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Studies on the Mechanism of Post-partum Amenorrhea:

Pituitary-Ovarian Axis during Post-partum Amenorrhea in Lactating Women*

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The aim of this study is to evaluate pituitary-ovarian function at different postpartum periods during the lactational amenorrhea in order to understand the mechanism by which puerperal lactation is associated with a protracted period of amenorrhea and natural infertility.

Ninety four lactating women and 119 lactating women with menstruation, aged between 21 and 38 years, volunteered for this study.

The pituitary was relatively insensitive to LH-RH during the first 3 weeks following delivery. The recovery of FSH responsiveness to LH-RH occurred earlier than that of LH. Normal FSH response resumed in the 2nd week while the LH response, although not normal, started at the 3rd week postpartum. Pituitary responsiveness after the 5th week postpartum was similar to that occurring in normally menstruating women, except that FSH response was exagerated. Serum prolactin levels were elevated above 160 ng/ml until the 5th week postpartum and decreased to 84.2 ng/ml in the 6th week postpartum.

It appears that at least one reason for anovulation during the first four weeks following delivery is the relative insensitivity of the pituitary to hypothalamic stimulation. Prolactin does not seem to modulate pituitary responsiveness to LH-RH.

In order to clarify hormonal profiles during the lactational amenorrhea beyond the 5th week puerperium, serum levels of LH, FSH, prolactin, estradiol and progesterone were determined during different postpartum periods. Serum FSH and LH levels during 1-10 months postpartum were similar to basal levels seen during the normal menstrual cycle. Serum estradiol concentrations throughout 1-10 months postpartum, however, were significantly decreased as compared with the levels during the follicular phase of the normal menstrual cycle. Serum prolactin levels were elevated throughout 1-10 months postpartum in lactating amenorrhic women but decreased as the postpartum period lengthened. As compared with lactating amenorrhic women, lactating women with resumed menstruation showed a decrease in prolactin levels from 89.20 ng/ml to 51.39 ng/ml at 1-3

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months, from 75.08 ng/ml to 49.99 ng/ml at 4-6 months, and from 54.73 ng/ml to 28.74 ng/ml at 7-10 months postpartum.

These results suggest that the apparent anovulation seen beyond 5th week postpartum during lactation was not due to pituitary insensitivity to LH-RH. Rather, prolactin-dependent mechanism interfering with cyclic activity may be operative during long term lactation.

Key Words: Pituitary-ovarian axis, Lactational amenorrhea.

Puerperal lactation is known to be associated with a protracted period of amenorrhea and natural infertility in postpartum women. Such infertility may be related to a temporal alteration in hypothalamic-pituitary-ovarian function.

Some investigators reported a lowered pituitary FSH and LH levels during the postpartum period (Crystle *et al.*, 1970; Reyes *et al.*, 1972), assuming that a suppressed gonadotropic function of the pituitary might be responsible for puerperal anovulation and amenorrhea. Low concentrations of gonadotropin in the puerperium might be due to decreased secretion of LH-RH and decreased pituitary responsiveness to LH-RH (del Pozo *et al.*, 1975; Nakano *et al.*, 1975; Key and Jaffe, 1976). Several authors, however, have opposed this hypothesis. They pointed out that there was no significant change in the levels of FSH and LH during the postpartum period, as compared with the levels during the normal menstrual cycle (Keettel and Bradbury, 1961; Faiman *et al.*, 1968).

Instead, decreased ovarian responsiveness to circulating gonadotropin rather than pituitary gonadotropic dysfunction has been reported during the puerperal anovulation and amenorrhea (Zarate *et al.*, 1972, 1974; Mrouch and Silerkhodr, 1976) although ovarian refractoriness to gonadotropin was denied in puerperium (Nakano *et al.*, 1975) and in non-puerperal hyperprolactinemia (Archer and Joshimovich, 1976; Caro and Woolf, 1980).

Thus, the endocrine function of pituitary-ovarian axis during the postpartum period remains open to speculation and has been discussed with controversy, especially with regard to the cause of puerperal anovulation and amenorrhea during lactation.

