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นิพนธ์ต้นฉบับ

Radioimmunoassay for Salivary Progesterone in Normal Menstrual Cycle and Pregnant Women

การศึกษาระดับฮอร์โมนโปรเจสเตอโรนในน้ำลายของสตรีที่มีรอบเดือนปกติและสตรีมีครรภ์ด้วยวิธีเรดิโออิมมิวโนเอสเสย์

*Kalayanee Tunsaringkarn**
*Sukanya Werawatgoompa***
*Nikorn Dusitsin****

บทคัดย่อ : **กัลยาณี ตันศฤงฆาร, สุกัญญา วีรวัฒนะกุมพะ, นิกร ดุสิตสิน. 2531. การศึกษาระดับฮอร์โมนโปรเจสเตอโรนในน้ำลายของสตรีที่มีรอบเดือนปกติและสตรีมีครรภ์ด้วยวิธีเรดิโออิมมิวโนเอสเสย์. วารสารวิจัยวิทยาศาสตร์การแพทย์ 2(2) : 77-84**

การศึกษาหาระดับฮอร์โมนโปรเจสเตอโรนในน้ำลายของสตรีที่มีรอบเดือนปกติ 10 ราย พบว่า สตรี 9 ราย มีระดับสูงสุดของโปรเจสเตอโรนในระหว่างวันที่ 4-12 ก่อนจะมีรอบเดือนครั้งต่อไป ยกเว้นสตรี 1 รายไม่พบระดับสูงสุดของโปรเจสเตอโรนอย่างชัดเจน ความเข้มข้นของโปรเจสเตอโรนในช่วง luteal phase มีค่าเฉลี่ย 328.42 ± 207.04 pmole/L และในช่วง follicular phase มีค่าเฉลี่ย 64.96 ± 96.33 pmole/L การศึกษาระดับฮอร์โมนโปรเจสเตอโรนและเอสโตรเจนในน้ำลายของสตรีมีครรภ์ 3 ราย พบว่าความเข้มข้นของฮอร์โมนทั้งสองชนิดจะเพิ่มขึ้นตามอายุการตั้งครรภ์ ความเข้มข้นของฮอร์โมนทั้งสองชนิดมีความสัมพันธ์กันอย่างมีนัยสำคัญทางสถิติ ($r = 0.9001024$) ความเข้มข้นสูงสุดของโปรเจสเตอโรนและเอสโตรเจนมีค่า 4072.86 ± 727.34 pmole/L และ 222.57 ± 4.57 pmole/L ในสัปดาห์ที่ 4 และ 3 ก่อนคลอดตามลำดับ น้ำหนักเด็กแรกเกิดมีค่าเฉลี่ย 3350.00 ± 304.14 กรัม

* *Institute of Health Research, Chulalongkorn University, Bangkok 10500.*
สถาบันวิจัยวิทยาศาสตร์การแพทย์ จุฬาลงกรณ์มหาวิทยาลัย กทม. 10500

** *Biochemistry Department, Faculty of Medicine, Chulalongkorn University, Bangkok 10500.*
ภาควิชาชีวเคมี คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กทม. 10500

*** *Dept of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Bangkok 10500.*
ภาควิชาสูติศาสตร์-นรีเวชวิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กท. 10500.

Abstract : **Kalayanee Tunsaringkarn, Sukanya Werawatgoompa, and Nikorn Dusitsin. 1988. Radioimmunoassay for salivary progesterone in normal menstrual cycle and pregnant women. Thai J Hlth Resch 2(2) : 77-84**

Salivary progesterone level has been measured in ten women with regular menstrual cycle. We found that nine subjects showed progesterone peak between day 4-12 before the next menstruation except one that showed the fluctuating pattern. The average progesterone concentration in luteal phase was 328.42 ± 207.04 pmole/L and in follicular phase was 64.96 ± 96.33 pmole/L. Salivary progesterone and oestradiol levels have been measured in three pregnant women. Their patterns were increased with advancing gestational age. There was good correlation between concentrations of progesterone and oestradiol (r = 0.9001024). The maximum progesterone and oestradiol concentration were 4072.86 ± 727.34 pmole/L and 222.57 ± 4.57 pmole/L at four and three weeks before labour respectively. The mean birthweight was 3350.00 ± 304.14 g.

