Human Reproduction vol.12 no.8 pp.1826–1831, 1997

Volumetric self-sampling of cervicovaginal fluid to determine potential fertility: a multicentre pre-effectiveness study of the Rovumeter™

A.M.Flynn[1], W.P.Collins[2,6], P.Royston[3], M.Barbato[4], P.Mena-Gonzalez[5] and M.E.Alliende[5]

[1]Natural Family Planning Unit, Birmingham Maternity Hospital, Birmingham, UK; [2]Academic Department of Obstetrics and Gynaecology, King's College School of Medicine and Dentistry, London, UK; [3]Department of Medical Statistics and Evaluation, Royal Postgraduate Medical School, Hammersmith Hospital, London, UK; [4]Department of Obstetrics and Gynecology, University of Milan, Milan, Italy and [5]Department of Obstetrics and Gynaecology, Hospital Clinico Universidade de Chile, Santiago, Chile

[6]To whom correspondence should be addressed at: Academic Department of Obstetrics and Gynaecology, King's College Hospital, Bessemer Road, London SE5 9PJ, UK

The aim of this study was to assess how effectively the Rovumeter™, designed for the volumetric self-sampling of cervicovaginal fluid (CVF), can be used to locate the minimum period of potential fertility (PPF) during ovulatory cycles. A multicentre, prospective study was undertaken of volunteers (attending natural family planning clinics) over three consecutive, apparently normal, menstrual cycles. All women collected daily samples of early morning urine and CVF and recorded the volumes (to the nearest 1.0 and 0.1 ml respectively). The concentrations of oestrone glucuronide (EG), luteinizing hormone (LH) and pregnanediol glucuronide (PG) were measured in all samples of early morning urine by immunoassay. A preliminary data set was used to optimize an algorithm to detect the start and end of potential fertility from the volumes of CVF. The end-points used were the normality of each menstrual cycle from its length, the length of luteal phase, and concentrations of EG, LH and PG, the start and end days of potential fertility from CVF volumes, and the minimum PPF, which was defined as the day of the LH peak minus 3 to day plus 2 inclusive. Overall, 72 women (median age 30 years, range 24–38) were recruited from three centres (23 from Birmingham, 24 from Milan, 25 from Santiago) and contributed data from 235 menstrual cycles (median length 28 days, range 23–44). The urinary LH peak was identified in 228 cycles (97%; median time, day 15 from day 1 of last menses, with range day 10 to day 35). The use of the Rovumeter™ gave start and end signals of potential fertility during 138 cycles (59%). The median length of the derived PPF was 8 days (range 4–18). The signals covered the defined, minimum PPF in 113 cycles [i.e. 50% of those with an LH peak; range 28% (Milan) to 62% (Birmingham)]. Overall 16/72 women (22%) had successful tests over three consecutive menstrual cycles [range 2/24 (8%; Milan) to 8/23 (35%; Birmingham)]. We conclude that signals from daily changes in the volume of CVF as determined by the use of the Rovumeter™ consistently locate the minimum period of potential fertility in only a small proportion of women.

Key words: cervicovaginal fluid/potential fertility/Rovumeter™/urinary LH peak

Introduction

The physiological basis for a discrete period of potential fertility (PPF) during the normal menstrual cycle has been reviewed (Burger, 1989; Collins, 1996). The change in consistency of cervical mucus is a crucial event, which allows and facilitates the passage of spermatozoa (Moghissi, 1984; Katz, 1991). Self-assessment of the presence and type of cervicovaginal fluid (CVF) at the vulva forms the basis of family planning by avoiding sexual intercourse during the PPF [World Health Organization (WHO), 1981a,b]. The failure rate of this method expressed as typical use is ~20% and 1–3% for perfect use (Hatcher *et al.*, 1994). These data suggest that the development of a test based on the measurement of a distinct change in CVF might improve the general acceptance and practice of this approach to family planning.

The volume and viscoelasticity of CVF are related variables which tend to increase during follicular development and decrease in the immediate postovulatory phase of the menstrual cycle (Viergiver and Pommerenke, 1946; Wolf *et al.*, 1978). However, initial attempts to use systematic changes in the volume of CVF to locate the PPF were unsuccessful (Moghissi, 1979; Wagner, 1979), and might be explained by the use of tampons to collect the samples (Godley, 1985). The development of a small, graduated aspirator (the Rovumeter™) has enabled women to take and read the volume of a daily sample of their CVF under standardized conditions (Usala and Schumacher, 1983). Preliminary data from the use of this device by experienced users of the symptothermal method of natural family planning have shown that changes in the volume of CVF might be used to develop an algorithm to predict ovulation and the start and end of potential fertility (Flynn *et al.*, 1988a,b).

