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The Hormonal Levels of the Short Luteal Phase in Korean Women (II): Change of Serum Prolactin

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- 국문초록 -

황체기단축현상을 가진 한국여성의 호르몬양(II)
- 혈청 Prolactin의 변동 -

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본 연구는 황체기 단축현상 (Short luteal phase, SLP)을 가진 한국여성의 월경주기내 Prolactin 호르몬의 농도변화를 정상월경주기 (Normal luteal phase, NLP)와 비교하고저 하였다. 한편, Circadian rhythm 내 PRL 농도의 변화를 비교하고 임신기간중 PRL의 농도변화를 방사면역측정법 (RIA)으로 조사하였다.

SLP 여성의 PRL 농도는 후기의 여포성숙기 (Late follicular phase) 및 초기 황체형성기 (Early luteal phase) 정상에 비하여 현저히 낮았다. 정상 월경주기에서는 PRL/Progesterone (mU/l/mg/ml)의 비율이 후기 여포성숙기까지 증가한후 LH Peak day 이후 감소하는데 반하여 SLP여성의 비율은 초기 여포성숙기부터 계속적인 감소현상을 보였다. 또한 취침중 (04:00 시) PRL의 농도는 SLP 여성에게는 현저히 낮았다. 임신주기중 PRL의 농도는 4개월째 1,077 mU/l 에서부터 9개월째 4,462 mU/l 까지 점진적인 증가를 하였다.

위의 결과로 보아 황체기단축현상은 PRL의 분비이상이 하나의 주요요인이 될 수 있으며, 특히 PRL/Progesterone의 농도비율이 SLP의 판정에 한 지표가 될 수 있을 것으로 사려된다.

INTRODUCTION

It has been known that the short luteal phase with cyclic menstrual breeding is due to the functional defect of corpus luteum (Jones, 1976; Andrew, 1979; diZerega and Hodgen, 1981). However, the aethiology and diagnosis for the treatment of this luteal phase defect are not clear yet.

Recently, many clinical studies have been concentrated on the various factors of producing an inadequately functioning corpus luteum: The ovarian factors such as the insufficient production of progesterone during the post-ovulatory period are known to induce an inappropriate endometrial development (Rosenfeld and Garcia, 1976; Wentz, 1980). The aspiration of the Graafian follicle or the induction of ovulation with HMG and HCG could also induce the luteal phase defect (Kreitman et al., 1981;

Feichtinger et al., 1982; Olson et al., 1983). However, a majority of these inadequately functioning corpus luteal appears to be due to the problems of the hypothalamo-pituitary ovarian axis. Those are suboptimal levels of follicle-stimulating hormone (FSH) in the cycle, inadequate lutenizing hormone (LH) surge, sublevel of FSH to LH ratio in follicular phase, and prostaglandins produced by the uterus, the endometrial progesterone receptor defects (Reviewed by Andrew, 1979; Daly et al., 1981; Yoon, 1981).

An additional factor, higher prolactin level in the patients with luteal insufficiency patients, has been reported (Seppala et al., 1976; Muhlenstedt et al., 1977). It has been known that highly increased prolactin concentration may impair ovarian steroid secretion and granulosa cell maturation, even though low prolactin may be necessary for the maintenance of luteal function (Del Pozo et al., 1979). These facts suggest that the elevated PRL level may be one of the causes of insufficient steroidogenesis in luteal phase defect. However, the exact role of prolactin on the regulation of human menstrual cycle and on the corpus luteum insufficiency is not clear yet.

Although a number of investigators have published data on the hormonal profile of the daily prolactin concentration during menstrual cycle (Vekemans et al., 1977; Lenton et al., 1979; Lee and Kim, 1983; Yu, 1983), the results are not in complete agreement.

The purpose of this study, therefore, was to define the distribution and range of serum prolactin concentrations found in the menstrual cycle of short luteal phase women compared to those in normal luteal phase one. The level of serum prolactin also studied in circadian rhythm and during pregnancy in Korean women as well.

