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EVALUATION OF SERUM ANTI-MULLERIAN HORMONE, FOLLICLE STIMULATING HORMONE AND PROLACTIN HORMONE IN WOMEN WITH INFERTILITY IN KANO METROPOLIS

Isah Suleiman Yahaya[1], *Emmanuel Ifeanyi Obeagu[2], Johnson Okwudili Nweke[3] and Aliyu Aishatu[1]

[1]Department of Medical Laboratory Science, Faculty of Allied Health Sciences, College of Health Sciences, Bayero University Kano, Nigeria.

[2]Department of Medical Laboratory Science, Imo State University, Owerri, Nigeria

[3]Diagnostic Laboratory Unit, Medical Centre, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Email : emmanuelobeagu@yahoo.com

ABSTRACT

The difficulty to conceive or subfertility constitutes a major social and psychological burden amongst couples especially in African women. The aim of the research is to evaluate Anti-Mullerian Hormone (AMH), Follicle Stimulating Hormone (FSH) and prolactin (PRL) hormone in infertile women in Kano metropolis. This was a comparative cross sectional study in which blood samples were taken from 50 fertile and 50 infertile women after administering a questionnaire and obtaining an informed consent. Serum level of FSH and PRL were assayed using sandwich-ELISA and serum level of AMH using Fine care plus rapid quantitative test kit. Based on these findings, higher frequency of 45 was observed in primary infertility, while lower frequency of 5 was observed in secondary infertility patients, higher frequency of 26 (52 %) was observed in infertility patients within the range of 18-28 years with (p=0.206). The mean serum FSH and PRL were significantly (p = 0.000) higher in patients group compared with controls., while no statistical significant was observed (p =0.949) in AHM between patients group compared with controls.No significant (p>0.05) correlation was established between serum AHM&FSH (r = -0.076, p=0.598), between AMH&PRL(r = -0.053, p=0.714) and PRL&FSH (r = -0.580, p=0.598) respectively. The result revealed significantly higher level of serum FSH and PRL in infertile patients with no significant difference in AMH among infertile patients. This study may help in representing a true step forward in rendering proper chemical pathology diagnosis and counselling in infertility care.

Keywords: *anti-mullerian hormone, follicle stimulating hormone, prolactin hormone, women with infertility*

INTRODUCTION

Infertility is a growing gynaecological problem in our environment with a rising number of women within reproductive age having difficulty becoming pregnant (Idrisa*et al*., 2008). Infertility has been defined as the inability to establish a child within a specified period of time, usually one year in couples of reproductive age who are having regular sexual intercourse without contraception (Adongo and Tabong, 2013). The definition of infertility has been extended to also include the inability to carry a pregnancy to the delivery of a live baby (Grunath *et al*., 2011). It could be primary in nature, i.e, when both partners have never conceived in their lifetime or secondary, i.e, inability of couples or partners to conceive after a year when one or both partners have previously had a child or children (Tamuno-Emine*et al.,* 2015). Primary infertility in a couple who have never had a child may include: being unable to conceive, being unable to maintain a pregnancy to full term or being unable to carry a pregnancy to a live birth (Wilson *et al.,* 2018).

In many cultures around the world, infertility has a very strong social stigma, especially in its relation to women, depending on the cultural context (Barros *et al.,* 2012).

Follicle stimulating hormone (FSH) is synthesized and secreted by the gonadotropic cells of the anterior pituitary gland and regulates the development, growth, pubertal maturation and reproductive processes of the body. Follicle stimulating hormone (FSH) and luteinizing hormone (LH) work together in the reproductive system (Bowen, 2019).The hypothalamus secretes GnRH, w hich stimulates the anterior pituitary to rele ase FSH and LH.

