Neuroendocrine mechanisms of lactational infertility in women

S DIAZ[1]*, M SERON-FERRE[1], HB CROXATTO[1]
and J VELDHUIS[2]

[1] Unidad de Reproducción y Desarrollo, Facultad de Ciencias Biológicas,
P. Universidad Católica de Chile, Santiago, Chile

[2] Department of Medicine, University of Virginia, Charlottesville, Virginia, USA

The current knowledge on the mechanisms of lactational infertility, discussed during a symposium of investigators in this subject, is reviewed. Three periods of lactation are examined: the first weeks postpartum, the period of extended lactational amenorrhea and the recovery of ovarian function. In the first postpartum weeks the inhibition of ovarian function is accounted by diminished pituitary response to GnRH, since exogenous GnRH fails to elicit a LH increase. Suckling can extend the period of ovarian inhibition for weeks, months or years, although it does not fully suppress pulsatile secretion of LH beyond the first weeks. Extended lactational amenorrhea is associated with low LH plasma levels, a great PRL increase in response to suckling, low basal E2 levels and a suppression of estrogen positive feedback. Decreased immunoreactive LH levels may result from partial suppression of the LH pulse generator and a smaller mass of GnRH released in each burst. The role of neurotransmitters, PRL and ovarian factors is discussed. After the recovery of ovulatory cycles suckling still has a residual infertility effect, associated to inadequate luteal function. The sources of variation among women and populations were recognized. Areas in which research is needed to improve the understanding of the mechanisms that sustain lactational amenorrhea are suggested.

Key terms: *breast feeding, lactational infertility, neuroendocrine mechanisms.*

INTRODUCTION

Lactation is an important component of the reproductive process. Breast feeding and lactational infertility are beneficial for the child and the mother, because of their significant contribution to infant health and as a natural birth spacing method. The vast majority of the families of the world live in developing countries where there are no alternatives to breast feeding for the support of infant growth, health and survival. In many of these countries provision and acceptance of modern contraceptives is a problem and the demographic effect of breast feeding cannot be replaced yet.

The physiological mechanisms underlying lactational infertility are not fully understood and the wisdom to manipulate them for increasing the advantages of breast feeding is lacking. To contribute to their understanding, we prepared this review which included the issues discussed in a Symposium (Table I), held in Santiago, Chile, November 23-26,

TABLE I

Participants in the
"Symposium on the Neuroendocrine Mechanism of Human Lactational Infertility",
held in Santiago, Chile, November 23-26, 1991

Name	Affiliation	Country
Hugo Cárdenas	Universidad de Santiago de Chile	Chile
Horacio B Croxatto	P. Universidad Católica de Chile	Chile
Soledad Díaz	P. Universidad Católica de Chile	Chile
Ann Glasier	Center for Reproductive Biology	United Kingdom
Barbara Gross	Westmead and Parramata Hospitals	Australia
Kathy Kennedy	Family Health International	USA
Miriam Labbok	Georgetown University	USA
Alan McNeilly	MRC Reproductive Biology Unit	United Kingdom
Alfredo Pérez	P. Universidad Católica de Chile	Chile
María Serón-Ferré	P. Universidad Católica de Chile	Chile
Mamdouh Shaaban	Assiut University	Egypt
Jean-Christophe Thalabard	Université Claude Bernard	France
Johannes Veldhuis	University of Virginia	USA
Robert Williams	The Howard and Georgeanna Jones Institute for Reproductive Medicine	USA

1991. The objectives of the Symposium were:

a) To review what is known about the neuroendocrine mechanisms involved in lactational infertility in the human female.
b) To identify the questions that remain unanswered and to establish their priority.
c) To agree upon research protocols that would answer the questions identified as most important.
d) To develop a collaborative research strategy to carry out these protocols.

