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# HORMONAL EVALUATIONS AND ENDOMETRIAL BIOPSY IN INFERTILE WOMEN IN KANO, NORTHERN NIGERIA: A COMPARATIVE STUDY

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***Key words:*** Infertility, hormones, endometrial biopsy

**Abstract**
***Background/Objectives****:* To determine the levels of prolactin, progesterone, follicle stimulating hormone and luteinizing hormone in infertile women in other to assess the contribution of certain endocrinopathies to infertility in the study population, and compared the performance characteristics of histological diagnosis of endometrial biopsies with hormonal evaluations.
***Methods****:* Five hundred and fifty consecutive infertile women over a period of five years (1999-2003) were evaluated. The hormones were determined using ELECSYS 1010 auto analyzer supplied by Roche, Germany, while the endometrial biopsy was performed using dating technique.
***Results****:* Ninety eight (17.8%) out of the five hundred and fifty subjects had measurable hormonal abnormalities. Of these ninety eight subjects, 26.5% had hypergonadotropic – hypogonadism, 19, 3% had hypogonadotropic – hypogonadism, 6.1% had hypergonadotropic hypergonadism and 48% had hyperprolactinaemia. 68.0% of the endometrial biopsies showed various stages of secretory pattern, 14.2% showed endometritis and 0.7% showed stroma-glandular dissociation. The performance characteristics of histological diagnosis of endometrial biopsies was compared with hormonal study.
***Conclusion:*** Endocrine abnormalities was involved in 17.8% of infertile women with 48% of them hyperprolactinaemic. Endometrial biopsy compared favourably well with hormonal evaluations. For effective diagnosis of the cause of infertility in women, endometrial biopsy study will still be complimentary to hormonal evaluations.

***Mots clés :*** l'Infertilité, les hormones, la biopsie endometriale

**Résumé**
***Fond/Objectifs :*** pour déterminer les niveaux de prolactine, la progestérone, l'hormone de follicule stimulant et l'hormone lutéinisante dans les femmes stériles àfin d'évaluer la contribution de certain endocrinopathies à l'infertilité dans la population d'étude et de comparer les caractéristiques de performance de diagnose histologique de biopsies endometriales avec les évaluations hormonales.
***Méthode :*** Cinq cent cinquante femmes stériles consécutives pour une période de cinq ans (1999-2003) ont été évaluées. Les hormones ont été déterminées en utilisant ELECSYS 1010 analyseur automatique fourni par Roche en Allemagne, pendant que la biopsie endometriale a été exécutée en utilisant la technique de datation.
***Résultats :*** Quatre-vingt-dix-huit (17,8 %) des cinq cent cinquante sujets avait des anomalies hormonales mesurables. De ces quatre-vingt-dix-huit sujets, 26,5 % avaient hypergonadotropic - hypogonadisme, 19,3 % avaient hypogonadotropic - hypogonadisme, 6,1 % avaient hypergonadotropic-hypergonadisme et 48 % avaient hyperprolactinémie. 68,0 % des biopsies

endometriales a montré des étapes différents de modèle sécrétoire, 14,2 % a montré l'endométrite et 0,7 % a montré la dissociation stroma-glandulaire. Les caractéristiques de performance de diagnose histologique de biopsies endometriales étaient par rapport à l'étude hormonale.

***Conclusion*** *:* les anomalies endocrines ont été impliquées à 17,8 % de femmes stériles. 48 % d'entre eux avait l' hyperprolactinémie. La biopsie endometriale a comparée favorablement bien avec les évaluations hormonales. Pour la diagnose efficace de la cause d'infertilité dans les femmes, l'étude de biopsie endometriale sera toujours complémentaire aux évaluations hormonales.

## Introduction

Infertility due to endocrine dysfunction was previously thought to be uncommon in African women but with the introduction of immunoassay and other sophisticated technologies in gynecological practice in Africa, this problem has been found to constitute an in important cause of infertility in African women. [1 - 3] The causes of infertility women are many and approximately 40% of couples will have more than one cause for their infertility. [4, 5] Various ovulatory disorders ranging from luteal phase deficiency to anovulation are estimated to account for up to 15% of all infertility problems. [5] Recent advances in endocrinology points to the fact that the hypothalamus – pituitary –ovarian axis is essential for the smooth operation of the mechanism of the endocrine function which leads to ovulation, [6] and priming of the endometrium for implantation of the zygote which has to pass through a normal fallopian tube following fertilization. [7] failure of any of these complex process leads to infertility.

