Prediction of Normal Ovulation by Sonographic Folliculometry Involving Natural Cycles among Women in Ojo, Southwest Nigeria

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ABSTRACT

Background: *Accurate prediction of ovulation is important in the management of female infertility.*
Aim: *To determine the sonographic sensitivity of reduction in follicular size and disappearance of ovarian follicle as predictors of imminent ovulation.*
Methods: *This was a longitudinal study involving 100 women between the ages of 18 and 35 years. Transvaginal sonography with 6.5 MHz probe frequency was performed with a General Electric (RT 2800) ultrasound machine. Dominant follicles were identified and measured in both the longitudinal and transverse planes and their disappearance was monitored prior to ovulation. Laboratory luteinizing hormone test strips were used to test serum samples collected daily from each patient to confirm the time of ovulation.*
Results: *Pre-ovulation follicular size among the subjects was in the range of 18–36 mm while the mean follicular size was 26.78 ± 4.03 mm. Prior to ovulation, disappearance and reduction in follicular size was noted in 59% and 41% of subjects, respectively. Luteinizing hormone test was also positive and peaked prior to ovulation in 92% of the subjects among whom follicles disappeared in 37% while their size reduced in 55%. There was no statistically significant difference between sonographic and laboratory findings ($p > 0.05$). Age, height, weight and body mass index do not have significant influence ($p > 0.05$) on follicular size and ovulation.*
Conclusion: *Sonographic observation of complete disappearance of a dominant follicle and reduction in follicular size of surrogate follicles after follicular rupture appeared to be a reliable predictor of imminent ovulation.*

Keywords: Female infertility, folliculometry, Nigerian women, ovulation, sonography

Predicción de la Ovulación Normal Mediante Foliculometría Sonográfica Relacionada con los Ciclos Naturales entre las Mujeres en Ojo, Nigeria Sudoccidental

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RESUMEN

Objetivo: *La predicción precisa de la ovulación es importante en el tratamiento de la infertilidad femenina. El objetivo de este estudio fue determinar la sensibilidad sonográfica de la reducción en el tamaño folicular y la desaparición del folículo ovárico como predictores de la ovulación inminente.*
Métodos: *Se trató de un estudio longitudinal que abarcó 100 mujeres entre las edades de 18 y 35 años. La sonografía transvaginal con frecuencia de sonda 6.5 MHz fue realizada con un aparato de ultrasonido General Electric (RT 2800). Los folículos dominantes fueron identificados y medidos en los planos longitudinales y transversales, y su desaparición fue monitoreada antes de la ovulación. Tiras reactivas de hormona luteinizante de laboratorio, se utilizaron para probar las muestras séricas recogidas cada día de cada paciente a fin de confirmar el momento de la ovulación.*

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Resultados: *El tamaño folicular en la fase de preovulación entre las sujetos osciló en el rango de 18-36 mm, mientras que el tamaño folicular promedio fue 26.78 ± 4.03 mm. Antes de la ovulación, se observaron desaparición y reducción en el tamaño folicular en 59% y 41% de las sujetos, respectivamente. La prueba de la hormona luteinizante fue también positiva, y alcanzó su máximo antes de la ovulación en 92% de las sujetos, entre las cuales los folículos desaparecieron en un 37%, mientras que su tamaño se redujo en 55%. No hubo diferencias estadísticamente significativas entre los hallazgos de laboratorio y los sonográficos (*p > 0.05). El índice de masa corporal, la altura, el peso, y la edad no tuvieron influencia significativa (*p > 0.05) en el tamaño folicular y la ovulación.*
Conclusión: *La observación sonográfica de la completa desaparición de un folículo dominante y la reducción del tamaño folicular de los folículos sustitutos después de la ruptura folicular, parece ser un predictor fiable de la ovulación inminente.*

Palabras claves: Infertilidad femenina, foliculometría, mujeres nigerianas, ovulación, sonografía

INTRODUCTION

Accurate prediction of ovulation is an important requirement in the management of female infertility (1). Such prediction would aid in the precise timing of artificial insemination, oocyte retrieval for *in vitro* fertilization, administration of human chorionic gonadotropin and intercourse for natural conception (1). Presumptive evidence of ovulation may be obtained by steroid or gonadotropin hormone assays in the blood/urine or by serial ultrasound scanning in the preovulatory period (2).