REVIEW OF CURRENT KNOWLEDGE

*Structures and mechanisms involved*

Multiple components contribute to the maintenance of lactational infertility in humans. Unfortunately, data are extremely limited regarding the precise nature, importance, and prevalence of the specific neuroendocrine mechanisms involved. At least the following should be considered: increase in the tonic inhibitory effects of hypothalamic centers on GnRH secretion; lack of activation of the brain pathways that stimulate GnRH secretion; enhanced inhibitory feedback effects of gonadal products on hypothalamic centers; failure of estradiol positive feedback in initiating a midcycle preovulatory LH surge; diminished number or transducing efficacy of GnRH receptors in the anterior pituitary gonadotrophs; defects in the synthesis, processing, and effective exocytosis of biologically active LH; subresponsive ovarian compartments that fail to support the developing Graafian follicle or normal oocyte maturation and ovulation; and impairment of fertilization, preimplantation development or implantation of the blastocyst. The structures and likely pathways of interaction among these factors are summarized in Fig 1.

Stages of postpartum infertility

During the perinatal period, signals between the child and the mother influence both the reproductive functions of the mother and the
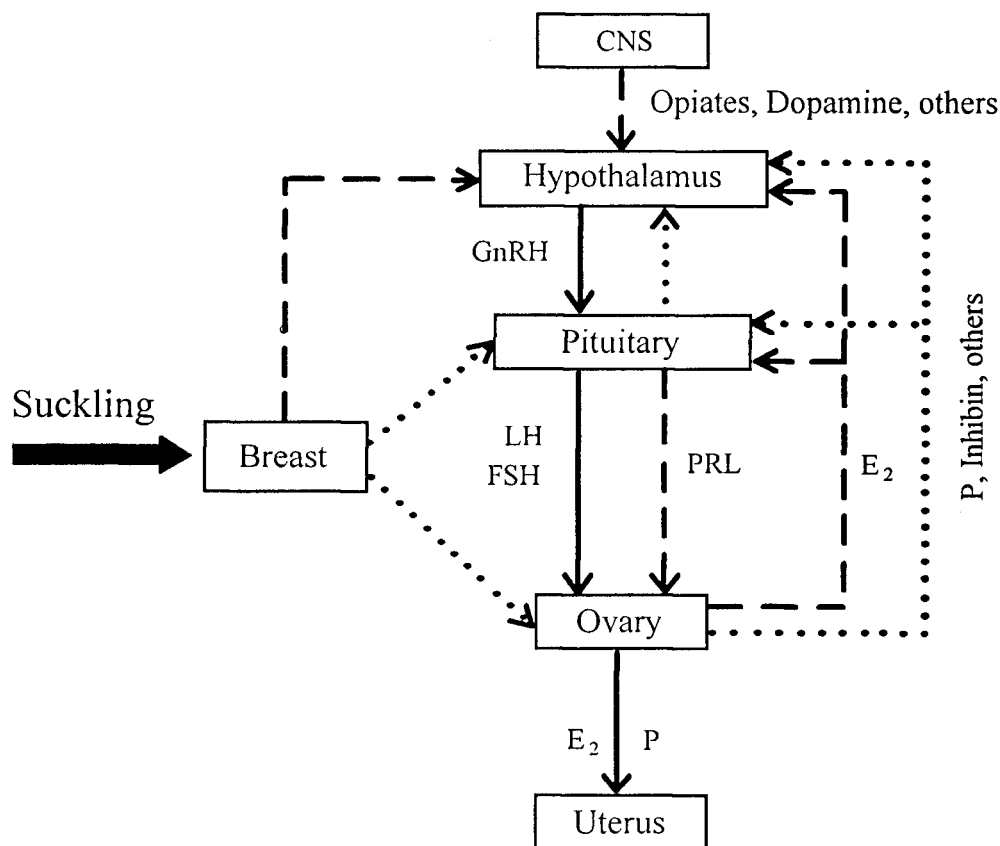
Fig 1. Structures involved in the maintenance of lactational amenorrhea and likely interactions among them. Lines indicate stimulatory or inhibitory influences conveyed via nervous pathways and/or blood-borne substances. Solid lines indicate positive influences. Broken lines indicate known predominantly inhibitory influences. Dotted lines indicate hypothetical inhibitory influences. In some of the above the mediators are unknown. CNS, central nervous system; E_2, estradiol; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; P, progesterone; PRL, prolactin.

growth of the child. These signals do not remain in a steady state during pregnancy and the postpartum period. Therefore, the mechanisms blocking the reproductive function of the mother change over time, in nature and intensity.

