

ORIGINAL RESEARCH ARTICLE

Measurement of ovarian reserve in infertile versus fertile women: A case control study in Edo State, Nigeria

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Abstract

The reproductive potential in a woman is age related. Nevertheless, the female reproductive system undergoes ovarian follicular development, resulting in ovulation of matured ovum for fertilization. Consequently, female reproductive aging parallels the depletion of the store of follicles until menopause is attained. This is the essential reason for evaluating ovarian reserve in women of reproductive age for infertility screening. The objective of the present study is to compare the serum FSH, LH, estradiol and progesterone levels in infertile women with fertile controls. The study was designed as a case control descriptive study conducted in Benin City Edo State, Nigeria. Data were obtained through Questionnaire interview, while ELISA technique was used in the hormone analysis. Seventy-one women participated, of which 42 of them were infertile; and 29 age-matched fertile women (as controls). Secondary infertility was higher (64.3%) with 35% of them married between 3 and 5 years. The infertile patients had a significantly higher BMI than the controls ($p < 0.001$). There was a significant difference in the serum levels of FSH and LH of the infertile women compared to the controls (p -value = 0.001 and < 0.001) respectively. Similarly, day 3 and day 21 serum progesterone levels of controls were significantly higher than those of the infertile women (p -value = 0.001 and 0.001) respectively. Though mean serum estradiol levels were higher in controls than the infertile women it was however not statistically significant ($P = 0.191$). Sexually transmitted infections / pelvic inflammatory disease was identified to be treated in 52% of the infertile women. In conclusion, measurement of serum FSH, LH, Estradiol, Days 3 and 21 Progesterone collectively or FSH / LH ratio could be useful as markers for the assessment of ovarian reserve in women with infertility. (*Afr J Reprod Health* 2023; 27 [3]: 25-31).

Keywords: Infertility, reproductive age, menopause, ovarian function, hormones

Résumé

Le potentiel de reproduction chez une femme est lié à l'âge. Néanmoins, le système reproducteur féminin subit un développement folliculaire ovarien, entraînant l'ovulation de l'ovule mature pour la fécondation. Par conséquent, le vieillissement reproducteur féminin est parallèle à l'épuisement de la réserve de follicules jusqu'à ce que la ménopause soit atteinte. C'est la raison essentielle de l'évaluation de la réserve ovarienne chez les femmes en âge de procréer pour le dépistage de l'infertilité. L'objectif de la présente étude est de comparer les taux sériques de FSH, de LH, d'œstradiol et de progestérone chez des femmes infertiles avec des témoins fertiles. L'étude a été conçue comme une étude descriptive cas-témoin menée dans l'État de Benin City Edo, au Nigéria. Les données ont été obtenues par le biais d'un entretien par questionnaire, tandis que la technique ELISA a été utilisée dans l'analyse hormonale. Soixante et onze femmes ont participé, dont 42 étaient infertiles ; et 29 femmes fertiles du même âge (témoins). L'infertilité secondaire était plus élevée (64,3 %) avec 35 % d'entre elles mariées entre 3 et 5 ans. Les patientes infertiles avaient un IMC significativement plus élevé que les témoins ($p < 0,001$). Il y avait une différence significative dans les taux sériques de FSH et de LH des femmes infertiles par rapport aux témoins (valeur de $p = 0,001$ et $< 0,001$) respectivement. De même, les taux de progestérone sérique au jour 3 et au jour 21 des témoins étaient significativement plus élevés que ceux des femmes infertiles (valeur de $p = 0,001$ et $0,001$), respectivement. Bien que les taux sériques moyens d'œstradiol aient été plus élevés chez les témoins que chez les femmes infertiles, ils n'étaient cependant pas statistiquement significatifs ($P = 0,191$). Les infections sexuellement transmissibles/maladies inflammatoires pelviennes ont été identifiées comme étant traitées chez 52 % des femmes infertiles. En conclusion, la mesure de la FSH sérique, de la LH, de l'œstradiol, de la Progestérone aux jours 3 et 21 collectivement ou du rapport FSH/LH pourrait être utile comme marqueurs pour l'évaluation de la réserve ovarienne chez les femmes infertiles. (*Afr J Reprod Health* 2023; 27 [3]: 25-31).

Mots-clés: Infertilité, âge de procréer, ménopause, fonction ovarienne, hormones

Introduction

Infertility, defined as the failure to achieve a conception after twelve months of regular unprotected sexual intercourse¹ is a global public health problem affecting millions of people of reproductive age. Infertility could be classified as primary when there is inability to achieve any conception, or secondary when there is inability to achieve pregnancy after previous successful conception(s). It is associated with a lot of adverse social consequences including stigmatization, deprivation and neglect, violence, marital disharmony, psychosocial and mental health issues^{2,3}. Globally, it is estimated to occur in 5- 8 % of couples (about 48 million couples)². Prevalence is higher in Sub-Saharan Africa, with about 10 – 30% of couples affected in Nigeria⁴.

