered to be the best endocrine indicator of follicular response to ovarian stimulation compared to other commonly used markers such as FSH,

Oestradiol, Inhibin, patient's age alone.[21] Also, AMH has been reported to be more strongly associated with age.[21] AMH levels decrease before increase in FSH can be detected;[21] therefore, serum AMH has been considered to be a more sensitive marker of ovarian reserve than FSH.

FSH showed a statistically significant positive correlation with age, in this study. LH also showed a positive correlation with age which was statistically significant. Several authors[3,13,18,20,23] have reported similar findings in their various studies on infertile women. The consistency of these findings', especially of increasing levels of FSH with increasing age, supports its use as one of the markers of ovarian reserves to some extent. LH/FSH ratio did not show a statistically significant correlation with age in our study, hence its use in evaluation of ovarian reserve and response may be limited in our study population. Correlation of Oestradiol with age was not statistically significant. Its usefulness also may be limited.

Conclusion

Serum AMH levels decreased significantly with increasing age and showed a statistically significant negative correlation with age. Strength of correlation with age was stronger than that demonstrated with FSH, which also showed a statistically significant increase with age and positive correlation with age. AMH may be a more reliable marker for evaluation of ovarian reserve and response to assisted reproduction stimulation protocols compared with other hormones studied.

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