Folliculometry has been defined as "the process of using ultrasound scan to track and measure ovarian follicular development and to subsequently predict ovulation" (3). Follicular tracking is normally best performed with transvaginal scan, from day 9 of the onset of menstrual cycle (day 1 being the first day of menstrual bleeding) to the 11th day because, according to Gurevich (4), the ovulation period usually lasts 48 hours. Mikolajczyk *et al* (5) reported that the time when ovulation is likely to occur can be estimated if the follicle measures about 20 mm in size.

Several sonographic features have been reported to be indicators of imminent ovulation such as disappearance and reduction in follicular size at ovulation, visualization of cumulus oophorus projecting from the wall of the dominant follicle and presence of considerable amount of fluid in the pouch of Douglas (6). Earlier studies focussed solely on describing the growth curve in terms of mean values of follicular diameter on days prior to ovulation (7). In general, wide variations have been documented in follicular diameter prior to ovulation (7). Improved visualization of ovarian morphology has become possible recently with the use of high resolution transvaginal transducers.

Infertility is a major global, social and medical problem (8). Incidence of female infertility tends to increase as age increases (9). Population-based studies, for instance, have estimated prevalence rates of infertility to be 5.5%, 9.4% and 19.7% at 25–29 years, 30–34 years and 35–39 years age range, respectively (9). In the United States of America, more than 10% of women aged 15–44 years report one form of fecundity problem or the other, whereas in the United Kingdom, it has also been established that one in seven couples has infertility problem (8, 9). Infertility is even a worse clinico-social issue in sub-Saharan Africa where an estimated 10–20% of women of reproductive age have the problem (10). In Nigeria, female infertility is particularly regarded as a calamity and is often the cause of either divorce or polygamy (11).

The definition of infertility varies considerably. Investigation of infertility is, however, undertaken by most clinicians if a couple fails to achieve pregnancy after a period of one year of regular unprotected sex (12). Prominent among causes of female infertility, according to Rastogi (13), are ovarian factors such as follicular and ovulation abnormalities. Regular menstrual cycles within the 26–28 days range are usually indicative of ovulation; however, about 9% of regular menstrual cycles are anovulatory (13). Anovulation and infrequent ovulation are in fact, known to account for more than 21% of all infertility cases globally (11). Assisted reproduction physicians usually require accurate menstrual history from infertile and subfertile women seeking medical assistance. For instance, among women with regular menstrual cycles and who are sure of the date of onset of their last menstrual period (LMP), clinicians can predict to an appreciable level of accuracy, the likely ovulation period of such women by applying Naegele's formula to the first day of the LMP (14). Unfortunately, many women are often not sure of the date of onset of their LMP (14). This uncertainty makes it imperative to seek alternative ways of ascertaining the presence of mature follicles as the basis for assisting subfertile women achieve pregnancy.

Assessment of ovulatory function is a key component of the work-up of infertile women (13). Laboratory assessment of ovulation is often the initial/primary investigation (2) to monitor changes in the level of luteinizing hormone (LH) in the serum which surges to a peak when ovulation is imminent. A mid-luteal progesterone level > 3 ng/mL obtained at about one week before menstruation (possibly on day 21 of a 28-day cycle) is indicative of recent ovulation (2). Furthermore, an alternative to laboratory assessment is the use of commercially

available urinary ovulation prediction home kit which detects LH surge as a predictor of ovulation (2). Since home kits (often preferred by most women for convenience and cost-effectiveness) have greater than 10% false positive and false negative rates, serum confirmation is still necessary in patients who are unable to detect a urinary surge of LH (2).

