Original Research Article

Hormonal profile of women of reproductive age investigated for infertility in Bida Metropolis, Niger State, Nigeria

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Abstract: Infertility is a major gynecological challenge for many women within the reproductive age and may arise as a result of hormonal dysfunction of the hypothalamic-pituitary-gonadal axis. The aim of this cross-sectional study was to evaluate the hormonal profile of infertile women in Bida metropolis, Niger State, Nigeria with a view to assess the contribution of certain endocrinopathies to infertility in the study population. Serum concentration of Leutinizing Hormone (LH), Follicle stimulating hormone (FSH) and Prolactin (PRL) were measured by the microwell enzyme linked immunosorbent assay (ELISA) technique based on the non competitive sandwich principle, while Progesterone and Estradiol were measured based on competitive ELISA principle in 150 infertile women (test) and 50 fertile women (control). The serum LH, Estradiol and Progesterone levels were significantly lower ($P<0.05$) in the infertile group (10.2 ± 3.1 mlu/ml, 80.0 ± 10.6 pg/ml and 5.4 ± 2.2 ng/ml, respectively) compared to the control group (46.3 ± 2.3 mlu/ml, 176.6 ± 15.8 pg/ml and 17.58 ± 5.3 ng/ml, respectively). While serum Prolactin was significantly higher ($P<0.05$) in infertile group (29.6 ± 10.3 ng/ml) than in the control group (16.2 ± 9.2 ng/ml), the FSH level of infertile group (11.2 ± 4.8 mlu/ml) was not significantly different ($P>0.05$) from that of the control group (10.4 ± 3.7 mlu/ml). 16.7% of the infertile women studied had normal hormonal levels; the remaining 83.3% had abnormal hormonal pattern comprising of: hyperprolactinemia (6.7%), hyper gonadotropic hypogonadism (13.3%), isolated increase in Oestrogen (13.3%) and hyperprolactinemia/hypogonadotropic hypogonadism (20%). Hypogonadotropic hypogonadism was found to have the highest incidence (30%) of all the hormonal disorders recorded among the infertile women under study and was also found to coexist with hyperprolactinemia. This study underscores the clinical significance and contribution of these hormones during reproductive stage of life.

Keywords: Reproductive age, Gynecological problem, Infertility, Endocrinopathies, Hormonal profile

INTRODUCTION

Infertility is a growing gynaecological problem in our environment with a rising number of women within reproductive age having difficulty becoming pregnant [1]. 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 [2]. The definition of infertility has been extended to also include the inability to carry a pregnancy to the delivery of a live baby [3]. 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 [4, 5]. 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 [6].

The reproductive age for women is considered to be between 15 and 45 years of age. Demographically, one can be determined infertile after 5 years exposure of consistent sexual intercourse without the use of contraceptive and being non-lactating [6]. In many cultures around the world, infertility has a very strong social stigma, especially in its relation to women, depending on the cultural context [7]. Reproduction, a basic human instinct, represents a continuation of the family and the survival of the species. Therefore fertility is respected and almost revered in most cultures around the world [8]. Although infertility is not a disease in the classic sense, it is an extremely important personal concern for many couples and a significant health problem for the medical profession. The psycho-social consequences of infertility include stress, anxiety, depression, marital difficulties, physical violence and social discrimination [4, 7, 9]. In Nigeria for example, motherhood can define a woman's social worth, her treatment in a community, her economic status, and her own self worth.

Available evidence suggests that the social consequences of infertility are particularly profound for African women as compared to men. Recent studies from various parts of Africa have demonstrated higher rates of Human Immuno deficiency virus (HIV) infection in infertile women compared to fertile controls [10], suggesting a greater vulnerability of infertile women to the social factors that predispose to HIV [11]. The fear of infertility has also been identified as the most important reason why women often do not use contraceptives, believing that these could lead to infertility. Until recently, studies have focused predominantly on patients in industrialized countries, while the experience of infertility in the developing world has received comparatively little attention [12].

