FEASIBILITY OF DETERMINATION OF FERTILE PERIOD IN INDIAN WOMEN BASED ON URINARY HORMONE ESTIMATIONS

Meena Desai, U.M. Donde and M. Ikram Khatkhatay

Department of Immunodiagnostics, Institute for Research in Reproduction (ICMR), J.M. Street, Parel, Mumbai - 400 012

ABSTRACT

Baseline data available on the excretory profiles of estrone glucuronide (E₁G), pregnanediol glucuronide (PdG) and luteinising hormone (LH) on human menstrual cycles (n = 104) was retrospectively analysed for identifying the limits of fertile period (FP) to be used as natural method of family planning. The limits of fertile period are suggested based on centile distribution of E₁G and PdG levels during defined phase of menstrual cycle. Two approaches, which do not involve any mathematical calculation are suggested. In approach A, fertile period is said to have started when E₁G value of 35 ng/ml is reached and is said to have ended when the PdG value of 2 µg/ml on two consecutive days is obtained. The criteria were applied to 30 test cycles in whom authentic fertile period was identified based on excretory profiles of E₁G, PdG and LH throughout the menstrual cycles. When approach A was followed the authentic fertile period was covered in 27 cycles giving an accuracy of 90% with a mean fertile period length of 9.11 ± 1.9 days. In approach B, the cut off limit of E₁G value was increased to 55 ng/ml in order to reduce the days of abstinence. Though the length of the fertile period was reduced to 7.2 ± 1.5 days the accuracy of the approach was 66.6%. Thus the approach A which has accuracy of 90% may appeal to determined couples who wish to practice family planning by periodic abstinence or restrict the use of barrier methods.

KEY WORDS : Estrone glucuronide, pregnanediol glucuronide, fertile period, natural family planning.

INTRODUCTION

Natural family planning (NFP) methods are based on the identification of the potentially fertile period of the ovulatory menstrual cycle termed as the window of fertility and is defined as that period during which a woman can conceive (1, 2). The fertile period (FP) usually lasts for five days and takes into account the fertilizing life of the sperm and that of ovum (3). Since it depends on many factors in each individual menstrual cycle, its accurate determination becomes difficult. Yet a number of methods have been described to identify the approximate limits of the fertile period as it has many applications in the management of fertility and infertility (4, 5, 6).

Author for correspondence :

Dr. M.I. Khatkhatay, Research Officer, at the above address, Tel. No. 4132111-12-16-17, Fax No. 4139412

Fertile period is usually determined by serial ultrasound scans of the ovary or from patterns of secretion of luteinising hormone (LH) and the ovarian hormones in blood or by the rate of excretion of their metabolites in urine (7, 8). Among these methods for identification of FP, impending or recently occurred ovulation as revealed by levels of LH, estrone glucuronide (E₁G) and pregnanediol glucuronide (PdG) in urine offer interesting leads. It is generally agreed that the first rise in urinary E₁G above a preceding baseline, signals start of the FP while that of PdG the end of FP for which a variety of statistical approaches have been described (9, 10). Since they involve mathematical calculations or graphical illustrations it is feared that they may not become popular among Indian women (11, 12, 13). Earlier we had accumulated a database containing complete daily excretory profiles of E₁G, PdG and LH from 108 normal menstrual

cycles collected from 78 Indian women of proven fertility. The rationale of the present paper is to analyse the data and suggest an approach to identify fertile period without involving any calculations keeping in view the needs of most of the Indian women.

MATERIALS AND METHODS

A. Subjects

The present study comprises of data collected over a decade (1989-1999) **Group I (Reference Group)** : The reference group consisted of 108 normal menstrual cycles contributed by 78 women of proven fertility, aged between 20 and 35 years with cycle lengths varying from 24-32 days. **Group II (Test Group)** : The test group consisted of 30 women of reproductive age group (23-32 years) with normal menstrual cycles planning to achieve a pregnancy. These women contributed 30 cycles.

The database on hormone profiles obtained from women in group I was used for initial development of an algorithm while group II data was used to validate the developed algorithm.

B. Collection of samples :

Women in both the groups collected early morning urine (EMU) samples throughout their menstrual cycles starting from 5th day of the cycle. EMU represented urine samples from the time of going to bed to rising. Small aliquots of urine samples were brought daily to the laboratory and preserved with 0.1% NaNO₃ at -20°C till analysed (assayed within a month).