We now report the main findings from a multicentre study designed to provide information about the potential value of using the Rovumeter™ to locate the minimum PPF (as defined by established hormonal and biological indices) in women from different cultures during consecutive menstrual cycles.

Materials and methods

Design

The aim was to study the use and acceptability of the Rovumeter™ in three centres located in different countries with laboratory facilities

for the measurement of hormone metabolites in urine (Birmingham, UK; Milan, Italy; Santiago, Chile). The plan was to recruit 25 women of reproductive age from each centre and study three consecutive menstrual cycles. Established indices of hormone production were to be used to define minimum periods of potential fertility (Collins *et al.*, 1979; WHO, 1982, 1983a). After the last day of menses, all volunteers were to: (i) take a daily sample of their CVF with the Rovumeter™ and record the volume, (ii) collect a daily sample of early morning urine (for the subsequent analysis of urinary hormone metabolites) and record the time since last urination and volume, (iii) record traditional signs of potential fertility on a symptothermal chart, and (iv) complete a questionnaire for assessing the acceptability of the device. Data from items (iii) and (iv) would only be processed if the results from items (i) and (ii) for the whole study were sufficiently encouraging.

The project co-ordinator (A.M.F.) was located in Birmingham. All centres had a Principal Investigator and a Chart Co-ordinator and followed the same protocol (including the use of identical report forms).

Criteria for subject selection

The volunteers had to be experienced users of the symptothermal method of natural family planning and had to fulfil the following criteria: (i) be aged between 25 and 39 years inclusive (the study period had to finish before the 40th birthday), (ii) have regular menstrual cycles (length 25–35 days for the last six cycles), (iii) have a symptothermal chart for the last cycle which illustrated a PPF, (iv) not have used any form of hormonal contraception or intrauterine device for the previous six cycles and not have an intention of using such methods of contraception during the study period, (v) have no history or evidence of liver or kidney disease or dysfunction (which might affect the urinary excretion of hormone metabolites), nor any recent pathological discharge from the vagina, (vi) not be receiving any form of chronic drug therapy, (vii) not be working night shifts, (viii) not planning a pregnancy during the study period, (ix) be willing to make daily aspirates of their CVF and record the volumes on a diary sheet, (x) be willing to collect daily timed and measured samples of early morning urine (and store them for a limited period in their freezer), (xi) be willing to record the signals of the symptothermal method on a diary sheet, and (xii) be willing to provide information on when barrier methods of contraception or spermicides were used during the study period.

Cervicovaginal fluid aspiration

A disposable, sterile, plastic volumetric aspirator (the Rovumeter™; A & O Pharmavertries, Munich, Germany) was used to make the daily collection of CVF from the upper vagina and posterior fornix. The aspirator is a graduated, syringe-like device (135 mm in length) with a blunt, bulb-headed, flat, elliptically shaped mouthpiece with an outer gutter. The inner diameter of the bulb head is 5 mm. A photograph of the device has been published (Flynn *et al.*, 1988a,b).

The sampling time was chosen by the individual women (in the morning before any act of intercourse) and used consistently for a complete menstrual cycle. Instructions for using the device have been published (Usula and Schumacher, 1983). Briefly, each volunteer was told to: (i) hold the aspirator so that the opening is directed towards the vagina with the largest diameter of the bulb in a horizontal position and the longitudinal gutter on the upper side; (ii) determine the most convenient position to use the device (standing, sitting or lying on the back); (iii) gently insert the aspirator along the posterior wall of the vagina until it will go no further (ensuring that it is behind the lips of the cervix/neck of the womb); (iv) withdraw the aspirator slowly so that movement of the plunger does not suck up the vaginal wall; (v) aspirate with the right hand while maintaining the device firmly in position with the left hand; (vi) ensure that all the fluid is removed by gently moving the aspirator from side to side as the plunger is withdrawn to the maximum; (vii) hold the aspirator vertically with the end uppermost and expel air by gently pressing the plunger; (viii) read volume of fluid to the nearest 0.1 ml. All volunteers were given personal instruction by an experienced user of the device.

