



The Determination and Comparative Study of Basic Hormonal Assessment in Polycystic Ovary Syndrome (PCOS) Women Undergoing Assisted Reproduction Treatment in Southern Part of Nigeria

Ngozi O. Ibadin^{a*}, Rachael O Okojie^b, Kennedy O Ibadin^c

^{a,b}Department of Microbiology, University of Benin, Benin City Nigeria

^cHuman Reproduction Research Program Department of Obstetrics and Gynecology University of Benin Teaching Hospital Benin City Nigeria

^aEmail: ngozioj99@gmail.com, ^bEmail: kenbadin3@gmail.com

Abstract

Polycystic Ovarian Syndrome (PCOS) is a heterogenous disorder frequently diagnosed in women attending fertility clinics in Nigeria. It is characterized by anovulation, hyper androgenism and polycystic ovary, several factors have been associated with the pathophysiology of this disorder. A prospective cross-sectional study was conducted in four selected fertility clinics in South-South part of Nigeria. The purposive sampling technique was used to recruit participants. A total of 206 women were recruited in this study, 101 (49.1%) women fulfilled the Rotterdam criteria for the diagnosis of PCOS, while 105 women (50.9%) without PCOS were included as control. Biochemical hyper-androgenism as described by the Rotterdam criteria was observed in 69(68%) of women with PCOS, Elevated Anti-mullerian hormone (AMH) 87(86%) and elevated Luteinizing hormone (LH) 76(75%) were observed in women with PCOS. The aim of the study was to Determine and Compare levels of Luteinizing Hormone (LH) Follicle Stimulating Hormone (FSH) Anti-mullerian Hormone (AMH) Testosterone in the follicular fluid of women with PCOS undergoing Assisted Reproduction Treatment.

Keywords: Hormonal Profile; Polycystic Ovarian Syndrome.

Received: 11/3/2023

Accepted: 1/3/2024

Published: 1/13/2024

* Corresponding author.

1. Introduction

The follicular fluid is a clear liquid located in the ovary, it is in this fluid that complex physiological mechanism required for optimum development and release of the oocyte takes place, the follicular fluid contains the key metabolite and controls a myriad of complex signaling pathways in the ovary [1] the follicular fluid holds the oocyte and it is in this fluid that the oocyte develops to full maturity, this is vital also in the preceding process of oocyte fertilization and development of a healthy embryo. The cocktail of intracellular mediators which includes proteins, growth factors, hormones, chemokines, and cytokines determine the quality of oocyte and eventually the resulting oocyte of which practically in assisted reproduction technology, determines treatment outcome [2]. Therefore, the molecular dialogue between the follicles, cumulus cells, theca cells and the locally produced agents in direct response to steroid hormones directly or indirectly determines the oocyte quality [3]. The ovary is the most well-studied tissue regarding AMH expression and function. The ovarian AMH expression is detected in granulosa cells of activated primordial follicles and is highest in preantral and small antral follicles. AMH expression is absent in follicular stages following follicle-stimulating hormone (FSH)-dependent selection, although some expression remains in cumulus cells of pre-ovulatory follicles [4]. Expression of the AMH-specific type II receptor (AMHR2) coincides with AMH expression, albeit that AMHR2 expression is also detected in the cells. Thus, AMH may affect both granulosa and the cell function. Studies using AMH knockout (AMHKO) mouse models revealed that AMH inhibits the primordial follicle recruitment and selection of follicles for dominance, two major steps in folliculogenesis. In the absence of AMH, more primordial follicles are recruited and FSH sensitivity was increased [5] furthermore, studies in the AMHKO mice suggest that AMH may act as an intraovarian inhibitor of follicular atresia [6]. The effect of AMH on selection of follicles for dominance seems consistent across species. However, species differences may exist regarding preantral follicular growth. In non-human primates, Xu and his colleagues showed that in vitro treatment of macaque secondary follicles with AMH during the first 3 weeks of culture advanced follicle antrum formation within a week, whereas treatment with an AMH neutralizing antibody delayed this process. Consistent with the increased growth, estradiol (E2) production of these secondary follicles was also increased. In contrast, in mice, AMH mostly acts as a survival factor for small preantral follicles. Importantly, blocking AMH action in vitro through intra-ovarian infusion of an AMH neutralizing antibody for 4 days resulted in the growth of multiple antral follicles in most animals. In both the vitro and in vivo experiments, blocking AMH action in antral follicle increased E2 levels [7]. This study focuses on comparing AMH expression and AMH follicular fluid levels in women with PCOS, we also compare levels of luteinizing hormone (LH) and testosterone to investigate the relationship between hyperandrogenism, LH and AMH in PCOS. Many studies in the field of reproductive biology are serum based and investigate mainly the outcome of fertility treatment in women with PCOS or the metabolic characteristic of PCOS and its pathological consequence, however, few studies have focused on follicular fluid hormonal markers of PCOS. Since the follicular fluid is central in the physiological communications between cells of the female reproductive system, it seems its study may hold great potentials in adding to the knowledge on the pathophysiology of PCOS and improve its treatment.