MATERIALS AND METHODS

Subjects and Sampling of Blood Sera

Twelve women (aged 20-27 years) with short luteal phase were bled between 10:00 and 12:00 hours throughout an periovulatory cycle and every other day before or after ovulatory cycle from the ante cubital vein by sterilized disposable syringes (Plastic pak). After transferring to test tube and being allowed to clot, sera were separated by centrifugation at 1,000 Xg for 10 minutes. All aliquots (0.5ml) of the sera were stored separately at below $-20^{\circ}C$ until analysis. Normal and short luteal cycles were defined by the criteria as shown in previous reports (Yoon, 1981).

ASSAY METHODS FOR LH, FSH, ESTRADIOL-17BETA AND PROGESTERONE

The amounts of the above hormones in sera were determined by the previous method with WHO Matched Reagents (Yoon, 1981).

ASSAY METHOD FOR PROLACTIN

Prolactin assays were established by radioimmunoassay method as follows:

Assay Buffer (pH 7.2–7.4); 0.1M sodium phosphate buffer containing 8.5% NaCl, 0.1% merthiolate, 0.5% bovine serum albumin (BSA, Sigma Chem. Co., RIA grade), and 0.025M EDTA.

Tracer diluent; Assay buffer as above and added 1% normal rabbit serum.

Standards; Human prolactin (75/504).

Immunoadsorbent; Goat anti-rabbit gamma globulin (Anti-bodies Inc.) at 1/26 dilution.

Antiserum; Prolactin antiserum was generated in rabbit and distributed by WHO Matched Reagent Programme (final dilution 1/400,000). The cross reactions of this antiserum with human growth hormone was 0.05%.

Tracer; Iodine 125-PRL (W7901-8 Series, Spec. act. 17uCi/vial, Swiss Federal Reactor Institute).

Repurification of hormone tracers; Labelled tracers of prolactin hormones were repurified regulary at one week interval through the column (AcA 54, 1.6 x 25cm, Ultrogel, LKB product).

Assay procedure

One hundred microliter of standard series and unknown sample with 100μl of antisera (final dilution, 1/400,000), 100μl of tracer, and 400μl of assay buffer were mixed and incubated for 48 hours at 4°C as shown in Table 1 After initial incubation, 200μl of second antibody (Goat anti-rabbit gamma globulin) was added and incubated for another 18-24 hours at 4°C. After this second incubation and centrifugation at 4°C for 30 minutes at 2,000 Xg with refrigerated centrifuge (IEC, Model, PRC 6,000), the supernatant was discarded and the precipitate was counted with a gammacounter (Packard, Tri-Carb, Scintillation Spectrometer, Model 5210). For the standard series, the range of PRL measurement in this assay was 78-2, 500 m U/litre. The final values of dose-response were plotted on the semilog paper by the bound per initial bound (B/Bo) against logarithmic dose, or plotted using Logit-log paper. The results of this prolactin concentration are expressed in terms of the 1st International Reference Preparation (1st IRP 75/504) issued by the NIBSC. The conversion factor to ng/ml order is 30.77.

Student's t-test of unpaired observations was used.

Table 1. The Flow sheet of PRL radioimmunoassay.

Prolactin Standard or Serum Sample

+ Prolactin Antiserum

+ ^{125}I–Prolactin

First incubation for 48 hrs at 4°C

+ Goat Anti–Rabbit Serum

2nd incubation for 18-24 hrs at 4°C

Centrifuge for 30 min at 2,000 Xg

Remove the supernatant

Count the radioactivity using Packard Spectrometer (Model 5210, Packard Tri-Carb)

Data calculation using programmed calculator (Hewlett Packard H.P. 67 Model)

RESULTS

I. On the level of prolactin during the menstrual cycle of short (SLP) and normal luteal phase (NLP) women:

1) Distribution of the determined PRL concentration.

Frequencies of serum PRL during the menstrual cycle are demonstrated in Figure 1 This

