Monitoring of ovarian activity by daily measurement of urinary excretion rates of oestrone glucuronide and pregnanediol glucuronide using the Ovarian Monitor, Part III: Variability of normal menstrual cycle profiles

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STUDY QUESTION: What are the characteristics of, and how variable are, individual normal menstrual cycle profiles of excretion rates for the urinary metabolites oestrone glucuronide (E1G) and pregnanediol glucuronide (PdG)?

SUMMARY ANSWER: There is a continuum of menstrual cycle profiles that differ from standard textbook profiles but which can be understood simply in terms of growth, atresia and ovulation of ovarian follicles.

WHAT IS KNOWN ALREADY: Point-of-care assays with the Ovarian Monitor pre-coated assay tubes, using urine samples diluted to a constant volume per unit time, give laboratory accurate clinical data for individual menstrual cycles. Lay operators can perform the point-of-care assay system at home to achieve reliable and reproducible results, which can be used for natural family planning.

STUDY DESIGN, SIZE, DURATION: This prospective study involved 62 women, with normal menstrual cycles, recruited from three centres: Palmerston North, New Zealand, Sydney, Australia and Santiago, Chile. The study lasted 3 years.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Women collected daily urine samples and determined their E1G and PdG rates with a pre-coated enzyme assay system known as the Ovarian Monitor. For two cycles, the assays were repeated in a study centre and the results were averaged to give 113 individual menstrual cycles for analysis. The cycles were displayed individually in a proprietary database program.

MAIN RESULTS AND THE ROLE OF CHANCE: The individual normal hormonal profiles were more complex than the classic composite curves for 40% of the cycles. Of 113 ostensibly normal cycles, only 91 were potentially fertile and 22 had some luteal phase defect. The oestrone glucuronide and PdG excretion rates were reliable and informative in the non-invasive elucidation of ovulation and ovarian function for both simple and complex profiles. Daily monitoring revealed the variability of normal menstrual cycle profiles. The LH peaks were variable and ambiguous markers for ovulation.

LIMITATIONS, REASONS FOR CAUTION: The study consisted of cycles only from women with regular cycles of 20–40 days duration. All the women were intending to avoid a pregnancy during the study thus the limits of the fertile window were not tested.

WIDER IMPLICATIONS OF THE FINDINGS: The principles established in this study should apply to cycles of any length. All peaks in oestrone glucuronide excretion should be tested by concurrent measurements of PdG, which gives a positive indication of the fate of the follicle it represents. The Ovarian Monitor provides a useful addition for practitioners of natural family planning.
STUDY FUNDING/COMPETING INTEREST(S): Financial support for this study was obtained from the UNDP/UNFPA/World Bank/WHO Special Programme of Research, Development and Research Training in Human Reproduction (HPR). D.G.C. is currently employed by and holds stock in Manawatu Diagnostics Ltd, a company in the development phase of a potentially competing product. The remaining authors have nothing to declare.

Key words: oestrone glucuronide / menstrual cycle profile / pregnanediol glucuronide / urine / home test

Introduction

The results of a multi-centre study of the Hormonal Definition of the Fertile Days of the Cycle by Home Monitoring for Natural Family Planning (UNDP/UNFPA/WHO/World Bank protocol #90905) were used to evaluate the suitability of point-of-care assays for determining the pattern of oestrone glucuronide (E1G) and pregnanediol glucuronide (PdG) excretion in normal menstrual cycles. In the first two parts of this study, it was shown that (i) the point-of-care assays with the Ovarian Monitor pre-coated assay tubes, using urine samples diluted according to time, give laboratory accurate clinical data for individual menstrual cycles (Blackwell et al., 2003) and (ii) lay operators can perform the point-of-care assay system at home to achieve reliable and reproducible results (Blackwell et al., 2012). The second part of the study has provided a valuable collection of 113 individual menstrual cycle profiles of daily measurements of urinary E1G and PdG excretion rates for analysis (Blackwell et al., 2012). This paper presents the results of this analysis.

