Stratification of fertility potential according to cervical mucus symptoms: achieving pregnancy in fertile and infertile couples

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Stratification of fertility potential according to cervical mucus symptoms:
achieving pregnancy in fertile and infertile couples

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ABSTRACT
Women wishing to conceive are largely unaware of fertility symptoms at the time of ovulation. This study investigated the effectiveness of fertility-awareness in achieving pregnancy, particularly fertile mucus pattern, in the context of infertility. The 384 eligible participants were taken from consecutive women desiring pregnancy who attended 17 Australian Billings Ovulation Method® clinics from 1999–2003. This cohort included couples with infertility ≥12 months (51%) and female age >35 years (28%). Under fertility-awareness instruction, pregnancy was achieved by 240 couples (62.5%) after maximum follow-up of two years. Mucus symptom observations effectively stratified ‘low pregnancy-potential’ (35.2%) and ‘high pregnancy-potential’ groups. Pregnancy rates were ~30% higher in the latter group (44.4% versus 72.3%) in addition to consistent effects observed on pregnancy achievements within subgroups defined by prognostic factors such as duration of infertility (p = 0.001) and increasing female age (p = 0.04). Fertile symptoms were also associated with significantly shorter time to conception (4.2 versus 6.4 months) in a survival analysis (p = 0.003). Billings Ovulation Method® observations strongly predicted successful conception. This has the capacity to provide a rapid, reliable and cost-effective approach to stratifying fertility potential, including directing timely and targeted investigations/management, and is accessible for women who may be remote from primary or specialist care.

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KEYWORDS
Infertility; menstrual cycle; reproduction

Introduction
Infertility is defined as failure to conceive after one year of regular uncontracepted sexual intercourse, thus allowing for timely evaluation of potentially treatable causes of infertility (National Institute for Health and Care Excellence, 2013; Practice Committee of the American Society for Reproductive Medicine, 2013). Clinical features independently associated with low probability of natural conception include increasing duration of infertility beyond one year and increasing female age above 35 years (Gnoth et al., 2005; van der Steeg et al., 2007).

The Billings Ovulation Method® is a clinical, validated fertility awareness method (FAM) that teaches couples to identify patterns of fertility and infertility by an awareness of physiological symptoms. This empowers couples to time intercourse with a view to either avoiding or achieving pregnancy. It does not rely on menstrual cycle timing, instead determination of day-specific fertility/infertility is made based on daily vulval sensation and cervical mucus observations. The rising oestrogen levels of the fertile phase correlate with activation of different cervical crypts prior to ovulation, producing a changing, developing pattern of vulval sensation and discharge leading to a vulval slippery sensation. Disappearance of the slippery sensation reflects the beginning of the progesterone rise and allows identification of the ‘Peak’ symptom and of ovulation. Detailed physiological and biochemical aspects of such fertility-awareness, including clinical application, have been described elsewhere (Brown, 2011; Vigil, Blackwell, & Cortes, 2012).

Numerous studies have demonstrated increased probability of conception from intercourse close to the time of ovulation during the ‘fertile window’ (Evans-Hoeker et al., 2013; Mu & Fehring, 2014; Wilcox, Weinberg, & Baird, 1995). The standard fertile window is 6 days up to and including ovulation (Ecochard, Duterque, Leiva, Bouchard, & Vigil, 2015). This window can be reliably identified by the presence of oestrogenic-type cervical mucus (Odeblad, 1997) even when menstrual cycles are irregular (Billings, 1991). Although
the physiological timing of ovulation can be delayed by up to 1–2 days after the Peak mucus symptom, from a clinical perspective the day-specific probability of conception is highest on Peak day (Scarpa, Dunson, & Colombo, 2006; World Health Organization, 1983). Thus, observation of vulval sensations combined with the pattern and quality of mucus provides a useful strategy for optimising timing of intercourse to achieve pregnancy. In spite of this, women wishing to conceive are largely unaware of fertility symptoms, while more than 90% who have difficulty conceiving believe women should receive fertility-awareness education when they first report such difficulty (Hampton & Mazza, 2015). The concerning issue is that General Practitioners receive little training and have low confidence and inadequate knowledge to advise on fertility-awareness matters (Hampton & Newton, 2016). The Billings Ovulation Method® has specific guidelines in order to achieve pregnancy (Billings & Westmore, 2011) and instruction is available in person or by tele-health, using either paper-based or online charting. This FAM is thus available to women in primary care in metropolitan or rural/remote areas, either directly by the clinician or as an adjunct to family planning consultations.