Introduction

It has been known that plasma progesterone bound to plasma proteins in biologically inactive (Baker and Forbes, 1949) but the only unbound fraction can interact with the specific tissue receptors. There are several methods used to separate bound and unbound steroid hormones but no simple technique is available for measuring routine unbound progesterone in plasma. The present steroid hormones can be detected in saliva by radioimmunoassay (Evans *et al.,* 1980 ; Luisi *et al.,* 1981 ; Walker *et al.,* 1981 ; Tunsaringkarn *et al.,* 1987). In some studies there was good correlation between plasma and salivary levels of the hormones. (Wang *et al.,* 1981 ; Choe *et al.,* 1983 ; Boever *et al.,* 1986 ; Evans, 1986). So the measurement of salivary progesterone by radioimmunoassay may be simple, sensitive and noninvasive method for study physiological in normal ovulatory and pregnant women.

Materials and Methods

Ten healthy volunteer women having regular menstrual cycles, ages between 17-35 years and 25-35 days of menstrual cycle, were studied.

Three healthy volunteer pregnant women, age between 32-34 years with a history of normal menstrual cycles, were allocated for study.

Each subject did not have any medication or any hormonal steroid treatment.

Whole saliva was collected from each subject between 9.00-11.00 AM about 10 ml by splitting directly into glass bottle and stored at -20° C until required for assay. The saliva samples were collect daily in normal cycle women and weekly in pregnant women. Progesterone and oestradiol were measured by radioimmunoassay technique utilizing the methods and reagents of WHO Special Programme of Research Development and Research Training in Human Reproduction 1987.

Specificity

Table I. *The specificity of the progesterone antiserum.*

Substance	% cross reaction
Progesterone	100%
Cortisol	0.002%
Testosterone	0.02 %
17 Hydroxyprogesterone	1.6 %
20 Dihydroprogesterone	0.04 %

Table II. *The specificity of the estradiol antiserum.*

Substance	% cross reaction
Estradiol	100 %
Estriol	0.8 %
Estrone	< 0.02 %
Cortisol	< 0.02 %
Progesterone	0.02 %
Testosterone	0.02 %

(Cross reactions estimated by noting relative potencies at B/B_0 of 50%)

From the WHO Special Programme of Research Development and Research Training in Human Reproduction, Method Manual, Eleventh Edition, January 1987.

Result

The sensitivity of salivary progesterone was 31 fmole/tube and salivary oestradiol was 21 fmole/tube.

The percentage of accuracy of salivary progesterone was 86.71 ± 20.62, 84.27 ± 13.04 and 87.87 ± 12.35 at concentration 156, 655 and 2500 pmole/L respectively.

The percentage of accuracy of salivary oestradiol was 87.45 ± 11.56, 84.45 ± 8.76 and 90.09 ± 14.69 at concentration 93.75, 375 and 1500 pmole/L respectively.

The pattern of salivary progesterone during normal menstrual cycle of ten subjects were varied as shown in Fig I. The nine subjects (number 1-9) showed variable in follicular phase and showed progesterone peak in luteal phase (if we assumed that luteal phase was consistently 14-days). The progesterone peak was between day 4-12 of the next menstruation (Fig II). The average concentration of salivary progesterone in luteal phase was 328.42 ± 207.04 pmole/L and in follicular phase was 64.96 ± 96.33 pmole/L. The subject number 10 (having a 27 days of cycle) demonstrated fluctuation. There was no peak of progesterone in luteal phase which indicated abnormal ovarian function.

In three pregnant women salivary progesterone and oestradiol patterns were rose with advancing gestational age. The Pearson's correlation coefficient calculated between progesterone and oestradiol concentrations found significantly different than 0 ($r = 0.9001024$) at 5 to 37 weeks of gestational age (Fig III & IV). The maximum concentration of salivary progesterone and oestradiol were 4072.86 ± 727.34 and 222.57 ± 4.57 pmole/L respectively (Table III). The mean birthweight was 3350.00 ± 304.14 g.