Urinary hormone immunoassay

The frozen urine samples were collected from each volunteer by the Chart Co-ordinator and taken to a local laboratory for the analysis of oestrone glucuronide (EG), luteinizing hormone (LH) and pregnanediol glucuronide (PG) by non-competitive radioimmunoassays. The reagents and assay protocols were supplied by the Matched Reagents Programme of the WHO (Queen Charlottes and Chelsea Hospitals, Goldhawk Road, London, UK). The results for this aspect of the study were expressed as nmol/l for EG, U/l for LH, and μmol/l for PG. Each laboratory implemented their own internal quality control procedures and participated in a programme of external quality assessment coordinated by the London centre mentioned above.

Definitions

The following definitions were used: (i) day 1 of cycle: the first day of menstruation (the day starting after midnight on which there is an appreciable flow of blood through the vagina), (ii) last day of cycle: the day previous to day 1 of next cycle, (iii) a normal menstrual cycle: a cycle with sequential changes in the concentrations of urinary EG, LH and PG, which are consistent with a PPF, (iv) peak day of urinary LH: the day with the highest numerical value for LH between days 10 and 35 of the cycle with an appropriate temporal relationship to the rise and fall in the concentration of EG and the rise in the concentration of PG, (v) the minimum PPF: the day of the LH peak minus 3 to day plus 2 inclusive, (vi) a signal: the indicator of the start of the PPF or infertile period, and (vii) length of PPF: the day of the start signal to day of signal for infertile period minus 1.

Data analysis

The clinical report forms from all centres were sent to the Project Co-ordinator for checking before entry into a computer by a Data Entry Clerk. The data were rechecked and analysed by P.R. Initially, plots were made of the concentrations of urinary EG, LH and PG for each volunteer by day of cycle for visual inspection of normality. Similarly, the volumes of CVF for each woman for all three cycles were plotted on the same graph, and then combined by centre and overall.

Optimization of algorithm

A set of preliminary rules to determine signals for the start and end of a PPF were kindly supplied by Dr G.F.B.Schumacher (Department of Obstetrics and Gynecology, The University of Chicago Pritzer School of Medicine, Chicago, IL, USA). These rules were adapted slightly for electronic data processing, and optimized by application to a preliminary data set (mainly to improve the number and timing of start signals for the infertile period).

The finalized algorithm was as follows: (i) identify the highest value (max) for the volume of CVF from the first three recorded values (if all three values are the same, add 0.05 ml to max); (ii) the first of the following days when the volume is greater than or equal to max is the signal (day) for the start of a PPF. The volume must increase to greater than max + 0.25 ml then decrease for a signal to occur for the start of the infertile period. The second consecutive day of values less than or equal to max + 0.25 ml is noted and the fourth day after that day is the signal (day) for the start of the infertile period.

Table I. Some characteristics of the subjects and menstrual cycles by centre

Variable	Centre[a]	No. patients	Mean (SD)	Median	Minimum	Maximum
Age (years)	B	23	32 (4)	32	26	38
	M	24	31 (4)	30	30	37
	S	25	30 (4)	30	24	37
	All	**72**	**30 (4)**	**30**	**24**	**38**
Cycle length (days)	B	81	29 (3)	29	23	44
	M	72	29 (4)	28	23	40
	S	82	28 (2)	28	23	35
	All	**235**	**29 (3)**	**28**	**23**	**44**
Urinary LH peak day, relative to day 1 of menses	B	81	16 (3)	15	10	30
	M	69	16 (4)	16	11	35
	S	78	15 (3)	15	11	24
	All	**228**	**16 (3)**	**15**	**10**	**35**
Luteal phase length (days)	B	81	14 (2)	14	8	18
	M	69	14 (3)	14	7	23
	S	78	14 (2)	14	11	18
	All	**228**	**14 (2)**	**14**	**7**	**23**

[a]B = Birmingham; M = Milan; S = Santiago.
LH = luteinizing hormone.

The success rate of the method was calculated as the proportion of cycles in which the algorithm successfully delineated the minimum PPF based on the urinary LH peak day, and as the proportion of women in whom the algorithm successfully delineated the minimum PPF over three consecutive cycles.