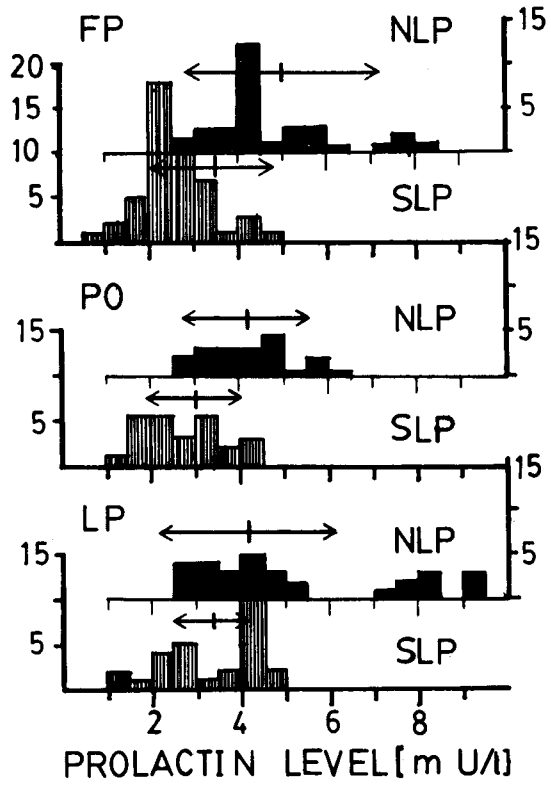

Figure 1. The frequency distribution of prolactin concentration in short and normal luteal phase during menstrual cycle.

The horizontal lines with the arrows represent mean ± standard error. The follicular phase (FP) corresponded to the days −14 to −4 before LH peak and periovulatory period (PO), −3 to 3 days and luteal phase (LP), the remaining days of the cycle. The histograms with block are the frequency in normal cycle (NLP) but those with vertical lines, the frequency in short luteal phase (SLP) cycle.

figure shows that there were very large variations during the menstrual cycle. This figure also shows that the greatest frequency of SLP was found at the level of 250mU/l during the follicular phase (FP) and periovulatory period (M), whereas it was at 400mU/l during luteal phase.

The largest frequencies of NLP were found at 430-480m U/l level throughout the menstrual cycle. Only one sample was recorded with a concentration of less than 100mU/l in the follicular phase of SLP women and three samples had a concentration greater than 900mU/l in NLP cycle.

The present result also shows that the frequency distribution was skewed to the right and suggests that the sample was not homogeneous and further division of the sample was necessary.

2) The daily prolactin levels

The profiles of day-to-day PRL concentration from individuals of SLP and NLP women are demonstrated in Figure 2.

This figure shows that PRL concentrations vary from day-to-day and that profiles do not show any consistent trends. However, a tendency can be found that PRL level was increased during the ovulatory and early luteal phase in normal cycle ($P < 0.01$), but in SLP, no significant elevation of PRL level could be found in early luteal phase compared to the values of follicular phase.

This figure also demonstrates that the PRL concentrations of SLP during late follicular and early luteal phase were lower than those of NLP and that there was no significant differences between the PRL levels of SLP and of NLP during the follicular phase.

3) The concentrations of peptide and steroid hormones during the menstrual cycle of short and normal luteal phase.

In order to compare the hormonal levels of each period, the menstrual cycle were grouped into seven subcategories: (1) bleeding period (BP), (2) three subcategories in the follicular period such as early (EF, more than -11th day before LH surge), middle (MF, -10th to -6th day) and late (-5th to -1st day), (3) peak day of middle cycle (M), and (4) two subcategories in luteal period such as early (EL, +1st to +4th day after LH peak) and late (LL, more than +5th day) luteal phase.

The present study shows the several characteristics of SLP as shown in Figure 3. First, the serum LH levels in the follicular phase and on the midcycle day of SLP were not different from those of NLP but the LH values in the early luteal phase of SLP were slightly lower than those of NLP (Figure 3). Secondly, the levels of the FSH in the mid-cycle peak and early luteal phase of the SLP were significantly lower than those of NLP (Figure 3). Thirdly, the levels of estrodiol-17 beta of SLP were significantly lower than those of NLP on the day of LH surge and luteal phase (Figure 3). Fourthly the progesterone concentrations in the luteal phase of SLP were significantly lower than those of NLP. Finally the ratio of FSH to LH was significantly lower after the day of LH surge through luteal phase of SLP cycle.

In order to compare the PRL levels of SLP to those of NLP women in each period of the menstrual cycle, the values are also grouped into 7 subcategories as described previously (Figure 4).

