

## The clinical utility of early follicular phase lh/fsh ratio as a presumptive evidence of ovulation in the management of infertile women in Enugu, Nigeria

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### ABSTRACT

Follicular maturation is initiated by follicle stimulating hormone (Fsh) and later assisted by luteinizing hormone (Lh). In the early phase of follicular maturation, the concentration of these hormones is in a particular equilibrium that ensures adequate ovulation. The extent of ovulatory dysfunction that will occur when this equilibrium is altered is variable. The objective of the study was to determine whether early follicular phase Lh/Fsh ratio can be used as a presumptive evidence of ovulation in a resource limited setting. This is a cross sectional study involving 110 women undergoing infertility treatment in Enugu between January 2007 and June 2008. Early follicular phase Fsh, Lh and midluteal phase progesterone was assayed using standard methods. The data was analyzed by descriptive and inferential statistics using the statistical package SPSS for windows version 13. Twenty six women (23.6%) had Lh/Fsh ratio of less than one with a mean progesterone of  $10.2 \pm 2.4$  ng/ml. Twenty two (20%) had Lh/Fsh ratio of 1-2 with mean progesterone of  $13.9 \pm 3.4$  ng/ml. Majority, sixty two (56.4%) had Lh/Fsh ratio of more than 2, with mean progesterone of  $5.5 \pm 1.5$  ng/ml. There is a statistically significant difference between Lh/Fsh ratios and the levels of progesterone ( $F = 13.169, P = 0.000$ ). Early follicular phase Lh/Fsh ratio of more than two may indicate anovulation. Thus in a resource limited setting, treatment can be instituted without recourse for further expensive investigation.

**Key words:** Lh/Fsh ratio, Infertility, Anovulation, Resource limited setting.

### INTRODUCTION

Infertility has remained a significant factor that determines the cordiality between married couples in our environment. Childlessness brings unhappiness and unfulfilled dreams; more so in settings where great importance is attached to child bearing. It is very common, occurring in about 1 in every 10 couple's worldwide<sup>1</sup>. The prevalence is particularly high in sub-Saharan Africa, ranging from 20-40% in some parts of West Africa<sup>1</sup> and between 36.7% and 63.3% in Nigeria<sup>2</sup>.

Infertility in the female, which constitute between 30% and 48% of cases<sup>3,4</sup>, could result from ovulatory dysfunction, abnormalities of the cervix,

uterus and tubes<sup>5,6</sup>. In the Western countries, inability to ovulate occurs in approximately 20% of infertile women<sup>7</sup> while it is approximately 25% in most centers in sub-Saharan Africa<sup>4</sup>. Ovulatory dysfunction results commonly from polycystic ovarian syndrome (PCOS) accounting for 70% of cases<sup>7</sup>. Less common causes include, hypogonadotropic hypogonadism, hyperprolactinaemia and premature ovarian failure (hypergonadotropic hypogonadism)<sup>7</sup>.

The natural process that occurs during ovulation is regulated by the complex interplay of hypothalamic, pituitary and ovarian hormones. The hypothalamus produces the gonadotropin releasing hormone (GnRh), which stimulates the pituitary to

produce follicle stimulating hormone (Fsh) and luteinizing hormone (Lh). These hormones are responsible for development and growth of Graffian follicles and indeed ovulation. Serum Fsh and Lh are at their lowest levels just before the start of follicular phase. In the early follicular phase, the level of Fsh begins to rise but the rise in Lh begins later and continues slowly and steadily. At this stage therefore Fsh is either higher or at the same level as Lh. The maintenance of normal Fsh to Lh ratio at this point is necessary in the determination of adequate follicular maturation and ovulation. Indeed some studies have highlighted that early follicular phase Fsh/Lh ratio can be used as a significant indicator for follicular maturation both for natural and stimulated cycles<sup>8,9,10</sup>. Furthermore, abnormal Fsh/Lh ratio has also been implicated in unexplained recurrent pregnancy loss<sup>11</sup>.

Prior to the introduction of hormone assay facilities in our environment, diagnosis of ovulatory dysfunction was made by histological examination of a 21 day endometrial biopsy. Currently this has largely been replaced by assay of reproductive hormones whose cost can hardly be afforded by an average Nigerian. This makes the management of infertility in a resource limited setting very frustrating to the gynaecologist.