The present study was performed to evaluate pituitary-ovarian function at different postpartum periods during lactation.

MATERIALS AND METHODS

1) Subjects

Ninety four nursing women and 119 nursing women with resumed menstruation, aged between 21 and 38 years, volunteered to be subjects for this study. There were no known complications during their antepartum course, labor, or delivery. None of the women received hormones during pregnancy or the puerperium other than those used in the protocol for this study.

Twenty six lactating women at 1 week, 1½ weeks, 3 weeks, 5 weeks, and 6 weeks puerperium received an intravenous injection of 100 ug synthetic LH-RH (Hoechst, A.G.,W. Germany). Blood samples were obtained by venipuncture just prior to LH-RH injection and at 30 min., 60 min., and 120 min. following LH-RH administration.

The second group of 68 lactating amenorrhic women was studied at 1-3 months, 4-6 months, and 7-10 months postpartum. One hundred nineteen lactating women with

resumed menstruation were also studied. Five ml of blood was collected at 1-2 hr following the last breast feeding. Serum was separated and kept frozen at -50°C until assayed.

2) Methods

Serum levels of FSH and LH were measured by a double antibody radioimmunoassay, employing RIA kits provided by Daiichi Radioisotope Laboratory (Tokyo, Japan). Prolactin was also measured by a double antibody radioimmunoassay, using RIA kits provided by Abbott Laboratory (Chicago, Ill., U.S.A.). Radioimmunoassay kits from Daiichi Laboratory (Tokyo, Japan) were employed to determine serum estradiol and progesterone. Student t-test was used for the statistical analysis.

RESULTS

The base line LH concentrations during 1 to 6 weeks puerperium are shown in Table 1. The high levels of LH at 1 and 1½ weeks resulted from the cross-reactivity with HCG in the assay system. From the 2nd week puerperium base line LH concentrations were similar to those seen during the follicular phase of the menstrual cycle. The base line concentrations of FSH were low during the first 1½ weeks following delivery, as compared with levels of FSH during follicular phase of the menstrual cycle. From 2nd week, basal levels of FSH reached levels similar to those seen in the follicular phase of the normal cycle (Table 1).

The LH response to LH-RH during the first 2 weeks puerperium was not marked. Even at the 3rd week, LH response was less than those of women in the early follicular phase. Following this period of relative refractoriness to LH-RH, normal LH response was resumed at 5th week postpartum (Fig. 1). FSH started to respond to LH-RH at 2 weeks following delivery when no LH response was shown. At 5 weeks, FSH response to LH-RH was exaggerated above the response of LH. Exaggerated FSH response also occurred at 6 weeks but it was lower than the LH response (Fig. 1).

The patterns of LH and FSH responses to LH-RH were similar to those reported previously in normally menstruating subjects. LH concentrations rose rapidly reaching the maximum at 30 minutes following LH-RH administration and gradually declined thereafter. On the other hand, FSH did not reach the peak response until 60 minutes following LH-RH injection.

As shown in Table 1, serum prolactin levels were more than 160 ng/ml before 5 weeks and dropped to 84.2±26.4 ng/ml at 6 weeks puerperium.

In order to clarify hormonal profiles during the lactational amenorrhea beyond early puer-

Table 1. Basal levels of serum LH, FSH, and prolactin in nursing women during early puerperium

		Week after delivery					
		1	1½	2	3	5	6
LH	(mIU/ml)	76.0±9.0	33.5±3.7	16.0±0.8	18.0±4.1	21.0±3.4	17.5±0.9
FSH	(mIU/ml)	5.5±1.5	4.8±1.1	6.9±0.9	8.4±1.5	17.5±1.5	16.0±1.0
Prolactin	(ng/ml)	> 160	> 160	> 160	> 160	> 160	84.2±26.4
*n		4	4	4	6	5	6

* "n" refers to number of subject.

perium, serum levels of LH, FSH, prolactin, estradiol, and Progesterone were determined during different postpartum periods. Serum LH levels were 17.6, 24.5, and 19.6 mIU/ml respectively at 1-3 months, 4-6 months, and 7-10 months post-partum and these values were not significantly different from basal levels shown during the normal menstrual cycle. There was also no change in FSH levels during 1-10 months post-partum, and these levels were similar to basal levels of FSH during the normal menstrual cycle.