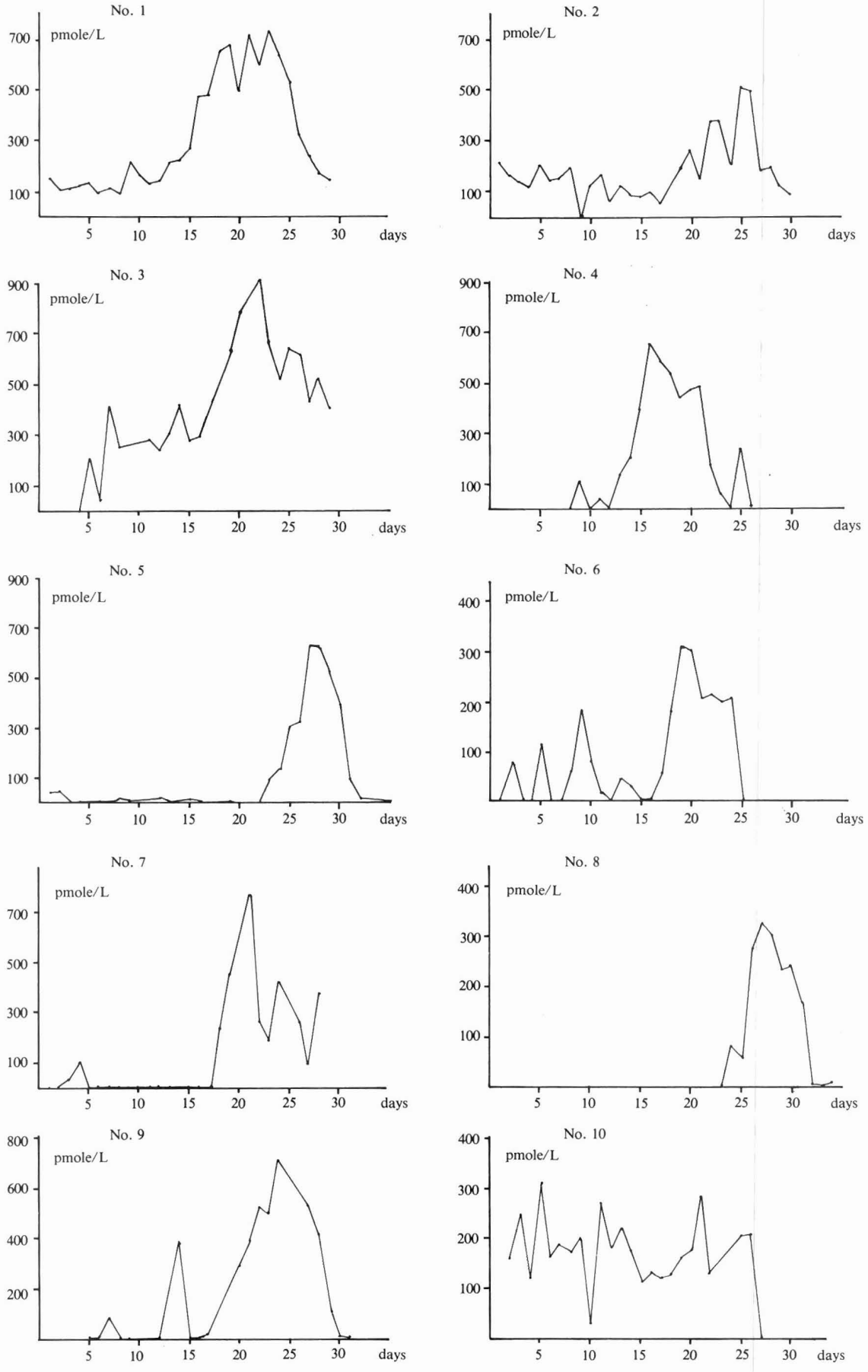


Fig. 1 Concentrations of salivary progesterone of ten subjects during menstrual cycle.