Averaging of individual cycle data into mean profiles before analysis is commonly done and, while producing smoother curves, this step has the added consequence of averaging away many non-classical variations (Dodson et al., 1975; Brown et al., 1988; Brown et al., 1989; Brown et al., 1991; Munro et al., 1991; Lewis et al., 1994; Alliende, 2002; Blackwell et al., 2012). There also has been a tendency to discard the results of the cycles whose patterns differ from the typical textbook patterns as being abnormal or due to assay errors, and once again important information has been lost (Renaud et al., 1980). Brown (2011) showed that a continuum of possibilities for menstrual cycle types exists throughout reproductive life; this ovarian continuum ranges from no activity at all to fully fertile cycles. Complex cycle patterns may be expected even in normal cycles, hence it is important that women who are identifying their fertile periods understand that continuation of current patterns of ovarian activity cannot be assumed and vigilance is required to detect the next onset and end of fertility. Since the changes in hormone levels just before and after ovulation are rapid (30–50% per day), it is also necessary to be able to monitor ovarian activity daily. The individual cycles obtained in this study with self-monitoring provide a unique opportunity to determine the occurrence and frequency of variations in cycle profiles for normal cycles. This is the first time to our knowledge that it has been possible for a study of fertility to be carried out based entirely on the results of individual cycles obtained by home monitoring.

The objective of the present study was to determine the characteristics and variability of individual normal menstrual cycle profiles from daily self-determination of urinary E1G and PdG excretion rates.

Materials and Methods

The Ovarian Monitor study

The details of the women (mean age ± SD: 34 ± 6 years; mean cycle length ± SD: 28.1 ± 3.1 days) have been described previously (Blackwell et al., 2003; Blackwell et al., 2012). The majority of the women (94%, 58/62) had at least one previous pregnancy (times pregnant mean ± SD: 3.6 ± 1.98). The four who had not been pregnant were assumed to be fertile based on their previous cervical mucus and basal body temperature (BBT) patterns. All participants signed an informed consent form before enrolling in the study and the protocols followed the WHO ethical guidelines for research involving human subjects. The details of the urine collection and dilution procedures are given in Cooke et al. (2007). The urine samples were collected once a day at any convenient time providing the collection time was 3 h or greater (usually 3–10 h) and the results were expressed as moles per 24 h after correcting for the rate of urine production (time dilution). The women measured their E1G and PdG excretion rates at home daily on their freshly collected and time-diluted urine samples for their first two cycles (Blackwell et al., 2012) using pre-coated assay tubes (Brown et al., 1989; Brown et al., 1991), and the samples were re-analysed in batches at the local centres by non-technical personnel (Blackwell et al., 2012). The Ovarian Monitor uses the principle of homogeneous enzyme immunoassay (Brown et al., 1988, 1989, 1991; Blackwell et al., 2003), and the details of the pre-coated tube assays and their validation are given in parts I (Blackwell et al., 2003) and II (Blackwell et al., 2012) of this series. The data were reported by the women simply as transmission change, $\Delta T = T_2 - T_1$ for 5 min (PdG) or 20 min (E1G), where $T_2$ is the turbidity of the assay tube at time t and $T_1$ is the turbidity of the assay tube at time = 0. However, for the analysis in the present study, after removal of a small percentage (<5%) of rogue results (Blackwell et al., 2012), the $\Delta T$ values were converted into nmol/24 h for E1G and pmol/24 h for PdG using the standard curves provided with each batch by the production unit in Melbourne. For 113 complete individual cycles, the daily averages of the valid duplicate home and centre excretion rates were used to construct hormonal profiles. The cycles were normal by accepted criteria such as cycle length and ovulatory patterns of cervical mucus secretion and/or BBT (Blackwell et al., 2012).

LH measurements

LH levels were provided by local commercial laboratories on the undiluted urine samples (Blackwell et al., 2003). For the Palmerston North centre, the results were provided by the biochemistry laboratory of the Palmerston North Hospital, which used a Boehringer-Mannheim Immunodiagnostics Enzymum LH test. Details of the assays were not available for the other two centres.

Cycle definitions used for analysis of the menstrual cycle profiles

A computer database program (NewDB) was written to display the cycles individually and calculate key parameters for each cycle from the daily...
changes in the excretion rates of both E1G and PdG (Blackwell et al., 2012). The definitions used by the program NewDB for determining the key cycle parameters are given below and were derived by visual inspection of the collection of individual cycles in the database. The PdG thresholds applied in this study were based on the pregnanediol (Pd) excretion rates given by Brown (2011) according to his conversion: μmol/24 h PdG = 4.5 × mg/24 h Pd.

Start of the menstrual cycle

The start of the menstrual cycle was the first day of significant bleeding.

The E1G baseline

An E1G baseline was present if there were three or more consecutive days of constant low pre-ovulatory E1G values that showed no consistent trend (Blackwell and Brown, 1992) and had a coefficient of variation of <15%. The baseline need not necessarily commence on the first day of the cycle.