Results

A total of 72 women were recruited; 58 (81%) provided data from three consecutive cycles (one, nine, two and two women provided data from two, four, five and six consecutive cycles respectively). Overall, 235 cycles were studied and 228 (97%) had an identifiable peak of urinary LH. The contribution of data from each centre and some characteristics of the menstrual cycles are shown in Table I. There was no significant difference between the three centres for the age of the volunteers, the length of the cycles, the day of the urinary LH peak or the length of the luteal phase.

Volume of CVF

The number of CVF volumes that was recorded by day of cycle is shown, by centre, in Figure 1. There was a gradual increase in the number of observations at all centres until day 8 and a corresponding reduction around day 25. The volume of CVF by day relative to the day of the urinary LH peak (day 0), by centre, is shown in Figure 2. The median values on day 0 were 0.187 ml for Milan, 0.375 ml for Santiago and 0.438 ml for Birmingham.

Time of signals

The number of start and end signals for the PPF relative to day 1 of menses and the day of the urinary LH peak are shown in Table II. Overall, there were 226 start signals from 235 cycles (96%), but only 138 end signals (59%). Seven cycles did not have a urinary LH peak; of those that did, 99% had a start signal and 61% an end signal for the PPF. The mean (and median) day for the start signal was day of urinary LH peak minus 5; the corresponding day for the end signal was day of urinary LH peak plus 3.

Duration of derived PPF

The length of the derived PPF from the 138 cycles with start and end signals are shown, by centre, in Table III. The overall mean (and median) was 8 days (range 4–18) and the values were similar at each centre. The number of samples of CVF that were tested ranged from 8 to 29 (median 14).

Success rates

The number (and percentage) of successful tests per cycle, i.e. where signals for the start and end of the PPF covered the defined minimum PPF, are shown by centre and overall in Table IV. Overall, the test was successful in 113/228 cycles (50%) and the value ranged from 28% at Milan to 62% at Birmingham.

The number of women in whom the test was successful or unsuccessful over three consecutive cycles is shown in Table V. Overall, the test was successful in 15/71 (21%) of women and unsuccessful in 20/71 (28%). The success rates were 32, 8 and 24% at Birmingham, Milan and Santiago respectively. The relationship between the successful use of the test (by women from Birmingham and Santiago) and the mean volume of CVF for days 10–18 over consecutive menstrual cycles is shown in Table VI. The mean (SD) volume of CVF associated with successful use, 0.37 (0.17), was significantly higher ($P < 0.001$, Student's *t*-test) than the corresponding value, 0.20 (0.13), associated with unsuccessful use.

Discussion

A preliminary testing of the Rovumeter at one centre (Birmingham) had indicated that serial changes in the volume of CVF reflect oestrogenic activity and the values might therefore be used in a refined algorithm to delineate the PPF during a normal menstrual cycle (Flynn *et al.*, 1988a,b). The findings were sufficiently encouraging to initiate a multicentre study designed to provide additional and complementary data over consecutive menstrual cycles from different groups of women, who were experienced users of the symptothermal