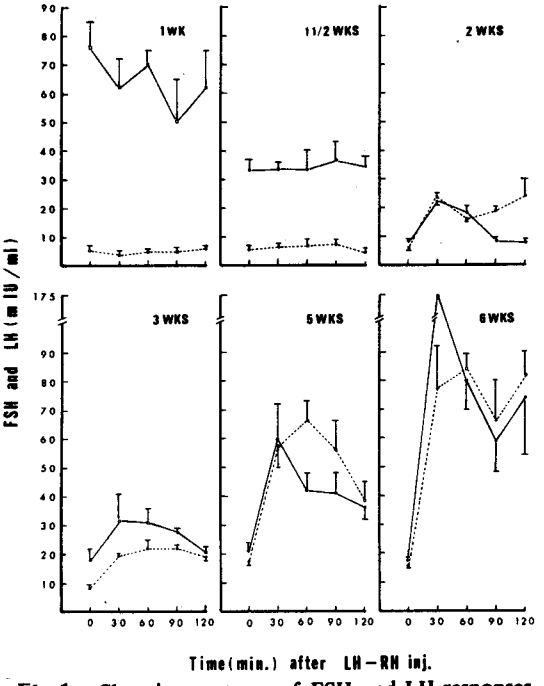

Fig. 1. Changing patterns of FSH and LH responses to LH-RH during the early puerperium in lactating women.

Serum estradiol concentrations at 1-10 months postpartum during lactational amenorrhea were significantly decreased as compared with levels at the follicular phase of normal menstrual cycle while there were no changes in progesterone concentrations (Table 3).

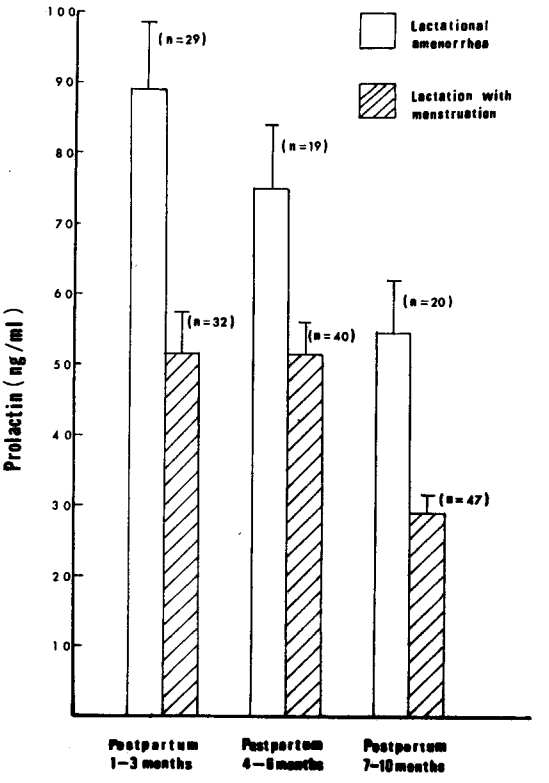

Fig. 2. Serum prolactin levels in lactating women and lactating women with menstruation resumed at different postpartum periods.

Table 2. Basal levels of serum LH, FSH, and prolactin (Mean ± S.E.) in amenorrhic women during lactation at different postpartum period

Post partum months	LH (mIU/ml)	FSH (mIU/ml)	Prolactin (ng/ml)
1-3	17.6±2.8	14.6±1.0	89.2±9.1
	*(n = 31)	(n = 26)	(n = 29)
4-6	24.5±3.2	14.9±1.2	75.1±8.7
	(n = 17)	(n = 16)	(n = 19)
7-10	19.6±2.2	13.3±0.8	54.7±7.4
	(n = 21)	(n = 21)	(n = 20)
Non-puerperal	24.5±1.6	14.2±0.9	13.7±0.6
	(n = 92)	(n = 91)	(n = 32)

* parentheses refer to number of subject.

