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## Cervicovaginal fluid changes to detect ovulation accurately

María Elena Alliende, MD, MSc,\* Carlos Cabezón, MD, PhD, Horacio Figueroa, MD, MSc, Cristián Kottmann, MD

*Departamento de Obstetricia y Ginecología, Facultad de Medicina, Universidad de los Andes, Santiago, Chile*

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### KEY WORDS

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**Objective:** The purpose of this study was to evaluate changes in cervicovaginal fluid characteristics to identify ovulation.

**Study design:** Several ovulation indicators were studied in a university-based natural family planning center. Fifteen parous women during 29 ovulatory cycles detected cervicovaginal fluid at the vulva. They self-aspirated their upper vaginal fluid, described it, and kept it for later checking. They also took basal body temperature, collected timed first morning urine samples for estrone and pregnanediol glucuronide enzyme immunoassays, and submitted to serial ovarian transvaginal ultrasound scans.

**Results:** Considering a  $\pm$  1-day period since ultrasound ovulation detection or allowing an extra day ( $-1$  to  $+2$ ), women perceived ovulation from cervicovaginal fluid at the vulva in 76% or 97% of cycles, on the basis of their visual description of vaginally extracted fluid in 76% or 90%, which rose to 90% or 97% for the instructor's description, and in 76% or 86% with a rapid drop in glucuronide ratio. Basal body temperature was less precise (71% or 79%).

**Conclusion:** Evaluation of cervicovaginal fluid changes is an accurate ovulation indicator.

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The detection of ovulation simply and precisely is essential for natural family planning (NFP) methods and is useful in cases of infertility. Most cycles in fertile women show a typical pattern of increasing preovulatory estrogen levels that is associated with a luteinizing hormone (LH) surge and a subsequent progesterone rise. However, we have observed individual differences

in this typical pattern,<sup>1</sup> which sometimes may make accurate ovulation detection difficult. Most NFP methods consider these personal variants.

In different studies of many cycles in healthy volunteers, urine LH, although highly concordant with serum LH, does not always show a clear LH surge, because of multiple or ill-defined surges.<sup>2</sup> We have had the same experience,<sup>1</sup> and a European multicenter study has confirmed this finding with ultrasound studies in a large population.<sup>3</sup> Baird et al<sup>2</sup> have established, in their fertile window studies, that a day of luteal transfer, after a rapid drop in the ratio of estrogen and progesterone urinary metabolites, is a better ovulation indicator than the LH surge. Reliable and accurate measures of estrone

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\* Reprint requests: María Elena Alliende, Department of Obstetrics and Gynecology, Universidad de los Andes, Camino La Posada 13920, Las Condes, código postal 7591434, Santiago, Chile.

E-mail: mealliende@uandes.cl

and pregnanediol glucuronides with simple and cheap methods that consider urinary concentrations currently are available.<sup>4</sup>

Women perceive cervicovaginal fluid (CVF) changes at the vulva. After receiving training, they can determine their fertile period. CVF extraction by self-aspiration from the upper vagina has been reported<sup>5</sup> and introduces another way of observing and checking a woman's CVF description over time.

This study evaluates the reliability of a simple appreciation of periovulatory CVF change at different levels as an ovulation indicator and correlates it with basal body temperature (BBT), hormonal parameters of ovulation, and ultrasound imaging.

## Methods

Our institution approved the study, and informed consent was obtained from 15 NFP users who were willing to participate at the Universidad de los Andes NFP clinic in San Bernardo on the outskirts of Santiago, Chile.

All of the women were multiparous, previously cycling regularly, with an ovulatory pattern according to NFP (clear peak and postpeak period). None of them used barrier contraception or spermicides, and none of them had a history of liver or kidney disease or dysfunction or of any recent pathologic vaginal discharge.

## CVF determination

The women were periodic abstinence NFP users, and they identified their fertile period by perceiving changes in their CVF at the vulva, using a variant<sup>6</sup> of the ovulation method.<sup>7</sup> They observed, as usual throughout an entire day, noting the most fertile sign and symptom on their NFP chart at the end of each day, before carrying out vaginal aspiration.