The objectives of the present study were to: (i) identify factors associated with achievement of pregnancy based on fertility-awareness charting in the context of infertility; and (ii) demonstrate utility of the Billings Ovulation Method® for achieving pregnancy.

Materials and methods

Study design and participants

Records were collected from all consecutive women desiring to achieve pregnancy who attended 17 Billings Ovulation Method® clinics situated in all Australian states and the Northern Territory from January 1999 to December 2003. Each participant was followed up until clinical pregnancy was achieved or for a maximum of 24 months. Institutional Human Research Ethics Approval was granted by the Behavioural and Social Sciences Ethical Review Committee of The University of Queensland (approval number 2015001735).

Data collection

Data taken from case history records and Billings Ovulation Method® charts were de-identified at each local clinic and submitted centrally to the Ovulation Method Research and Reference Centre of Australia (OMR&RCA) where all de-identified data were stored. Information gathered included clinical and reproductive history as well as demographic data. Data collated from charts included: (i) mucus patterns; (ii) Peak symptom; (iii) bleeding; (iv) luteal phase length; and (v) timing of intercourse.

A study questionnaire, identified only by code number and dates of birth, was posted to each of the 449 qualifying participants by the local Billings Ovulation Method® teacher. Responses were returned directly by participants to OMR&RCA. Responses were blinded as to whether or not pregnancy was achieved and then classified according to fertility symptoms.

Instructions regarding peak fertility-awareness

Participants kept a daily record of vulval sensation and any visible discharge observed in the normal course of daily activity, following established Billings Ovulation Method® criteria (Billings & Westmore, 2011), as well as acts of intercourse. Instructions were given that a changing, developing pattern indicated hormonal activity and potential fertility, while the sensation of vulval wetness or slipperiness occurring after this indicated the Peak symptom and the day corresponding to optimum fertility.

Statistical analysis

Participants were classified into groups according to duration of attempting conception: (i) normal fertility (<12 months); (ii) infertility (12–24 months); and (iii) prolonged infertility (>24 months). Distributions of continuous variables are presented as mean and standard deviations (SD). Group comparisons utilized one-way analysis of variance (ANOVA) with Bonferroni correction for multiple comparisons as appropriate. Univariate and multivariate logistic regression analyses were also undertaken utilizing demographic, clinical and fertility chart-derived variables as predictors of pregnancy, with results presented as β-coefficients (β) with standard error (SE) estimates. Time-dependent data (time to conception) were assessed using Kaplan–Meier survival analysis, utilising log rank (Mantel-Cox) tests for equality of survival distributions. Statistical analyses were performed using SPSS version 15.0. Unless stated otherwise, all reported results were derived from an intention-to-treat approach in which unknown pregnancy achievements were assumed to be negative, thus providing a conservative estimate of factors associated with achievement of pregnancy in this cohort.
Table 1. Clinical risk factors, fertility symptoms, and pregnancy rates.