Furthermore, infertility is the leading reason for gynaecological consultation in Nigeria and couples seeking help can place a heavy burden on limited health care resources [13]. Since the desire for reproduction is a basic human instinct; it is not surprising that infertility is causing serious distress to many couples. The causes of infertility vary from country to country and for different social groups [14]. Among other causes, infertility may arise as a result of hormonal dysfunction of the hypothalamic-pituitary-gonadal axis. Measurement of peptide and steroid hormones in the serum is therefore an essential aspect of the evaluation of infertility. While, Follicle-stimulating hormone (FSH) stimulates the growth of the follicles, luteinizing hormone (LH) stimulates ovulation; both of which are produce in the pituitary gland. Prolactin, also produce in

the pituitary gland, interacts with the breasts and ovaries. It stimulates the growth of the mammary glands during pregnancy, as well as milk production after birth. Too high a level of prolactin (hyperprolactinemia) may inhibit ovulation. Oestradiol, on the other hand is produced in the ovaries. It encourages growth of the endometrium during menstrual cycle. An elevated oestradiol level mid-cycle causes an increase in LH, which triggers ovulation. Progesterone together with oestradiol helps to prepare the endometrium to receive the fertilized egg. And during pregnancy, the same progesterone supports the endometrium so that it isn't shed [8, 15].

Unlike many other causes of infertility in black community especially in Bida, hormonal factor has been least considered, researched, or even discussed among professionals within the health care community. More worrisome is that the assessment of the contribution of certain endocrinopathies in infertile women is neglected in the management of patients in most part of Nigeria including Bida. Against this background, the aim of this present study was therefore to evaluate the hormonal profile (Mean±S.D value of FSH, LH, progesterone, estradiol and prolactin levels) of infertile women living in Bida, Niger state as compared to the control group.

MATERIALS AND METHODS
Study Area and Population

The study was carried out at Umaru Sanda General Hospital, Bida, and a tertiary health care centre in Niger state. The state is named after the River Niger. Two of Nigeria's major hydroelectric power stations, the Kainji Dam and the Shiroro Dam, are located in Niger State. The state capital is Minna, and other major cities are Bida, Kontagora and Suleja. Bida is the traditional City of the Nupes, a Local Government Area (51 km^2) located South-West of Minna, Niger State, coordinates: 9°05′N 6°01′E in Central Northern Nigeria. It is a dry and arid town with an estimated population of 178, 840 [16]. The major ethnic groups are Nupes, Yorubas, and Igbos. Inter-marriage between these tribes occurs quite frequently especially in Bida town. Majority of the inhabitants are farmers, traders and civil servants. Social amenities available in Bida include primary schools, post primary institutions, tertiary institutions, primary health care centers, secondary health care centers and tertiary health care centers.

Sample size

A total of 200 blood specimens were randomly collected from interested 200 female subjects living in

Bida metropolis, Niger state. They consisted of 150 infertile women and 50 apparently healthy fertile women of reproductive age with normal menstrual cycle (range 28-30 days) as control.

Ethical consideration

Ethical approval was sought for and obtained from the Igbinedion University Health Research Ethics Committee. Also, administrative permission for the study was obtained from the Management, Umaru Sanda General Hospital, Bida, Niger state.

Eligibility of Subjects
Inclusion Criteria

Consenting female subjects within reproductive age (15-45years) that are unable to conceive after one year despite regular and unprotected sexual intercourse, as well as apparently healthy subjects who were married and having children were included in the study.

Exclusion Criteria

Subjects who were below 15years and above 45years, as well as infertile women who were currently on hormonal therapy for the treatment of their infertility were excluded from the study. Pregnant and lactating women, fertile women using hormonal injectable or oral contraceptives and those whose years of last child birth were more than five years were excluded from the control group.

Informed Consent

The purpose and protocol of the study were clearly explained to each patient and all participants were requested to voluntarily sign the consent forms in their own handwriting as proof of willingness to provide samples for the tests.

Data Collection

Prior to specimen collection, demographic and gynaecological information of the participants were obtained through administration of prepared questionnaires. Interpreter was provided for translation in local dialect where necessary. Each questionnaire had a unique participant identification number (PIDN). The first part of the questionnaires contained the biodata of the patients e.g. sex, age etc. Second part includes gynaecological data such as age at menarch, menstrual status, duration of menstrual cycles, duration of menstrual flow, duration of infertility, date of last child birth, history of use of contraception etc. For reasons of privacy, all data were kept confidential in accordance with World Medical Association declaration of Helsinki [17]. For each participant, only the PIDN was recorded on the laboratory forms (no names). All the filled questionnaires were destroyed after data entry has been completed.

Specimen Collection

About 10ml of blood specimen was collected from a peripheral vein (antecubital venepunture) of each participant in fasting state. This was transferred into a plain bottle and allowed to clot in about 30min. It was centrifuged for 5minutes at 3000 revolutions per minute. The serum was separated from the cells, transferred to another bottle and then stored frozen at -20°C until the time for analysis.