Ovulation may be evaluated by basal body temperature (BBT) charts, unfortunately such charts lack sensitivity[8]. Endocrine evaluations and endometrial biopsy are more reliable methods of assessing ovulation function. When endometrium is dated, functional abnormalities of the pituitary-ovarian-endometrial axis may be detected as well as other endometrial causes of infertility such as infection/endometritis. [9] However, some authors have reported that the place of endometrial biopsy in the diagnosis of infertility is doubtful because of inconsistency of their findings, error in interpretation, [10] and hazard of pregnancy interruption during the luteal phase as well as the limited therapeutic benefits of the procedure. [11]

The objectives of this study therefore was to determine the levels of prolactin, progesterone, follicle stimulating hormone(FSH) luteinizing hormone (LH) in infertile women which will help to assess the contribution of endocrine abnormalities to infertility in the study population and compare the performance characteristics of histological diagnosis of endometrial biopsy with endocrine evaluations.

## Materials and Methods

The study population are women who were referred to the chemical pathology laboratory from the infertility clinics of Aminu Kano Teaching Hospital, Kano and other hospitals in the catchment areas over a five year period (1999 – 2003). These were patients who complained of inability to conceive for more than one year despite unprotected and regular coitus. Their ages ranged from 25 to 44 years with a mean of 30.1 year. Two hundred and five subjects had primary infertility which three hundred and forty five had secondary infertility.

Five milliliters of blood was collected from the antecubital vein on the 21$^{st}$ day of the menstrual cycle. This was done to assess the luteal phase of the cycle. The blood was allowed to clot for 2hours at room temperature. The sera were obtained after centrifugation at 3000 rpm for 10minutes. The sera were kept frozen at – 20$^0$C until analysed.

FSH, LH, Prolactin and progesterone were analysed using ELECSYS 1010 auto analyzer supplied by Roche, Germany. The analyzer uses the principle of electrochemiluminescence immunoassay technique.

Endometrial biopsies were done on the same day. Each biopsy specimen was fixed in 10% buffered formol-saline, processed routinely and embedded in paratin-wax. The sections were stained in haematoxylin-Eosin. The sections were examined for evidence of secretory activity; the histologic age of the secretory endometrium was compared with the chronological menstrual age. A diagnosis of anovulation was made when the endometrium showed a non secretory pattern despite the fact that the chronological menstrual age was compatible with the luteal phase. While luteal phase deficiency was diagnosed when the histologic age lagged behind the chronological age by 72 or more hours. Any section showing the significant presence of inflammatory cells were classified as endometritis. In stroma-glandular cell dissociation, while the glandular cells are showing evidence of being in the secretory phase, the stroma cells show proliferative pattern.

## Results

The hormonal values and endometrial biopsy results of five hundred and fifty infertile women are presented in tables 1, 2 and 3. Of the five hundred and fifty subjects, ninety eight (17.8%) had measurable hormonal abnormalities while four hundred and fifty two (82.2%) had their hormonal values within the normal limits.

Of the ninety eight who had abnormal hormonal levels, twenty six (26.5%) has elevated levels of FSH, LH, diminished level of progesterone and normal prolactin level. This was classified as hypergonadotropic-hypogonadism. Nineteen (19.3%) had diminished levels of FSH, LH and progesterone and normal protactin level (hypogonadotropic-hypogonadism). Six subjects (6.1%) had elevated levels of FSH, LH, progesterone and normal prolactin level (hypergonadotropic-hypergonadism). While forty seven subjects (48%) had elevated level of prolactin, low limit of normal FSH and normal levels of LH and progesterone (Table 1).

Table 2 shows the histological diagnosis of endometrial biopsy in the study group. Of the total number, three hundred and seventy four (68.0%) showed various stages of secretory patterns ranging from early secretory phase (25.5%), mid-secretory phase (28.0%) and late secretory phase (14.5%). Twenty (3.6%) biopsies showed proliferative phase, seventy eight (14.2%) showed endometrial hyperplasia, forty six biopsies (8.4%) showed inflammatory endometrium (endometritis) and four (0.7%) showed stroma-glandular dissociation. Twenty eight (5.1%) showed decidual reaction.