In males, FSH receptors are found in the Ser toli cells of the testes, FSH fuctions in induction and maintenance of spermatogenesis. In females, FSH receptors are located in the granulosa cells of the ovaries, FSH fuctions in estrogen production and follicular development (Barbieri, 2014). Follicle stimulating hormone (FSH) is used to stimulate multiple follicles in the ovaries in order to harvest multiple eggs which aids in fertilization, it has been used successfully for infertility treatment of patients with hypogonadotropic hypogonadism for more than 50 years, initially with urinary menopausal gonadotropins having FSH activity (Orlowski, 2018). Follicle stimulating hormone (FSH)and Anti-Müllerian hormone (AMH) are individually widely used to assess functional ovarian reserve but demonstrate discrepancies in efficacy (Gleicher *et al.,* 2013).High levels of FSH indicate that the normal restricting feedback from the gonad is absent, leading to an unrestricted pituitary FSH production (Kim *et al.,* 2011). Conditions with abnormal FSH levels include premature menopause, poor ovarian reserve, gonadal dysgenesis, castration, testicular failure, lupus (McMurray *et al.,* 2005).

Anti-Müllerian hormone (AMH) is a glycoprotein, which circulates as a dimer composed of two identical 72 kDa monomers that are linked by disulfide bridges, it belongs to the transforming growth factor-β family (Donahoe *et al.,* 2001). It is named for its first described function in fetal sexual differentiation, a regression of the Müllerian ducts in males

during early fetal life. In males, AMH is secreted by Sertoli cells of the testes. Anti-Müllerian hormone (AMH) concentrations are high until puberty and then decrease slowly to residual post-puberty levels (Picard *et al.,* 2013). This decline of AMH production during puberty is related to the pubertal development stage (Grinspon and Rey, 2010).In the early development of the female foetus, the absence of AMH allows the Müllerian ducts to further develop, resulting in the internal female anatomy (Macklon *et al*., 2009). In females, AMH expression has been observed at approximately 36 weeks gestation in granulosa cells of preantral ovarian follicles and is produced by these cells until menopause (Themmen *et al.,* 2004). Its concentrations in adult women reflect the number of small antral and preantral follicles entering the growth phase of their life cycle, which is proportional to the number of primordial follicles that still remain in the ovary or the ovarian reserve (Ledger *et al.,* 2010). Anti-Müllerian hormone (AMH) decreases throughout a woman's reproductive life, which reflects the continuous decline of the oocyte/follicle pool with age and according to ovarian aging (Gracia *et al.,* 2012). Although AMH concentrations decrease with age, studies have shown that the day-to-day variability of AMH concentrations in menstruating women is low (Griesinger *et al.,* 2013). Anti-Müllerian hormone (AMH) has been used in the evaluation of ovarian reserve (Fleming *et al.,* 2009).

Follicle Stimulating Hormone (FSH) and Anti-Müllerian hormone (AMH) are two different hormones and predict ovarian reserve at two different stages of follicular development. FSH levels reflect antral and post antral follicular development while AMH values are representative of post primordial preantral follicular pool (Barad, 2010).

Prolactin is a polypeptide hormone that is responsible for lactation, breast development, and hundreds of other actions needed to maintain homeostasis. The chemical structures prolactin is similar to the structure of growth hormone and placental lactogen hormone. Together, they form the "prolactin/growth hormone/placental lactogen" family, which is characterized by a conserved helix bundle protein composition. All hormones in this family derive from a common ancestral gene (Al-Chalabi, 2021).

Prolactin is composed of 199 amino acids after the proteolytic cleavage of the signal peptide from the prolactin prohormone (pre-prolactin) and posttranslationally modified. The anterior pituitary synthesizes and secretes prolactin and dopamine-mediated hypothalamic regulation; however, the central nervous system, the immune system, the uterus, and the mammary glands all are capable of producing prolactin. Nipple stimulation, light, olfaction, and stress can all contribute to the initiation of prolactin synthesis in these tissues (Bass, 2021). Other factors that stimulate prolactin production include thyrotropin-releasing hormone (TRH), estrogen (pregnancy), and dopamine antagonists (antipsychotics). Males have characteristically low levels of prolactin. Abnormal elevation in males is suggestive of a potential underlying

pathological process like a pituitary adenoma or a medication adverse effect, which warrants further evaluation. Prolactin is low in males and non-lactating non-pregnant females (Alsalman, 2021).