<table>
<thead>
<tr>
<th>Study groups</th>
<th>Normal (≤12 months)</th>
<th>Infertile (12–24 months)</th>
<th>Prolonged Infertile (&gt;24 months)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (% of group)</td>
<td>Pregnancy</td>
<td>n (% of group)</td>
<td>Pregnancy</td>
</tr>
<tr>
<td>Number</td>
<td>188</td>
<td>156</td>
<td>40</td>
<td>33.1 (4.5)</td>
</tr>
<tr>
<td>Female age, years (mean, SD)</td>
<td>32.8 (4.3)</td>
<td>33.1 (4.6)</td>
<td>34.9 (4.0)</td>
<td>30.3 (4.5)</td>
</tr>
<tr>
<td>Months attempting (mean, SD)</td>
<td>5.2 (3.4)</td>
<td>18.0 (6.4)</td>
<td>46.6 (16.5)</td>
<td>14.0 (6.5)</td>
</tr>
<tr>
<td>Pregnancy documented (n, %)</td>
<td>137 (72.9%)</td>
<td>82 (52.6%)</td>
<td>21 (52.5%)</td>
<td>240 (62.5%)</td>
</tr>
<tr>
<td>No pregnancy (n, %)</td>
<td>22 (11.7%)</td>
<td>46 (29.6%)</td>
<td>12 (30.0%)</td>
<td>80 (20.8%)</td>
</tr>
<tr>
<td>Unknown (n, %)</td>
<td>29 (15.4%)</td>
<td>28 (17.9%)</td>
<td>7 (17.5%)</td>
<td>64 (16.7%)</td>
</tr>
</tbody>
</table>

**Fertility chart results**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>% (%)</th>
<th>% (%)</th>
<th>% (%)</th>
<th>% (%)</th>
<th>% (%)</th>
<th>% (%)</th>
<th>% (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent symptom</td>
<td>14 (7.4%)</td>
<td>13 (92.9%)</td>
<td>16 (103%)</td>
<td>12 (75.0%)</td>
<td>3 (75.0%)</td>
<td>14 (7.4%)</td>
<td>13 (92.9%)</td>
<td>16 (103%)</td>
</tr>
<tr>
<td>Inadequate/deranged</td>
<td>44 (23.4%)</td>
<td>25 (56.8%)</td>
<td>55 (35.3%)</td>
<td>24 (43.6%)</td>
<td>16 (40.0%)</td>
<td>7 (43.8%)</td>
<td>11 (65.0%)</td>
<td>5 (32.5%)</td>
</tr>
<tr>
<td>Short symptom</td>
<td>21 (11.2%)</td>
<td>16 (76.2%)</td>
<td>7 (34.3%)</td>
<td>6 (52.6%)</td>
<td>20 (50.0%)</td>
<td>12 (75.0%)</td>
<td>10 (60.0%)</td>
<td>6 (30.0%)</td>
</tr>
<tr>
<td>Obvious peak</td>
<td>101 (53.7%)</td>
<td>82 (81.2%)</td>
<td>67 (69.1%)</td>
<td>40 (52.6%)</td>
<td>20 (50.0%)</td>
<td>13 (65.0%)</td>
<td>40 (52.6%)</td>
<td>13 (65.0%)</td>
</tr>
<tr>
<td>&lt;30 years</td>
<td>43 (22.9%)</td>
<td>35 (81.4%)</td>
<td>37 (23.7%)</td>
<td>27 (73.0%)</td>
<td>3 (75.0%)</td>
<td>2 (66.7%)</td>
<td>43 (22.9%)</td>
<td>35 (81.4%)</td>
</tr>
<tr>
<td>30–35 years</td>
<td>97 (51.6%)</td>
<td>67 (69.1%)</td>
<td>76 (48.7%)</td>
<td>40 (52.6%)</td>
<td>20 (50.0%)</td>
<td>13 (65.0%)</td>
<td>97 (51.6%)</td>
<td>67 (69.1%)</td>
</tr>
<tr>
<td>&gt;35 years</td>
<td>48 (25.5%)</td>
<td>35 (72.9%)</td>
<td>43 (27.6%)</td>
<td>43 (34.9%)</td>
<td>17 (42.5%)</td>
<td>6 (35.3%)</td>
<td>48 (25.5%)</td>
<td>35 (72.9%)</td>
</tr>
</tbody>
</table>

**Results**

There were 521 consecutive women enrolled in the study initially, with 72 excluded due to: non-return after initial instruction, already being pregnant, and couples trying to avoid pregnancy. The remaining 449 couples were surveyed, with complete data from the Billings Ovulation Method® charts available for 384 (85.5%). From this group, 62.5% achieved pregnancy while 20.8% did not (Table 1).