Hormonal Assay

The serum LH, FSH and Prolactin were measured spectrophotometrically by the microwell enzyme linked immunosorbent assay (ELISA) technique based on the non competitive sandwich principle, while the serum Progesterone and Estradiol were measured based on competitive ELISA principle in accordance with the methods provided by the diagnostic reagent kits supplied by Syntron Bioresearch, California, USA.

STATISTICAL ANALYSIS

The demographic and gynaecological characteristics of the participants were expressed as mean values and standard deviation. Differences in serum hormone levels between the groups of fertile and infertile women were tested by student t-test. P values <0.05 were considered statistically significant [18]. The statistical package for the social science (SPSS) for window version 18.0 was used for all calculations and statistical analysis.

RESULTS

The present study examined the hormonal profile of women within reproductive age in Bida metropolis, Niger state, Nigeria. A total of 200 subjects (150 infertile women and 50 fertile women) with mean age of 30±2.6 years were used for the study. The mean and standard deviation of the demographic and gynecological characteristics of the infertile and fertile women are presented in Table 1. The infertile women (Test) had a mean±SD age of 31±3.8 years, while the fertile women (Control) had a mean age of 33.6±3.2 years. The mean±SD age, age at menarch and duration of menstrual flow of the infertile women were not statistically different from those of the fertile women (P>0.05). However, the duration of menstrual cycles was significantly higher (P<0.05) in infertile women (60.5±7.4 days) than in fertile women (25.8±2.9 days).

Table 1: Demographic and Gynecological Characteristics (mean±SD) of infertile and fertile women

Characteristics	Test (n=150)	Control (n=50)	P-values
Age	31.7±3.8	33.6±3.2	>0.05
Age at menarch	14.2± 2.3	14.4±2.8	>0.05
Duration of menstrual cycles (Days)	60.5±7.4	25.8±2.9	<0.05
Duration of menstrual flow (Days)	4.3±0.4	4.6±0.6	>0.05

Key: *SD = Standard Deviation. P<0.05 is considered significant.*

The serum hormonal profile of the infertile and fertile women is presented in Table 2. On one hand, the serum LH, Estradiol and Progesterone were significantly lower (P<0.05) in the infertile group (10.2±3.1 mlu/ml, 80.0±10.6 pg/ml and 5.4±2.2 ng/ml, respectively) compared to the control group (46.3±2.3 mlu/ml, 176.6±15.8 pg/ml and 17.58±5.3 ng/ml). On the other hand, serum Prolactin was significantly higher (P<0.05) in infertile women (29.6±10.3 ng/ml) than in the control group (16.2±9.2 ng/ml). The FSH level of the infertile group (11.2±4.8 mlu/ml) was not significantly different (P>0.05) from that of the control group (10.4±3.7 mlu/ml).

Table 2: Serum hormonal profile (mean±SD) of infertile and fertile women

Hormone (unit)	Normal value	Test (n=150)	Control (n=50)	P-values
LH (mlu/ml)	0.5-10.5	10.2±3.1	46.3±2.3	<0.05
FSH (mlu/ml)	2.0-12.0	11.2±4.8	10.4±3.7	>0.05
PRL (ng/ml)	1.2-19.5	29.6±10.3	16.2±9.2	<0.05
PROG (ng/ml)	2.0-25.0	5.4±2.2	17.58±5.3	<0.05
E2 (pg/ml)	44-196	80.0±10.6	176.6±15.8	<0.05

Key: *FSH= Follicle stimulating hormone, LH = Leutinizing Hormone, E2 = Estradiol, PROG = Progesterone, PRL = Prolactin, SD = Standard Deviation. P<0.05 is considered significant.*

The comparison of the serum hormonal profile of menstruating and non-menstruating infertile women is presented in Table 3. The serum FSH, Estradiol and Progesterone levels were significantly lower (P<0.05) in non-menstruating infertile women (7.3±3.6 mlu/ml, 100.8±62 pg/ml and 0.7±0.1 ng/ml, respectively) than in menstruating infertile women (17.8±6.2 mlu/ml, 290.3± 176 pg/ml and 5.0±1.0 ng/ml, respectively), while the serum Prolactin was significantly higher (P<0.05) in non-menstruating infertile women (49.4±5.1 ng/ml) compared to the menstruating infertile women (30.9±3.6 ng/ml). There was no significant difference (P>0.05) in the LH level of the menstruating (9.2±2.7 mlu/ml) and non-menstruating infertile women (8.8±4.2 mlu/ml).