After this, every night before going to bed, once menstrual spotting had ceased and until the third day after the vulvar CVF peak, they collected a CVF sample from the vagina as described previously.<sup>5</sup> The volunteers gently inserted a graduated syringe-like device (Rovumeter; A&O Pharmavertries, Munich, Germany) with a blunt bulb-headed mouthpiece, into the upper vagina and posterior fornix and slowly withdrew it. They observed the CVF sample and placed it in a tube, which they labeled and kept refrigerated. The women described the extracted fluid on a special study chart, as they did for the CVF that was obtained at the vulva. They also indicated the aspiration time and time of any intercourse. Each week a NFP instructor collected the aspirated CVF samples and did an independent visual inspection, in the same way as the women observed at

home, using the same criteria. In addition, the women measured and recorded BBT during the study period.

## Hormonal determination

Beginning at menstruation, the women daily collected their first morning urine in a graduated jar. They recorded the urine volume, the time of collection, and the time of the last urine voided. After this, they transferred a small urine sample to a vial that they labeled and placed in their freezers at home. All the urine samples were collected weekly and stored at  $-20^{\circ}\text{C}$ , until cycle completion, for analysis. Different urine concentrations were corrected before analysis, by the addition of distilled water to the samples to a constant volume of 150 mL per hour of collection. The same person measured estrone glucuronide (E1G) and pregnanediol glucuronide (PdG) in batches, as described by Blackwell et al,<sup>4</sup> with the Ovarian Monitor (St. Michael NFP Services Pty Ltd, Victoria, Australia), which was developed for home, clinical, and laboratory use. The monitor uses homogeneous enzyme immunoassays and measures changes in light transmission over fixed periods. Results for E1G were expressed in nanomoles per 24 hours and for PdG were expressed in micromoles per 24 hours.

## Ultrasound determination

Serial ultrasonic transvaginal examination was performed during the early and late preovulatory phase, ending with daily pelvic scanning as ovulation approached. Ultrasound scanning was not ended systematically immediately after ovulation was recognized, to better evaluate the postovulation images and also to avoid influencing the women's detection of ovulation. A condom was used to contain gel that was used during the evaluation, so it would not interfere with CVF observations. An ultrasonic scanner (Sienna Doppler color; Siemens Medical Systems Inc USG, Issaquah, Wash) with a 4.2 to 6.5 MHz vaginal probe was used. Ultrasound ovulation follow-up protocol included follicular volume, follicular disappearance or decrease, irregular follicular walls, internal follicular echoes, free fluid in cul-de-sac,<sup>8</sup> endometrial length and type (endometrial interface).<sup>9</sup> The ecographers defined a day of ovulation according to the ultrasonic pattern.

## Definitions

*Vulvar CVF expected day of ovulation (EDO; woman's vulvar CVF-EDO).* The peak day of a woman's vulvar CVF perception was the last day of wet, slippery vulvar sensation, and/or any degree of clear, bloody and/or stretchy ( $\geq 3$  cm) CVF.

*Vaginal CVF-EDO (woman's or instructor's vaginal CVF-EDO).* The peak day of vaginally extracted CVF was the last day of any degree of clear, bloody, and/or stretchy ( $\geq 3$  cm) CVF.

*BBT-EDO.* The day before a sustained rise for 3 consecutive days above the highest of 6 previous BBTs.

*E1G to PdG ratio EDO (E1G/PdG-EDO).* The ratio was determined according to the algorithm of Baird et al<sup>2</sup> that identifies a day of luteal transference after a rapid drop in the E1G/PdG ratio.

*Ultrasound day of ovulation (US-DO).* The day of maximal follicular enlargement followed by evidence of rupture.

The woman's description of vulvar CVF was done previously and did not consider vaginally aspirated CVF, which was described separately. During the study, women were not informed of the NFP instructor's fluid descriptions or ultrasonic or hormonal findings. The NFP instructor knew only the date and intercourse time when inspecting a woman's aspirated fluid. The ecographers were not informed of the CVF, BBT, or hormonal findings.

## Statistics

Sensitivity was calculated for each method compared with the US-DO. Fisher's exact test was used to assess the accuracy of the ovulation diagnosis between the different methods. Data were analyzed with Stata software (version 7.0; Stata Corporation, College Station, Tex).

## Results

Of 33 cycles in 15 women, 3 incomplete cycles and 1 cycle without US-DO were eliminated from the analysis. Of the 29 study cycles, 3 women supplied 3 cycles, 8 women contributed 2 cycles, and 4 women provided 1 cycle. The women were a mean of  $33.2 \pm 4.2$  years old, and the youngest child was  $5.0 \pm 2.9$  years old. Cycles lasted  $27.9 \pm 3.5$  days.

