The African Journal of Medicine and Medical Sciences

Volume 17
1988

BLACKWELL SCIENTIFIC PUBLICATIONS
Oxford London Edinburgh Boston Palo Alto Melbourne

Afr. J. Med. med. Sci. (1988) 17, 77–82.

Correlation of ovarian follicle size and the urinary excretion of oestrone glucuronide, luteinizing hormone and pregnanediol glucuronide in spontaneous cycles

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Summary

Ovarian follicular development was investigated in 17 apparently normal women in whom ovulation was dated using real-time ultrasonography. Ultrasonic determination of follicular growth was performed on a daily basis with a real-time sector scanner, and correlated with daily concentrations of oestrone-3-glucuronide (E_1-3-G), luteinizing hormone (LH) and the ratio of E_1-3-G to pregnanediol-3α-glucuronide (Pd-3α-G) in early morning urine. The main results from ultrasonography showed that all patients except one developed follicles greater than 1.8 cm, with a mean growth rate of 0.18 cm/day. There was a statistically significant correlation between the mean follicular diameter and the mean concentrations of urinary E_1-3-G ($r = 0.95$, $P < 0.001$) and ratio of E_1-3-G to Pd-3α-G ($r = 0.97$, $P < 0.001$) during the 8 days prior to ovulation.

Résumé

L'enquête a été faite sur le développement folliculaire ovarien en 17 femmes normales apparemment dans lesquelles l'ovulation a été datée en utilisant un temps réel d'ultra-sonographie. La détermination ultra-sonique de croissance folliculaire était demontrée à base journalière avec un secteur de temps réel et de corrélation avec des concentrations journalières d'oestrone-3-glucuronide (E_1-3-G), hormone lutéinisant (LH) et la proportion de E_1-3-G à pregnanédiole-3α-glucuronide (Pd-3α-G) dans l'urine maternale de bonne heure. Les résultats principaux d'ultra-sonographie a montré que les malades, sauf un, avaient développé des follicles plus que 1.8 cm avec un taux moyen de croissance de 0.18 cm par jour. Il y avait une corrélation importante statistiquement entre le diamètre folliculaire moyen et la concentration urinaire moyenne E_1-3-G ($r = 0.95$, $P < 0.001$) et en raison de E_1-3-G à Pd-3α-G ($r = 0.97$, $P < 0.001$) de 8 jours antérieurement à l'ovulation.

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Introduction

Ultrasound monitoring of ovarian structural changes has become a widely accepted method for the evaluation of ovarian function and its accuracy in this regard has been confirmed [1, 2]. There are increasing reports that these readily identifiable morphological changes reflect the physiological process occurring within the ovary [3]. Most of these reports have been on plasma oestradiol. However, steroidal hormones are characterized by large day-to-day variations, and frequent samples are often required to identify their trend [4, 5]. As frequent blood samples are impractical in routine clinical practice, other biological fluids that are easily collected at home by the patient may be preferable to daily serum hormone assays.

Results have shown that the measurement of oestrone-3-glucuronide (E_1-3-G) in daily samples of early morning urine (EMU) could be used to monitor follicular development, predict ovulation, and locate the probable start and

finish of the fertile period in healthy women and in infertile women undergoing therapy to induce ovulation [6]. The measurement of pregnanediol-3α-glucuronide (Pd-3α-G) could be used to confirm the presence of a corpus luteum. This study attempts to determine the temporal relationships between ultrasonic morphologic measurement of follicular diameter and urinary hormone metabolites as indices of ovarian function in spontaneous cycles.

Patients and methods

A total of 17 women (aged 20–30 years) were studied. They were selected from patients on the waiting list for artificial insemination by donor (AID). These women had all experienced regular menstrual cycles (24–33 days) for at least 3 months prior to the study. Furthermore, all were in good general health and were not on a special diet or receiving any drug therapy. Particular care was taken to check that there was no history or evidence of liver or kidney disease or dysfunction.

An ATL Mark 100 real-time sector scanner with a 720 ultrasonic scanhead was used for echographic studies. The maximum diameters of the follicle were measured in both the longitudinal and transverse planes. The means of these measurements were obtained. Each patient was scanned daily from day 5 until there was confirmatory ultrasonic evidence of ovulation. Ovulation was assumed to have occurred when one or more of the following phenomena were observed with ultrasound: appearance of internal echoes in a mature follicle; collapse of follicle, either in shape and/or reduction in size; disappearance of a mature follicle. This was earlier described by Queenan *et al.* [1].