2. Methods

2.1 Study area

This study was carried out in selected fertility clinics across South-South and South-East Nigeria and were independent private or public organization and were equipped with needed facilities and expertise for in-vitro fertilization treatment and PCOS diagnosis. Ethical clearance was obtained from the university of Benin Teaching Hospital Ethnical and Research committee. Consent was obtained from study participants and was voluntary, the confidentiality and privacy of all the participants was respected as they were assured that there was no penalty for refusal or withdrawal from participation. Information on medications, menstrual and clinical history was collected by administering a structured questionnaire. The purposive sampling technique was utilized in this study, this involved identifying and selecting study participant who fit the inclusion criteria for this study and recruiting them based on their availability and willingness to participate in the study.

2.2 Study population

The study group was taken from patient receiving in-vitro fertilization treatment in the selected hospital spread across the two geographical zone of Nigeria.

2.3 Inclusion criteria

The inclusion criteria as based on the definition of PCOS adopted at the joint consensus meeting of the American Society for Reproduction Medicine and the European Society for Human Reproduction and Embryology (ASRM/ESHRE) criteria.

- Oligo-and /or anovulation
- Hyperandrogenism (clinical and /or biochemical) and
- Polycystic ovaries with the exclusion of other etiologies.

2.4 Exclusion criteria

This study excluded women who has any of the following condition:

- History of chronic hypertension.
- Known autoimmune disorder.
- Women who did not give constant.
- Diabetes mellitus or treatment with oral glucocorticoids
- Congenital adrenal hyperplasia.

2.5 Follicular fluid sample collection

About 15 ml of follicular fluid samples were collected from the first follicular perforate at the start of each oocyte

pick-up procedure, only clear follicular fluid samples without blood contaminants were collected. Follicles were multiplied in each participant using the short antagonist method of ovarian hyperstimulation, then on the 12th day of ovarian cycle dominant follicles were measured using ultrasonographic method and when the dominant follicle measured 17mm or higher, follicular rupture was induced using recombinant human chorionic gonadotropin (HCG Pregnyl, Baxter, USA). Oocytes retrieval was done 36 hours following HCG ovulation trigger. Aspirated follicular fluid was aseptically transferred into a sterile plate for oocyte pick-up, the residual follicular fluid was aseptically transferred into sterile 15ml Falcon tubes for culture and then stored at -20°C for assay.