The study was done to evaluate Anti-Mullerian Hormone (AMH), Follicle Stimulating Hormone (FSH) and Prolactin (PRL) in infertile women in Kano metropolis.

MATERIALS AND METHOD

Study Area

This study was carried out at several hospitals within Kano metropolis.

Study Design

This is a comparative cross-sectional study design.

Study Population

A total of 100 participants were recruited for the study. Out of which 50 were infertile women attending several hospitals in Kano metropolis and 50 were used as the control group.

Sample size determination

The sample size was calculated using the following formula (Cochran, 1977);

$n = \frac{Z^2 P(1-P)}{d^2}$

Where;

n =sample size

z=statistics for level of confidence at 90%=1.64

p=prevalence of female infertility 4% (Abiodun *et al.,* 2007) = 0.04

d= allowable error 5%=0.05

$$n = \frac{1.64^2 \times 0.04\ (1 - 0.04)}{0.05^2}$$

n= 41

Sampling Technique

Simple random sampling technique was employed for this study.

Ethical Approval

Ethical clearance to conduct the research was obtained from research ethical committee Kano State Ministry of Health (NHREC Approval Number: NHREC/17/03/2018). A written informed consent was obtained from the study participants after a verbal explanation is made to each participant. The provision of Helsinki declaration was respected at every step of the study.

Inclusion Criteria

1. Infertile women between the age range of 18 to 45 years who agreed to participate in the study.
2. Infertile women who have no underlying disease condition known to affect FSH, AMH and PRL such as cancer, systemic lupus erythematosus, pelvic infections, endometriosis and tubal diseases.

Exclusion Criteria

1. Patients on contraceptives.
2. Patients who were known to have hysterectomy

Data Collection

Socio demographic data was collected using questionnaire as shown in Appendix II. Detailed information about the study was given to the client and consent to participate was also obtained before patient sample and data was taken.

Sample Collection, Processing and Preservation

About 5mls of venous blood sample was collected aseptically from the ante-cubital fossa of each participant using sterile syringe and needle, into a vacutainer tube gel activator. The sample was allowed to clot and then centrifuged at 3000rpm for 5 minutes. The separated serum was transferred into pre-labelled serum

containers and will be stored at -40C until ready for assay.

Equipment

The following equipment was used; centrifuge: microplate ELISA reader, mircoplates ELISA washer: Rayto RT-2100C, tourniquet, test tube rack, timer, calculator, and water bath: diagen, Germany, and disposable tips: Micro points Nigeria. AMH Test device, AMH desiccant pouch, AMH test device ID chip, detection buffer, PRL and FSH Calibrators, PRL and FSH Enzyme reagent, Streptavidin coated plate, wash solution concentrate, stop solution, substrate A and B.

Laboratory assay methods

Serum level of FSH and PRL were assayed using sandwich-ELISA (Accu Bind, USA). Serum level of AMH was assayed using finecare AMH rapid quantitative test (Guangzhou Wondo Biotech co., Ltd China).

Serum FSH Determination

Procedure of the assay

Before proceeding with the assay, all reagents, serum, reference calibrators and controls were brought to room temperature. Microplate wells were formatted for each serum reference calibrator, control and patient specimen to be assayed in duplicate. 50ul of appropriate serum reference calibrator, control or specimen was pipetted into the assigned well. 100ul of FSH-Enzyme reagent solution was added to all wells. Microplate was swirled gently for 20-30 seconds to mix and cover. Incubated 60 minutes at room temperature. Content of the microplate was discarded by decantation and blotted dry with absorbent paper. 350ul of wash buffer was added and decanted. 100ul of working substrate solution was added to all wells and incubated for 15 minutes at room temperature 50ul of stop solution was added to each well and gently mixed for 15-20 seconds. Absorbance in each well was read at 450nm in a microplate reader.