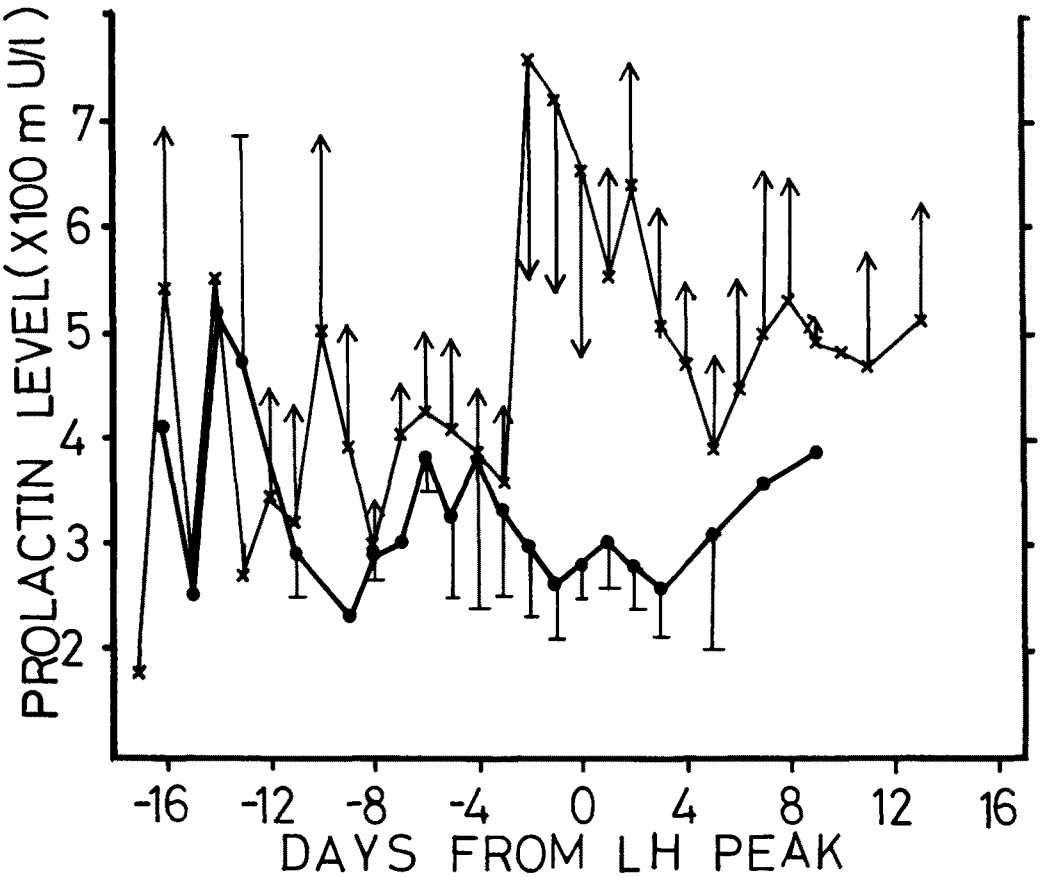

Figure 2. Mean daily prolactin concentrations throughout the menstrual cycle of short and normal luteal phase women.

Data are expressed as mean ± standard deviation. Prolactin concentrations were determined in the serial samples of the short (SLP, thick solid line with the closed encircles) and of the normal (NLP, thin line with X marks). Values obtained in individual subjects of the both groups are synchronized with reference to the day of the LH peak.

There was no statistically significant difference between the levels of serum PRL during the follicular phase and those during the luteal phase in both SLP and NLP (Table 2).

There is a tendency of PRL elevation during the periovulatory period only in total population even though the statistical significancy is not found at the 99% confidence ($P < 0.01$).

On the other hand, the PRL concentrations of SLP were significantly lower than those of NLP in the periods of late follicular and luteal phase and also on the day of LH surge ($P < 0.01$ in LFP and $P < 0.05$ in ELP) when the day-to-day profiles of the PRL concentrations were group into 7 subcatories.

4) PRL/Progesterone Ratio

The mean values with 1 standard error of the ratios of PRL (mU/l) to progestrone (P_4, ng/ml) during the menstrual cycle are summarized in Figure 5.

This figure depicts that the ratio rises until late follicular phase and then falls down to the mid luteal phase when the P4 level is increased. The ratios of late follicular phase of SLP were slightly lower than those of NLP ($P < 0.1$).