An US-DO was established in 29 cycles (Table). No ultrasonic signs that indicated ovulation could be seen in 1 cycle, except for a change in endometrial characteristics to secretory type.<sup>9</sup> This cycle had an ovulatory pattern in accordance with the other indicators, but the second half of the cycle showed low PdG values, which were  $< 8 \mu\text{mol}/24$  hours.<sup>4</sup>

Each EDO that was based on CVF was detected in every cycle (woman's vulvar, woman's vaginal, and instructor's vaginal CVF-EDO). No CVF-EDO was detected before US-DO  $-1$ ; some CVF-EDOs were detected on day  $+2$  and rarely thereafter.

A BBT-EDO was observed in 28 cycles. One cycle had an incomplete BBT curve but showed a high temperature during the second half of the cycle. Two

of the 4 cycles with BBT-EDO before US-DO-2 belonged to the same woman.

An E1G/PdG-EDO was determined in all the cycles. One cycle had a slow E1G/PdG drop (E1G/PdG-EDO on US-DO  $+5$ ). This cycle, however, did not show a delay for any EDO that was based on CVF.

Sensitivities for the period  $-1$  to  $+1$  days from the US-DO for the woman's vulvar, woman's vaginal, instructor's vaginal CVF-EDO, E1G/PdG-EDO, and BBT-EDO were 75.9%, 75.9%, 89.7%, 75.9%, and 71.4%, respectively. Sensitivities for the extended period  $-1$  to  $+2$  days from US-DO were 96.6%, 89.6%, 96.6%, 86.2%, and 78.6%, respectively (Table).

Woman's vulvar CVF-EDO and instructor's vaginal CVF-EDO were significantly better than BBT-EDO for days  $-1$  to  $+2$  from US-DO. The instructor's vaginal CVF-EDO suggested better than BBT-EDO for days  $-1$  to  $+1$ , but this did not reach statistical significance ( $P = .07$ ).

## Comment

Our results indicate that simple appreciation of peri-ovulatory CVF change is an accurate ovulation indicator, better than BBT and similar to the E1G/PdG ratio.

Differences in evaluation times of the various ovulation indicators that were studied must be considered to interpret their relationship. A woman's detection of vulvar CVF requires observation during the course of a whole day. At the end of the day, the most fertile signs and symptoms that are observed are recorded. The upper vaginal CVF sample was taken at night, after the last observation of vulvar CVF. Ultrasound imaging was performed once a day, in the morning or in the early afternoon, whereas estrogen and progesterone metabolites were measured in the first morning urine samples.

As a fair estimation of the time of ovulation, a span of 1 day ( $\pm 1$  day) can be considered from the US-DO, given the differences in measurement times discussed earlier. The detection of ovulation too early, before US-DO  $-2$ , could lead to a pregnancy; the detection of ovulation on US-DO  $+2$ , or later, would imply a longer period of sexual abstinence.

On this basis, there were no cases of ovulation that were detected too early by any of the CVF methods, before US-DO  $-2$ , in a potentially fertile period. Some authors have also found this with woman's vulvar CVF-EDO<sup>10,11</sup>; other authors have observed cases of early ovulation detection.<sup>3,12,13</sup> On the other hand, there were some cases of CVF-EDO on days  $+2$  and rarely later, which implies a low rate of unnecessary sexual abstinence. Regarding late ovulation detection, other authors have obtained similar results<sup>10</sup> or better<sup>3,11,13</sup> with woman's vulvar CVF-EDO.

**Table** Time lag between day of ovulation by ultrasound scan and estimated by different methods

	Lag (d)*	Postovulation (d)*†	Day from ultrasound scan–determined ovulation‡									
			−4	−3	−2	−1	0	+1	+2	+3	+4	+5
US-DO		13.0 ± 1.5					29 (100%)					
Woman's vulvar CVF-EDO	0.93 ± 0.8	12.3 ± 2.0				4 (14%)	10 (34%)	8 (28%)	6 (21%)	1 (3%)		
Woman's vaginal CVF-EDO	0.89 ± 1.1	12.2 ± 2.2				4 (14%)	13 (45%)	5 (17%)	4 (14%)	1 (3%)	1 (3%)	1 (3%)
Instructor's vaginal CVF-EDO	0.62 ± 0.8	12.9 ± 1.6				8 (28%)	15 (52%)	3 (10%)	2 (7%)	1 (3%)		
BBT-EDO§	1.25 ± 1.2	13.0 ± 2.5	1 (4%)	3 (11%)		4 (14%)	8 (29%)	8 (29%)	2 (7%)	2 (7%)		
E1G/PdG-EDO	1.00 ± 1.1	12.6 ± 2.1			3 (10%)	3 (10%)	10 (35%)	9 (31%)	3 (10%)			1 (3%)

Data are from 29 cycles for each indicator, except for BBT (n = 28).