Regarding negative prognostic factors, the prevalence of unintended infertility of >12 months was 51.0% (n=196) and female age >35 years in 28.1% (n=108). A specific history of female and/or male infertility factors was reported and included without formal verification by 58 couples (15.1%). These included polycystic ovary syndrome (PCOS), unilateral Fallopian tube patency, previous chlamydia infection, known endometriosis, and sperm abnormality. There was a higher prevalence found in the prolonged infertility group (p=0.03).

**Symptoms of peak fertility**

Among those with mucus symptoms suggestive of peak fertility (n=249, including 34 cases with unknown results for this variable), achievement of pregnancy (72.3%) was independently associated with duration of infertility (β =−0.85, SE 0.26, p =0.001) and age group (β =−0.57, SE 0.27, p =0.04) in multivariate analyses. Adequate symptoms of fertility according to Billings Ovulation Method® criteria (including observations of a ‘short’ ovulatory phase) were consistently associated with an approximately 30% increase in pregnancy rates in the study (Table 2). This effect was broadly observed and evident within risk groups defined by female age and duration of infertility.

**Symptoms of reduced fertility**

Specific mucus symptoms indicating reduced or absent fertility (Brown, 2011) according to Billings Ovulation Method® criteria (inadequate/deranged symptoms (115) or absent fertility symptoms (20)) were identified in 135 women (35.2%) (Table 1). Although abnormal fertility symptoms were noted in 27.7% of women in the normal fertility group, they were more common among those with infertility (p=0.009).

Women with reduced fertility symptoms had lower rates of pregnancy (44.4%) compared with couples who had normal fertility symptoms (72.3%), a difference that was highly statistically significant (p<0.001). These differences remained statistically significant between the normal fertility and infertile subgroups (p<0.01).

**Determinants of pregnancy rates in multivariate regression and survival analyses**

Pregnancy rates within study subgroups classified according to fertility symptoms are presented in Table 2. Data presented excludes couples with self-reported infertility risk factors to demonstrate stratification based on fertility symptoms alone, although all cases were considered in the analyses. In univariate regression analyses, the factors most highly correlated with achievement of pregnancy were symptoms of peak fertility (β =1.18, SE 0.22, p <0.001), duration of infertility as defined by study groups (β =−0.62, SE
Evidence of timing intercourse to coincide with peak fertility symptoms was also found to be statistically significant ($\beta = 0.54$, SE 0.24, $p = 0.03$). These covariates remained independently significant and retained very similar effect size when incorporated into a multivariate regression model: fertility symptoms ($p < 0.001$), age group ($p < 0.001$), duration of infertility ($p = 0.003$), and timed intercourse to peak fertility ($p = 0.03$).

### Time to conception analysis

In a Kaplan–Meier survival analyses of known conceived pregnancies ($n = 240$), mucus symptoms of peak fertility were associated with a significantly shorter mean time to conception compared to those with absent or disordered mucus symptoms (4.2 months [95% CI 3.5–4.9], versus 6.4 months [95% CI 5.0–7.8], $p = 0.003$) (Figure 1). Adjusting for this effect revealed no independent effect of age group ($p = 0.35$) or duration of infertility ($p = 0.54$) on time to conception in the study. The majority (185/240, 77.1%) of pregnancies that were achieved did so within the first six months, while 92.5% (222/240) of pregnancies were achieved within the first 12 months.

### Pregnancy-potential stratification based on peak fertility symptoms

From our findings, a diagnostic approach could be to stratify ‘pregnancy-potential’ based on initial assessment of the mucus symptoms of peak fertility. Taking this approach with the study population, this would classify 135 couples (35.2%) into the ‘low pregnancy-potential’ group with evidence of reduced fertility. Pregnancy was achieved by 60 of these couples (44.4%), indicating a recovery of fertility (Brown, 2011).

Logistic regression analysis within this group revealed a strong association between achievement of pregnancy and younger age group ($\beta = 0.73$, SE 0.27, $p = 0.007$), although no additional significant contribution was associated with duration of infertility ($p = 0.45$).