2.6 Hormonal profile analysis

Follicular fluid FSH, LH, AMH, and Testosterone were measured using automated immunoassay analyzer (mini VIDAS BioMerieux, France). FSH, LH AMH and testosterone assay for VIDAS Biomerieux combines any enzyme immunoassay sandwich method and a final fluorescent detection and this combination of methods is known as Enzyme Linked Fluorescent Assay (ELFA). The solid phase Receptacle (SPR) serves as the solid phase as well as the pipetting device for assay. Reagent for these assays is ready-to-use and pre-dispensed in a sealed reagent. Stored serum sample and reagents were brought to room temperature and sample was transferred into the well containing alkaline phosphatase-labelled antibodies (conjugate). The sample/conjugate mixture was cycled in and out of the SPR several times to increase the reaction speed, then the antigen binds to antibodies coated on the SPR and to the conjugate forming a 'sandwich'. Unbound components are eliminated during the washing steps. The final detection steps require the substrate (4-Methyl-umbelliferyl phosphate) to be cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-methyl-umbelliferone). The fluorescence of which is measured at 450nm. Then the intensity of the fluorescence is proportional to the concentration of antigen present in the sample. At the end of the assay, results were automatically calculated by the instrument in relation to the calibration curve stored in memory and then printed out. The external instrument barcode reader was used to scan the barcode for each lot of reagent kit and samples were gently mixed and 100µL (200µL for AMH) of sample were pipetted into wells in the reagent strip. The SPR and strips for each sample was inserted into the VIDAS instrument making sure that the color labels with the assay code on SPR and the reagent strip matches, and the assay was performed automatically, 12 samples at a run by the VIDAS instrument

3. Result

A total of 206 women attending fertility clinics in 4 facilities across the study area were recruited for this study and the study subjects were divided into two groups according to the Rotterdam diagnostic criteria for PCOS. 101(49%) women who met the Rotterdam criteria for diagnosis of PCOS were grouped as the PCOS group while, 106 (51%) apparently healthy women who had male factor infertility were recruited and grouped as the control group. The baseline demographic characteristic of the study of population is shown in Table 1. The study group age ranged from 26 to 53 yrs. and there was no significant difference between the mean ages of the PCOS and the control group with mean \pm SD which was 29.46 \pm 4.3 for PCOS group vs 30.44 \pm 3.1 for the control group. The mean body mass index (BMI) in the PCOS (25.37 \pm 5.9). group were significantly higher than mean BMI of the control group (24.29 \pm 3.8) on the other hand, the mean number of menstrual cycle/yrs. in the PCOS group (5 \pm 4.63)

were significantly lower than that of the control group (5±4.63 and 9±3.04).

Table 2 shows the hormonal profile analysis in the PCOS and control group, follicle stimulating hormone (FSH) levels showed no difference in the two groups, this was not so for the other hormone of interest analyzed. Serum levels of Anti-mullerian hormone (AMH), Luteinizing hormone (LM) and Testosterone were all significantly higher in the PCOS group. The standard error of mean for AMH levels (4.420± 0.679) were significantly higher than the control group (1.462±0.241) with a high significant level of p<0.001.

Table 1: Demographic characteristics of study subjects

Demographic Characteristics	PCOS (Mean ±SD)	Control (Mean ± SD)	P value
Mean age (years)	29.46±4.6	30.44±3.1	0.124
Number of menstrual cycle/year	5±4.63	9±3.04	0.001*
Body Mass index (kg/m ²)	25.37±5.9	24.29±3.8	0.021*

Values are mean± standard deviation (n=206) p< 0.05 = significant.

Table 2: Follicular fluid hormonal profile of study population

HORMONE	PCOS (Mean ± SEM)	CONTROL (Mean ± SEM)
AMH	4.420± 0.679 ^a	1.462±0.241
FSH	13.33±2.501 ^b	11.96±1.625
LH	27.54± 1.978 ^a	12.11±1.109
TESTOSTERONE	2.574± 0.356 ^a	0.75±0.207

P<0.001= very high significant difference, P> 0.05 = no significant difference, ^asignificant, ^b no significance. LH: Luteinizing Hormone; FSH: Follicular Stimulating Hormone; AMH: Anti-Mullerian Hormone

Table 3 shows the result of logistic regression analysis of hormones in women with PCOS, a positive and significant relationship was observed between AMH and testosterone (r= 0.838; P = 0.001). There was no correlation found between FSH levels and testosterone in women with PCOS, on the contrary, a negative correlation was observed between FSH and LH levels. When compared with proinflammatory cytokine IL6, using linear regression analysis with IL-6 as the response variable and AMH or testosterone as predictors, serum AMH concentration determined 54.7% of follicular fluid IL-6 levels (R²=0.354).