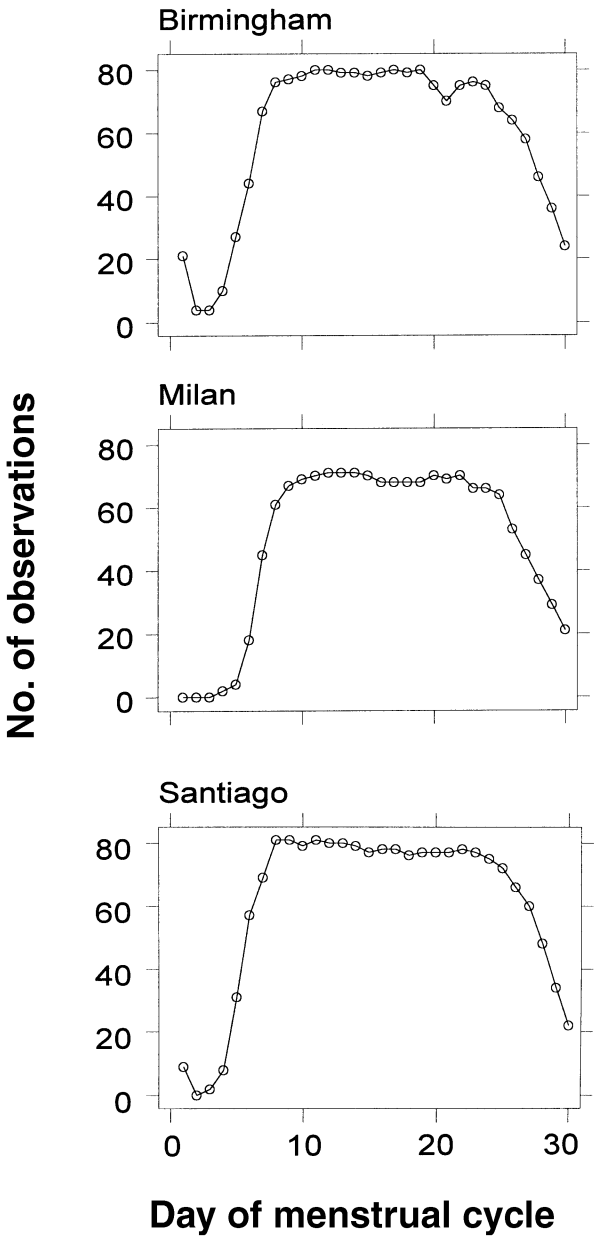

Figure 1. Number of times the volume of cervicovaginal fluid was recorded on each day of the menstrual cycle by centre.

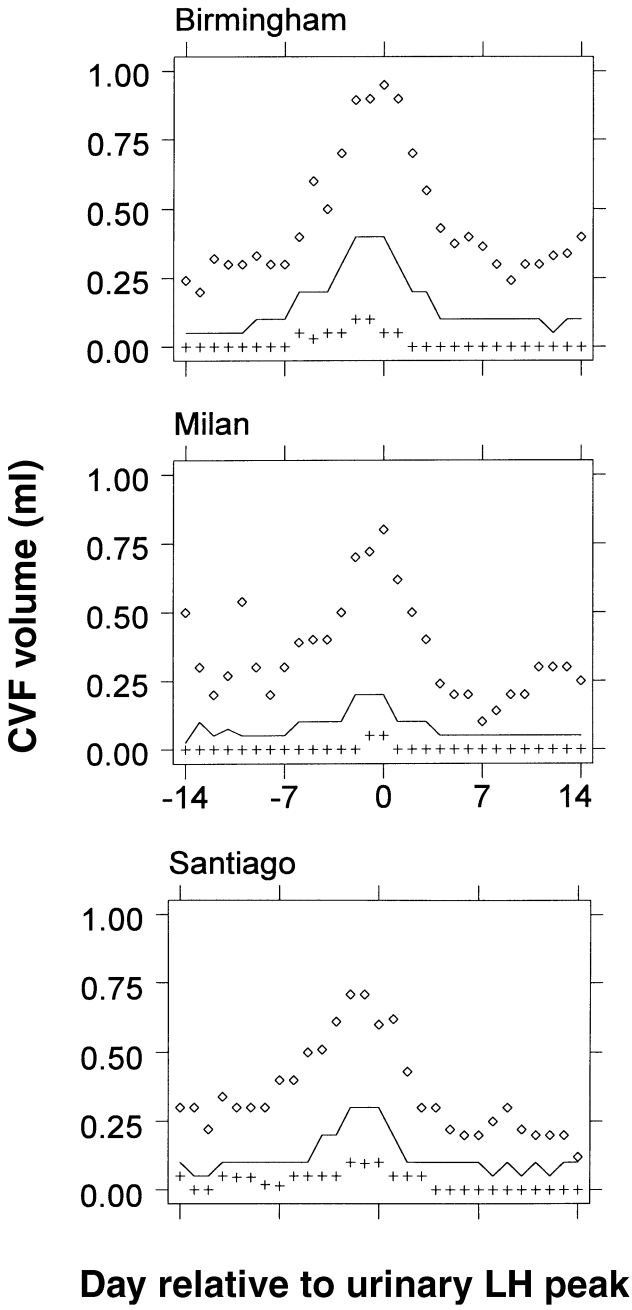

Figure 2. The volume of cervicovaginal fluid [10th (+), 50th (—) and 90th (◇) centiles] relative to the day of the urinary luteinizing hormone peak by centre.

method of natural family planning. The daily concentrations of urinary EG, LH and PG were used as additional indices to assess the normality of each study cycle. The day of the LH peak was used as a reference point for impending ovulation and the minimum PPF (Collins, 1989).