Full ovarian function is suppressed during pregnancy. Although not well established, pulsatile GnRH secretion is putatively inhibited by the high levels of steroids present in the circulation. In addition, the possibility exists that placental GnRH and hCG present in the maternal circulation, may desensitize the hypophysis and the ovary to GnRH and LH, respectively.

After pregnancy, suckling and other signals sent by the child change along time, exerting a progressively diminished inhibitory effect on maternal fertility. Three stages can be individualized: the early postpartum

period, the period of extended lactational anovulation and the first menstrual cycles postpartum. The duration of pregnancy and of the early postpartum stage is very similar among all women. In contrast, the duration of the extended lactational amenorrhea varies between women and between population. In some populations the proportion of women who are cycling while fully nursing is around 50% at 6 months postpartum [4] while in others amenorrhea lasts up to 4 years [25,42]. Not only the length of lactational amenorrhea and infertility are variable but the characteristics of the first postpartum cycles also differ between women and populations. These differences are not fully explained by the breast feeding behavior since they occur even among women who are fully nursing with similar suckling frequency and duration and similar infant growth rate.

Early postpartum period

The impact of the inhibitory mechanisms present during pregnancy lasts for about eight weeks after delivery both in nursing and non-nursing women [19, 22, 32]. The main mechanism of ovarian suppression in the early postpartum weeks seems to be a diminished pituitary response to GnRH. The contribution of a reduced activity of the GnRH pulse generator is less clear in the human.

GnRH administration, either as an i.v. or s.c. bolus fails to elicit a LH increase in plasma during the first postpartum month in non nursing [3, 12, 24] and nursing women [3, 23]. Such findings indicate that either the amount of LH secreted immediately postpartum in response to GnRH is significantly attenuated or that the isoforms of LH secreted are not recognized with equal avidity in the immunoassays employed, and/or that the metabolic clearance rate of LH is markedly accelerated at this time. Attenuated gonadotroph responses to GnRH could result from: i) prolonged withdrawal of endogenous GnRH by suppressed hypothalamic GnRH neurons during pregnancy and the immediate postpartum period [11, 26]; ii) decreased pituitary GnRH receptors, possibly secondary to the decrease in hypothalamic GnRH release [38]; and iii) post-GnRH receptor alterations in gonadotroph cell synthesis, post-translational modification, and exocytosis of bioactive LH [15]. It is not known which mechanisms pertain to the human.

There is uncertainty regarding the pattern of spontaneous LH secretion in lactating women during this period. Three studies [14, 30, 40] show extremely low LH plasma levels and no pulsatility. Others detected a normal frequency of LH pulses of low amplitude and, consequently, low LH levels during the third postpartum week [31]. This suggests that the activity of the GnRH-LH pulse generator during breast feeding may vary among women or populations. Differences in LH assay, pulse identification or sampling conditions may also contribute to the discrepancy.

The time at which pituitary function is fully recovered in lactating women has been examined through long-term i.v. adminis-

tration of pulsatile GnRH by pump in two studies done during the second postpartum month [15; Zinaman MJ, personal communication]. At this time, the pituitary responds with FSH and LH secretion that stimulates follicular growth, ovulation and corpus luteum activity. The response vanishes when the pump is discontinued. In addition, the fact that some fully nursing women get pregnant in the second and third postpartum months [4], suggests that the system may fully recover spontaneously. Therefore, just a few weeks after delivery, the pituitary insensitivity to GnRH is no longer an obligatory major factor in ovarian suppression during nursing and other mechanisms keep LH secretion and ovulation depressed.

Extended lactational anovulation

In nursing women, ovulation is inhibited for a long and variable period [4, 22, 32, 33, 37]. Low immunoactive LH levels, as those of the early follicular phase, have been described during lactational amenorrhea [5, 13]. LH pulses similar to those of the early follicular phase have been described from the third month postpartum onwards in three studies [14, 31, Díaz S, unpublished results], although not all women showed pulses and LH pulses when present exhibited large intra and inter individual variability both in frequency and amplitude.