Table 3: Correlation between hormones in women with PCOS

Hormone	Testosterone (r)	p-value	LH(r)	p-value
AMH	0.838	0.001*	0.86	0.053
FSH	0.018	0.624	-0.047	0.001*

r: correlation coefficient; ^asignificant correlation; ^bNo significant correlation

4. Discussion

This study was undertaken to determine and compare levels of Luteinizing Hormone, Follicle Stimulating Hormone, Anti-mullerian Hormone and Testosterone in the follicular fluid of women with PCOS undergoing Assisted Reproduction Treatment. Since the follicular fluid is central in the physiological communications between cells of the female reproductive system, it seems its study may hold great potentials in adding to what is already known about the pathophysiology of infertility in PCOS and improve its treatment outcome. Regarding clinical presentation of women investigated in this study, elevated levels of testosterone was observed in 68% of women with PCOS which indicates androgen excess, elevated androgen concentration has been studied in relation to PCOS and is found to play a key role in its pathophysiology and development. This study estimated (41.6%) of oligomenorrhea in women with PCOS, this can be easily compared to 23.8% of oligomenorrhea reported by a study among Australian women [8]. In this study, 66.8% of women with PCOS were overweight with BMI >32, women in the PCOS group had higher median BMI which was statistically significant when compared to women in the control group. It is also worthy of note that 69.2% of our study population who were overweight or obese also had polycystic ovary morphology. This result is comparably higher than the BMI results from the work of Pasquali and his colleagues who revealed that about 35% of the women who were overweight or obese were also diagnosed with PCOS [9]. It appears that the increased incidence of high BMI reported in this study may be due to the predominance of fatty or starchy foods in the Nigerian local staple foods and the culture of lack of exercise amongst women in Southern Nigerian cities. Regarding Antimullerian Hormone (AMH), AMH concentrations were significantly elevated in women with PCOS compared to the control group in this present study, data from this study adds to existing information regarding AMH and its role in the etiology of PCOS by showing a remarkable association between AMH levels and hyperandrogenism in women with PCOS. AMH expression and follicular fluid AMH levels decline in gonadotropin-dependent follicles in normal ovulatory women, whereas this did not occur in PCOS patients. Likewise, the coincided increase in E2 levels was absent in PCOS patients [10]. This altered AMH expression may be the result of intrinsic granulosa cell dysregulation in PCOS. Both the cell and the granulosa cells of small antral follicles express higher levels of the luteinizing hormone (LH) receptor in PCOS women compared to normoovulatory women [11]. Combined with the elevated LH level in PCOS, this leads to hyperstimulation of the cells and premature luteinization of granulosa cells. Interestingly, LH stimulation increased AMH expression in granulosa cells of PCOS women but not in normal ovulatory women [12]. Treatment with 5- α -dihydrotestosterone yielded similar results. Furthermore, although estrogens suppress AMH expression, mediated via estrogen receptor β , in normoovulatory women, this suppression was not observed in granulosa cells of anovulatory PCOS women [13]. Combined these results suggest a failure in the downregulation of AMH expression in gonadotropin-dependent follicular stage in PCOS, which may contribute to a failure in follicular growth. From our results, one can deduce that the marked increase in AMH concentration may be directly linked to the altered immunological compounds and chronic low grade follicular inflammation detected in women with PCOS in this study. Also elevated level of AMH as detected in this study may not be surprising given that one may link this phenomenon to the fact that women with PCOS also present with early follicle excess, and it is known that AMH production corresponds to the number of follicles present in the ovaries at any given point during their reproductive years. This study revealed that elevated levels of AMH also correlated with elevated