The decline in fertility can be linked to multiple events associated with advancing age, decline in follicular quantity and quality, frequency and efficiency of ovulation, sexual function, uterine diseases and complications of pregnancy (e.g. hypertensive disease and gestational diabetes). In the female, it may be caused by a range of abnormalities of the external or internal genital systems, the metabolic system or of endocrine origin. Recent social, demographic and career related trends have led to an increased tendency for women to delay childbearing thereby increasing the incidence of age-related infertility⁵.

The endocrine function plays a major role by its contribution to the ovarian reserve which correlates with age and is determined by the quantity and quality of the primordial follicular pool. Genetic factors, social habits such as smoking, pelvic surgeries and infections are also implicated in reduced ovarian reserve in older women⁶. Nevertheless, there is considerable variation in ovarian reserve among women of the same chronologic age. Reduced ovarian reserve remains an important aetiology of infertility in women. Recent data shows that about 10% of women requiring treatment of infertility have reduced ovarian reserve compared with fertile women of same age⁷.

Currently, anti-mullerian hormone (AMH), follicle stimulating hormone (FSH), luteinizing hormone (LH), Estradiol (E2) and Progesterone are used to assess ovarian reserve. However, an ideal ovarian reserve parameter should be easily

measurable, minimally invasive, affordable and have good predictive value⁸. 'Day 3' serum FSH level is widely used to determine ovarian reserve in women at risk of infertility irrespective of their chronological age^{9,10}. Studies have shown that the levels of FSH and LH are higher in infertile and menopausal women than the normal fertile women^{11,12}. This may emanate from reduced FSH and LH response to gonadotropin releasing hormone¹³. Hence, the desire for using the analytes as good biomarkers of ovarian function. A further study however reported that although FSH was a good predictor of poor follicular yield, anti-mullerian hormone was observed to be a better predictor¹⁴.

Progesterone levels are < 2ng/ml prior to ovulation, and > 5ng/ml after ovulation approximately 7 days before expected menstruation while in Luteal phase it varies according to the cycle which therefore makes it useful to predicting ovulation¹⁵. Estradiol levels provide useful information for the evaluation of ovarian reserve. Early elevations in Estradiol levels are in tandem with follicular development and can predict selection of a dominant follicle. The levels also provide a guide on the commencement of gonadotropin stimulation^{16,17}.

This study aimed at the measurement of fertility hormones such as follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2) and progesterone as markers for the assessment of ovarian reserve in infertile Nigerian women who reside in Edo State and to determine the most useful predictors of infertility that may be applicable in resource constraint settings.

Methods

This was a case control descriptive study carried out from February to November 2019 in a tertiary referral hospital that has medical and surgical specialties in Benin City. The patients were women who attended gynaecology clinic of the University of Benin Teaching Hospital with complain of inability to conceive. The controls were age-matched married female members of staff of the hospital who had no difficulty in getting pregnant, selected randomly. Women below 18 years and above 45 years, breastfeeding mothers, those on contraception and those who refused to give consent were excluded from the study.

Sample size was calculated using the formula¹⁸

$$n = 2\sigma^2 (Z \text{ power} + Z^{\alpha 2})^2 / d^2$$

Where n = sample size in groups (equal size groups)

σ = standard deviation of the outcome variable = 3.56

Z power = desired power (typically 0.84 for 80% power) = 0.84

Z α 2 = desired level of statistical significance (typically 1.96) = 1.96

d = Difference in the mean of infertile and control women = 3.66 – 5.62

Mathematically, mean of infertile and control women is 3.66 and 5.62 respectively

$$n = 2(3.56)^2(0.84 + 1.96)^2 / (3.66 - 5.62)^2$$

$$n = 25.34 \times 7.84 / 3.84$$

$$n = 51.7$$

With a factor of 10% attrition the final sample size for infertile participants and control was estimated at 57 respectively.

A self-administered structured questionnaire was used to obtain information on biodata, health related factors and socio-economic status from women who consented to the study.

Participants heights were measured using stadiometer, and weights measured with the women wearing light cloths on the bathroom weighing scale. Body Mass Index was calculated using the weight divided by the square of the height (kg/m²). 5 ml of venous blood was drawn from patients and controls following routine aseptic procedure on days 3 and 21 of their menstrual cycle. Blood samples were dispensed into plain bottles, allowed to clot over 20 to 60 minutes before centrifuged at 3000 revolutions per minute for 15 minutes to yield clear serum. The serum was aliquoted for storage at minus 20° Celcius (-20°c). Serum was analyzed in batches for day 3 FSH, LH, oestradiol and progesterone; and day 21 progesterone using DRG diagnostic ELISA kits (Germany)¹⁹.