Serum Anti-Müllerian hormone (AMH) Determination

Procedure of the assay

Step1: Preparation

The test cassette, detection buffer and specimen were allowed to equilibrate to room temperature prior to testing. The ID chip was taken out and made sure that the test cassette lot number matches with ID chip lot number. The ID chip was inserted into the chip port of meters.

Step2: Sampling

75ul of plasma was pipetted and added into the detection buffer tube.

Step3: Mixing

The lid of detection buffer tube was closed and mixed with the sample thoroughly by shaking it well.

Step4: Loading

75ul of sample mixture was pipetted into the sample well of the test cassette.

Step5: Testing

Timer was set to count down right after adding sample mixture into the sample well and left at room temperature for 15 minutes. The test cassette was inserted onto the test cassette holder of Meter. "Test" was pressed to start testing. Meter started to scan the sample-loaded test cassette immediately.

Step6: Reading results

Results were displayed on the main screen of Meter and printed out

Step7: Withdraw

The used test cassette was discarded after released from the instrument (as shown in Appendix VI and VII).

Serum PRL Determination

Procedure of the assay

Before proceeding with the assay, all reagents, serum, reference calibrators and controls were brought to room temperature. Microplate wells were formatted for each serum reference calibrator, control and patient specimen to be assayed in duplicate. 25ul of appropriate serum reference calibrator, control or specimen was pipetted into the assigned well. 100ul of PRL-Enzyme reagent solution was added to all wells. Microplate was swirled gently for 20-30 seconds to mix and cover. Incubated 60minutes at room temperature. Content of the microplate was discarded by decantation and blotted dry with absorbent paper. 350ul of wash buffer was added and decanted. 100ul of working substrate solution was added to all wells and incubated for 15 minutes at room temperature. 50ul of stop solution was added to each well and gently mixed for 15-20 seconds. Absorbance in each well was read at 450nm in a microplate reader.

Statistical analysis

Data obtained was evaluated using SPSS version 16.0 package by suitable statistical analyses. The mean and standard deviation was computed and results will be expressed as mean± SD. Student t-test was used to compare differences between means. Correlation was performed by Pearson's correlation coefficient. Statistical significance was set at $p<0.05$. Result was presented in the form of Figures and Tables.

RESULTS

The results obtained from this study are presented in tables 1 to 4 respectively.

Table 1 shows the distribution of infertile patients' across age groups and types, the higher frequency was observed in age 18-28 years with percentage frequency 52%, while the lower frequency was observed in age 39-48 with percentage frequency 14%.

Table 2 shows the distribution of AMH level in infertile patients across age groups, higher AMH level was observed in age group 18-28 with (Mean ± SD) of (2.95 ± 2.43) while lower AMH level was observed in age group 39-48 with (Mean ± SD)of**(**1.80 ± 2.80).

Table 3 shows AMH, PRL and FSH(Mean ± SD)in infertile patients and control group revealed (2.57 ± 2.88), (592.83 ± 332.12) and (14.91 ± 24.36) as compared to control (2.60 ± 1.44), (219.08 ± 90.85) and (6.42 ± 3.39) respectively.

Table 4 shows correlation of (AMH & PRL), (AMH & FSH), (PRL & FSH),and (FSH & PRL) in infertile patients which revealed CI (95%) of (498.44 – 687.22), (7.99 – 21.83), (7.99 – 21.83), (498.44 – 687.22) respectively.