On the other hand the ratios of ML in SLP women was significantly higher than those of NLP ones even though the number of the samples was too small to calculate the statistical significances.

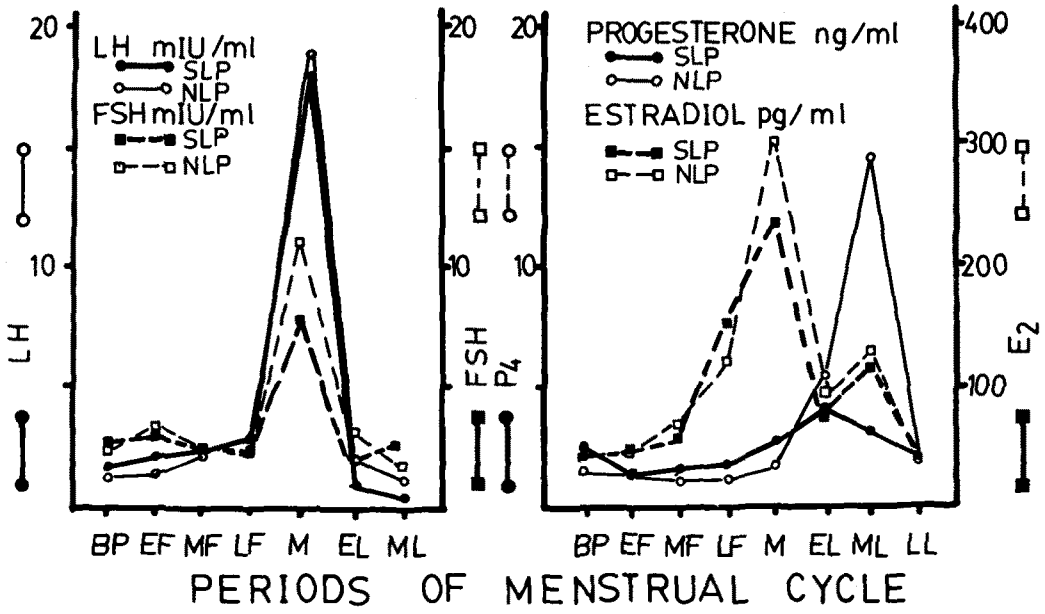

Figure 3. Comparison of the secretory patterns of serum LH, FSH, estradiol-17 beta and progesterone during the menstrual cycle of SLP to those of NLP women.

The period of menstrual cycle were subdivided into 7 subcategories (See the text). Hormone concentrations of SLP are presented as the thick lines with the closed encircles or cubes and those of NLP as the thin lines with the open circles or cubes.

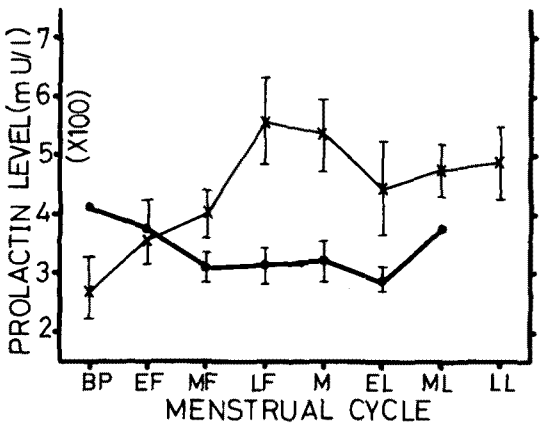

Figure 4. Mean prolactin concentrations throughout the menstrual cycle of short and normal luteal phase women.

The values obtained from the individual subjects in both groups are synchronized with reference to LH surge day and then grouped into 7 subcategories. Thick solid line with the closed encircles represents the concentration of the short luteal phase (SLP) and thin line with X marks, that of normal luteal phase women (NLP). Data are expressed as means ± standard error.

Table 2. Serum prolactin concentrations during menstrual cycle of short and normal luteal phase women.

Group cycle	Total	NLP	SLP
follicular	384.49	430.76	340.00
phase	±18.34	±27.15	±32.39
< −3 days	(76)	(25)	(16)
periovulatory	482.91	449.58	293.68
phase	±33.80	±51.58	±18.97
−3 to +3 days	(73)	(17)	(19)
luteal phase	424.98	356.42	297.27
> ±3 days	±25.79	±25.82	±30.87
	(56)	(35)	(11)

Mean hormone concentrations and standard errors are given in Table. The numbers of first column represent the days from the day of LH peak and those in the parenthese mean the number of the samples.