The patients were scanned between 08.30 h and 10.30 h daily, and the examination time was less than 5 min per patient. The full bladder technique was employed. Each patient collected early morning urine samples from day 1 of the menstrual cycle to the day preceding the onset of the next menstrual bleeding, except when the patient became pregnant and urine samples were collected until day 42. Oestrone-3-glucuronide and Pd-3α-G were measured by liquid-phase radioimmunoassay (RIA) using tritiated antigens as described by Collins *et al.* [6]. Luteinizing hormone was measured by chemiluminescence immunoassay using an activated ester of a hemisuccinyl derivative of 6-(N-4-aminobutyl-N-ethyl)amino-2,3-dihydrophthalasine-1,4 dione (ABEI-H), as previously described by Brockelbank *et al.* [7]. An evaluation of the methods gave the following values: sensitivity of calibration curve: 2.78 mol/l for E_1-3-G, 0.24 mol/l for Pd-3α-G and 0.2 iu/l for LH. The intra-assay precision (CV%) was 6.1% for E_1-3-G, 4.3% for Pd-3α-G and 25.7% for LH. The between-batch precision or routine conditions variance (RCV%) was 11.4% for E_1-3-G, 12.1% for Pd-3α-G and 5.8% for LH. The mean bias was 17% for E_1-3-G, −15% for Pd-3α-G and + 5.8% for LH.

The first significant rise in the concentration of a urinary metabolite was defined as the first amount that was 1.5 times the mean of the three preceding values. The peak excretion was the highest amount that was at least twice the mean concentration for the three preceding samples. For statistical analysis, the value for each metabolite on every day of the ovarian cycle, relative to the day of follicular rupture, was best described by the mean transformation of the results to Log_{10}.

Results

The daily time scale for both the hormonal and ultrasonic variations was related to the day immediately after the largest follicular diameter (i.e., the day of follicular rupture), which was defined as day 0. Two patients became pregnant during the study.

Table 1 shows the mean follicular diameter (dominant follicle) relative to the day of follicular rupture and the increase in mean follicular size. Steady follicular growth occurred in all patients reaching a mean maximum diameter of 2.05 (± 0.19) cm. In the two patients that became pregnant, the pre-ovulatory follicular diameter was 1.8 and 2.0 cm, respectively. The mean increase in follicular size relative to the day of follicular rupture was 0.18 ± 0.04 cm/day. In all cases, the dominant follicle ovulated within 20–30 h of reaching its mean peak diameter.

The days on which the rise and peak value for each steroid glucuronide and urinary LH occurred, relative to the day of follicular rupture, were identified, and the total number of sub-

Table 1. Follicular diameters in spontaneous cycles (mature follicles)

Days relative to day of follicular rupture	−8	−7	−6	−5	−4	−3	−2	−1
Mean follicular diameter (cm)*	0.82	0.93	1.10	1.24	1.46	1.67	1.90	2.05
Standard deviation	0.25	0.22	0.20	0.23	0.22	0.18	0.16	0.19
Increase in mean follicular size (cm)†		0.11	0.17	0.14	0.22	0.21	0.23	0.15

*Maximum diameters of the dominant follicles were measured in both the longitudinal and transverse planes over the period of maximum follicular growth (day −8 to day 0) in 17 subjects.

†Increase in mean follicular diameter (cm/day) was 0.18 (± 0.04).

jects with identifiable rise and peak values for each analyte is presented in Fig. 1. A peak of LH could be defined in 14 (82.3%) of the patients within 24 h of follicular rupture, as observed by ultrasound. These results provide further evidence that an initial rise or peak in the concentration of urinary LH may be used to detect ovulation. A peak of E_1-3-G was identified in 16 (94.1%) of the patients about the time of ovulation (i.e. ± 24 h of follicular rupture). Using the initial rise in Pd-3α-G values, ovulation was detected in 10 (58.9%) of the 17 patients studied within 48 h of follicular rupture. Table 2 compares the values of all the hormones assayed as a useful index for the immediate detection of ovulation. The shortest mean interval to the estimated time of ovulation (ETO) are the rise values for LH and the peak values for E_1-3-G, and the ratio of E_1-3-G/Pd-3α-G. Using the rise values as the sole criterion for ovulation, less than 25% of the patients studied failed to meet this criterion.

Figure 2 is a scatter diagram showing the relationships between the log mean concentration of urinary E_1-3-G and the mean follicular diameter. There is a significant correlation between the urinary E_1-3-G and mean follicular diameter ($r = 0.95$, $P < 0.001$). On a linear regression analysis of urinary oestrogen (y) on mean follicular diameter (x), the regression equation was as shown in the figure. The regression line is significant ($P < 0.001$) and the fraction of the total sum of squares explained