Table 1: Distribution of infertile patients across age groups and types

Age group (years)	Primary (n)	Secondary(n)	frequency n(%)	p value
18 – 28	24	2	26(52)	0.206
29 – 38	16	1	17(34)	
39 – 48	5	2	7(14)	
Total	45	5		

$P < 0.05$ is statistically significant at 95% Confidencelevel. *chi square test; *n=Number of Subjects; %= percentage*

Table 2: Distribution of AMH level in infertile patients across age groups

	Age group (n=50)	AMH (Mean±SD)	Reference range
	18 – 28	2.95 ± 2.43	0.88 – 10.35
	29 – 38	1.97± 1.50	0.31 – 7.86
	39 – 48	1.80 ± 2.80	≤5.07

%=percentage; n=*Number of Subject*

Table 3 : AMH, PRL and FSH(Mean±SD) in infertile patientsand controls

Parameter	Patients	Controls	t-value	p-value
AMH (ng/ml)	2.57 ± 2.88	2.60 ± 1.44	-0.065	0.949
PRL(ng/mol)	592.83 ± 332.12	219.08 ± 90.85	7.675	0.001*
FSH (uiu/ml)	14.91 ± 24.36	6.42 ± 3.39	2.441	0.016*

%=percentage; n=*Number of Subject*

Table 4: Correlation of AMH, PRL and FSH in infertile patients

Parameters	r – value	Cl(95%)	p-value
AMH & PRL	-0.053	498.44 – 687.22	0.714^{Ns}
AMH & FSH	-0.076	7.99 – 21.83	0.598^{Ns}
PRL &FSH	-0.064	8.16 – 17.40	0.580^{Ns}

N = Total number of infertile patients, r = Pearson correlation coefficient, NS = Not significant, CI = confidence interval;

DISCUSSION

Infertility remains a major challenge for many women. For a long time, the diagnosis of infertility was mainly based on imaging tests for examining the uterus and fallopian tubes include ultrasound (particularly saline-infusion sonohysterography), hysterosalpingography, hysteroscopy, fertiliscopy, and laparoscopy. An endometrial biopsy to verify ovulation and Pap smear test are done to view pelvic organs and check for signs of infection. However, these do not give a proper infertility management and therefore the need for hormonal assay in diagnosis of infertility is essential.

In the present study, the mean and standard deviation of age group 19-28 was higher than the other age groups though they were all found to be within the reference ranges. This disagrees with the findings of Digban *et al.* (2017) who observed infertility in age group 27-35. This might be due to patient selection in their study, which consisted of older women.

In these findings, subfertile women had statistically significant ($P < 0.05$) higher basal serum FSH levels. This result agrees with the result reported by Kalaiselvi *et al*. (2012) and disagrees with result reported by Digban *et al.* (2017), who found no significant difference in basal serum FSH between infertile group and control group. This might be due to unresponsive gonads or hyperfuctioning pituitary adenomas.

In this study, serum Prolactin (PRL) was significantly higher ($P < 0.05$) in infertile group than in the control group. This agrees with result reported by Digban *et al.* (2017). This can be as a result of several effects that can interfere with ovulation leading to infertility; this includes decrease of Gonadotrophin Releasing Hormone (GnRH), inhibition of LH and FSH release and inhibition of both Oestrogen and Progesterone secretion in the ovary (Evers *et al*., 2002).

Current study reveals that, the mean distribution of AMH is slightly lower in infertile group than in control group. However,the difference in AMH is not statistically significant ($P > 0.05$). This result is in agreement with the findings of Bozkurt *et al.* (2016) and disagress with the findings of Kalaiselvi *et al*. (2012) who found significant lower random serum AMH in the subfertile women. This might be as a result ofreversible suppressive subtle effect of current use of oral contraceptives on AMH (Dolleman *et al.,* 2013).

CONCLUSION

Based on this study's findings, it can be concluded that infertility is commonly observed in age group 19-28.Infertile patients have high level of serum follicle stimulating hormone. Subfertile patients have high level of serum prolactin hormone. Hormonal assessment should be part of routine investigation of infertile women in Nigeria in order to assess the aetiology of hormonal abnormalities and determine the treatable groups.

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