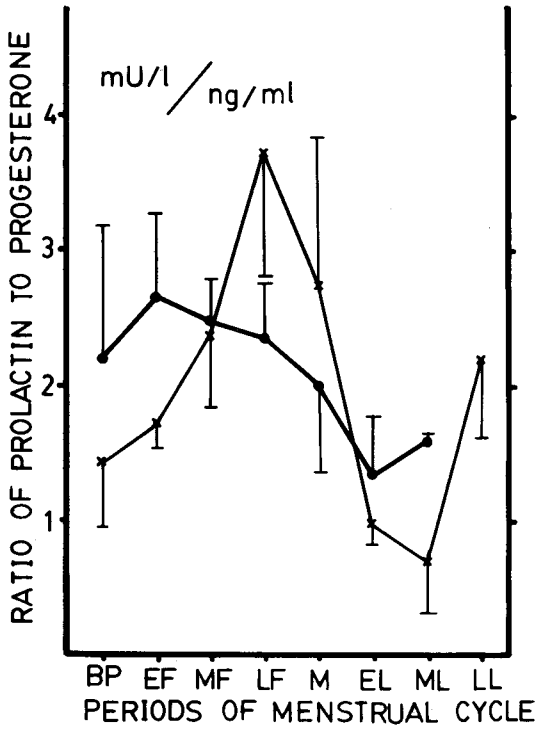

Figure 5. Changes of PRL/ progesterone ratio throughout the menstrual cycle of the short and normal luteal phase women.

Legends are the same as shown in Fig. 4. The concentration of PRL (m U/L) was divided directly by the level of progesterone (ng/ml), which were determined in the same sample. Data are expressed as mean ± standard error. Thick solid line with the closed encircles (•——•), the ratios in SLP; thin line with the X marks (X —— X), that in NLP.

II. PRL Concentration during Circadian Rhythm and Normal Pregnancy.

The 24-hour PRL secretory patterns of SLP and NLP can be seen in Figure 6.

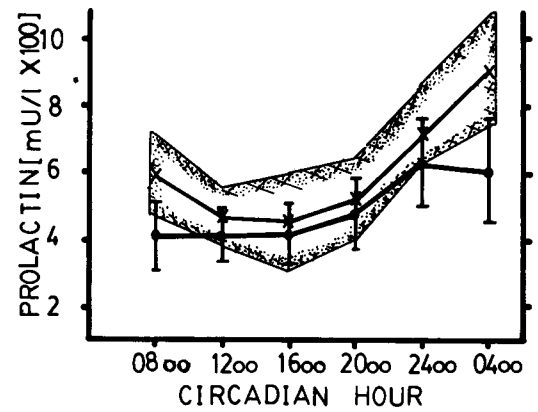

Figure 6. Serum prolactin concentrations in 24 hour period.

Each control point represents the mean of 5 subjects of normal luteal phase and of 3 subjects of SLP. Sleeping after 12:00 hour was confirmed in order to carry out blood collection without awakening from the extension tubing. The shaded area represented mean (thin line with X marks) + standard deviation of the normal luteal phase.

This figure depicts that the PRL values from 10:00 to 12:00 o'clock were constantly secreted at the level of 350-550mU/l and then increased significantly to more than 700mU/l after sleeping. However, the mean PRL values fell down to the normal levels after awakening. The mean PRL values during day times (12:00 to 20:00) of SLP women was also slightly lower than those of NLP but those during night (24:00 to 08:00) of SLP were significantly lower than those of NLP.

Figure 7 shows the range of serum prolactin level according to the months of pregnancy in 157 determinations in 35 normal pregnant women. The PRL concentration was constantly increased from the level of 1076.9±461.6mU/l at 4 months to the 4461.7±450.5mU/l on 9 months. PRL concentration seems to be decreased slightly just before the term (paturition).