level of testosterone in PCOS, this agrees with a previous study of women with PCOS, in which AMH was positively correlated to androstenedione and testosterone levels in the serum of women with PCOS. This study propose that the association between AMH and androgen excess i.e testosterone excess may be as a result of the catalytic effect of follicles testosterone and other androgen on follicles inducing multiple growth of primordial follicles which in turn induces excess production of AMH in the respective developing follicles. In this study, results established undeniable relationship between androgen and AMH which appears to be discriminatory to women with PCOS as this pattern was not observed in the healthy controls. Furthermore, using data from our results, we also propose that elevated levels of AMH observed in this study may be directly linked to the absence of aromatase activity induced by FSH which is attributed to induce the follicular growth arrest in PCOS, elevated levels of AMH observed in this study agrees with the work of Cook and his colleagues, who successfully correlated decreased serum FSH with elevated AMH in women with PCOS [8]. Concerning Luteinizing hormone (LH), in the study, it was observed that there may be a link between LH and elevated AMH levels in PCOS, this study, noticed a marked increase in LH levels more than 3 times in contrast to the LH levels measured for the control group. Till data there are unanswered questions about the correlation between LH and AMH, it is unclear if elevated levels of AMH directly affects the production of LH in women with PCOS [14]. Notwithstanding, recent studies have observed that elevation or amplification of pulsatile LH in the theca/granulosa cells of growing follicles can be the cause of follicular arrest of growing follicles typical in PCOS. LH receptor specific for LH have been discovered mostly on the surfaces of small sized ovaries predominant in women with PCOS, these receptor were not found in larger ovaries of women without PCOS, this phenomenon could be the main physiological component responsible for follicular growth arrest [15]. However, this explanation of follicular growth arrest is still debatable given that some studies have disputed this claim and recorded no evidence of association between follicular arrest elevated LH levels. On the other hand, some studies successfully established a statistical significance between AMH and LH levels, this study also found clear statistical difference between AMH and LH in women with PCOS whereby elevated AMH levels corresponded with LH elevations in PCOS its was independent of circulating FSH levels. The elevated levels of serum LH in PCOS group on this study can be said to have been a factor in increasing AMH levels. However, in this study no significant correlation was observed between follicular fluid AMH and serum LH levels in both the PCOS and control group. Similarly, there was no significant association found between AMH levels and elevated levels of LH in this study. Although our statistical logistical regression analysis found no association between AMH levels and LH, it is still probable that there could be a link found between these two biological entities in a larger study. Nonetheless, this study noted a statistically significant elevation in AMH levels in the PCOS group. Indicating that the elevations in serum AMH previously recorded in other studies may not be only as a result of multiple AMH producing small follicles seen in PCOS. Irrespective of the relationship between AMH and other circulating hormones. This study observes that women with PCOS have an altered AMH levels different from normal ovulating women and this could be admissible physiologically. AMH in humans acts as a gatekeeper of follicle growth by preventing premature selection and E2 production of small antral follicles. Species differences in FSH-dependency of preantral follicles may explain the observed differences in AMH effects on follicular growth. Although cultured macaque preantral follicles require FSH for survival These species differences should be taken into consideration when translating results to human, particularly when there is an implication of AMH in the pathophysiology of PCOS. In PCOS, where AMH levels are increased, the AMH effect on follicular growth/survival and FSH sensitivity may be

exacerbated leading to increased follicle number combined with follicular arrest. Several studies showed that the increased serum AMH level in PCOS is not only explained by the increased follicle number but also by increased hormonal secretion per follicle compared to normal ovaries.