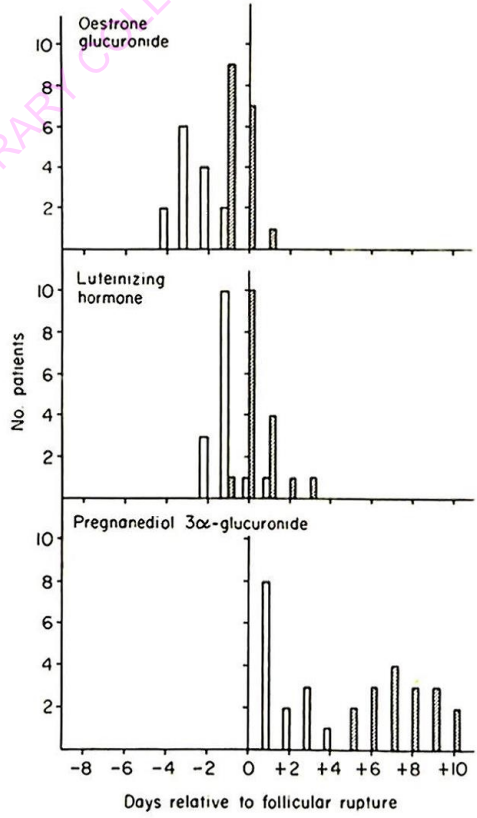

Fig. 1. Rise (□) and peak (▨) values of oestrone glucuronide, LH and pregnanediol glucuronide relative to day of follicular rupture.

Table 2. A comparison of urinary hormone assays for the immediate prediction of ovulation*

Assay†		No. patients who fail to meet criterion (%)	Mean interval (days) from follicular rupture to estimated time of ovulation by analyte (mean ± s.d.)
E_1-3-G			
	Rise	3 (17.6)	−2.57 (0.94)
	Peak	—	−0.52 (0.62)
LH			
	Rise	2 (11.76)	−1.0 (0.76)
	Peak	—	+0.47 (0.94)
Pd-3α-G			
	Rise	3 (17.76)	+1.79 (1.05)
	Peak	—	+7.47 (1.59)
Ratio E_1-3-G to Pd-3α-G			
	Rise	4 (23.53)	−4.62 (1.50)
	Peak	—	−1.18 (1.13)

*Early morning urine samples were collected on a daily basis and assayed for each metabolite in 17 menstrual cycles (17 subjects).
†The first significant rise in the concentration of a urinary metabolite was defined as the amount that was 1.5 times the mean of three preceding values. The peak excretion was the highest amount that was at least twice the mean concentration for the three preceding samples.

by the regression line is 0.91. Figure 3 is a scatter diagram showing the relationship between the mean follicular diameter and the ratio of E_1-3-G/Pd-3α-G. There is a significant correlation between ratio of E_1-3-G/Pd-3α-G and mean follicular diameter ($r = 0.97$, $P < 0.001$). On a linear regression analysis of ratio of E_1-3-G/Pd-3α-G (y) on follicular diameter (x) the regression was shown in the figure. The regression is significant ($P < 0.001$) and the fraction of the total sum of squares explained by the regression line is 0.93.

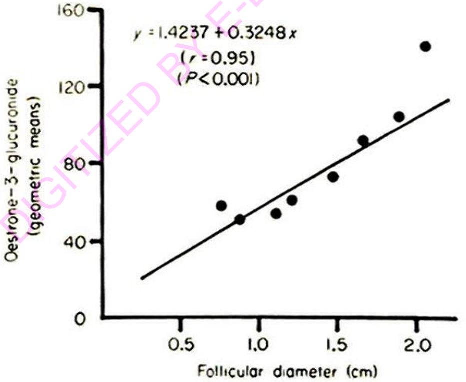

Fig. 2. Correlation between the mean of urinary oestrone-3-glucuronide and mean follicular diameter.

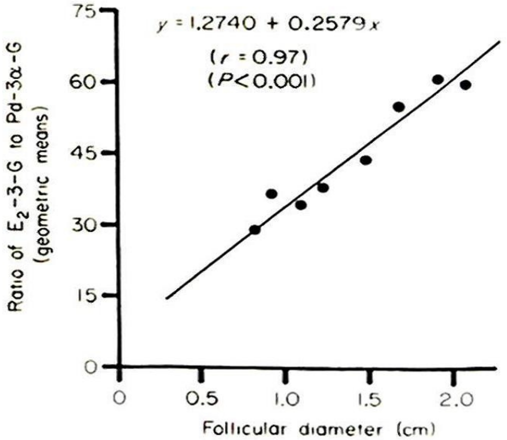

Fig. 3. Correlation between ratio of oestrone glucuronide (E_1-3-G) to pregnanediol glucuronide (Pd-3α-G) and the mean follicular diameter.