C. Methods

Assay Methodology :

Urinary LH, E₁G and PdG were estimated by competitive enzyme linked immunosorbent assays (ELISA) using in-house reagents as described earlier (14,15,16). In brief, to microtitre ELISA plate adsorbed with appropriately diluted antisera, 100 µl of diluted urine sample or standard or buffer and 100 µl of required dilution of enzyme-labelled analyte were added. After incubation at 37°C for 2 hrs plates were washed with 0.15M NaCl containing .05% Tween-20

and enzyme activity was estimated in the bound fraction.

Data Analysis :

The data were analysed by determining threshold level of each of the hormones. The threshold level for each hormone was determined taking into consideration excretory profile of the individual hormone throughout the menstrual cycle and significant changes occurring in its level in relation to the event of ovulation.

The threshold level of LH was determined studying scatterograms of values of LH obtained in 104 ovulatory cycles from regularly menstruating women. Values of each cycle were synchronised with respect to day of LH peak considered as day 0. The data revealed that the 95th centile of values obtained during the early and mid follicular phase (day - 9 and day - 2) was 40 mIU/ml and on day - 1 and day 0, LH levels were always > 40 mIU/ml. Hence LH value of 40 mIU/ml was considered as threshold level.

For determining the threshold level for E₁G, the follicular phase was divided into 3 subphases namely (i) early follicular phase when E₁G levels were static, (ii) active phase when there was active growth of follicle as evident from marked rise in E₁G excretion and (iii) peak activity phase when there is peak excretion of E₁G levels. These observations were compared with ovarian follicular ultrasonography in 13 cycle's (15). Earlier, Bettendroff had also suggested division of follicular phase into sub-phases (17). The values corresponding to 10th, 20th, 50th, 80th and 95th centiles of E₁G of the above three phases are given in Table I. Based on the centile distribution of subphases, when E₁G value of 35 ng/ml (90th centile of early follicular phase) is reached, it was presumed that follicle had entered into active phase of growth. Therefore a threshold level of 35 ng/ml (90th centile) was assigned as a cut off value for the start of fertile period. In another approach, 98th centile (55 ng/ml) of early follicular phase (EFP) was considered as cut off limit in order to reduce the length of fertile period.

The threshold level of PdG was used as a signal for end of fertile period. Through scatterogram of PdG

Table 1. Centile distribution of E₁G levels during subphases of follicular phase

Centile	E ₁ G levels (ng/ml) in subphases of follicular phase		
	Early	Active	Peak
10th	12	34	55
20th	18	45	74
50th	24	60	117
80th	29	73	135
90th	35	88	149
95th	43	107	160
98th	53	133	175

estimations it was observed that PdG levels were always < 2 µg/ml during follicular phase. On the day of ovulation and a day after it was observed in 51 cycles out of 104 (50th centile), levels of PdG were > 2 µg/ml whereas levels of PdG were > 2 µg/ml in all the 104 cycles on day + 2. Therefore, it was considered that PdG level of > 2 µg/ml on two consecutive days indicated end of fertile period.

Based on the above analysis two approaches were suggested and in each test cycle it was checked whether its authentic FP coincided or is enveloped within the proposed FP. The authentic fertile period in 30 test cycles was identified based on excretory profile of E₁G, PdG and LH throughout the menstrual cycle. Two days prior to day of LH peak and two days after LH peak including day of LH peak was considered as authentic fertile period.

RESULTS AND DISCUSSION

Of the 108 menstrual cycles of the reference group 104 were considered to be ovulatory as they showed a distinct midcycle urinary LH peak preceded by an E₁G surge and followed by a rise in E₁G and PdG during the luteal phase. The duration of cycle of reference group ranged from 24 to 32 days (28.9 ± 2.64 days) with follicular phase ranging between 11 and 20 days (15.35 ± 2.1 days) and luteal phase ranging from 12 to 14 days (13.2 ± 1.9 days). The four cycles in which midcycle LH peak and subsequent rise in E₁G and PdG levels were not observed were excluded from the study as they were

considered anovulatory. All the cycles were referenced to the day of urinary LH peak designated as day zero in each case. In the present study two approaches which do not involve any mathematical calculations for determining the start and end of fertile period based on the threshold levels of hormones are suggested. For the first time centile distribution of the data have been used in determining the threshold levels.