For the 219 couples (57%) with favourable mucus symptoms and without self-reported infertility factors, pregnancy rates were generally greater than 60% across strata defined by age or duration of fertility (Table 2), and >70% when including only known pregnancy achievements (data not shown).

### Table 2. Combined fertility symptoms and pregnancy rates.

<table>
<thead>
<tr>
<th></th>
<th>Normal (&lt;12 months)</th>
<th>Infertile (12–24 months)</th>
<th>Prolonged Infertile (&gt;24 months)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$ (% of group)</td>
<td>Pregnancy ($n$ (% of group))</td>
<td>Pregnancy ($n$ (% of group))</td>
<td>Pregnancy ($n$ (% of group))</td>
</tr>
<tr>
<td><strong>Favourable symptoms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age &lt;30 years</td>
<td>27 (26.9%)</td>
<td>26 (96.3%)</td>
<td>16 (14 (87.5%))</td>
<td>43 (40.0%)</td>
</tr>
<tr>
<td>Age 30–35 years</td>
<td>63 (51.0%)</td>
<td>51 (81.0%)</td>
<td>24 (58.5%)</td>
<td>82 (71.9%)</td>
</tr>
<tr>
<td>Age &gt;35 years</td>
<td>34 (28.4%)</td>
<td>13 (61.9%)</td>
<td>13 (61.9%)</td>
<td>62 (51.0%)</td>
</tr>
<tr>
<td>Overall</td>
<td>124 (65.4%)</td>
<td>105 (84.7%)</td>
<td>78 (50.0%)</td>
<td>219 (57.0%)</td>
</tr>
<tr>
<td><strong>Unfavourable symptoms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age &lt;30 years</td>
<td>13 (8 (61.5%))</td>
<td>16 (10 (62.5%))</td>
<td>2 (1)</td>
<td>31 (19 (61.3%))</td>
</tr>
<tr>
<td>Age 30–35 years</td>
<td>22 (9 (40.9%))</td>
<td>21 (8 (38.1%))</td>
<td>4 (3)</td>
<td>47 (20 (42.6%))</td>
</tr>
<tr>
<td>Age &gt;35 years</td>
<td>8 (5 (62.5%))</td>
<td>1 (1)</td>
<td>7 (2)</td>
<td>8 (27.6%)</td>
</tr>
<tr>
<td>Overall</td>
<td>43 (22.9%)</td>
<td>22 (51.2%)</td>
<td>19 (32.7%)</td>
<td>107 (27.9%)</td>
</tr>
</tbody>
</table>

*Bold for emphasis represent broad/total figures from the data.

*Data for couples with known infertility risk factors not presented.*

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**Figure 1.** Kaplan–Meier survival analysis of time to conception among subjects achieving pregnancy ($n = 240$). Adjusting for the significant effect of the presence or absence of favourable mucus symptoms ($p = 0.003$), no independent effect of fertility group or female age group was identified (both $p > 0.2$).
Discussion

The major finding from these data is that identification of fertile and infertile mucus pattern variants is a powerful predictor of successful conception among the known prognostic categories of increasing age and duration of infertility. Knowledge of peak fertility symptoms also permitted effective stratification of pregnancy-potential in this study population, based on evidence of a highly statistically significant effect on pregnancy rates as well as time to conception.

The diagnostic potential of this approach is best illustrated by the performance of mucus symptom observations as a predictor of female fertility compared with known clinical prognostic factors. Oestrogenic-type mucus is critical to creation of a reproductive environment supportive of sperm survival and transport to the ovum (Dunson, Bigelow, & Colombo, 2005; Menárguez, Pastor, & Odeblad, 2003) and is clinically predictive of ovulation and successful conception (Bigelow et al., 2004; Ecochard et al., 2015). However, even when ovulation can be reliably detected, the timing of intercourse to this event will not guarantee conception if appropriate mucus is absent or the ovulatory mechanism does not operate satisfactorily, causing an infertile ovulation indicated by the short/deficient luteal phase. Adequate production of oestrogenic-type mucus followed by an obvious Peak symptom and a good length luteal phase shows that the woman is then currently in the fertile phase of the ‘continuum’ (Brown, 2011). A determination of fertility potential is therefore available from the woman’s chart.