The first rise in E1G excretion rate

If the cycle has a follicular phase baseline period, the first E1G rise day is the day that the rate of E1G excretion increases to exceed the baseline mean + 2 SD. If there is no early baseline period, the first E1G rise is taken as the first day of the rising E1G values that defines the earliest E1G peak. The first day of the rise in E1G excretion rate is the first day of potential fertility.

A pre-ovulatory E1G peak day

A pre-ovulatory E1G peak day occurs if there are at least three consecutive rising values followed by a distinct fall without a following rise in the PdG excretion rate above the PdG threshold (Blackwell et al., 1998) to mark the end of the fertile window. More than one pre-ovulatory E1G peak per cycle may occur, particularly in long cycles. These pre-ovulatory E1G peaks generally show increasing heights with each subsequent peak but none reach the height of the ovulatory peak (Brown, 2011).

Ovulatory E1G peak types

Four distinct ovulatory E1G peak types were recognized in the peri-ovulatory period as shown in Figure 1. All ovulatory E1G peak types are characterized by a distinct fall in the excretion rates immediately before the rapid rise in PdG excretion rate.

- **Type I**: A single day with a high value for the E1G excretion rate flanked by lower values (Fig. 1A); the classical peak pattern.
- **Type II**: Two days with similar or identical high E1G excretion rates in the peri-ovulatory period (Fig. 1B).
- **Type III**: Two days with high E1G excretion rates in the peri-ovulatory period separated by a single intervening day with a lower E1G excretion rate (Fig. 1C), or three days with approximately equal excretion rates.
- **Type IV**: A plateau with 4 or more consecutive days with similar high E1G excretion rates in the peri-ovulatory period (Fig. 1D).

The ovulatory E1G peak day

The ovulatory E1G peak day was defined as the last day with a high value for the E1G excretion rate which was followed closely by a rise in the PdG excretion rate to equal or exceed the PdG threshold for the end of the fertile window within 6 days. It was not necessarily the highest E1G excretion rate in the cycle (see for example Fig. 2A where the highest E1G value was in the luteal phase).

The LH peak day

The LH peak day was defined as the day with the highest LH value closest to the E1G peak day and before the PdG values rose to exceed the PdG threshold for the end of the fertile window (Fig. 2).

The PdG threshold days

The PdG threshold days are the first cycle days that the PdG excretion rate equals or exceeds a threshold value following an E1G peak day. The key thresholds were as follows: end of the fertile window, 7 μmol PdG/24 h; biochemical proof of ovulation, 9 μmol PdG/24 h and a critical PdG value which must be equalled or exceeded within 6 days of the ovulatory E1G peak for potential fertility, 13.5 μmol PdG/24 h (Brown, 2011). Elevated PdG excretion rate days at the beginning of the cycle are excluded as falls from the previous cycle (as in Fig. 1A and C).

Luteal phase length

The luteal phase length is the number of days from the day of the ovulatory E1G peak to the day before the beginning of the next menstrual bleed, inclusive.

Potentially fertile cycle

A potentially fertile cycle has an E1G peak day which within 6 days is followed by a rapid rise in the PdG excretion rate to equal or exceed the critical value for fertility (Brown, 2011), and at least 2 days in the luteal phase when the PdG excretion rate exceeds this critical value. The luteal phase lasts 11–17 days (e.g. as in Fig. 1A–D).

Subfertile cycle types

The subfertile cycle types were as previously defined (Brown, 2011).

- **Short luteal phase**: a cycle where all other parameters are the same as for a potentially fertile cycle but the luteal phase length is 10 days or less.
- **Deficient luteal phase**: a cycle with a normal length luteal phase but the PdG excretion rate does not equal or exceed the critical value for fertility (13.5 μmol/24 h) within 6 days of the E1G peak (Fig. 3B).
- **Luteinized unruptured follicle (LUF)**: a cycle where the PdG excretion rate exceeds the PdG threshold for the end of the fertile window but no day has a PdG excretion rate exceeding the value indicating ovulation (9 μmol/24 h) before the next menstrual bleed.
- **Anovulatory cycle**: a cycle with an E1G peak day but no rise in PdG excretion.

Statistical methods

The arithmetic means and SD of parameters such as differences between E1G peak days and LH peak days were all calculated by the column statistics package of GraphPad Prism, v. 5 (GraphPad Software, Inc., La Jolla, CA, USA). Statistical significance was calculated by comparison of pairs of means and SD using the unpaired Student’s t-test package of Prism which yielded a two-tailed P-value in each case. The significance of differences between percentages of various cycle events in the Ovarian Monitor immunoassay data was assessed by a χ² calculation using the CHITEST function of Excel. Significance was determined at P < 0.05.