The serum levels of some reproductive hormones; early follicular phase Fsh, Lh and mid-luteal phase progesterone determine the ovulatory functions and thus cyclical changes that occur in the endometrium. Studies have shown that a rise in the mid-luteal phase progesterone of more than 10ng/ml is a presumptive evidence of ovulation<sup>12-15</sup>.

In this study therefore we decided to find out whether there is any relationship between early follicular phase Fsh/Lh ratio and mid-luteal phase progesterone as a presumptive evidence for ovulation.

## **Subjects and methods**

### **Patient Characteristics**

This is a cross sectional study involving 110 apparently normal women aged between 20-40 years. They are being managed for infertility between January 2007 and June 2008 and were recruited from the university of Nigeria Teaching

Hospital, Enugu and some private hospitals in Enugu and environs. Ethical approval was obtained from the relevant local authority and oral consent was obtained from the subjects.

Inclusion criteria were normal tubal patency test, normal uterus and cervix, normal male fertility test and normal menstrual cycle. Those with BMI more than 30 kg/m<sup>2</sup> were excluded. Very high Lh and Fsh level of 35miu/ml or more were not included in the analysis. This was to avoid subjects tending towards premature ovarian failure. Subjects that met the above criteria were recruited and some reproductive hormones assayed.

### **Laboratory methods**

The investigations were done at Amblin laboratories Enugu. Amblin was chosen because the laboratory is one of the first that started hormone assay in South East Nigeria; no doubt many gynaecologists in the region patronize them. They have good quality control which ensures the production of standardized results.

Fasting blood samples were taken on day three of the menstrual cycle for the measurement of Fsh and Lh while the samples for progesterone were taken at the midluteal phase (day 21 of the menstrual cycle). For all the cases, fasting venous blood was collected from the antecubital vein into sterile plain bottles. Samples were allowed to stand for about 30 minutes to clot and then centrifuged at 3,500 rpm for ten minutes. Serum samples were analyzed within three days of sample collection using the Microwell immunoassay kit, SYNTRON Bioresearch incorporated USA. Samples not analyzed immediately were refrigerated at a temperature of 2-8 degrees centigrade.

### **FSH assay**

For precision, the intra-assay coefficient of variation (C.V) was 7.5 % while the inter-assay C.V was 5.6%. The sensitivity was less than 1.95mIU/ml. The early follicular phase reference range used was 3-20 mIU/ml standardized against World Health Organization 2nd international reference preparation (IRP) 94/632(78/549).

### **LH assay**

The intra-assay coefficient of variation

(C.V) was 5.5% and while the inter- assay C.V was 6.3%.The sensitivity was 5.5mIU/ml. The early follicular phase reference range used was 5-20 mIU/ml standardized against World Health Organization 1st international reference preparation (IRP) 68/40.

#### Progesterone assay

The intra-assay coefficient of variation (C.V) was 5.7% while the inter- assay C.V was 6.7% .The sensitivity is 0.1ng/ml. The luteal phase reference range used was 2.5-32ng/ml with a cross-reactivity of less than 0.8% with all major steroid hormones.

#### Statistical analysis

This was done using the statistical package SPSS for windows version 13. Results were presented as mean and standard deviation. The Lh/ Fsh ratios were calculated and the findings were used to group subjects into three; a) Lh/Fsh ratios

less than one,b) Lh/Fsh ratios between one and two,c) Lh/Fsh ratios of more than two. The mean Lh,Fsh and progesterone values was calculated for each group. Test for significant was done using Anova and values less than or equal to 0.05 were considered significant.

### RESULTS

The age range of the patients was 20 to 40 years, with a mean age of 29.9 ±3.4years. The minimum parity was 0 while the maximum parity was 2. Primary infertility occurred in 30.2%, while 69.8% had secondary infertility. Twenty six (23.6%) had Lh/Fsh ratio of less than one with a mean Lh of 3.2±0.6miu/ml,Fsh of 5.9±1.8miu/ml and progesterone of 10.2±2.4 ng/ml.Twenty two (20%) had Lh/Fsh ratio of 1-2 with mean Lh of 11.2±3.6 miu/ml,Fsh of 5.8± 1.8 miu/ml and progesterone of 13.9±3.4ng/ml.Majority,sixty two (56.4%) had Lh/