Alternative algorithms for identifying the PPF have been reviewed (Royston, 1991). Initially, cumulative sum (CUSUM) analysis with a peak selecting algorithm was applied to CVF volumes to determine the start and end of a PPF (Flynn *et al.*, 1988b). This approach failed to produce an appropriate end signal in 50% of 16 cycles where the day of maximum follicular diameter (as determined by pelvic ultrasonography) was used as the reference point for ovulation and potential fertility. Accordingly, a computer programme was written for a manual algorithm developed by the designer of the Rovumeter. This algorithm was optimized by a systematic evaluation of the rules when applied to a subset of the data acquired from the multicentre study. The principal aim was to improve the number and timing of the 'off' signals without compromising the number and timing of the 'on' signals and the length of the derived PPF.

The main findings were firstly that the test only covered the minimum PPF in 50% of cycles, and secondly that only 21% of women had a consistently successful test result over three consecutive cycles. There were, however, considerable differences in the success rates between centres (i.e. the success rate per cycle varied between 28% at Milan and 62% at Birmingham, and the success rate per woman for three cycles

Table II. Days of start and end signals for the period of potential fertility relative to day 1 of menses and day of urinary luteinizing hormone (U-LH) peak

Variable	Centre[a]	No. cycles	Mean (SD)	Median	Minimum	Maximum
Start signal relative to day 1 of menses	B	79	11 (3)	10	7	26
	M	68	12 (2)	11	8	20
	S	79	10 (3)	10	6	20
	All	**226**	**11 (3)**	**11**	**6**	**26**
End signal relative to day 1 of menses	B	56	20 (4)	19	12	33
	M	28	19 (4)	18	15	30
	S	54	18 (3)	18	13	27
	All	**138**	**19 (4)**	**18**	**12**	**33**
Start signal relative to day of U-LH peak	B	79	–5 (3)	–5	–13	2
	M	68	–4 (4)	–4	–22	6
	S	78	–5 (3)	–5	–12	4
	All	**225**	**–5 (4)**	**–5**	**–22**	**6**
End signal relative to day of U-LH peak	B	56	3 (2)	3	3	8
	M	28	3 (3)	4	–4	6
	S	54	3 (3)	2	–7	14
	All	**138**	**3 (3)**	**3**	**–7**	**14**

[a]B = Birmingham; M = Milan; S = Santiago.

Table III. Length of derived periods of potential fertility

Centre	Cycles[a] No.	Mean (SD)	Duration of derived period		
			Median	Minimum	Maximum
Birmingham	56	9 (4)	8	4	18
Milan	28	7 (3)	7	4	17
Santiago	54	8 (3)	8	4	18
All	138	8 (3)	8	4	18

[a]With start and end signals.

Table IV. Number (and percentage) of successful tests per cycle, i.e. where end-points from the algorithm covered the minimum theoretical period of potential fertility (i.e. day of urinary LH peak minus 3 to day plus 2)

Centre	No. of cycles[a]	No. of tests[b] unsuccessful	No. of tests[b] successful (%)
Birmingham	81	6	50 (62)
Milan	69	9	19 (28)
Santiago	78	10	44 (56)
All	228	25	113 (50)

[a]With urinary LH peak.
[b]With start and end signals of potential fertility.

Table V. Number of women with three consecutive cycles where the test for the minimum period of potential fertility was either consistently unsuccessful, variably successful, or consistently successful

Centre	Results (no. of women)			
	Unsuccessful	Variably successful	Successful	All (%)
Birmingham	3	12	7	22[a] (31)
Milan	13	9	2	24 (34)
Santiago	4	15	6	25 (35)
All (%)	20 (28)	36 (51)	15 (21)	71 (100)

[a]One woman provided data for two cycles only.