Discussion

The ability to monitor accurately the growth of human Graafian follicles using non-invasive procedures, such as ultrasonography, represents a considerable advance in studies of human fertility. In this study, both ultrasound and measurement of urinary hormone metabolites provided similar clinical information concerning follicular development. Other workers have attempted to correlate ultrasound findings with hormone levels. Initial reports were on plasma oestradiol and they demonstrated little or no correlation between serum oestradiol (E_2) levels and follicular diameter [8], until Hackeloer *et al.* [3] reported an excellent correlation between follicular diameter and plasma oestradiol levels in spontaneous cycles ($r = 0.968$, $n = 15$). Subsequently, Vargyas *et al.* [9] demonstrated a close correlation of real-time ultrasound imaging of ovarian follicle growth and E_2 secretion in ovulatory patients following clomiphene citrate for in-vitro fertilization.

In our study, when hormonal data were compared and contrasted with ultrasonic follicular diameters over a period of maximum follicular growth and development (day −8 to day 0), there was a statistically significant correlation between follicular diameter and urinary E_1-3-G levels, and the ratio of E_1-3-G to Pd-3α-G. Furthermore, our study shows that the shortest mean intervals to the estimated time of ovulation (ETO) were the mean rise value for LH and the mean peak values for E_1-3-G, and the E_1-3-G/Pd-3α-G ratio. The mean peak value for LH and the mean rise value for Pd-3α-G, although 0.50 day and 0.86 day, respectively, occurred after the ETO. This fact may exclude their use as a practical method for ovulation prediction. However, it must be borne in mind that as there was a 24-h interval between successive ultrasound examinations, the ETO in our study would spread over 24 h, i.e. between the time the maximum follicular diameter was observed and the time the ultrasonic evidence of ovulation was noted. Furthermore, the peak value is only known in retrospect and in our definition can only be known at least 3 days after its occurrence.

A peak of LH was identified in 14 out of 17 patients used in this study within 24 h of follicular rupture, as observed by ultrasonography. Previously, Renauld *et al.* [10] found that in three of 10 normal women volunteers with regular cycles and no gynaecological abnormalities, disappearance of the follicles by ultrasound scanning occurred on the day of maximum plasma LH values. In the remaining cycles, the follicles disappeared on the day following the maximum LH value. There are two components to the half-life of LH in peripheral circulation — one of approximately 20 min and the other of approximately 3 h [11], and the main metabolite is rapidly excreted in urine. Consequently, the measurement of LH in daily samples of urine is a practical reference method for the detection of ovulation. Similarly, the kinetics and mode of oestradiol metabolism have been studied using isotopic tracer techniques [12]. Oestradiol rapidly disappears from the circulation; both the half-life (defined as the time taken for the total blood hormone level to fall by half should endogenous production cease completely) and the turnover time (defined as the time taken to replace as much hormone as is present in the circulatory blood at any one instant), have values between 5 min and 25 min. The urinary oestrogen levels, as used in our study, may be a good reflection of plasma concentration and thus the close correlation obtained in the study.

The mean rise value for Pd-3α-G (0.50 day) in this study occurred after the ETO, and thus excludes its use for ovulation prediction. However, the rising progesterone concentration, being a marker of follicular response to LH surge, may be a useful index for the immediate detection of ovulation. In fact, using the initial rise in Pd-3α-G values, ovulation was detected in about 60% of our subjects within 48 h of follicular rupture. The results from numerous studies have shown that the urinary excretion of metabolites of progesterone starts immediately after an injection of labelled progesterone. Approximately 1% of the total dose accumulates in the urine during the first 30 min after injection, 9% by 4 h [13], 29% by 24 h, 37% by 48 h and 41% by 72 h [14]. Other studies have shown that up to 27% of radiolabelled progesterone are excreted in the urine as pregnanediol-3α-glucuronide [15, 16]. Thus, the concentration of this metabolite can be used as an index of luteal function. The ratio of E_1-3-G/Pd-3α-G as used in this study is considered important, as the pre-ovulatory levels of progesterone are fairly constant, and so may

be used to 'standardize' the E_1-3-G reading for the concentration of urine samples [17].

In conclusion, ultrasonography provides adequate information on Graafian follicle size and growth. Considering the wide biological variations that occur in human reproductive hormone levels, the findings of this study represent a good correlation between estimates of function and morphology. To predict and detect ovulation rapidly would be of great value for the treatment of infertility as well as natural birth control.

Acknowledgments

We thank Dr P. Bamigboye of University College Hospital, Ibadan, for his assistance in statistical analysis, and Dr P. A. Ibeziako for his helpful discussion. We would like to thank Dr J. B. Kim, Miss Janet Brockelbank and Miss Wendy Gooch of the King's College Hospital, London for the measurement of urinary LH by chemiluminescence immunoassay. This work received financial support from a Fellowship from the Nigerian Medical College Postgraduate Training Programme for Dr A. O. Adekunle.

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(*Accepted* 1 *April* 1987)