Table 3: Serum hormonal profile (mean ± SD) of infertile women that are menstruating compared to infertile women that are not menstruating

Hormone (Unit)	Normal value	Menstruating (n=145)	Non-menstruating (n=5)	p-value
LH (mlu/ml)	0.5-10.5	9.2±2.7	8.8±4.2	>0.05
FSH (mlu/ml)	2.0-12.0	17.8±6.2	7.3±3.6	<0.05
PRL (ng/ml)	1.2-19.5	30.9±3.6	49.4±5.1	<0.05
PROG (ng/ml)	2.0-25.0	5.0±1.0	0.7±0.1	<0.05
E2 (pg/ml)	44-196	290.3± 176	100.8±62	<0.05

Key: *FSH= Follicle stimulating hormone, LH = Leutinizing Hormone, PRL = Prolactin, PROG = Progesterone, E2 = Estradiol, SD = Standard Deviation. P<0.05 is considered significant.*

The relationship between the hormonal abnormalities found in infertile women and their mean duration of infertility is presented in Table 4. Out of the 150 infertile females examined, 25 (16.7%) had normal hormonal levels; while the remaining 125 (83.3%) had abnormal hormonal levels. 10 (6.7%) had

hyperprolactinemia with mean duration of infertility of 11.0±2.4 years. 20 (13.3%) had hypergonadotropic hypogonadism with mean duration of infertility of 4.2±1.2 years. 45 (30%) had hypogonadotropic hypogonadism with a mean duration of infertility of

6.8±1.9 years. 20 (13.3%) had isolated increase in Oestrogen with a mean duration of infertility of 3.3±1.6 years, while 30 (20%) had hyperprolactinemia/ Hypogonadotropic hypogonadism with a mean duration of infertility of 7.5±2.8 years.

Table 4: Relationship between the hormonal abnormalities of infertile women and the duration of infertility

Group	n (%)	Age (Yrs)	LH (mlu/ml)	FSH (mlu/ml)	PRL (ng/ml)	PROG (ng/ml)	E2 (pg/ml)	DI (Yrs)
Hyperprolactinemia	10 (6.7)	43.2± 3.7	2.0±0.6	2.6±0.3	41.5±2.4	1.6±0.8	40.0±8.3	11.0±2.4
Hypergonadotropic hypogonadism	20 (13.3)	28.0± 2.2	18.5±3.8	33.3±6.2	7.3±2.8	0.8±0.2	43.5±10.6	4.2±1.2
Hypogonadotropic hypogonadism	45 (30.0)	30.2± 3.6	0.3±0.9	1.5±0.8	25.7±6.8	1.7±1.2	41.0±9.8	6.8±1.9
Isolated increase in oestrogen level	20 (13.3)	32.0± 4.2	2.2±0.6	1.7±0.4	19.9±8.2	10.6±2.8	50.1±80.3	3.3±1.6
Hyperprolactinemia /Hypogonadotropic hypogonadism	30 (20.0)	30.7± 3.8	8.9±2.8	11.9±3.1	51.5±7.2	9.1±2.0	33.9±5.3	7.5±2.8
Normal	25 (16.7)	31.6± 3.2	4.0±1.2	6.5±2.8	13.8±4.6	6.4±2.3	88.4±23.2	7.4±2.2

Key: FSH= Follicle stimulating hormone, LH = Leutinizing Hormone, PRL = Prolactin, PROG, Progesterone, E2 = Estradiol, DI = Duration of Infertility

DISCUSSION

Infertility remains a major challenge for many women within the reproductive age (15-45 years). According to [14], fertility in women is at its maximum in the mid-twenties and declines after the age of 30 years. [19] opined that fertility is halved in women who are 35 years or above and declines sharply after the age of 37. The mean age of the infertile women (31.7±3.8 yrs) investigated in Bida, North-Central Nigeria in this present study was comparable with 34.52 ± 6.5 yrs reported by [20], for infertile women investigated in Shagumu, South-Western Nigeria.

The mean duration of infertility among women investigated in this study was 6.8 years. This result agrees with the work of [21], who reported a mean duration of infertility among women in Zaria to be 5.0 years. According to [19], it is customary for many couples to defer investigation until after one year of unprotected intercourse, but it is essential to start early investigation (*i.e.*, after six months after unprotected intercourse) in women above 35 years of age. [22] observed that 46% of couples in developed countries sought medical evaluation for infertility before waiting 2½ years, while over 2/3 in developing countries (70% in Africa) had been trying to conceive for more than 2½ years. Furthermore; in this current study, the mean serum Prolactin level was significantly higher (P<0.05)

in infertile women than in the control group; while the serum LH and progesterone levels were significantly lower (P<0.05) in the infertile group compared to the control group. The serum FSH, Estradiol and Progesterone levels were significantly lower (P<0.05) in non-menstruating infertile women than in menstruating infertile women, while the serum Prolactin was significantly higher (P<0.05) in non-menstruating infertile women compared to the menstruating infertile women. Howbeit, there was no significant difference (P>0.05) between the LH level of the menstruating and non-menstruating infertile women. These results both agree and disagree with other previous studies.