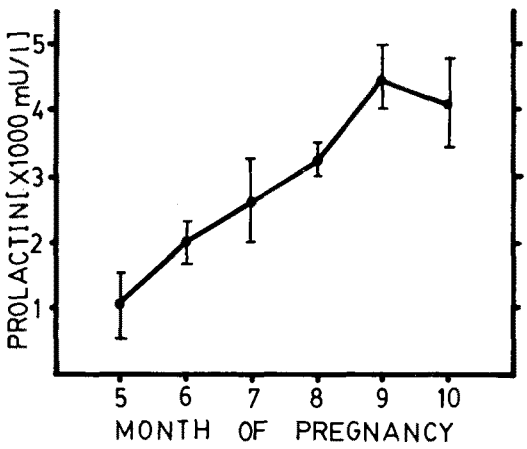

Figure 7. The range of serum prolactin during pregnancy.

Each point demonstrates the mean ± standard error. Prolactin concentration was determined in 157 samples from 35 normal pregnant women. The numbers in parentheses mean the determination of serum PRL on that period.

DISCUSSION

The range of individual PRL level found in the present study was very wide (approximately 60-950mIU/l). It is known that most data on the mean daily PRL concentration during the menstrual cycle demonstrated the normal values with zero or negative limits at 95% confidence level and were not in complete agreement (L'Hermite, 1973; Franchimont et al., 1976; Lenton, 1979; Yu, 1983). These PRL estimations might be due to the variations in techniques. In another point, variations of PRL concentration could be derived from the stress of vein puncture during the sampling, malnutrition, the heavy excise or the sampling time in a day (Koninkz, 1978; Chakravarty et al., 1982; Hale et al., 1983). These make it difficult either to define the normal range precisely or to establish the importances of PRL in regulating reproductive function until more information is available on the ranges of concentrations found in each reproductive stage.

It has been reported that there is a small but significant difference between the PRL level in the follicular phase and remainder of the cycle. This difference may also be physiologically important (Tamura et al., 1973; Franchimont, 1976; Vekemans et al., 1977; Healy and Burger, 1983; Yu, 1983). However, great variability between individual cycles was seen with random short term fluctuations (McNeilly and Chard, 1974) and also no mean PRL values showed significant changes during the menstrual cycle (Del Pozo et al., 1974; Lenton et al., 1979). The present study showed that only PRL levels in periovulatory period (from -2 day before LH peak to 3rd day after LH surge) were higher than those of other period during the menstrual cycle. This result is consistent with that of Aksel (1980, 1981). However the total PRL values of luteal phase were not statistically different from those of follicular phase. This seems to be due to the fluctuation of PRL concentrations during the early follicular and late luteal phase during the menstrual cycle.

It is thought that a gradual elevation in PRL level leads to the characterized luteal phase defect (LPD), which is known to atributed to inadequate FSH secretion during the follicular phase, and to an insufficient progesterone secretion during the luteal phase (Corenblum et al., 1976; Seppala et al., 1976). Contrary to this hypothesis, no abnormality of PRL concentration in LPD were reported (Del Pozo et al., 1975; Aksel et al., 1976; Cetel et al., 1982; Balash et al., 1983; Andersen et al., 1984).

However, the present result demonstrates another hypothesis that the PRL concentrations during the periovulatory of LPD is significantly reduced and that low PRL/progesterone ratio during this period is due to the lower production of progesterone by the suppressed prolactin and LH secretion. This latter assumption can be supported by the previous report (Yoon, 1981) and the facts that the production of PRL by

explants of secretory endometrium obtained from LPD is significantly lower than that from normal cycling tissue (Daly et al., 1981). In addition, it is supported by the results of Levy et al. (1980) that a number of progesterone receptors was decreased in the endometrium of LPD women, and by the report of Veldhuis et al., (1980) about the stimulatory action of PRL on the production of progesterone.

Nyctohemeral rhythms of PRL have been observed in non pregnant or pregnant women and men (Sassin et al., 1972; Parker, 1973) and also in polycystic ovarian syndrom (Mattox et al., 1984). No one has reported circadian PRL secretory pattern in SLP women to the best of our knowledge. The present study suggests that the production or secretion of PRL is suppressed during the sleeping period compared to that of NLP women.

The present data on PRL concentrations during the normal pregnancy are in good agreement with the previous (Sadovsky et al., 1977; Andersen et al., 1984). Prolactin values rise progressively from 1077mU/l to 4462mU/l at 9 months of gestation.

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