References

- [1]. A. Gougeon. "Dynamics of follicular growth in the human: a model from preliminary results." *Hum Reprod.* vol 1: pp 81-77, 1986.
- [2]. J.G. Thompson, M. Lane, R.B. Gilchrist." Metabolism of the bovine cumulus-oocyte complex and influence on subsequent developmental competence." *Soc Reprod Fertil Suppl.* vol. 64, pp 179-190, 2007.
- [3]. R.B Gilchrist, J.G. Thompson. "Oocyte maturation: emerging concepts and technologies to improve developmental potential in vitro." *Theriogenology.* vol. 67, pp 6-15, Jan 2007.
- [4]. J.A. Visser, F.H. de Jong, J.S. Laven and A.P. Themmen. "Anti-Mullerian hormone: a new marker for ovarian function." *Reproduction.* vol. 131, pp 1-9 2006.
- [5]. A.L. Durlinger, B. Kramer, F.H. de Jong, J.T. Uilenbroek, J.A. Grootegoed et al. "Control of primordial follicle recruitment by anti-mullerian hormone in the mouse ovary." *Endocrinology* vol. 140, pp 5789-5796, 2009.
- [6]. J.A. Visser, A.L. Durlinger, I.J. Peters, E.R. van den Heuvel, U.M. Rose, P. Kramer, F.H. de Jong and A.P. Themmen. "Increased oocyte degeneration and follicular atresia during the estrous cycle in anti-Mullerian hormone null mice." *Endocrinology.* vol. 148, pp. 2301-2308, 2007.
- [7]. M.P. Grossman, S.T. Nakajima, M.E. Fallat and Y. Sioy. "Mullerian-inhabiting substance inhibits cytochrome P450 aromatase activity in human granulosa lutein cell culture." *Fertility and Sterility.* vol. 89, pp. 1364-1370, 2008.
- [8]. L.C. Varanasi, A. Subasinghe, Y.L. Jayasinghe, E.T. Callegari, S.M. Garland, A. Gorelik, J.D. Wark. "Polycystic ovarian syndrome: Prevalence and impact on the wellbeing of Australian women aged 16-29 years." *Aust NZ J Obstet Gynaecol.* 2018 Apr; vol. 58 issue 2, pp. 222-233, 2018.
- [9]. R. Pasquali, A. Gambineri, U. Pagotto. "The impact of obesity on reproduction in women with polycystic ovary syndrome." *BJOG.* vol. 113, pp. 1148-1159, Oct 2006.
- [10]. P. Pigny, E. Merlen, Y. Robert, C. Cortet-Rudelli, C. Decanter, S. Jonard, D. Dewailly. "Elevated serum level of anti-mullerian hormone in patients with polycystic ovary syndrome: relationship to the ovarian follicle excess and to the follicular arrest." *J Clin Endocrinol Metab.* 2003 Dec vol. 88, pp 5957-5962, Dec 2003.
- [11]. Robin Geoffroy, Marie Deknuydt, Anne-Laure Barbotin, Pascal Pigny, Sophie Catteau-Jonard, Didier Dewailly. "Anti-Müllerian hormone as a driving force of polycystic ovary syndrome, independently from insulin resistance." *Reproductive Biomedicine Online.* Vol 42, issue 5, pp 1023-1031, 2021.
- [12]. A.J. Jakimiuk, S.R. Weitsman, A. Navab, D.A. Magoffin. "Luteinizing hormone receptor, steroidogenesis acute regulatory protein, and steroidogenic enzyme messenger ribonucleic acids are overexpressed in thecal and granulosa cells from polycystic ovaries." *J Clin Endocrinol Metab.* vol. 86, pp. 1318-1323, Mar 2001.
- [13]. S.L. Mumford, R.S. Legro, M.P. Diamond, C. Coutifaris, A.Z. Steiner, W.D. Schlaff, R. Alvero, G.M.

- Christman, P.R. Casson, H. Huang, N. Santoro, E. Eisenberg, H. Zhang, M.I. Cedars. "Baseline AMH Level Associated With Ovulation Following Ovulation Induction in Women With Polycystic Ovary Syndrome." *J Clin Endocrinol Metab*, vol. 101, pp. 3288-3296, Sep. 2016.
- [14]. A. Pierre, M. Peigné, M. Grynberg, N. Arouche, J. Taieb, L. Hesters, J. Gonzalès, J.Y. Picard, D. Dewailly, R. Fanchin, S. Catteau-Jonard, N. di Clemente. "Loss of LH-induced down-regulation of anti-Müllerian hormone receptor expression may contribute to anovulation in women with polycystic ovary syndrome." *Hum Reprod*, vol. 28, pp. 762-769, Mar. 2013.
- [15]. R. Deswal, N. Sanda, A.S. Dang. "Association of Luteinizing hormone and LH receptor gene polymorphism with susceptibility of Polycystic ovary syndrome." *Syst Biol Reprod Med*, vol. 65, pp. 400-408, Oct. 2013.
- [16]. S. Catteau-Jonard, P. Pigny, A.C. Reyss, C. Decanter, E. Poncelet and D. Dewailly. "Changes in serum anti-Müllerian hormone level during low-dose recombination follicular stimulating hormone therapy for anovulation in polycystic ovary syndrome." *Journal of Clinical Endocrinology and Metabolism* vol. 92, pp. 4138-4143, 2007.