**Tables 1: Average values for Lh/Fsh ratio and progesterone**

Lh/Fsh ratio	Lh(miu/ml)	Fsh(miu/ml)	Progesterone (ng/ml)
< 1(n=26) 23.6%	3.2±0.6	5.9±1.8	10.2±2.4
1-2(n=22) 20%	11.2±3.6.	5.8±1.8	13.9±3.4
>2.(n=62)56.4%	24.3±8.2	10.2±2.9	5.5±1.5

F = 13.169,P=000

Fsh ratio of more than 2 ,with mean Lh of 24.3±8.2 miu/ml,Fsh of 10.2±2.9 miu/ml and a progesterone of 5.5±1.5ng/ml.There is a statistically significant difference between Lh/Fsh ratios and the levels of progesterone (F = 13.169,P=000).These findings are illustrated in the table presented.

### DISCUSSION

In this study, prevalence of Lh/Fsh ratio of more than two was higher than the ratios of less than two. This was also the situation in a previous study where we recorded a high prevalence of elevated Lh/Fsh ratio<sup>16</sup>.We also observed that there was an inverse relationship between the level of

progesterone and the Lh/Fsh ratio, with the progesterone levels lowest in the group that had the highest Lh/Fsh ratio. The mean progesterone level in the group with Lh/Fsh ratio of more than two is in the non ovulatory range. Further more, the same group of subjects with high Lh/Fsh ratio had an average Fsh of more than 10 miu/ml.This situation has been proved to be an indication of poor ovarian reserve and difficulty in stimulation<sup>17,18</sup>.Although it has been widely believed that the early follicular phase Fsh drawn on day 3 of menstrual cycle has a normal ranges of about 3-20 miu/ml,the absolute levels have often been used as a gauge of ovarian reserve. In general, under 6 is excellent, 6-9 is good, 9-10 fair, 10-13 is

diminished reserve and, 13+ very hard to stimulate. Indeed studies have shown that a single Fsh determination of 10 miu/mL on the 3<sup>rd</sup> day of the cycle for the prediction of a poor ovarian response showed 87% sensitivity and, 100% specificity<sup>19</sup>. The utilization of Lh/Fsh ratio for the diagnosis of polycystic ovarian disease has been a subject of controversy. Although the incidence of elevated Lh/Fsh ratio in PCOS is high<sup>20</sup>, the utilization of this singular parameter in diagnosis is still a subject of controversy. Previously some authors believed strongly in this phenomenon<sup>21,22</sup>. However, more recent studies have continued to query its significance in diagnosis<sup>20,23,24</sup>. Generally speaking, the ratio is usually close to 1:1, and any thing higher than 2 is accepted as abnormal<sup>20</sup>, and may be one possible though not conclusive indication of PCOS.

In this study we have been able to show that abnormal Lh /Fsh ratio of more than two was associated with low progesterone level. Studies have shown that progesterone levels in this range may indicate anovulation<sup>12-15</sup>. While taking these arguments into consideration, we should consider the peculiar socioeconomic conditions of our environment. Most of our people cannot afford the cost of infertility investigation and treatment. The most important consideration here should be that since we have established a relationship between high Lh/Fsh ratio to anovulatory levels of progesterone, the issue of whether this is as a result of PCOS should be a matter of academic exercise and arguments should be reserved for further

studies. We therefore advocate initiation of treatment for anovulation in our poor socioeconomic environment when early follicular phase Lh/Fsh ratio is more than two. Further studies should aim at tracking the follicles of these subjects with elevated Lh/Fsh ratio to determine whether ovulation occurred or not. The subjects should also be followed up to determine pregnancy rates and outcome.

Polycystic ovarian syndrome, hyperprolactinaemia, anovulation and defective luteal function are common endocrine disorders that plague our infertile women. Hormone assay, though a significant tool in contemporary management of infertility has remained unaffordable by most of our subjects. If a proactive action is not taken, it will continue to hinder our efforts towards providing optimal infertility care. Reproductive endocrinology is yet to be fully developed in our environment and until this is done, diagnosis and treatment of ovulatory dysfunction should as a matter of fact involve basic modifications to suite our peculiar socioeconomic circumstances<sup>16</sup>.

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