In spite of the observed pulsatility, LH production or secretion does not progress to a preovulatory surge in the majority of women. Besides a diminished pituitary responsiveness to GnRH, other possible mechanisms involved are described in the following paragraphs.

Suppression of the hypothalamic GnRH pulse generator. Central opiatergic pathways stimulated during suckling [16] may contribute to the partial suppression of the pulse generator. However, experiments in which naloxone was administered to nursing women [41] and castrated monkeys [17] during the early postpartum period, have shown no acute increase in LH secretion suggesting that opioids are not involved in the inhibitory process. Since there are no studies with opiate-receptor antagonist in the

period of established lactation, one cannot exclude that such agents can normalize the hypothalamo-pituitary ovarian axis at this stage.

Other CNS and hypothalamic neurotransmitter pathways might also be involved in the suppression of GnRH pulse generator activity, at least in women who show altered pulsatility with respect to either amplitude or frequency. It is not known if a decrease in central dopaminergic tone contributes to the inhibition of pulsatile LH release during lactation in humans. Whether serotoninergic, cholinergic, and/or corticotropin-releasing hormone-dependent pathways contribute to decrease LH release during lactation is not clear.

Enhanced negative feedback effects on the hypothalamo- pituitary unit by one or more products secreted by the ovary. The ovary regulates the pituitary gland by the secretion of both steroidal and non-steroidal products, which are capable of altering hypothalamic control of the pituitary gland and the response of the pituitary gland to hypothalamic releasing factors. For example, estrogens and androgens exert negative feedback effects on hypothalamic GnRH secretion, as well as negative and positive effects on the responsiveness of the anterior pituitary gonadotrophs to GnRH.

The role of the ovary during lactational amenorrhea in the human is not known. Follicular development up to 5 mm is independent of gonadotropins [18]. Two studies found waves of follicular development during lactational amenorrhea and before the first ovulatory cycle [6, Shaaban MM, unpublished results]. These follicles, or the ovarian stroma, may contribute hormones (estrogens, inhibin, etc.) that may exert a negative feedback by themselves. Suckling may increase the sensitivity of the hypothalamo-pituitary unit to such negative feedback.

In the rhesus monkey [47], the ovary exerts a negative feedback upon gonadotropin release in the period of extended amenorrhea. Ovariectomized lactating females show a prompt increase in LH plasma levels in spite of a suckling behavior that keeps LH secretion low in intact females. In humans, LH and FSH levels in plasma were suppressed to a greater extent in lactating than in non lactating women by administration of E_2, suggesting that the negative feedback of estrogens is enhanced during breast feeding [1].

Failure of positive-feedback actions of estrogen on the preovulatory LH surge. The ability of increasing concentrations of estradiol to elicit a gonadotropin surge (positive feedback of estradiol) is impaired during lactational amenorrhea in women and rhesus monkeys [1, 29, 36]. Although the exact mechanisms are not known in detail, an important component of estrogen's facilitatory effect on LH release appears to be an enhancement of GnRH action. Estrogen exposure in experimental animals and in the human amplifies the release of LH in response to successive GnRH stimuli which has been referred to as the self-priming effect of GnRH. Such observations permit the speculation that the estrogen-poor postpartum milieu may cause a loss of the GnRH self-priming phenomenon.

Lack of stimulation of hypothalamic systems that activate pulsatile LH release. In the rat, both excitatory aminoacidergic and noradrenergic pathways, as well as various other peptidergic pathways (*e.g.* galanin, neuropeptide Y) can participate in activating GnRH secretion and, therefore, support pulsatile LH release. Similar data are not currently available in humans. Studies with potent alpha-receptor antagonists, such as phenoxybenzamine and phentolamine, administered in pharmacological doses in a small number of clinical experiments, have failed to implicate alpha-adrenergic pathways in the stimulation of pulsatile LH release in man [43]. Controversy exists regarding the effects of dopamine in humans, although infused dopamine can suppress the pituitary response to GnRH. In addition, alpha methyldopa, a noradrenergic transmission inhibitor, fails to alter pulsatile LH in humans [46]. Consequently, in the subset of women who may exhibit hypopulsatile LH profiles in lactational amenorrhea, the available clinical literature cannot point to a specific relevant CNS neurotransmitter pathway.