Table 3. Serum estradiol and progesterone concentrations (Mean ± S.E.) in amenorrhic women during lactation at different post-partum period

Post partum months	Estradiol (pg/ml)	Progesterone (ng/ml)
1-3	45.6±7.4	2.2±0.9
	*(n = 24)	(n = 23)
4-6	51.6±8.3	2.9±1.2
	(n = 18)	(n = 17)
7-10	41.7±7.7	2.1±1.1
	(n = 18)	(n = 17)
Non-puerperal follicular phase	99.4±11.0	2.1±0.2
	(n = 47)	(n = 47)

* parentheses refer to number of subject.

Serum prolactin levels were elevated throughout 1-10 months postpartum in lactating amenorrhic women but decreased as the postpartum period advanced (Table 2 and Fig. 2). Prolactin levels dropped from 89.20 ng/ml at 1-3 months to 75.08 ng/ml at 4-6 months and 54.73 ng/ml at 7-10 months postpartum ($P<0.01$). As compared with lactating amenorrhic women, lactating women with regular menstruation showed a significant decrease in prolactin levels (Fig. 2); 51.39 ng/ml at 1-3 months ($P<0.01$), 49.99 ng/ml at 4-6 months ($P<0.05$), and 28.74 ng/ml at 7-10 months postpartum ($P<0.01$).

DISCUSSION

The present result on pituitary responsiveness to LH-RH in early puerperium may be summarized as; (a) the pituitary was relatively insensi tive to LH-RH during the first 3 weeks following delivery; (b) the recovery of FSH responsiveness to LH-RH occurred earlier than that of LH. Normal FSH response was resumed at 2 weeks while LH response although not normal, started at 3 weeks postpartum; (c) pituitary responsiveness beyond 5 weeks postpartum was similar to that which occurred in normally menstruating women, except that the FSH response was exaggerated; (d) prolactin did not seem to modulate pituitary responsiveness to LH-RH.

Similar results have been obtained by other investigators utilizing LH-RH (Jequier *et al.*, 1973; Canales, *et al.*, 1974; Jeppsson *et al.*, 1974; Le Maire *et al.*, 1974; Reyes *et al.*, 1972). It has been proposed that the recovery of pituitary FSH secretion following delivery requires approximately 10-12 days (Canales *et al.*, 1974). Thereafter, FSH levels increase rapidly whereas the recovery of pituitary LH function is slower, resulting in a high FSH/LH ratio (Reyes, *et al.*, 1972). As HCG disappeared during the first 2 weeks, LH and FSH basal levels thereafter lay within the range of the basal levels during the normal menstrual cycle. However, FSH response to LH-RH was greater during puerperium than during a menstrual cycle. In contrast, LH response to LH-RH was lower at 2 weeks postpartum than during the follicular phase. There was an augmented FSH secretion coinciding with a diminished LH response to LH-RH administration at 2 weeks after delivery.

By 5 weeks postpartum, the pituitary has reached a normal response similar to that shown during the normal menstrual cycle. It should, however, be mentioned that the responsiveness was not yet normal, because the FSH response was greater than that of LH in contrast with what was seen during various phases of the menstrual cycle.

This changing gonadotropin responsiveness during early puerperium may be due to the changing milieu of sex steroids and protein hormones during puerperium. For example, pituitary insensitivity in the immediate postpartum period may be due to the high levels of sex steroids in late pregnancy and early puerperium (Keye and Jaffe, 1976). The suppressive

effect of estrogen on gonadotropin response to LH-RH has also been demonstrated in amenorrhic women (Thompson *et al.*, 1973; Taymor *et al.*, 1974; Yen *et al.*, 1974). Combined oral contraceptive steroids also suppressed gonadotropin response to LH-RH in normally menstruating and lactating-menstruating women (Ryu *et al.*, unpublished). The possible role of progesterone in the suppression of pituitary response to LH-RH has not been determined.