Serial ultrasonography can be used to determine ovulation by following the development and eventual disappearance of a follicle (15). The accuracy of ultrasonographic monitoring and estimation of follicular growth has been confirmed by laparoscopic studies at which time the follicles were aspirated and follicular diameters were directly measured or estimated from the amount of fluid aspirated (16). In spite of this, many are still skeptical about the ability of sonography to predict ovulation (2). According to Arthur and Moghissi (2), pelvic ultrasonography provides a good presumptive, but not definitive, evidence of ovulation.

Ultrasound is easily available in Nigeria; it does not involve ionizing radiation and it is less expensive than computed tomography and magnetic resonance imaging. It is also less expensive than laboratory serum analysis.

Ovaries appear on sonographic images as well defined echo-free or hypoechoic areas surrounded by echogenic rings (17). Usually, three or four growing follicles are found in the ovary during one cycle and one or two dominant follicles may be present. Dominant and other follicles may remain the same size till days 8–12 of the cycle at which point the growth of dominant follicles exceeds others (17). While surrogate follicles may not grow beyond 14 mm, a dominant follicle usually reaches 20–24 mm prior to ovulation (2, 17).

The aim of this study was to use ultrasound to track and monitor changes in follicular sizes as a means of predicting ovulation among women in Ojo municipality in Lagos state, southwest Nigeria. Consequently, we therefore determined the greatest mean follicular size prior to ovulation in the population studied. Furthermore, we compared sonographically detected reduction in follicular size and or disappearance of ovarian follicles as predictors of imminent ovulation with laboratory positive findings of ovulation. The relationship between mean follicular size and age, height, weight and body mass index (BMI) was also determined.

SUBJECTS AND METHODS

This prospective longitudinal study was carried out between May 2012 and July 2013. The study protocol was approved by the local committee on research and ethics, while written consents were obtained from subjects recruited for the study. A convenience sample of 100 eligible women was consecutively recruited from the gynaecology clinic attendees at Badagry General Hospital, Ojo in Lagos state, Nigeria.

Inclusion criteria: Normal women who had normal baseline pelvic scan, those within the age range of 18–35 years, those with regular menstrual cycle and those with cycles not less than 22 days or not more than 34 days.

Exclusion criteria: Women with irregular menstrual cycle, those with dysfunctional uterine bleeding, those with amenorrhea, presence of pelvic or ovarian masses and those with endocrine disorder or pelvic inflammatory disease.

Equipment: Sonographic examinations were performed with a General Electric RT 2800 high resolution ultrasound machine with 6.5 MHz transvaginal probe. The machine is a 2D real-time scanner with both on screen electronic callipers for measurement and a freeze button for still image capture. All sonographic examinations and measurements were performed by an experienced sonographer with 15 years' experience in gynaecology sonography. To reduce observer errors, each subject was scanned about the same time on study days (18).

Patient preparation, positioning and laboratory techniques
The technique and procedure of the investigation was explained to each subject prior to the commencement of the study. The date of onset of LMP was used as a guide in giving appointments for the study. Following the British Medical Society guidelines for such studies, each patient was booked for study only from day 10 of the menstrual cycle (19). Anthropometric data such as subject's age, weight and height were recorded before scanning started.

Ovulation in each subject was established using laboratory parameters. A serum sample was collected daily from each subject in the pre-ovulatory period. Commercially available (LH one-step) ovulation test strips with sensitivity of 20 mIU/mL and specificity of 98.8% were used to detect pre-ovulating surge of LH in order to confirm the time of ovulation. Each strip was dipped in each patient's serum about three to five minutes, in line with laboratory procedure for LH test. An experienced medical laboratory scientist subsequently read and interpreted the results.

Scanning techniques
Transvaginal sonography (TVS) was performed for each patient after the patient had been duly informed of the procedure and informed consent obtained from her. Each subject emptied her bladder shortly before the commencement of the study. Subjects were scanned lying in the lithotomy position and a chaperone was present in each case, in line with the departmental protocol for TVS. Coupling gel was applied on the transducer which was then covered with a latex condom and air bubbles expelled from it (17). The transducer was then gently introduced into the vagina. The gain and magnification were adjusted for optimum resolution. Different manoeuvres such as tilting, angling, rotation, pushing and pulling of the probe handle were deployed in a systematic manner to enhance the visualization of pelvic structures.

