The present study aims at determining the scientific reliability of salivary ferning pattern as a method to detect ovulation in normal women. The saliva was collected from unmarried women in the age group of 20-30 years. The saliva, collected at three different phases (i.e., preovulatory (6\(^{th}\)-12\(^{th}\) day), ovulatory (13\(^{th}\)-14\(^{th}\) day) and postovulatory (15\(^{th}\)-28\(^{th}\) day)), was smeared in the glass slide and observed under microscope. The salivary fern pattern was compared with the different phases of the menstrual cycle. In the ovulation phase, estrogen crystals which formed clearly were observed in saliva, whereas in other phases few crystalline structures were visible. The results suggest that the appearance of fern formation may be due to the appearance of surge of estrogenic activity. This is confirmed by the estimation of electrolytes namely sodium, potassium, and subsequently estrogen. The present study emphasizes, that the salivary fern pattern can be used for ovulation prediction, and the test may, therefore, be clinically useful in the prediction of ovulation after suitable modification.

**Keywords:** Saliva, Fern pattern, Ovulation, Woman.

**Introduction**

Identification of the period of ovulation in women is critical in the treatment of infertility. Success in *in vitro* fertilization and embryo transfer has been associated with the exact time of ovulation. A variety of techniques have been used to assess the time of ovulation, including temperature charts, cervical mucous scoring, and measurement of plasma and urinary hormonal profiles (Kerin, 1982). Several methods such as hormone tests, ultrasound, and basal body temperature, individually or in combination, have been adopted to predict the fertile period in women (Moghissi, 1976; and Martinez, 1997). These methods one way or the other have several disadvantages, and the results are also inconsistent. Further, identification of this fertile period using nonclinical or traditional methods appears to be
inaccurate, especially those who have irregular cycles. Therefore, a simple reliable method is needed to predict and detect ovulation in women.

In recent years, attention has been paid to the biochemical importance of saliva. Hormones which are measured in blood have now been estimated in saliva, though the quantities are comparatively less (Braat et al., 1998). Hence, saliva is considered as the best non-invasive source for chemical and biochemical study (Freundl et al., 1996). Reports showed that saliva was a very good source of both hormones and enzymes and their levels changed in accordance with the menstrual cycle (Flynn and Lynch, 1976). Appearance of "Fern Pattern" was established in bovine vaginal mucus to predict the time of ovulation (Etchepareborda et al., 1983; Affandi et al., 1985; Wagrowska-Danilewicz and Danilewicz, 1986; and Rajanarayanan, 2004), and the technique was then extended in human vaginal mucus (Moghissi, 1986). Eventhough studies concerning salivary ferning have been conducted comparing with other fertility markers, the results were not convincing (Guida et al., 1993; Fehring and Gaska, 1996). Hence, evidence for the occurrence of salivary ferning viewed under a microscope during the midcycle is presented which may be considered as a reliable parameter to predict ovulation. Further, mineral constituents such as sodium, potassium, and Sodium-Potassium ratio (Na/K) of the saliva produced in different phases of menstrual cycle assessed were assessed.

Materials and Methods
A total of 48 women volunteers between 20 and 30 years was considered for the present study. The volunteers were from women's hostel, Bharathidasan University as well as town and rural areas in and around Tiruchirappalli, Tamilnadu, India. The saliva sample was categorized into three phases, viz., preovulatory, ovulatory, and postovulatory phases. The volunteers were carefully cautioned not to use any contraceptives pills or natural medicines during the course of study. The samples were then collected in a sterile vial during morning hours and brought to the laboratory for microscopic examination. At least two to three cycles were observed to confirm the length of the period from the volunteers prior to sample collection. The women who exhibited normal 28 days cycle were taken as volunteers and the saliva sample was collected. The fern pattern was analyzed using glass slide, by adding one drop of saliva, which was smeared by other slide; the fern like crystals were formed in each stage of menstrual cycle.

Biochemical Analysis
1 ml aliquots of saliva were centrifuged for 10 min at 4000 g at 4 °C. The clear supernatant was concentrated and used for determination of estrogen (Worthman et al., 1990) sodium and potassium in normal menstrual cycle (Dawes, 1969).
Results

The result of the fern pattern in saliva is summarized in Figure 1. The examination of fern pattern in preovulatory phase exhibited few crystalline structures (Figure 1A). By contrast, the exploration of fern pattern studies in ovulatory phase discerned with clearly formed estrogen crystals (Figure 1B). The postovulatory fern pattern was found to show pebbly structures of indefinable form (Figure 1C). Interestingly, the saliva of ovulatory phase had accommodated with maximum number of clearly formed estrogen crystals (72.91%) when compared to other phases. By contrast, no fern like structure was formed in the preovulatory and postovulatory phases. Salivary estrogen, electrolytes like sodium and potassium and Na/K+ ratio levels during various phases of menstrual cycle are summarized in Table 1 and represented graphically in Figures 2A, 2B, 2C, and 2D. Salivary sodium concentration was highest at the time of ovulation and lowest at postovulatory phase (Table 1, Figure 2A). The difference between these two values was significant. Salivary electrolytes showed little variation throughout the menstrual cycle, except that the potassium concentration was increased significantly at ovulation when compared to other reproductive periods (Figure 2B). The Sodium-Potassium ratio (Na/K) in the saliva was computed for the three periods (Figure 2C) and comparison of Na/K ratio during preovulatory, postovulatory, ovulatory phases indicated a significant decrease in the ratio at ovulation.
(2.07±2.89; \( P < 0.01 \)) as compared to that of preovulatory (2.79±4.65), and postovulatory (2.99±2.65) phases. The level of estrogen showed maximum activity during the ovulatory period in contrast to other periods (Figure 2D).