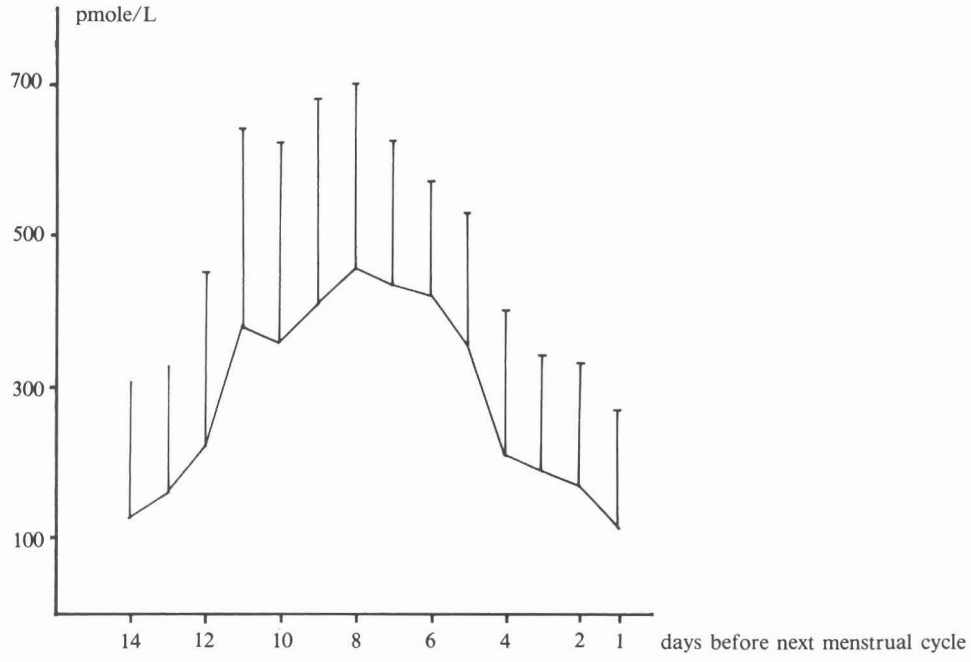


Fig. 2 The mean concentrations of salivary progesterone in luteal phase.