Approach A : Method is based on the cut off limits for E₁G and PdG :

This method takes into account E₁G and PdG levels of EMU samples throughout menstrual cycle as described earlier. It proposes estimation of these metabolites in urine samples collected from day 8 of the cycle. The day 8 was selected as it will be the same day of the following week when menstruation had begun during the current cycle and hence easy to remember. Based on above two observations following protocol is suggested. i) Assess levels of E₁G from day 8 of the cycle, ii) Fertile phase is considered to have commenced when a value of 35 ng/ml is reached and iii) Fertile phase is believed to have ended when PdG levels in samples of two consecutive days are >2 ug/ml.

When it was applied to 30 test cycles in 2 cycles (Cycle No.6 and 28), the approach did not signal start of fertile period in time and in one cycle (Cycle No.15) it signaled allied end of fertile period inaccurately i.e. one day before the end of authentic fertile period (Fig. 1). Thus, out of 30 cycles, authentic fertile period was enveloped in 27 cycles giving an accuracy of 90%. The mean length of predicted fertile period 9.11 ± 1.9 days and ranged from 5-13 days. The approach could signal end of fertile period more precisely (in 29 out of 30 cycles except in cycle No.15). The overall sensitivity of the approach is 90%.

Approach B :

With the approach A, the days of abstinence ranged from 5 to 13 days, which may not be acceptable to most of the couples. It was therefore decided to increase the cut off limit of E₁G value from 35 ng/ml to 55 ng/ml with an aim to reduce the number of days of

abstinence. When applied to the 30 test cycles, the average length of fertile phase was observed in 7.2 ± 1.5 days, but the accuracy of the method reduced to 66.6% as in 10 out of the 30 cycles the warning signals occurred in the probable fertile period (Fig. 1).

A number of parameters have been suggested to delineate fertile period but none have so far proved

Fig. 1 : Length of the fertile period (indicated by horizontal lines and numbered 1 to 30) of 30 test cycles as predicted by approach A and approach B. All cycles have been synchronised with the day of LH peak. The shaded cylinder indicates the authentic fertile period.

sufficiently reliable, and/or practical, to be used. Traditional markers, such as basal body temperature and characteristics of cervical mucus, are subjective and physiologically unreliable for the determination of fertile period (18, 19). Urine provides a convenient sampling medium for the patient herself and correlation of levels of the urinary steroid metabolites with levels of circulating estradiol and progesterone makes it a more attractive choice medium for determination of fertile period. The levels of these steroid glucuronides for this purpose have been advocated in the past (20). However, the primary objective of the present study is to see feasibility of determining fertile period based on measurement of the steroid glucuronide by simple methods which necessarily do not involve any

mathematical calculation as prospective user's will be Indian housewives. The approaches suggested here are based on retrospective analysis of data collected over period of 10 years.

For the first time, centile distribution of the data has been used in determining the threshold levels. The use of higher centile (90th and 98th centile) value in case of E1G as a signal for the start of fertile period and lower centile value (5th centile) in case of PdG as a signal for the end of fertile period in the present study approximates to upper and lower confidence interval of these two parameters collectively. The centile distribution takes into account the expected variations in the estimates. The study group also includes six women who contributed consecutive cycles in order to take into account cycle to cycle variations in secretion of these metabolites of hormones.

The approaches suggested here are easy to follow and well within reasonable limits of accuracy. The approaches suggested here were not validated prospectively as assays are yet to be modified into a home test. The accuracy mentioned herein refers only to the correctness in identifying the fertile period based on estimation of urinary hormones. Therefore, they need to be counter checked to see whether conception rate increases in desirous couples when asked to keep relations during the identified fertile period. The period of abstinence is relatively longer in the approach B which may appeal to determined couples who wish to practice family planning by periodic abstinence or restrict the use of barrier methods. The practicability of these approaches on large scale is possible only if a simple instrument is devised suitably for home testing which indicates rise in E₁G level in urine above 35 or 55 ng/ml and that of PdG about 2 ug/ml.

Acknowledgement

The authors are extremely grateful to the Director, Dr. H.S. Juneja for his keen interest, constant encouragement and valuable suggestions during the course of the study. Our sincere thanks to Mr. D.K. Parthi for the technical assistance provided and Mr. P. Chavan for typing this manuscript.