Table 1: Serum hormonal levels in infertile women

| Group | Mean age (Years) | Mean ± SEM | | | |
|---|---|---|---|---|---|
| | | Prolactin Reference range: 72-511 miu/ml | FSH Reference range: 3.6-7.7 miu/ml | LH Reference range: 4.3-11.4 miu/ml | Progesterone Reference range: 1.7-27ng/ml |
| Hypergonadotropic– hypogonadism (n=26) | 28.6 | 276.4± 5.32 | 27.8± 3.48 | 20.09± 1.49 | 0.614± 0.02 |
| Hypogonadotropic – hypogonadism (n=19) | 30.8 | 470.6± 5.29 | 2.26± 0.12 | 2.40± 0.17 | 0.602± 0.04 |
| Hypergonadotropic - hypergonadism (n=6) | 30.2 | 316.3± 48.1 | 25.8± 12.4 | 20.1± 8.41 | 50.29± 3.50 |
| Hyperprolactinaemia (n=47) | 33.8 | 855.4± 71.8 | 3.52± 0.25 | 6.00± 0.57 | 13.43± 2.44 |
| Normal hormonal levels (n = 452) | 36.7 | 270.6± 5.39 | 5.36± 0.05 | 7.16± 0.09 | 10.81± 0.31 |

Table 2: Endometrial biopsies in infertile women

| No. (n=550) | Mean age (years) | Histological diagnosis | % | Indication |
|---|---|---|---|---|
| 20 | 29.0 | Proliferative phase | 3.6 | Preovulatory (anovulation) |
| 140 | 29.2 | Early secretory phase | 25.5 | Post ovulation |
| 154 | 30.1 | Mid secretory phase | 28.0 | Post ovulation |
| 80 | 31.2 | Late secretory phase | 14.5 | Post ovulation |
| 28 | 30.8 | Decidual reaction | 5.1 | Evidence of pregnancy |
| 46 | 31.2 | Inflammatory endometrium (endometritis) | 8.4 | Evidence of infection |
| 28 | 30.9 | Endometrial hyperplasia | 5.1 | Anovulation |
| 4 | 28.2 | Stroma glandular dissociation | 0.7 | Anovulation |

Table 3: Comparison of hormonal levels with histological diagnosis of endometrial biopsies in infertile women

| Hormonal groups | Prolactin miu/ml | FSH miu/ml | LH miu/ml | Progesterone ng/ml | Histological diagnosis of endometrial biopsy |
|---|---|---|---|---|---|
| Hypergonadotropic-hypogonadism n=26 | 276.4±5.32 | 27.8±3.48 | 20.09±1.49 | 0.614±0.02 | Endometrial hyperplasia, n=6<br>proliferative endometrium ,n=20 |
| Hypogonadotropic-hypogonadism n=19 | 470.6±0.29 | 2.26+0.12 | 2.40±0.17 | 0.602±0.04 | Endometrial hyperplasia, n=19 |
| Hypergonadotropic-hypergonadism n=6 | 316.3±48.1 | 25.8±12.4 | 20.1±8.41 | 50.29±3.59 | Secretory endometrium, n=4<br>stroma glandular dissociation, n=2 |
| Hyperprolactinaemia n=47 | 855.4±71.8 | 3.52±0.25 | 6.00± 0.57 | 13.43± 2.44 | Secretory endometrium, n=6<br>Anovulation, n=37<br>inflammatory endometrium, n=2<br>stroma-glandular dissociation, n=2 |
| Normal hormonal levels n=452 | 270.6±5.39 | 5.36±0.05 | 7.16±0.09 | 10.81±0.31 | Decidual reaction, n=28<br>secretory endometrium, n=364<br>inflammatory endometrium, n=44<br>endometrial hyperplasia, n=11 |

Table 3 shows the endometrial diagnosis of biopsies in the various groups to which the subjects are stratified according to their hormonal evaluations. Of the twenty six subjects who had hypergonadotropic-hypogonadism, endometrial hyperplasia was observed in six biopsies, while proliferative endometrium was observed in twenty biopsies. Endometrial hyperplasia was observed in all nineteen biopsies in nineteen subjects with hypogonadotropic-hypogonadism. Of the six subjects with hypergonadotropic hypergonadism, four biopsies showed secretory endometrium and two showed stroma-glandular dissociation. While out of the forty seven with hyperprolactinaemia, only six showed secretory endometrium, thirty seven biopsies showed hyperplastic endometrium while two each showed inflammatory endometrium and stroma-glandular dissociation. Those whose hormonal levels were within normal limits, twenty eight biopsies showed decidual reaction, three hundred and sixty showed secretory endometrium, forty four showed endometritis and endometrial hyperplasia was observed in eleven biopsies.