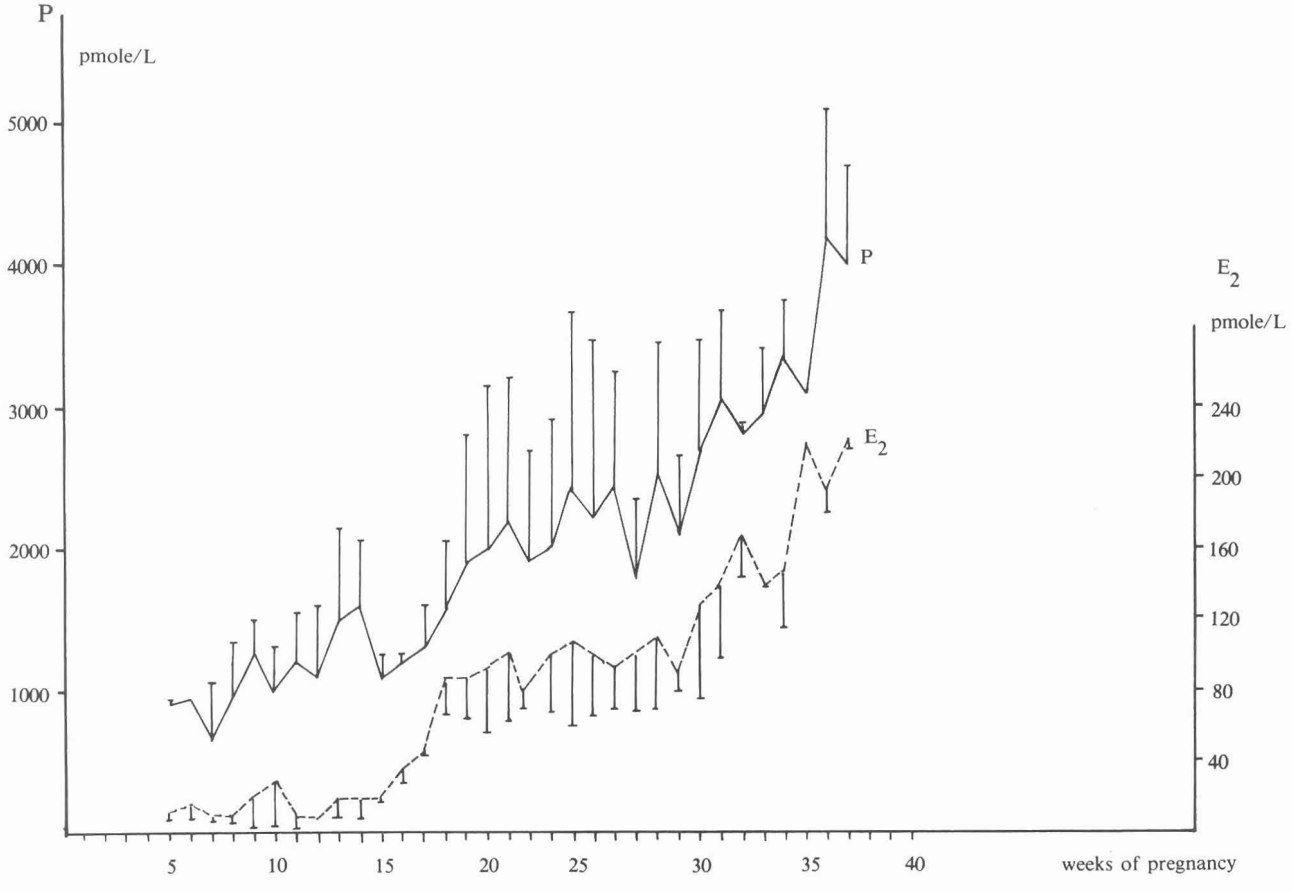


Fig. 3 The mean concentration of salivary progesterone (●—●) and oestrogen (●----●) in three prognant woman.