\* Values are means ± SD.

† Cycle days after each method's EDO.

‡ Values under each day from ultrasound scanning are the number of cycles.

§  $P = .04$  for days −1 to +2 versus instructor's vaginal CVF-EDO and women's vulvar CVF-EDO (Fisher's exact test).

In our study many women (75.9%) detected ovulation by CVF (women's vulvar and vaginal CVF-EDO) very close to US-DO ( $\pm 1$  day), which is similar to the findings of the European study (74.4%) with woman's vulvar CVF-EDO<sup>3</sup> and lower than what others describe.<sup>11,13</sup> We detected ovulation by ultrasound scanning 1 day before to 2 days after (−1 to +2) woman's vulvar CVF-EDO in 96.6% of cycles, whereas Hilgers et al<sup>14</sup> have estimated ovulation from serum progesterone levels in 95.4% cycles within 2 days ( $\pm 2$  days) of woman's vulvar CVF-EDO.

More ovulations (90%) were detected by the NFP instructor's inspection of vaginal CVF that was self-aspirated by women within  $\pm 1$  day from US-DO than by the women's observation of the same aspirated CVF samples (76%). This may show that a woman's CVF observation can improve further with training. If we allowed 1 extra day (−1 to +2 from US-DO), the woman's vulvar CVF-EDO perception and observation would have a similar sensitivity to the instructor's observation of vaginal CVF-EDO (97% for both).

We stress that we consider all EDO that is based on CVF as the last day of any fertile sign and/or symptom, which is according to usual NFP standards for vulvar CVF peak detection. CVF extraction from the upper vagina can be used to check the woman's descriptions during the training period or when the vulvar findings do not show a clear pattern. This technique could be useful for short periods in women without cervicovaginal inflammation. Vaginal aspiration should be performed at the end of the day, not to interfere with a woman's vulvar CVF detection.

In our experience, BBT was less accurate than the other methods to detect ovulation. Although BBT-EDO detected ovulation at 71.4% for days  $\pm 1$  from US-DO, 4 cases were observed with BBT-EDO on days −3 and

−4, in a potentially fertile period. Some women may tend to experience these early BBT rises. Our spread of BBT-EDO around US-DO was lower than what other authors have observed.<sup>3,15</sup> The reliability of BBT measurements requires strict compliance with instructions; nevertheless, occasionally there is only a slight BBT rise, which increases the frequency of monophasic and uninterpretable charts. This frequency is much lower in studies with highly motivated NFP subjects. All these factors support BBT use only when there are other fertile period indicators.

The E1G/PdG ratio indicated ovulation within  $\pm 1$  day from US-DO in 76% of cycles, comparable to 73% in the European study.<sup>3</sup> This is similar to the women's vulvar and vaginal CVF-EDO results. In our study, no cases of E1G/PdG-EDO occurred before US-DO −2, although the European study found some cases before this.<sup>3</sup> The E1G/PdG ratio only detects the peak that is related to ovulation when it is too late, which is problematic for use in NFP. Some cycles present other ratio peak(s) before the ovulatory peak, which complicates making a distinction. Other authors who have used the E1G/PdG ratio to establish ovulation have described this as well.<sup>16,17</sup> In these cases, absolute levels of E1G and PdG may be preferable.<sup>4</sup> All these aspects make the clinical determination of the fertile period end difficult with E1G/PdG ratio. Although E1G levels and E1G/PdG ratios vary in women and among different populations,<sup>17</sup> a PdG threshold could be useful.<sup>4</sup>

Dunson et al in a statistical analysis of error in ovulation estimation, conclude that the probability that E1G/PdG ratio correctly identified the day of ovulation was .65.<sup>18</sup> They consider that BBT is more error prone than the E1G/PdG ratio.<sup>19</sup> Our results seem to point in the same direction but were not statistically significant. In addition, they note that BBT as an ovulation

indicator is substantially different from the mucus peak (woman's vulvar CVF-EDO), which suggests that at least 1 of the markers must be error prone.<sup>18</sup> We have found that BBT is less accurate.

This study shows that simple appreciation of peri-ovulatory CVF change is an accurate ovulation indicator. Hormonal ovulation detection that measures urine LH<sup>2</sup> and E1G/PdG ratio does not seem to be better. All these findings can have clinical application in family planning and infertility.

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