## Discussion

Even though there information exists about hormonal levels in infertile women in other regions of Nigeria[11 - 13] such information is scanty in Kano and none has compared histological endometrial biopsy diagnosis with hormonal levels in infertile women. The causes of infertility in the female are numerous. Rhythmic changes occur in the hormones secretion at all levels of the reproductive system. Anovulation is becoming frequently diagnosed in patients with infertility and menstrual cycle irregularity. [14] The physiology of ovulation and management of ovulation dysfunction are important in the treatment of female infertility. After adequate diagnostic evaluation of the cause of anovulation, several treatment modalities are available that restore ovulation function and menstrual cycle. Ovarian inactivity is indicated when there is a low FSH, LH and progesterone level which means that there is failure at the hypothalamus or pituitary (hypogonadotropic-hypogonadism) whereas high FSH, LH and low progesterone indicates that the primary failure lies in the ovaries (hypergonadotropic-hypogonadism). [14]

Our results indicate that 26.5% of those with hormonal abnormalities had hypergonadotropic-hypogonadism, an indication that failure to conceive may lie in the ovaries. This was corroborated by histological endometrial biopsy, which showed endometrial hyperplasia and proliferative endometrium.Hypogonadotropic-hypogonadism which was found in 19.3% of the patients indicates that there may be dysfunction of the hypothalamus or the pituitary which are unable to secrete adequate gonadotropins to stimulate the ovary. The endometrial biopsy also supported this finding by the diagnosis of anovulation.

High LH levels with corresponding low FSH values is indicative of polycystic ovarian disease. This was not observed in the population studied, it is advisable to evaluate serum estradiol in polycystic ovarian disease for effective diagnosis. Luteal phase deficiency was found in 12.8% of the study group who had hyperprolactinaemia but normal FSH, LH and low progesterone levels. This may be due to defective progesterone secretion by luteal cells. Other investigators[15] had reported that 19% fertile women have occasional cycles that display luteal phase deficiency, only those individuals who have recurrent abnormalities should be treated.

The endometrial biopsy showed that the endometrial secretory pattern lagged behind the chronological menstrual age by over 72hours.

Hyperprolactinaemia was seen in 8.5% of the total number of subjects evaluated. But it accounted for 48% of all subjects with abnormal hormonal levels. Whereas 37% was recorded in Lagos, Nigeria, [13] 33% was reported in Maiduguri and 31.6% was reported in Egypt, North Africa. [7] The mechanism by which hyperprolactinaemia leads to menstrual disorders and infertility have been described by several investigators.[16, 17] It is important to mention that studies have shown that increased prolactin may render the ovary less responsive to the effects of gonadotropin given exogenously to induce ovulation. Similarly, in animals, high levels of prolactin can directly inhibit ovulation. It has been demonstrated that hyperprolactinaemia severely curtails or even abolishes gonadotropin pulsatility. [18] Although the manner in which prolactin weakens gonadotropin releasing hormone (GnRH) neural output is not clear, it is possible that prolactin excess enhances (via short loop feedback) hypothalamic dopamine turn over and that increased levels of dopamine in turn inhibit GnRH synthesis and/or release.[1]

Endometritis is known to cause infertility either by disturbing the cyclic endometrial rhythm or as a result of the accompanying tubal inflammation and secondary anatomical abnormalities which are always present. [19] Endometrial inflammation was present in 8.4% of the study population. This finding is in agreement with that of Ojo et al[19] which observed 10.6% in Ife, south Western Nigeria. The causes of inflammation in these women mainly from the subpopulation with normal hormonal levels is not clear, but may be due largely to infections of the genital tract. Mycoplasma, chlamydiae and bacterial infections are recognized as the major causes of endometritis in infertile women. [4, 19, 20]

This study shows that hormonal abnormalities were present in 17.8% (n – 98) of infertile women and 48% of those with abnormal hormone levels had increased levels of prolactin. The endometrial biopsies of the patients compared favourably well with hormonal evaluations. For effective diagnosis of the causes of infertility in women, endometrium biopsy study will still be complimentary to hormonal evaluations especially in Africa where infection are known to contribute to infertility.

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