Quality control samples were analyzed simultaneously in each batch to ensure accuracy and precision. Inter- and intra-assay co-efficient of variation (CV) for FSH, and LH were 6.06% and 5.53%; and 6.23% and 5.58% respectively.

Data analysis

Data obtained from the study was analyzed using the Statistical Package for Social Sciences (SPSS) version 20.0 (SPSS Inc. Chicago, Illinois, USA).

Descriptive demographic data are presented in frequency distribution tables. The values of hormonal assays are presented as continuous variables. The mean in infertile and controls were compared using the Student's t-test. All statistics are based on 95% confidence interval with $p \leq 0.05$ considered significant.

Ethical consideration

Ethical approval (number: ADM/E22/A/VOL VII/908) was obtained from the Ethics and Research Committee of the University of Benin Teaching Hospital. Written informed consent was obtained from each participant after explaining the nature of the study, the time it will take, what samples were required and the sampling procedure. Participants were informed of their liberty to withdraw from the study without any consequences. The cost of the investigations were borne by the researchers.

Results

A total of 71 women consented to participate in the study. Forty-two were infertile patients and 29 age-matched controls. Of the 42 patients, 15 (35.7%) presented with primary infertility while 27 (64.3%) had secondary infertility. Fourteen (33.3%) of the infertile patients were married for about 2years, 15 (35.7%) married for 3–5 years, and 13 (31%) married for more than 5 years.

The mean age of the infertile women was 32.02 SD \pm 4.81 years with a range of 27 to 37 years while, the mean age of controls was 31.2 SD \pm 5.37 years with a range of 26 and 37 years. About 28 (66.7%) of the infertile patients completed secondary education and 8(19.0%) had tertiary education. Among the controls 17 (58.6%) completed secondary education and 12 (41.4%) had tertiary education (Table 1). Among the 42 patients, a total of 18 (42.9%) were unemployed, 14 (33.3%) were self-employed and 10 (23.8%) were civil servants. Among the patients, 11 (26.2%) of them had normal Body Mass Index (18-24kg/m²); 23 (54.8%) were overweight (BMI = 25 - 29kg/m²) and 8 (19%) were obese (BMI > 30kg/m²). Among the controls, 22 (75.9%) had normal BMI and 7 (24.1%) were overweight. The infertile patients had a significantly higher BMI than the controls ($p < 0.001$). Table 1.

Table 1: Socio-demographic characteristics of infertile patients and controls

	Patients n=42	Controls n=29	P - value
Age (years) Mean (SD)	32.02 ± 4.81	31.21 ± 5.37	0.505
Educational Status			
None	1 (2.4)	0 (0.0)	
Primary	5 (11.9)	0 (0.0)	
Secondary	28 (66.7)	17 (58.6)	
Tertiary	8 (19.0)	12 (41.4)	
Body Mass Index (Kg/m²)			
18 – 24.9	11 (26.2)	22 (75.9)	
25 – 29.9	23 (54.8)	7 (24.1)	< 0.001*
≥30	8 (19.0)	0(0.0)	

*Significance: $p \leq 0.05$

Table 2: Comparing mean differences in Hormones of infertile patients and controls

	Patients (n=42) Mean (SD)	Controls (n=29) Mean (SD)	p - value
SerumFSH (mIU/l)	16.0 ± 11.9	9.9±2.3	0.001*
Serum LH(mIU/L)	17.1 ± 8.1	9.11±3.1	< 0.001*
Serum Estradiol(pg/ml)	58.4 ± 6.7	76.2±13.0	0.191
Serum progesterone Day 3(ng/ml)	0.6 ± 0.1	2.1±0.2	0.001*
Serum progesterone Day 21(ng/ml)	4.4 ± 0.4	13.2±0.7	0.001*

*Significance: ($p \leq 0.05$), SD: Standard Deviation

Sexually transmitted infections / pelvic inflammatory disease was also identified in 22 of the patients for which treatment was offered. There was a significant difference in the serum levels of FSH and LH of the infertile women compared to the controls (p -value = 0.001 and <0.001) respectively. Similarly, day 3 and day 21 serum progesterone levels of controls were significantly higher than those of the infertile women (p -value = 0.001 and 0.001) respectively. Though mean serum estradiol levels were higher in controls than the infertile women it was however not statistically significant ($P=0.191$). See Table 2.