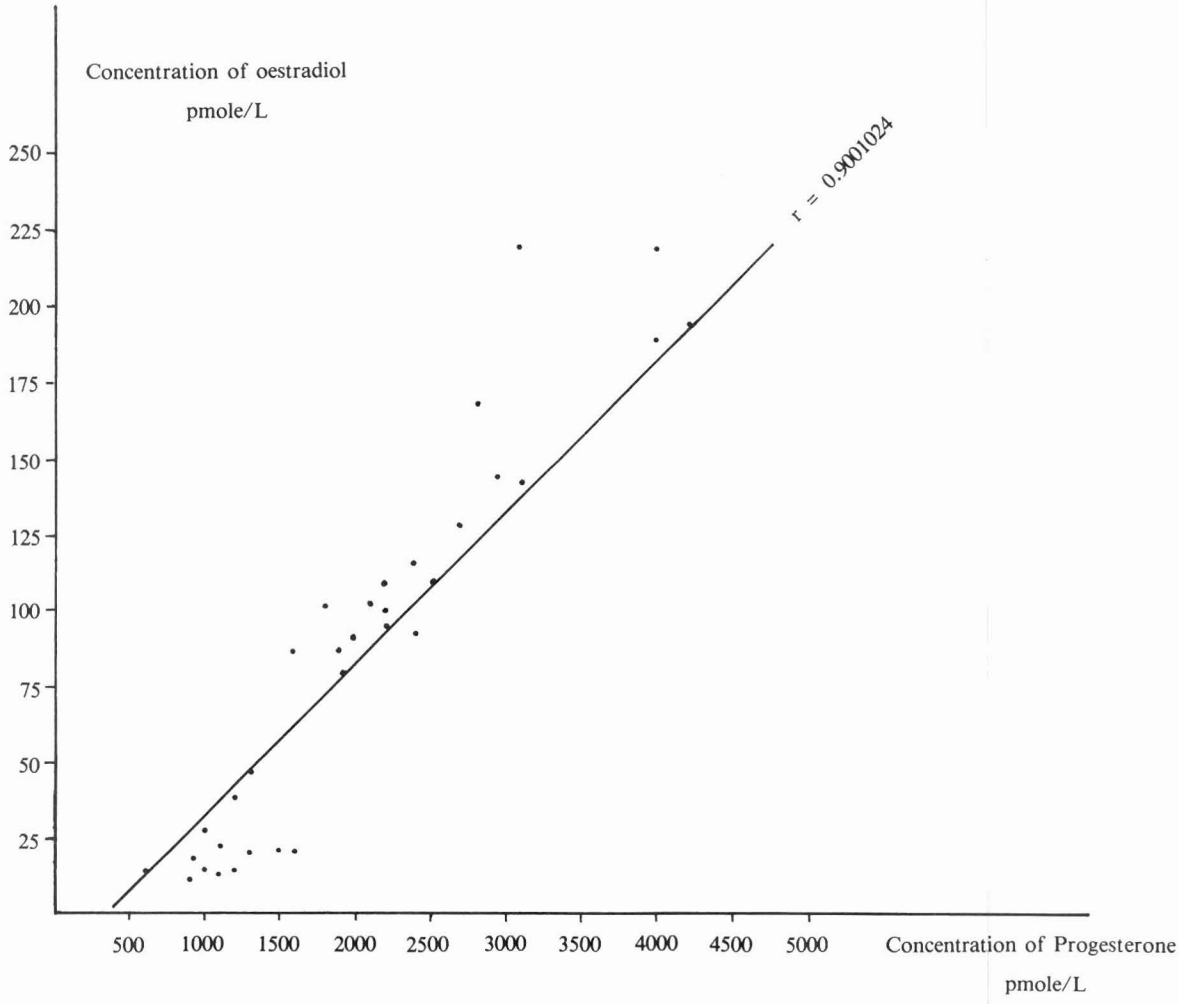


Fig. 4 Correlation between concentration of salivary progesterone and oestradiol in three pregnant women.

Table III. *The mean concentrations of salivary progesterone and oestradiol in late three pregnant women.*

Week before labour	Progesterone con^{n} pmole/L ($\bar{X}$ ± SD)	Oestradiol con^{n} pmole/L ($\bar{X}$ ± SD)
1	3764.80 ± 369.80	153.58 ± 87.93
2	3818.70 ± 296.45	202.87 ± 17.66
3	3807.43 ± 989.49	222.57 ± 4.97
4	4072.86 ± 272.34	171.84 ± 0.12
5	3193.04 ± 686.51	153.83 ± 12.00
6	2907.86 ± 115.20	153.52 ± 43.97
7	3155.03 ± 552.65	131.74 ± 14.88
8	2636.71 ± 412.93	111.37 ± 57.59
9	2490.65 ± 1143.40	107.13 ± 19.57
10	2604.83 ± 963.34	119.51 ± 42.37

Discussion

The salivary progesterone levels of nine subjects accepted normal ovarian function except one was shown abnormal ovarian function. Progesterone concentration in saliva during follicular phase was low (64.96 ± 96.33 pmole/L) and rose peak between day 4-12 before the next menstruation (328.42 ± 207.04 pmole/L). This result was corresponded with other reports (Walker *et al.*, 1979 ; Werawatgoompa *et al.*, 1981 ; Chearskul, 1982 ; Choe *et al.*, 1983 ; Boever *et al.*, 1986) and supported the result of Zorn *et al.* (1984). So salivary progesterone would be more simple than plasma progesterone for predicting the ovarian function and may be used instead of plasma progesterone in clinical work.

The salivary progesterone and oestradiol levels in pregnant women were rose with advancing gestational age. The concentration of progesterone was good correlated with oestrogen. Sybulski and Maughan (1972) reported that very low concentrations of estradiol in the maternal observed in the present cases were due to a primary placental deficiency severe enough to cause fetal death. So that the concentration of salivary progesterone and oestradiol would be correlated with birthweight (Joshi *et al,* 1976). This study found that the mean birthweight (3350.00 ± 304.14 g.) was higher than average normal Thai infant birthweight (3000.00 g). There were reports showed that concentration of progesterone and oestradiol in saliva correlated with plasma but level was lower (Tulchinsky *et al.*, 1982 ; Turnbull *et al.*, 1974 ; Goebelsmann, 1979). When studying concentration of hormones and time before labour, we found that salivary progesterone and oestradiol were meximum at four and three weeks before labour respectively. These results supported the report of Muthur *et al.* (1980). The rapid rise in oestrogen and rapid fall in progesterone concentrations (increase in ratio estrogen to progesterone) is the initial signal for time onset of parturition in several mammalian species. (Mcgarrigle and Lachelin, 1984 ; Liggins *et al.*, 1973 ; Puri and Garfield, 1982 ; Turbull *et al.*, 1974). So salivary progesterone and oestradiol could be used to assess foetal growth. Steriod hormones in plasma usually bound to plasma protein but unbound steroid hormone concentrations reflected to target organ. It has been suggested that measurement concentration of salivary steroid hormones would be correlated with metabolic status.

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