Results

Accuracy of the PdG excretion rate threshold data obtained by the lay users’ results with pre-coated PdG assay tubes

The distribution of the daily PdG excretion rates determined for each cycle day, relative to the E1G peak day as Day 0, is shown in Figure 3A for the 91 individual potentially fertile cycles. The percentile lines calculated from these data (10th, 50th and 90th) are shown together with the corresponding published normal ranges for Pd excretion rates derived from 140 different individual normal cycles in the Melbourne reference
database (Brown, 2011). These Pd normal range excretion rates (individual Pd data points not shown) were superimposed on the PdG data points after conversion into their equivalent PdG excretion rates according to the relationship given by Brown (2011). The 10th and 50th percentile lines overlapped for both assays but the 90th percentile lines for PdG showed a slightly broader profile.

### Classification of cycle types based on the PdG excretion rates

When the hormonal definitions given in the section Materials and methods were applied to the 113 individual cycles, 91 were potentially fertile and 22 were subfertile. Figure 1A–D show the E1G and PdG excretion rates for four cycles; all the four cycles were fertile in that they reached PdG values exceeding 13.5 μmol PdG/24 h within 6 days of the E1G peak day. The rapid rises in PdG excretion rates for these cycles were in marked contrast with the situation for a cycle classified as subfertile as shown in Figure 3B. For this cycle, no E1G baseline period was recorded since urine collection was only started on Day 4. There was a small pre-ovulatory E1G peak centred on Day 7 which was followed by rising E1G excretion rates starting from Day 13. The E1G excretion rate peaked on Day 17 (peak type I) and the luteal phase length calculated from this peak day was 13 days which was within the normal range. The PdG values began to rise on Day 17, to reach a peak value on Day 25 of

![Figure 1](https://example.com/fig1.png)
only 7.8 μmol/24 h. For the 22 subfertile cycles, 4 had an LUF, 17 had deficient luteal phases, 1 had a short luteal phase and there were no anovulatory cycles. The 22 subfertile cycles constituted 19.5% of the total and the cycles with deficient luteal phases made up 80% of the subfertile cycles.

**Menstrual cycle profiles for E1G and PdG**

For both the fertile and subfertile cycles, the E1G profiles were often complex (e.g., Figs 1 and 2) and consisted of many rises and falls in E1G excretion rates, from day to day throughout the cycle, that were greater than the experimental errors (Blackwell et al., 2012). Sometimes E1G and PdG levels were still falling from the previous cycle (see Fig. 1B and Fig. 2D). For cycles with only one pre-ovulatory E1G peak, or two pre-ovulatory peaks, the population mean first peak excretion rate was 220.2 ± 55.4 nmol/24 h (n = 45), which was less than the population mean ovulatory E1G peak value for the

**E1G baselines**

Only 48.7% of the E1G profiles (55/113) had a baseline period (e.g., Fig. 1B and Fig. 2D). For these cycles, the E1G baseline period averaged 4.4 ± 1.5 days in length and ran on average from cycle day 2.6 ± 1.8–7.0 ± 1.9. There was no significant difference in the baseline lengths when the cycles were divided into fertile (4.4 ± 1.5 days, n = 44) and subfertile (4.2 ± 1.8 days, n = 11) groups (P > 0.74).

**Pre-ovulatory E1G peaks**

For 40% (45/113) of the cycles there was at least one pre-ovulatory E1G peak (e.g., Fig. 1A, C and D) and for 11% (12/113) there were two pre-ovulatory E1G peaks in the same cycle. For cycles with only one pre-ovulatory E1G peak, or two pre-ovulatory peaks, the population mean first peak excretion rate was 220.2 ± 55.4 nmol/24 h (n = 45), which was less than the population mean ovulatory E1G peak value for the

![Figure 2](https://academic.oup.com/humrep/article-abstract/28/12/3306/691823)
potentially fertile cycles (339.1 ± 110.4 nmol/24 h; n = 91, P = 0.025). For the 12 cycles with two pre-ovulatory E1G peaks, the mean excretion rate for the second pre-ovulatory peak day was higher (261.5 ± 59.4 nmol/24 h) but still significantly less than the population mean ovulatory E1G peak value (P = 0.042).