The main limitation of this study was the incomplete data collected, including unknown pregnancy outcomes (16.7% of the evaluable cohort), and unknown mucus symptom observations (8.9%), although it is notable that unknown values were found to be proportionately distributed across study groups. Nevertheless, the intention-to-treat approach for data analysis provided conservative estimates of the study endpoints and effects of covariates. The data on self-reported infertility risk factors was not validated and may have influenced results to a greater or lesser degree depending on clinical severity/effect. Another source of bias may be the unknown contribution of the self-selection or referral of participants to the Billings Ovulation Method® clinics. While there was no formal control group in the current study, this cohort represented sequential real-world patients desiring pregnancy in the primary care setting. More than half the women in the study had experienced clinical infertility prior to using the Billings Ovulation Method® which, informally, may be considered a control situation for themselves.

Up to one in six couples is affected by infertility, a rate which is increasing over time (Inhorn & Patrizio, 2015). The use of Assisted Reproductive Technology (ART) is also increasing, with data from 2014 revealing that 1 in 25 children born in Australia, including 1 in 12 to women over 35 years, were conceived with ART (Chambers et al., 2017). Significant issues, however, include treatment-associated morbidity and substantial cost of ART, while the benefits for unexplained infertility are also variable (Bhattacharya et al., 2008). The strategy utilized in the current study incurs minimal cost, provides valuable information regarding potential fertility in those desiring pregnancy as well as those with known infertility and is accessible for women who may be remote from primary or specialist medical care.

Based on these results and without apparent impediments to fertility, when a couple seeks advice on achieving pregnancy they can be offered a targeted approach by applying mucus recognition as a sign of potential fertility. Such an individualized approach is more sound and scientifically rigorous than using an arbitrary or calculated time-interval-based method. The timing and nature of early investigations can be initiated according to whether or not the mucus is favourable, with further refinement according to age of the woman and duration of uncontracepted intercourse.

A woman under 30 years old with favourable mucus can be reassured that spontaneous pregnancy is quite likely (approximately 90%) within the next 6–9 months. If pregnancy does not occur within that time, further investigation is reasonable. Similarly, where the woman is 30 years or older and has favourable mucus, an initially conservative approach is appropriate provided the period of infertility is not prolonged. On the other hand, where the mucus is unfavourable, the occurrence of spontaneous pregnancy is significantly reduced, especially where the woman is older and/or has been infertile for longer. Consequently, it would be appropriate to commence investigations relatively early in these couples. This approach is similar to that described by others (de Sutter, 2006; Gnoth et al., 2005), is logical and easily explained to the couple, and may potentially avoid unnecessary investigations which could otherwise increase anxiety without materially contributing to resolution of the problem.

In conclusion, these findings indicate that the slippery sensation defining the Peak symptom is the critical indicator of favourability for achieving pregnancy.
with timed intercourse. A comprehensive approach to pregnancy achievement should thus incorporate an assessment of mucus symptoms in addition to a standard initial clinical review.

Acknowledgements

This paper is dedicated to the memory of Dr John Billings (2007) AM KCSG MD FRCP FRACP, Dr Evelyn Billings (2013) AM DCGS MBBS DCH, and Emeritus Professor James B Brown (2009) AM MSc PhD Dsc FRACOG. Dr Evelyn Billings and Prof James Brown contributed significantly to the design and evaluation of the study prior to submission for publication. The authors would like to thank all participants in the study, Dr David Nolan and Dr Monica Nolan for assistance with preparation of the manuscript, and Timothy Nolan and Katie Olivier for assistance with database design and preparation. The Melbourne Research Study Team of Gillian Barker, Kerry Bourke and Joan Clements are also gratefully acknowledged together with the Billings Ovulation Method© teachers from the 17 Australian Clinics who assisted in the gathering of data.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References