Folliculometry starts with a baseline ultrasound to see the baseline status of the ovaries on day two or three of the period (20). Then a series of ultrasound scans are done every few days to note changes in the dominant follicle; ending around day 12 to 17 (20). To obtain a baseline scan, the uterus was examined from fundus to cervix in both longitudinal and

transverse planes. The position, size, shape and echo texture of the uterus and ovaries were assessed and recorded. The pouch of Douglas was also examined to exclude free fluid collection.

Follicular measurement

Beginning on the 10th day of the menstrual cycle, the dominant follicle was measured. The follicle is measured in its longest diameter from wall to wall using the on screen callipers. The dominant follicular measurement was continued till the time of ovulation, which could be on or before the 17th day of the menstrual cycle (21). From these measurements, mean follicular diameters were obtained. In addition to follicular measurement, the diameters of the uterus, endometrial stripe and ovaries were measured.

Data analysis

Descriptive and inferential statistics were used for data analysis. Arithmetic mean of age (years), weight (kg), height (m), BMI (kg/m²) of each subject, and also initial (F1) and final (F2) mean follicular diameters (mm) were calculated. Z-test statistic was used to compare laboratory findings with sonographic results of disappearance and reduction in size of dominant ovarian follicles as predictors of ovulation. Z-test was also used to test the difference between reduction in mean follicular size and disappearance of ovarian follicle immediately before ovulation. Pearson's product moment coefficient of correlation was used to test the effect of age and BMI on follicular size prior to ovulation. SPSS version 17.0 for Windows software (SPSS Inc., Chicago, Illinois, USA) was used for data analysis. *P*-value at 0.05 was considered statistically significant in the study.

RESULTS

The mean age of the subjects as shown in Table 1 was 28.17 ± 4.74 years, while their average weight and height, respectively were 78.15 ± 10.58 kg and 1.66 ± 0.1 m. Table 1 also shows that the subjects studied had a mean BMI of 28.38 ± 4.4 m/kg and initial (F1) and final (F2) mean follicular sizes were 18.81 ± 2.39 mm and 26.78 ± 4.03 mm, respectively. The mean difference between the final and initial mean follicular size was 7.97 ± 3.29 mm.

Table 2 shows that complete disappearance of follicles was noted in 59 subjects (59%) while reduction in follicular size was seen in 41 subjects (41%). Table 2 further shows no significant difference between the two as indicators of ovulation ($p > 0.05$). On comparison of sonographic findings with laboratory findings, Table 3 shows that no statistically significant difference exists between them ($p > 0.05$). Pearson's product moment coefficient (Table 4) showed a weak but positive correlation between both age and BMI with mean follicular size. These correlations were, however, not significant ($p > 0.05$).

Table 1:   Range, mean and confidence values of the sample parameters

Parameter n = 100	Range	Mean ± SD	CV (%)	SE	Confidence interval	
					Lower	Upper
Age (years)	18–35	28.17 ± 4.74	16.83	0.474	27.241	29.099
Weight (kg)	58–108	78.15 ± 10.58	13.54	1.058	76.076	80.224
Height (m)	1.42–1.9	1.66 ± 0.1	6.02	0.010	1.640	1.680
Body mass index (kg/m²)	17.7–42.1	28.38 ± 4.4	15.50	0.440	27.518	29.242
Initial follicular size–F_1 (mm)	14–26	18.81 ± 2.39	12.71	0.239	18.571	19.049
Final follicular size–F_2 (mm)	18–36	26.78 ± 4.03	15.05	0.403	25.990	27.570
$F_2 – F_1$ (mm)	2–18	7.97 ± 3.29	47.28	0.329	7.641	8.299

SD: standard deviation; SE: standard error; CV: coefficient of variation; α: 0.05 at two-tailed