Table VI. Volume of cervicovaginal fluid (CVF) per woman (mean value for days 10–18 over three menstrual cycles) according to the outcome of tests to locate the minimum period of potential fertility over the same three cycles. Data from Birmingham and Santiago only are shown because changes in the daily volumes of CVF were more similar (see Figure 2)

Test results over three cycles	*n*	CVF volume (ml)			
		Mean (SD)	Median	Minimum	Maximum
Unsuccessful	7	0.20 (0.13)	0.19	0.06	0.46
Variably successful	27	0.27 (0.12)	0.26	0.10	0.66
Successful	14	0.37 (0.17)	0.34	0.17	0.75

varied between 8% at Milan and 32% at Birmingham. These findings might simply reflect differences in small, self-selected groups of women. Alternatively, the values might indicate real differences in the volume of CVF produced over a PPF by women from some cultural groups. This interpretation would support the finding that the length of the PPF as determined by the cervical mucus method of natural family planning is significantly different in women from some cultures (WHO, 1983b). Another explanation is that there were small differences in the sampling technique, although the women at all centres usually removed their CVF during the mid-morning period and recorded the same number of observations on different days of the menstrual cycle (see Figure 1).

The success rate of the test was undoubtedly related to the mean volume of CVF produced by each woman over days 10–18 of her cycles. Moreover, the low volumes of CVF produced during the early follicular phase of the cycle were difficult to measure accurately. The relationship between CVF volume and fertility is unknown. It is likely, however, that the type (or consistency) of CVF is more important than the volume (Odeblad, 1977). The potential use of measuring various constituents of CVF (including mucin and enzymes) has been reported (Wolf *et al.*, 1980; Van Koij *et al.*, 1980; Tsibris *et al.*, 1989). Consequently, a test that is based on a physicochemical characteristic of mucus (e.g. viscosity), or a ratio of two constituents (e.g. an enzyme/total protein), or the degree of hydration is likely to be more successful than volume in delineating the PPF.

The Rovumeter is undoubtedly cheap and useful for a small

proportion of women who are familiar with the symptothermal method of natural family planning in the context of a clinical trial. The success rate in delineating the PPF, however, is likely to be less in routine use by unselected women from the general population. Nevertheless, the Rovumeter offers an objective method of demarcating the PPF and may have a role in providing CVF for teaching some women the cervical mucus method of natural family planning. Defined changes in the volume of CVF may also help subfertile couples achieve a pregnancy by predicting the time of maximum potential fertility (Flynn *et al.*, 1988a,b). It is of interest, however, that there have been conflicting reports of the potential use of home tests for detecting the urinary LH surge by donor insemination services (Robinson *et al.*, 1992; Anderson *et al.*, 1996). There is also some preliminary evidence to suggest that the Rovumeter might be used to monitor treatment with human gonadotrophins (Pratt *et al.*, 1992). All of these possibilities need to be explored further by controlled clinical trials.

Acknowledgements

The authors are grateful to the World Health Organization, Special Programme of Research Development and Research Training in Human Reproduction for financial support and the provision of Rovumeters, the Chart Co-ordinators for their contribution at each centre, and the technical staff who performed the hormone assays.

References

Anderson, R.A., Eccles, S.M. and Irvine, D.S. (1996) Home ovulation testing in a donor insemination service. *Hum. Reprod.*, **11,** 1674–1677.

Burger, H.G. (1989) Estradiol: the physiological basis of the fertile period. *Int. J. Gynecol. Obstet.* **1** (Suppl.), 5–9.

Collins, W.P. (1989) Biochemical indices of potential fertility. *Int. J. Gynecol. Obstet.* **1** (Suppl.), 35–43.

Collins, W.P. (1996) Indicators of potential fertility: scientific principles. In Bonnar, J. (ed.), *Natural Contraception through Personal Hormone Monitoring*. Parthenon Publishing Group, New York, pp. 13–34.

Collins, W.P., Collins, P.O., Kilpatrick, M.J. *et al.* (1979) The measurement of urinary oestrone-3-glucuronide, LH and pregnanediol-3α-glucuronide as indices of ovarian function. *Acta Endocrinol.*, **90,** 336–348.

Flynn, A.M., McCarthy, N., Docker, M. and Royston, P. (1988a) The temporal relationship between vaginal fluid volumes obtained with the Rovumeter vaginal aspirator and the fertile phase of the cycle. *Hum. Reprod.*, **3,** 201–205.

Flynn, A.M., Docker, M.F., McCarthy, N. and Royston, P. (1988b) Detection of the fertile phase from changes in cervico-vaginal fluid volume. *Int. J. Fertil.* **1** (Suppl.), 17–23.