Discussion

Infertility is a multifactorial condition viewed by many as a distraction to families, which in many instances lead to blames and regrets. Historically, age is considered the single most important factor that determines the quality and quantity of ovarian reserve. In fact, both quality and quantity of ovarian follicles significantly decrease with advancing age. In view of the above findings, it is expected that women of the same chronologic age should have equal reproductive potentials, but this is not always the case in real life situation as depicted in this study

by the mean of infertile patients age 32.02 ± 4.81 years and controls age 31.2 ± 5.37 years. An earlier study by El-Toukhy *et al* argued that young age does not protect against adverse effect of reduced ovarian reserve²⁰.

The FSH in a normal woman contributes to good follicular development, ovulation and fertility. At molecular level, FSH ensures fertility by enhancing the response of the numerous mature follicles with granular cells to produce adequate estradiol through activation by aromatase, as well as induction of LH receptor to stimulate ovulation^{21,22}. Deficiency of these factors result in infertility in a woman. In order to address this problem studies have been focusing on in vitro fertilization programmes²³. Contributing to those programs this study focuses on women who presented with complains of infertility with the aim of assessing the ovarian reserve of these patients before providing appropriate management measures for them.

The result of this study shows that a greater proportion of women who presented with infertility were married for a minimum of 3 years and maximum of 5 years. Lack of awareness and misinformation may cause late presentation and diagnosis among these patients. Other factors

include traditional / cultural believe, unavailability or inability to access health facility. Treatment for infertility like other illnesses among Nigerians is characterized by first visit to traditional herbal practitioners and religious homes for prayers resulting in delay in seeking medical assistance.

The higher rate of secondary infertility (64.3%) in this study agrees with previous studies in northwestern, southwestern and North Eastern Nigeria, where 67.2%, 77.5% and 63.3% had secondary infertility respectively^{24,25}. African review of infertility identified pelvic inflammatory disease as a cause in 39.38% of the cases²⁶. Although this study did not stratify the infertile women according to body weight and relate with the status of ovarian reserve, 73.8% of 42 of them were overweight and obese. This was significant in the study. Going by observations from independent studies, body size was found to have effect on fertility in late reproductive age, on one hand^{27,28}. The reason is due to failure of plasma level of Luteinizing hormone (LH) to increase in obesity. On the other hand, some studies observed that overweight or obesity does not affect ovarian reserve²⁹.

High day 3 serum (basal) FSH and LH levels observed in infertile patients compared to controls in this study agrees with earlier study by Randolph et al. They observed, that serum FSH and LH were significantly higher in infertile patients than controls¹¹. Other studies recruited 48 regularly menstruating infertile women age < 40 years with age-matched controls. The women with infertility had significantly higher basal FSH, LH and follicular phase LH concentration³⁰. Smith et al in a review of the role of LH as a marker of ovarian reserve noted it to present an imprecise reason for its use for the testing despite its prominent role in the early follicular phase of the menstrual cycle³¹. In a related study, LH was found to promote follicular growth and maturation but not reliable in determining the status of the ovarian reserve as it is affected by several factors³². Following the above observations, it was concluded that FSH/LH ratio should be considered as a better marker of assessment. The establishment of the ratio is out of scope of this study.

Elevated serum FSH is associated with low serum levels of inhibin and activin, shortened follicular phase and decreased number of follicles (ovarian reserve) in the ovary. The predictive

importance and clinical impact of day 3 FSH in younger women (less than 35 years) exposed to gonadotropin in a study showed reduced chances of spontaneous and sustained pregnancy occurrence when FSH levels is 10U/L and above³³.

Low plasma levels of follicle stimulating hormone and luteinizing hormone equally cause infertility following failure of follicular development and ovulation. Estradiol and progesterone synthesis and function also depend on FSH and LH³⁴.

The estradiol level among the infertile women was observed to be low compared with the normal women with higher levels. This was similar to a study by De Pergola *et al*³⁵. The reason for this is explained in an in vitro study by Lin Q, et al where they observed that high leptin level in plasma of obese women is equally represented in the granulosa cells which cause a reduction in the synthesis of estradiol and progesterone³⁶.

This study has shown that, age alone is not a reliable index of ovarian reserve and conception. Contemporary researches on infertility are aimed at addressing the problem in women through in vitro fertilization programme^{23,36}. The question remains, "how does this benefit those in the resource poor economy?"

Conclusion

It was observed that FSH, LH, Estradiol and progesterone could be used collectively as markers for the assessment of ovarian function in women with infertility. Given the role of FSH and LH in the follicular phase of the menstrual cycle, FSH / LH ratio may be a better marker for assessing ovarian reserve in resource poor communities.

Limitation

The results may not be true representation of patients with infertility in the general population because of the small sample size.

Conflict of interest

None was declared.

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