Table 2:   Z-test comparison of means of the two sample groups

Parameter	Follicle disappearance		Reduction in follicular size		Calc. Z	CV of Z
	n	X ± SD	n	X ± SD		
Age (years)	59	28.27 ± 4.53	41	28.02 ± 5.09	0.25	1.96
Weight (kg)	59	79.14 ± 11.33	41	76.73 ± 9.36	1.15	1.96
Height (m)	59	1.67 ± 0.10	41	1.65 ± 0.11	0.93	1.96
BMI (kg/m²)	59	28.41 ± 4.12	41	28.34 ± 4.84	0.08	1.96
Initial follicular size–F1 (mm)	59	19.00 ± 2.00	41	19.00 ± 3.00	0.00	1.96
Final follicular size–F2 (mm)	59	26.93 ± 3.78	41	26.56 ± 4.40	0.44	1.96
F2 – F1 (mm)	59	8.00 ± 3.00	41	8.00 ± 3.00	0.00	1.96

BMI: body mass index; Calc. Z: calculated Z; CV: critical value of Z

Table 3:   Z-test comparison of sonographic result and laboratory findings

Parameter	Disappearance	Reduction	Calc. Z	Critical Z
Sonographic findings	59	41	0.999	1.96
Laboratory findings	57	35		

Calc. Z: calculated Z.

Table 4:   Pearson correlation values showing relationship between age, BMI and follicular sizes

		Correlation		
Parameter		BMI	F1	F2	F2–F1
Age	Pearson correlation	0.082	0.11	0.056	0.011
	Sig. (two-tailed)	0.417	0.276	0.58	0.914
	n	100	100	100	100
BMI	Pearson correlation	1	0.023	0.085	0.087
	Sig. (two-tailed)		0.82	0.4	0.389
	n		100	100	100

F1: initial follicular size prior to rupture; F2: final follicular size prior to rupture; F1–F2: difference between initial and final follicular size prior to rupture; BMI: body mass index.
Correlation is not significant at 0.05 level of alpha (α), two-tailed

Figures 1 and 2 are scatter plots further highlighting the weak association between both age and BMI with mean follicular size.
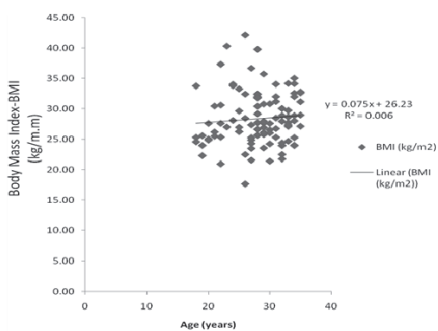

Fig. 1:   Scatter plot showing a weak positive relationship between age and body mass index.
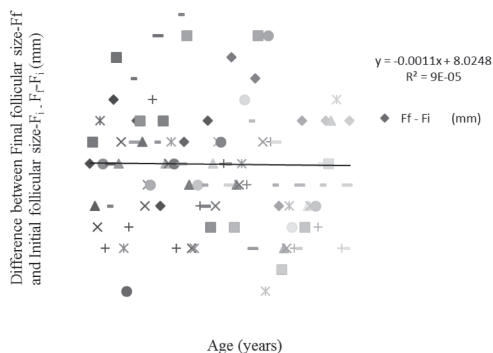

Fig. 2:   Scattered plot showing the relationship between age (years) of the 100 women and the changes in follicular size.

DISCUSSION

Variation in follicular size prior to ovulation has been well documented. According to Picker *et al* (22), such variation may be attributable to such factors as ethnicity, the woman's personal characteristics, or factors specific to a particular menstrual cycle. In the present study, follicular size ranged from 18.0–36.0 mm and the mean follicular size was 26.78 ± 4.03 mm (mean cycle length of 28 days; range 22–34 days) prior to imminent ovulation. These results agree with the results of Malhotra *et al* and Picker (7, 22) who had earlier reported variations in follicular size in their respective studies. Comparing the range of follicular size reported in the present study with 16.0–33.0 mm and 18.0–28.0 mm, respectively reported by Picker *et al* (22) and Malhotra *et al* (7), it would then be plausible to assert that possible racial variation does exist in follicular size across various population groups. The noted variation, however, seems to suggest that the usefulness of mean follicular size as a sole predictor of imminent ovulation may be limited.