It has been hypothesized that the insensitivity of the pituitary in the early puerperium may also be due to the inhibitory effect of prolactin on gonadotropin response to LH-RH (Seki *et al.*, 1974; Keyes and Jaffe, 1976). In the present study, gonadotropin response to LH-RH, however, augmented at 5-6 weeks when prolactin levels were still very high, suggesting that prolactin does not modify pituitary response to LH-RH. Also, the observation that the recovery of pituitary response to LH-RH is similar in lactating and nonlactating women does not support this conclusion (Jequier *et al.*, 1973; Le Maire, *et al.*, 1974).

From our data, it appears that at least one reason for anovulation during the first four weeks following dilivery is the relative insensitivity of pituitary to hypothalamic stimulation. As a result, gonadotropin concentrations may not rise to levels necessary to stimulate follicular maturation and ovulation. Alternatively, it has been suggested that ovarian insensitivity to gonadotropins, perhaps due to an inhibitory effect of prolactin on the ovary, is the major factor in anovulation and amenorrhea during the four weeks postpartum (Keettel and Bradbury, 1961; Tyson *et al.*, 1972a). This hypothesis is supported by the controlled studies of Zarate *et al.* (1972, 1974) which demonstrated ovarian refractoriness to LH and FSH during the first 20 days of the puerperium. However, Nakano *et al.* (1975) found a significant increase in urinary estrogen in 14 of 18 women who received exogenous gonadotropins during the first four weeks postpartum. Recently, Caro and Woolf (1980) reported that hyperprolactinemia did not inhibit ovarian estradiol secretion, supporting previous study using exogenous gonadotropin in high doses (Archer and Josimovich, 1976). This evidence suggests that the puerperal ovary is sensitive to gonadotropin stimulation and that decreased ovarian sensitivity to gonadotropins does not explain puerperal anovulation.

The apparent anovulation seen beyond 5 weeks postpartum during lactation in our study was not due to pituitary insensitivity to LH-RH. Instead, hypothalamic and/or ovarian factors may be the cause of anovulation. Then, what is responsible for modulating hypothalamus and/or ovary to cause anovulation? Mothers who breastfed their babies usually experienced a greater delay in resumption of menses than nonlactating women (El-Minawi *et al.*, 1971; Tollis *et al.*, 1974; Rolland *et al.*, 1975) and earlier restoration of ovarian function has been observed after prolactin inhibition with bromocryptine (Rolland, *et al.*, 1975). This suggests that in contrast to the early postpartum period, prolactin-dependent mechanisms interfering with cyclic activity may indeed be operative during long term lactation. In the present study, serum prolactin levels were elevated in lactating amenorrhic women throughout 1-10 months postpartum, although gradually decreasing as the postpartum period continued. Lactating women who resumed their menstruation, however, showed a significant decrease in prolactin levels as compared with prolactin values in lactating amenorrhic women. Prolactin elevations provoked with surpiride have led to decrease or abolition of the ovulatory LH peak and to a fall in plasma progesterone in normal women (Delvoye, 1974). The report that stimulation of

prolactin release with T-RH would suppress the pulsatile pattern of LH secretion (Bohnet *et al.*, 1974) is in agreement with data reported in pathologic hyperprolactinemia (Boyar, 1974) and strongly suggests an antigonadotropic action of prolactin. Recent in vitro work has shown that progesterone production by cultured human granulosa cells was inhibited when prolactin concentration in the medium was increased (McNatty, *et al.*, 1974) suggesting an action of prolactin on luteal function. Indeed, short luteal phase and low progesterone secretion were reported to be associated with elevated prolactin levels in some infertile women (del Pozo *et al.*, 1975).

The present study showed that lactating women who resumed their menstruation maintained still significantly higher prolactin levels as compared with nonpuerperal normal values of prolactin (less than 25 ng/ml) in our laboratory. Then, the question might be raised again regrading the levels of prolactin which start to restore the ovulatory cycle. Further study has to be attempted in order to elucidate the exact menchanism by which menstrual cycles are restored during lactation.

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