6.7% of the 125 infertile women with abnormal hormonal level investigated in this study had hyperprolactinemia. The result here was far lower than those reported by previous studies. For instance, [23] recorded 48% cases of hyperprolactinemia among the 98 infertile women with hormonal abnormality examined in Kano state [21]. Recorded as high as 50% cases of hyperprolactinemia among the 86 infertile women with hormonal abnormality studied in Zaria, Kaduna state, while [20] recorded 28% cases of hyperprolactinemia among the 50 infertile women studied in Shagamu, South-Western Nigeria.

According to [24], high level of prolactin (hyperprolactinaemia) has 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. Both LH and FSH are required for follicle development and oestrogen production, hence low levels of these hormones may mean that fewer numbers of follicles will develop and there will be no Graffian follicle formation [25]. It has also been documented that high level of prolactin and low levels of LH, FSH and progesterone may cause anovulation and hence infertility [20]. According to [26], typically, females with hyperprolactinemia will present with anovulation (lack of ovulation), amenorrhea (lack of menstruation) and sometimes galactorrhea (abnormal milk production). Hyperprolactinaemia may occur primarily as a result of normal body changes during pregnancy, breastfeeding, mental stress, sleep; diseases affecting the hypothalamus and pituitary gland; disruption of the normal regulation of prolactin levels by drugs and heavy metals; or secondary to disease of other organs such as the liver, kidneys, ovaries and thyroid [27, 28] suggested that progesterone appears to have critical role in implantation and development of normal pregnancy. He noted that limited exposure to progesterone may result in infertility in severe terms or mildly recurrent pregnancy loss. Also, according to [29], normal values of LH and FSH that involves presence of ovulation, release of eggs and proper fertilization of egg without the proper level of progesterone for the preparation of the endometrial lining and for implantation results in miscarriage seen as heavier monthly circle even before the uterus is implanted.

The outcome of the current study further shows that there was 13.3% occurrence of hypergonadotropic hypogonadism among the infertile women investigated which was found to be lower than that the 26.5% reported by [23] in Kano; but higher than 5.9% reported by [21] in Zaria. Female hypergonadotropic hypogonadism (*i.e,* a high FSH, LH and low Progesterone level) occurs as a result of primary ovarian hypofunction, an indication that failure to conceive may lie in the ovary. Other causes of primary female hypergonadotropic hypogonadism include Turners Syndrome, Menopause and Testicular Feminisation.

It was also observed in this study, that 30% of the infertile women examined had hypogonadotropic hypogonadism, which was found to be ten times higher than the 3% reported by [23] and almost eighteen times higher than the 1.7% reported by [21]. Hypogonadotropic hypogonadism (*i.e,* a low FSH, LH and progesterone level) suggests that there may be a dysfunction of the hypothalamus or the pituitary gland in the women and as a consequence, are unable to secrete adequate gonadotropins to stimulate the ovary. The genetic causes of hypogonadotropic hypogonadism include: Kallmans Syndrome (anosmia and gonadotropic releasing Hormones deficiency), Cerebral Tumor, Head trauma, cerebral infection, cerebral radiation and Malnutrition. Other causes include hyperprolactinemia, Diabetes mellitus and marijuana. The present study in addition, shows that hypogonadotropic hypogonadism had the highest incidence (30%) of all the hormonal abnormalities recorded and it was also found to coexist with some other hormonal abnormalities such as hyperprolactinemia.

CONCLUSION

The outcome of this study suggests that multiple endocrine disorders exist in some cases of infertility and that hormonal disorder, which is treatable, may be responsible for some cases of infertility among women within reproductive age. Hormonal assessment should therefore be part of routine investigation of infertile women in Nigeria in order to assess the aetiology of hormonal abnormalities and determine the treatable groups. These results should be assessed in conjunction with patient's history and clinical examination. This could be of help for diagnostic, therapeutic and prognostic purposes including provision of assisted reproductive techniques for infertile women and therefore, reduce the emotional stress and social strains on individuals and couples in order to bring about a considerable positive impact on the reproductive health of infertile women.

COMPETING INTERESTS
Authors have declared that no competing interests exist.

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