Table 1: Quantification of Salivary Electrolytes and Estrogen During the Menstrual Cycle of Women

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Preovulatory Phase (6\textsuperscript{th}-12\textsuperscript{th} Day)</th>
<th>Ovulatory Phase (13\textsuperscript{th}-14\textsuperscript{th} Day)</th>
<th>Postovulatory Phase (15\textsuperscript{th}-28\textsuperscript{th} Day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (Na\textsuperscript{+}) (meq/L)</td>
<td>146.83 ± 21.52</td>
<td>186.78 ± 25.77*</td>
<td>127.44 ± 20.73</td>
</tr>
<tr>
<td>Potassium (K\textsuperscript{+}) (meq/L)</td>
<td>52.61 ± 4.62</td>
<td>89.81 ± 8.91*</td>
<td>42.58 ± 7.82</td>
</tr>
<tr>
<td>Na/K ratio</td>
<td>2.79 ± 4.65</td>
<td>2.07 ± 2.89*</td>
<td>2.99 ± 2.65</td>
</tr>
<tr>
<td>Estrogen (pg/ml)</td>
<td>75.76 ± 7.27</td>
<td>138.28 ± 17.58*</td>
<td>58.15 ± 7.06</td>
</tr>
</tbody>
</table>

Note: The values are Mean±SE of four observations; Values are expressed in one way ANOVA shows statistically significant (Na\textsuperscript{+}, \( F = 2.126 \); K\textsuperscript{+}, \( F = 12.206 \); Na/K ratio, \( F = 0.174 \); Estrogen, \( F = 16.910, P < 0.001 \)) in ovulatory periods while compare to other reproductive stages.

Figure 2A: Salivary Sodium Concentration
Figure 2B: Salivary Potassium Concentration

Figure 2C: Salivary Sodium-Potassium Ratio
Discussion

The present study reveals that the nature of saliva considerably varied depending upon the reproductive status of women. It is noteworthy that salivary fern pattern is very prominent in ovulatory phase as compared to preovulatory and postovulatory phases (Figure 2A and 2C). Unlike the appearance of ferning in human cervical mucus (Moghissi, 1980) and bovine vaginal mucus (Ramesh Kumar, 2000; Rutllant et al., 2002), we did not find the appearance of ferning immediately while observing the saliva under microscope. A slight warm up about 8-10 minutes provided good ferning appearance in the present study. It indicates formation of crystallization in human saliva probably takes little more time. These fern structures are accurately dried salt or electrolyte crystals present as a result of the electrolyte build up prior to and during ovulation. Moreover, the ferning is caused by equal proportion of sodium and potassium ions, which cyclically increases under the influence of estrogen (Fernando et al., 1988). The ferning pattern of saliva is also helpful to detect the female ovulation period. The ferning is caused by NaCl, which cyclically increase under the influence of estrogen (Cockle and Harkness, 1978). These findings were similar to the result of Barbato et al. (1993) in which a strong relationship was found among salivary ferning patterns in menstrual cycle.

It is noteworthy that there was also a strong correlation between the occurrence of LH in the urine and appearance of vaginal mucus ferning and salivary ferning (Fehring and Gaska, 1998). It shows the interrelationship of hormone production and formation of ferning in saliva. It is important to note that collecting the cervical vaginal mucus sample from women
is not always possible. Identification of fertility markers for women is under active investigation. Developing a technique with salivary sample has several advantages because it is an easily accessible body fluid. Since appearance of salivary ferning provides promising results as observed in the present study, there is a possibility to develop potential markers for prediction of ovulation in women.

During the past several years there has been interest in the study of relationship between the minerals and ovarian hormonal activity. The coordinated sequence of hormonal changes during the normal menstrual cycle is well characterized, whether similar or parallel changes occur in preovulatory phases. Several studies have provided evidence for phase related changes in blood constituents during menstrual cycle (Frank and Carr, 1957; Pitkin et al., 1978; and Dadlani et al., 1982). The present study revealed distinct changes in salivary estrogen, sodium and potassium level during preovulatory, ovulatory, and postovulatory phases. Southam and Gonzaga (1965) reported that the results were analogues to the studies that reported serum calcium was in the highest concentration during ovulatory phase and in the lowest concentration during premenstrual phase. It is further possible that hormone induced changes in phosphorus may be compensated for active secretion of phosphorus into saliva. This hypothesis has been advanced (Ben-Aryeh et al., 1976) and seems tenable since phosphorus, in its association with intracellular messenger c-AMP, is involved in regulatory aspects of the menstrual cycle in several ways. As the level of sodium and potassium increases during ovulation, the level of salivary glucose (Davis and Balin, 1973) and phosphate (Ben-Aryeh et al., 1976) contents also increases. Further, Boyer and France (1976) reported that there is an increase in salivary alkaline phosphatase at the time of ovulation, whereas Cockle and Harkness (1978) did not find any changes in alkaline phosphatase at ovulatory phase. The contradictory observations are probably due to the variations in the method of collection and treatment procedures of saliva samples (Tandra and Bhattacharaya, 1989). Sodium, however, appeared to decrease progressively four days before ovulation and then increased.

**Conclusion**

The changes in ions and appearance of fern pattern in ovulatory phase may form the basis of estrogen circulation. Since the data obtained in this study indicates that salivary sodium and potassium remains within physiological limits, it is possible that the salivary gland or perhaps some other regulator may perform the function of maintaining normal salivary Na\(^+\), K\(^+\) concentrations across the menstrual cycle. In conclusion, although the cyclic changes in salivary sodium and potassium levels during the menstrual cycle, all physiological and biochemical changes occur only under the influence of ovarian hormones. Further testing of saliva with modified techniques would unravel the possibility to make a marker for detection of ovulation in women.

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References