Ovulatory E1G peak types
All of the cycles showed a mid-cycle E1G peak immediately prior to the PdG rise and could be categorized into four main ovulatory peak types (Fig. 1). The frequencies of the different E1G peak types shown in Table I were similar irrespective of the study centre or whether the cycles were fertile or subfertile (all P values >0.542). The percentages of the three non-classical E1G peak types were approximately equal (ca 10%). The classical type I mid-cycle estrogen peak with a single high E1G value flanked by 2 days with lower E1G values (as in Fig. 1A and 2D) was observed for only about 70% of the cycles. However, only about 60% of the cycles had the classical type I mid-cycle estrogen peak and no early E1G peaks (i.e. the classic textbook cycle pattern). For 12% of the fertile cycles (11/91), the highest E1G excretion rate occurred in the luteal phase (see Fig. 2A).

Ovulatory LH peaks
An LH peak day could be assigned by our definition for 89% (101/113) of the cycles even though more than half of these (64/101) also showed at least one extra peak of lower magnitude (e.g. Fig. 2B) and 10/101 had two or more large LH peaks. For the remaining 12/113 cycles (11%), two or more high LH peaks were present in five cycles. In 12/113 cycles, the highest LH peak occurred in the luteal phase (Fig. 2D), which starts on the day of the intersection between the line for the PdG end of fertility threshold excretion rate and the PdG profile as shown in Figures 1 and 2. These peaks were ignored for the purposes of this analysis. For five cycles (4.4%), although the LH level was elevated, it was difficult to discern a peak day, and for a further two cycles, there was no discernible LH peak even though the E1G and PdG profiles appeared perfectly normal in each case. Overall, only 31/113 cycles (27.4%) had the classic LH pattern. The ovulatory LH peaks occurred on average ~5 h after the E1G peak day (Table II) and there were no significant differences in this timing between the fertile and subfertile cycles.

First E1G rise days
The first significant rise in E1G excretion rates for the cycles with type I E1G peaks and no pre-ovulatory peaks (i.e. with a baseline period) occurred on average 3.5 ± 1.5 days (n = 41) before the E1G peak day with a range of −9 to −1 days relative to the E1G peak day. There was no significant difference (Table II) in the first E1G rise days between the fertile and subfertile cycles. If all of the E1G peak types were included (without pre-ovulatory peaks), the rise day was 3.6 ± 1.5 days (n = 65) which was not significantly different (P > 0.142) from that of the type I peak cycles. However, when all the cycles, both fertile and subfertile, including those with pre-ovulatory E1G peaks, were included in the analysis, the first rise in E1G excretion rate occurred on average 5.7 ± 3.4 days (n = 113) before the E1G peak day.

Timing of PdG threshold days
The mean time after the ovulatory E1G peak day that the PdG threshold for the end of the fertile window was equalled or exceeded for the potentially fertile cycles was 2.9 days (Table II). No end of fertility day was signalled on, or before, the E1G peak day using this threshold. There was no statistically significant difference between the means and SD when the potentially fertile cycle data were analysed by ovulatory E1G peak type (all P values >0.175) or by study centre (all P values >0.178). However, the corresponding mean and SD for the subfertile data set was greater by ~1.5 days (P < 0.0001) (Table II).

Effect of age on cycle parameters
The women were divided into two groups: those aged <35 years (n = 52) and those 35 years and older (n = 38). The older group was not over-represented in the fertile or subfertile populations, or for cycles
The classic textbook menstrual cycle pattern consists of a baseline of estrogen values which then increase monotonically to reach a maximum on a single day followed by a sharp fall before a steep rise in progesterone values (as in Fig. 2D). However, 40% of the individual E1G and PdG menstrual cycle profiles were more complex and did not follow this simple pattern. In addition, in many cycles, the E1G and PdG excretion rates were still falling from the previous cycle over the first 3 or 4 days (see Fig. 1A) and often no E1G baseline period was found since rises and falls in E1G excretion rate (>36 nmol/24 h) that were greater than the experimental errors (Blackwell et al., 2012) were common, indicating follicular growth and atresia. The highest E1G excretion rate was not always the E1G peak day since it could occur in the luteal phase (Fig. 2A) and in ~30% of the cycles more than one high E1G excretion rate day was found in the peri-ovulatory period (i.e. ovulatory E1G peak types II, III or IV). Few studies involving daily hormonal monitoring for individual cycles have been reported; however, some of the profile types reported here were seen also in earlier studies performed with complete 24 h urine specimens (Brown, 2011). Complex cycle profiles have also been shown in daily blood estradiol and progesterone measurements for six individual cycles (Dodson et al., 1975), and the complex estrogen peak types (Table I) reported in this study have been seen in other urinary studies of individual cycles (Alliende, 2002). There is no evidence that complex profiles are a barrier to conception (Brown, 2011).