In the present study, there was a statistically significant difference ($p < 0.05$) between complete disappearance of the dominant follicle (59%) and reduction in folliclar size (41%). This result agrees with the results of Jaffe *et al* (23) who reported 64% and 36% disappearance of and reduction in follicular size, respectively. These results suggest then that the disappearance of and/or reduction in size of ovarian follicle may be independent of follicular growth. It is therefore plausible to conclude that sonographic observation of either complete disappearance of the dominant follicle or reduction in follicular size of surrogate follicles after follicular rupture may be more reliable predictors of imminent ovulation than merely measuring follicular dimensions. Furthermore, there was no significant difference ($p > 0.05$) between disappearance of dominant follicle or reduction in size of a follicle as predictors of ovulation and laboratory analysis using LH test strips on subjects' serum. It was earlier reported in a similar study that where follicles either disappeared or were reduced in size, the patients all had positive serum LH tests (24). This result seems to suggest that sonographic folliculometry, like laboratory LH serum test, could be reliably used as a predictor of imminent ovulation. This assertion, however, needs further verification.

Opinion appears divided on the effect of the patient's weight on the growth of ovarian follicles. Rich-Edwards *et al* (25) defined normal weight as BMI < 25 kg/m^2, overweight as BMI > 25 kg/m^2 but < 30 kg/m^2, while obesity is BMI > 30 kg/m^2. In the present study, 21%, 46% and 33% of subjects were obese, overweight and normal in weight, respectively. Results of this study further showed that both age, weight and BMI do not have any significant effect on either the initial or final ovarian follicular diameters. There is a positive but weak correlation between follicular size, BMI and age in the present study as shown on the scatter diagrams. This seems to imply that ovarian follicular size prior to ovulation may not be significantly influenced by the patient's age, height, weight and BMI. This result supports the finding of Dodson *et al* (26)

who had earlier reported in a similar study no significant statistical difference in mean follicular size among normal weight, overweight and obese subjects.

According to Metwally *et al* (27), a patient's weight has a significant influence on follicular size during ovarian steroidal stimulation. They also reported that obese women had lower peak oestradiol level and required higher doses of gonadotrophins to achieve ovulation. Moreover, it has been suggested that weight loss regularizes menstrual cycles and increases the chance of spontenous ovulation and conception in anovulatory, overweight and obese women (28). Fedorcsak *et al* (29), however, investigated whether measures of ovarian reserve differed between obese and normal weight women. They hypothesized that all measures of ovarian reserve would be affected by body size. Their result did not support the notion that ovarian reserve is usually impaired in obese women. They further opined that although the antral follicle count was slightly lower and ovarian volume appeared clinically larger in these subjects compared to normal weight subjects, the differences were not statistically significant. Moreover, Santoro *et al* (30) showed that serum and ovarian measures of decreased ovarian reserve do not show consistent changes with body size, arguing that estimates are not ideal surrogates of ovarian reserves. In the present study, however, only women with normal unstimulated cycles were studied. It is our belief that body weight, age, height and BMI may not have any significant influence on follicular sizes and ovulation outcomes in women with normal unstimulated cycles.

A major limitation of this study would be the small sample size studied which is not totally representative of the population of Nigerian women. Furthermore, the quality of our results would have improved if such sophisticated ultrasound machines with Doppler facility and sonography based automated volume count (sono-AVC) were available in our centre.

CONCLUSION

Sonographic observation of complete disappearance of a dominant follicle and reduction in follicular size of surrogate follicles after follicular rupture appeared to be more reliable predictors of imminent ovulation than merely measuring follicular dimensions.

RECOMMENDATION

Sonographic folliculometry and laboratory methods for prediction of imminent ovulation should be combined for better results in patients with irregular menstrual history.

ACKNOWLEGEMENT

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