Godley, M.J. (1985) Quantitation of vaginal discharge in healthy volunteers. *Br. J. Obstet. Gynaecol.*, **92,** 739–742.

Hatcher, R.A., Trussell, J., Stewart, F. *et al.* (1994) The essentials of contraception: effectiveness, safety and personal considerations. In *Contraceptive Technology*, 16th edn. Irvington Publishers, New York, pp. 107–137.

Katz, D.F. (1991) Human cervical mucus: research update. *Am. J. Obstet. Gynecol.*, **165,** 1984–1986.

Moghissi, K.S. (1979) Vaginal fluid constituents. In Beller, F.K. and Schumacher, G.F.B. (eds), *The Biology of the Fluids of the Female Genital Tract.* Elsevier-North Holland, NY, USA, pp. 13–23.

Moghissi, K.S. (1984) The function of the cervix in human reproduction. *Curr. Probl. Obstet. Gynecol.*, **7,** 1–58.

Odeblad, E. (1977) Physical properties of cervical mucus. In *Mucus in Health and Disease. Adv. Exp. Med. Biol.*, **89,** 217–225.

Pratt, D.E., Vignovic, E., Holt, J.A. and Schumacher, G.F. (1992) Correlation of cervicovaginal fluid volume with serum estradiol levels and total follicular volume during human gonadotropin stimulation. *J. Assist. Reprod. Genet.*, **9,** 14–18.

Robinson, J.N., Lockwood, G.M., Dalton, J.D.E. *et al.* (1992) A randomised prospective study to assess the effect of the use of home urinary luteinizing hormone detection on the efficiency of donor insemination. *Hum. Reprod.*, **7,** 63–65.

Royston, P. (1991) Identifying the fertile phase of the human menstrual cycle. *Stat. Med.*, **10,** 221–240.

Tsibris, J.C.M., Lewis, V., Langenberg, P.W. *et al.* (1989) Cervical mucus enzymes as markers of the woman's fertile period. *Int. J. Gynecol. Obstet.* **1** (Suppl.) 73–82.

Usala, J.S. and Schumacher, G.F.B. (1983) Volumetric self-sampling of cervicovaginal fluid: a new approach to ovulation timing. *Fertil. Steril.*, **39,** 304–309.

Van Koij, R.J., Roelofs, H.J.M., Kathmann, G.A.M. and Kramer, M.F. (1980) Human cervical mucus and its mucus glycoprotein during the menstrual cycle. *Fertil. Steril.*, **34,** 226–233.

Viergiver, E. and Pommerenke, W.T. (1946) Cyclic variations in the viscosity of cervical mucus and its correlation with amount of secretion and basal temperature. *Am. J. Obstet. Gynecol.*, **51,** 192–196.

Wagner, G. (1979) Vaginal transudation. In Beller, F.K. and Schumacher, G.F.B. (eds), *The Biology of the Fluids of the Female Genital Tract.* Elsevier-North Holland, pp. 25–34.

Wolf, D.P., Blasco, L., Khan, M.A. and Litt, M. (1978) Human cervical mucus IV. Viscoelasticity and sperm penetrability during the ovulatory menstrual cycle. *Fertil. Steril.*, **30,** 163–169.

Wolf, D.P., Sokoloski, J.E. and Litt, M. (1980) Composition and function of human cervical mucus. *Biochim. Biophys. Acta*, **630,** 545–558.

World Health Organization (1981a) A prospective multicentre trial of the ovulation method of natural family planning. I. The teaching phase. *Fertil. Steril.*, **36,** 152–158.

World Health Organization (1981b) A prospective multicentre trial of the ovulation method of natural family planning. II. The effectiveness phase. *Fertil. Steril.*, **36,** 591–598.

World Health Organization (1982) The measurement of urinary steroid glucuronides as indices of the fertile period in women. *J. Steroid Biochem.*, **17,** 695–702.

World Health Organization (1983a) Temporal relationships between indices of the fertile period. *Fertil. Steril.*, **39,** 647–655.

World Health Organization (1983b) A prospective multicentre trial of the ovulation method of natural family planning. III. Characteristics of the menstrual cycle and of the fertile phase. *Fertil. Steril.*, **40,** 773–778.

Received on February 10, 1997; accepted on May 23, 1997