### Table I: Number of E1G peak types in fertile and subfertile cycles

<table>
<thead>
<tr>
<th>Data set</th>
<th>E1G peak type (%)</th>
<th>Type I</th>
<th>Type II</th>
<th>Type III</th>
<th>Type IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertile cycles</td>
<td>64/91 (70.3)</td>
<td>9/91 (9.9)</td>
<td>11/91 (12.1)</td>
<td>7/91 (7.7)</td>
<td></td>
</tr>
<tr>
<td>Subfertile cycles</td>
<td>14/22 (63.6)</td>
<td>3/22 (13.6)</td>
<td>3/22 (13.6)</td>
<td>2/22 (9.1)</td>
<td></td>
</tr>
</tbody>
</table>

*Fertile cycles are those which have an E1G peak (Types I to IV) followed within 6 days by a rapid rise in PdG excretion rate to equal the critical value (see Definitions in the section Materials and methods).

*Subfertile cycles have inadequate luteal phases as defined in the section Materials and methods.

*E1G peak types I to IV are defined in the section Materials and methods.

### Table II: Comparison in days between key parameters of the menstrual cycles by different groups

<table>
<thead>
<tr>
<th>E1G rise day</th>
<th>LH peak day</th>
<th>PdG threshold day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertile cycles</td>
<td>3.38 ± 1.60 (34)</td>
<td>0.23 ± 0.86 (87)</td>
</tr>
<tr>
<td>Subfertile cycles</td>
<td>3.86 ± 0.70 (7)</td>
<td>0.53 ± 0.93 (19)</td>
</tr>
</tbody>
</table>

*The relative day that these events occurred was calculated as day of the event minus the E1G peak day.

*The first E1G rise day was calculated for cycles with type I E1G peaks and without pre-ovulatory E1G peaks. The first E1G rise day was not statistically significantly different between the fertile and infertile cycles and the sum.

*Fertile cycles are those which have an E1G peak (Types I to IV) followed within 6 days by a rapid rise in PdG excretion rate to equal the critical value (see Definitions in the section Materials and methods).

*Subfertile cycles have inadequate luteal phases as defined in the section Materials and methods.

Discussion

This study demonstrates the necessity of frequent self-monitoring of ovarian steroids throughout individual menstrual cycles to recognize the sometimes complex changes in ovarian activity as they occur. The computer program NewDB allowed each individual cycle to be displayed and examined separately according to the definitions given in the section Materials and methods. Hence, many features of the normal cycle profiles that were not generally recognized in composite cycles were obvious in the individual cycles. The complex picture of the normal human menstrual cycle thus revealed is consistent with the ovarian continuum concept enunciated by Brown (2011). The various types of ovarian activity encountered here represent practical examples of the continuum operating in the lives of normally cycling women. Importantly, the periods of fertility and subfertility were recognized clearly by the women as their cycle unfolded daily from their home hormone measurements without recourse to any previous cycle history.

E1G and PdG excretion rates as functional tests

The interpretation of these complex cycle profiles by the home users depended on understanding that the E1G and PdG assays are functional tests of ovarian and corpus luteum activity, respectively. The pre-ovulatory E1G peaks represent real changes in ovarian activity, and hence in potential fertility. For example, when the E1G excretion rate increases significantly above baseline levels for two or more days, at least one ovarian follicle must be present and expressing aromatase activity in its granulosa cells (McNatty, 1981). Thus, significant changes in urinary E1G excretion rates (Blackwell et al., 2012) are the signature for the growth and ovulation or atresia of one, or more, ovarian follicles (for example the pre-ovulatory and ovulatory E1G peaks in Fig. 1A). The actual E1G profile for each menstrual cycle can be considered to result from a succession of separate or overlapping hormonal signatures for developing follicles which continues until a follicle ovulates. The actual sequence of follicular events constitutes a unique and sometimes complex pattern for that woman in that cycle as shown in Figure 1.
The distinction between pre-ovulatory follicles (non-ovulatory) and ovulatory follicles can be made conveniently by measurement of absolute excretion rates of PdG. Specific threshold values have been assigned to distinguish between fertile ovulatory cycles, cycles with deficient luteal phases or LUFs and anovulatory cycles. It is important in any study to demonstrate first that the PdG assay being used gives an accurate result which we define as returning the same threshold excretion rates as given by the reference Pd assay (Brown, 2011). The PdG assay used by the women at home in this study clearly met this criterion. The Pd database made an appropriate reference as it was collected using 24 h urine samples measured with a chemical assay of proven reliability and precision (Brown, 2011). The fact that the percentile lines for the reference Pd assay overlapped those calculated for the PdG assay data (Fig. 3A), and that the Pd and PdG excretion rates correlated with a correlation coefficient of 0.95 (Brown et al., 1989) gave further confidence that both assays give identical excretion rates within experimental error. The daily PdG excretion rates were the most important measurement available to help the women define their E1G and LH peak days and to define the end of their fertile window. When the PdG excretion rate increased to equal or exceed the various threshold levels given in the section Materials and methods, ovulation had already occurred and hence the E1G peak day and/or LH peak day had to occur beforehand even if a higher value occurred later in the cycle. Every peak in the E1G excretion rate should be tested therefore for ovulation by a concurrent PdG measurement. For example, the pre-ovulatory peak in E1G levels in Figure 1A was not accompanied by an increase in the PdG excretion rate, thus it represents a failed follicle. This failed follicle was replaced immediately by another follicle which ovulated. This possibility was recognized in the continuum concept (Brown, 2011) and is shown here by the daily rapidly rising values for the PdG excretion rate, which followed the second E1G peak.