REFERENCES

1. Brown, J.B., Blackwell, L.F., Billings, J.J., Conway, B., Cox, R.I., Garrett, G., Holmes, J. and Smith, M.A. (1987). Natural family planning. *Am. J. Obstet. Gynecol.* 15, 1082-1089.
2. Brown, J.B., Blackwell, L.F., Holmes, J. and Smyth, K. (1989) New assays for identifying the fertile period. *Int. J. Obstet. Gynecol.* 1, 111-122.
3. Austin, C.R. (1975) Sperm fertility, viability and persistence in the female tract. *J. Reprod. Fertil.* 22, 75-89.
4. Martinez, A.R., Zinaman, M.J., Jennings, V.H. and Lamprecht, V.M. (1995) Prediction and detection of the fertile period. *Int. J. Fertil.* 40, 130-155.
5. Collin, W.P. (1989) Biochemical indices of potential fertility. *Int J Obstet Gynec* 1, 35-43.
6. Aldercreutz, H., Brown, J., Collins, W., Goebelsman, U., Kellie, A., Campbell, H., Spieler, J. and Braissand, G. (1982) The measurement of urinary steroid glucuronides as indices of the fertile period in women. *J Steroid Biochem* 1, 695-702.
7. Lewis, J.G., Manley, L., Whitlow, J.C. and Elder, P.A. (1994) Re-examining steroid hormone metabolites as ovulation markers using monoclonal antibodies. *Steroids* 59, 288-291.
8. Lasley, B.L., Mobed, K. and Gold, E.B. (1994) The use of urinary hormonal assessments in human studies. *Annal. New York Acad Sci* 709, 299-311.
9. Blackwell, L.F. and Brown, J.B. (1992) Application of time series analysis for the recognition of increases in urinary estrogens as markers for the beginning of the potentially fertile period. *Steroids* 5, 554-562.
10. Blackwell, L.F., Brown, J.B. and Cooke, D. (1998) Definition of the potentially fertile period from urinary steroid excretion rates. Part II. A threshold value for pregnanediol glucuronide as a marker for the end of the potentially fertile period in human menstrual cycle. *Steroids* 63, 5-13.
11. Carter, R.L., Abrams, R.M. and Moghissi, K.S. (1982) A statistical approach to the determination of the fertile period. *Gynecologic and Obstetric Investigation* 13, 226-234.
12. World Health Organisation. (1983) Temporal relationships between the indices of the fertile period. *Fertility and Sterility* 39, 647-655.
13. Royston, P. (1991) Identifying the fertile phase of the human menstrual cycle. *Statistics in Medicine* 10, 221-240.
14. Desai, M.P., Khatkhayat, M.I., Sankolli, G.M., Meherji, P.K. and Joshi, U.M. (1989) ELISA for urinary gonadotropins using enzyme penicillinase as a marker. *Clinica Chimica Acta* 184, 315-322.
15. Khatkhayat, M.I., Sankolli, G.M., Meherji, P.K. and Joshi, U.M. (1988) Excretion of estrone-3-glucuronide in ovulatory and in induced cycles. *Int. J. of Fert.* 33, 181-187.
16. Khatkhayat, M.I., Sankolli, G.M., Meherji, P.K., Gogate, J., Choudhary, V. and Joshi, U.M. (1987) Application of penicillinase linked ELISA of pregnanediol glucuronide for detection of ovulation and assessment of corpus luteum function. *Endocrinol Japonica* 34, 465-472.
17. Bettendroff, G., Lehmann, F. and Leifemberger, F. (1981) Hormonal treatment of female infertility. In : *Regulation of human fertility WHO symposium E. Diczfalussy (ed.)* 76, Human Reproduction Programme, W.H.O. Switzerland, p. 155-179.
18. Royston, J.P. (1982) Basal body temperature, ovulation and risk of conception with special reference to the lifetimes of sperm and egg. *Biometrics* 38 : 397-406.
19. Fathallah, M.F., Rosenfield, A. and Indriso, C. (1989) Periodic abstinence in Family Planning Parthenon Publish. Group U.K. 2 : 128-141.
20. Baird, D.T. (1983) Prediction of ovulation : Biophysical, physiological and biochemical coordinate. In : *Ovulation : Methods for its prediction and detection*. Ed. S.L. Jaffovate, John Wiley and Sons Ltd. p. 1-17.