Once the PdG end of the fertile window threshold (7 μmol/24 h) has been reached after a mid-cycle E1G peak, the beginning of the period of post-ovulatory infertility (Figs 1, 2 and 3B) is indicated. However, it does not necessarily signify that the ovulation is fertile or indeed that the cycle is ovulatory (see Fig. 3B) as a PdG excretion rate of > 9 μmol/24 h needs to be reached as biochemical proof of ovulation (Pepperell et al., 1975). Also, analysis of pregnancy cycles (Brown, 2011) has shown that the PdG excretion rate must exceed 13.5 μmol/24 h within 6 days of the E1G peak day for a pregnancy to be maintained. Thus, the daily PdG excretion rate profile, including the three threshold values of 7 μmol/24 h (end of fertile window marker), 9 μmol/24 h (threshold for biochemical proof of ovulation) and 13.5 μmol/24 h (threshold for a fertile cycle), is an important tool to help the users manage their fertility.

A significant finding from this study was that despite being considered normal and fertile by properties such as cycle length, BBT and mucus patterns, nearly 20% of the cycles had either a deficient luteal phase, a short luteal phase or an LUF and thus were probably subfertile. The most common cause of subfertility was a deficient luteal phase. This could be identified clearly by the slow and deficient daily increase in the PdG excretion rate (Table II) for the 6 days following the E1G peak day. Deficient luteal phases could manifest themselves at any time even after a previous history of normal, ovulatory, fertile cycles. This high frequency was not unexpected as it is one of the first steps in the regression from the fertile ovulatory cycle in the continuum. The age of the women did not seem to be a factor in determining the occurrence of luteal phase deficiency. The ratio of women aged <35 years to those 35 years and over remained similar to the distribution for the whole population in the fertile and subfertile cycles and the cycles with deficient luteal phases. There was no evidence from the hormonal profiles that the shorter follicular phases for the older group were a barrier to fertility. Women desiring to get pregnant and who have a re-occurring PdG profile indicating subfertility can be treated successfully with clomiphene and gonadotrophins (Brown, 2011). A similar finding of deficient luteal phases in a supposedly normal population of athletic women was published with daily monitoring of E1G and PdG by De Souza et al. (2010).

Fertility monitoring with home assays for natural family planning

The women could detect the beginning of their fertile window by the first rise in E1G excretion rate, as defined in the section Materials and methods, irrespective of whether the profiles were simple or complex. Fertility has to be assumed whenever the E1G values are rising unless the PdG values have equaled or exceeded the end of fertility threshold already. For the avoidance of pregnancy, the interval between the first E1G rise signal and the ovulatory peak ideally should be 4–5 days to allow for the fertilizing life span of the spermatozoa which probably averages 72 h (Brown et al., 1989). Since ovulation is expected to occur in the 24–48 h period after the E1G peak day, the total warning of ovulation given by the first E1G rise for the cycles with textbook profiles was ~5–6 days which should be sufficient for pregnancy avoidance. This warning is similar to that reported previously with laboratory assays (Blackwell and Brown, 1992). For cycles with less warning, it was probably also adequate as the cervical mucus over the days with low E1G excretion rates would most likely be of the non-estrogenic type and hostile to sperm survival (Bigelow et al., 2004). For the more complex profiles, as in Figure 1A and C, the first rise in E1G excretion rate inevitably gave a longer warning of ovulation by ~2 days on average. Although such a long warning is more than sufficient for the avoidance of pregnancy, the increased abstinence that can result is not desirable.

Presently, distinguishing prospectively between pre-ovulatory E1G peaks and the ultimate ovulatory E1G peak (Fig. 1A and C for example) is an unsolved problem for natural family planning since there were no obvious differences in the E1G values during the follicular phase that could serve to discriminate between the fertile and subfertile cycles. Again, it is the increase, or lack of, in the PdG excretion rate following the E1G peaks that differentiates between them and defines the end of fertility for that cycle. The PdG threshold was a good marker for the end of the fertile window. It was definite and there were no false positives. The PdG thresholds also allowed assessment of the adequacy of the corpora lutea and identified deficient luteal phases simply according to our definitions. For example, the cycle shown in Figure 3B had a maximum PdG excretion rate that was below the 10th percentile for the potentially fertile cycles (Fig. 3A) and below the threshold for biochemical proof of ovulation (9 μmol/24 h). By our definitions (see section Materials and methods), this was therefore a cycle with a luteinized unruptured follicle.

In establishing the criteria for luteal phase deficiency it is necessary to have an accurate reference point for ovulation. In this study, the mid-cycle marker was supposed to be the LH peak. However, the expectation that every cycle would have an unambiguous LH peak day that could be used as the gold standard marker for ovulation was not realized (see Fig. 2) since only 31/113 (27%) cycles met this criterion. Multiple LH
peaks were common (Fig. 2) and the maximum value could occur after the PdG end of fertility threshold day. Thus, it was not possible to determine which LH peak was the pre-ovulatory one independently of the associated E1G peak and PdG rise days which were more reliable and much more informative. Multiple LH peaks have also been noted in other studies (Allende et al. 2002; Park et al., 2007; Direito et al., 2013). Furthermore, some cycles did not show any LH rise at all but all other indicators showed that ovulation had occurred. Hence, we referenced our data to the mid-cycle E1G peak day. It should be noted that some of these LH peaks in urine could be artefacts, as unlike the E1G and PdG assays, for the LH assays there is no correction for variations in urine volume by time dilution (Kesner et al., 1995).

We took the last day of high E1G excretion rate before the marked fall and the following rapidly rising PdG values as the definition of the E1G peak day and the mid-cycle marker for ovulation. This makes sense physiologically since there is evidence from primate studies that once the ovulatory follicle has ruptured there is an abrupt cessation in estrogen production (diZerega and Hodgen, 1981), which explains the characteristic fall in the E1G excretion rate used to define the peak day (Fig. 1). It also seems reasonable that if more than one follicle is of a size to produce estrogens (>4–10 mm) during the peri-ovulatory period (McNatty, 1981), the last of these immediately before the PdG rise should be the ovulatory one. This definition of the E1G peak day needs to be tested by daily ultrasound studies. However, the fact that no dependence on E1G peak type was found for any of the clinical parameters, suggests that it is clinically useful.

In conclusion, daily self determination of urinary E1G and PdG excretion rates gave the women valuable tools in assessing and understanding their personal fertility and defining their fertile window accurately, even in complex menstrual cycle profiles. A set of definitions that may be applied to the analysis of any individual menstrual cycle are given in the section Materials and methods. The picture of the normal variability of the menstrual cycle presented here on the basis of the hormonal data should be further verified by daily concomitant ultrasound measurements. Hormone measurements are a cheap and readily accessible alternative. In particular, the PdG excretion rates were vital in defining the periods of fertility and infertility in the complex menstrual cycle profiles. The E1G and PdG results have obvious relevance for natural family planning methods. Clearly, the availability of accurate and precise urinary tests, such as the Ovarian Monitor, would improve the clinical accessibility of this information.

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Authors’ roles

J.B.B. (deceased) conceived the study, produced the Monitor system, helped to write the original manuscript and analysed the data for the papers that arose from the study. L.F.B. assisted with the original application protocols for the study and was the principle investigator for the Palmerston North Centre. He drafted the article and developed the computer program used in the analysis and approved the final version of the manuscript. P.V. was the principle investigator for the Chilean centre and was involved with the collection, analysis and interpretation of the data. She helped to edit and revise the manuscript and approved the final version. D.C. was involved in the interpretation of the data and made a major contribution to the revision and editing of the manuscript with respect to its intellectual content. She approved the final version. C.A. was involved with the conception and design of the project and its overall management. She read the manuscripts and approved of the final version.

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Conflict of interest

D.G.C. is currently employed by and holds stock in Manawatu Diagnostics Ltd, a company in the development phase of a potentially competing product. The remaining authors have nothing to declare.

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