Decreased number or function of GnRH receptors. In the rat, suckling decreases the

number of GnRH receptors in the pituitary. The number of receptors is increased by GnRH and decreased by PRL [26, 38]. In addition, impaired coupling of GnRH receptors to intracellular synthesis, processing, and exocytosis of LH may occur during lactation, but has not been described yet.

Alterations in the release of biologically active FSH. FSH is a critical initiator and sustainer of early and mid-follicular growth and development, without which follicular maturation and adequate estradiol secretion to stimulate a preovulatory LH surge cannot be accomplished. In non nursing women, adequate FSH bioactivity is essential to initiate the proliferation of granulosa cells in Graafian follicles, to induce LH and FSH receptors, to promote aromatase activity and generate estradiol. However, FSH secretory patterns and bioactivity have not been investigated during lactational infertility. Plasma FSH concentrations return rapidly (two-three weeks postpartum) to values within the range in cycling non lactating women [3, 12, 24].

The scarce information available in lactating women shows higher concentrations of FSH than in non nursing women and a FSH/LH ratio greater than one [15]. The FSH response to GnRH is greater than the LH response in the first month postpartum [23] but the opposite occurs later on [15]. Although one could expect high FSH levels because of the low E2 levels occurring during lactation, they have not been observed in other studies [1, 5, 29].

Alterations in biological activity of LH. Decreased release of immunoactive LH may be accompanied by lower LH bioactivity [10, 44], and possibly the predominance of more acidic and less potent isoforms of LH in the circulation [21]. Extensive studies in experimental animals indicate that the half-life of gonadotropic hormones and hCG is dependent on the carbohydrate content of the gonadotropic hormone. For example, removal of carbohydrate residues, including sialic acid, from hCG results in a multi-fold decrease in its half-life [21].

Consequently, possible alterations in the post-translational modification of LH in lactational amenorrhea may influence, not only the molecular isoforms of LH present in

the blood, but also the kinetics of removal of LH. An increase in LH bioactivity, assessed by *in vitro* Leydig cell bioassay, occurs during the early stages of recovery of the hypothalamo-pituitary gonadal axis in lactating cows (44). Preliminary data in women show a decrease in the ratio of bioactive/immunoactive LH during breast feeding [Serón-Ferré M, unpublished results], but no difference in LH bioactivity was found between women with short (<180 days) or long (>180 days) periods of lactational amenorrhea.

The role of PRL. High PRL levels are present during lactational amenorrhea [7, 20, 22, 34]. However, women may ovulate spontaneously [4, 32] or when treated with a pulsatile infusion of LHRH [15, Zinaman M, personal communication] and may get pregnant during full nursing with high PRL levels [4]. This evidence suggests that PRL is not a major inhibitory factor but rather a marker of hypothalamic-pituitary response to suckling. On the other hand, women with prolonged lactational amenorrhea show higher PRL levels, a larger PRL response to suckling and a greater bioactivity of PRL in the postpartum period in comparison with women who experience short amenorrhea while fully nursing [2]. Therefore, PRL may act synergistically with other factors at the central level (e.g. reinforcing the negative feedback of estradiol or decreasing GnRH receptors), or contribute to a decreased ovarian response to gonadotropins [9, 45].

First menstrual cycles postpartum

When ovulatory cycles are recovered, luteal phase defects are frequent [8, 28, 37] and the ovulation/ pregnancy ratio per cycle is much higher than in non nursing women. The abnormal characteristics of the first cycles, in the presence of an apparently normal ovulatory gonadotropin pattern, suggest an inhibitory effect of suckling. This effect could be mediated by PRL (9, 45) or other factors at the ovarian level.

Sources of variation among women and populations. Some women reinitiate ovulatory cycles during breast feeding. In some populations, the proportion of women

experiencing menstrual cycles while fully nursing is as high as 50% at 6 months postpartum [4]. Since the first month postpartum, these women show higher estradiol levels, decreased PRL response to suckling, lower LH levels [5], similar LH pulse frequency [Díaz S, unpublished results] and similar ratio bio/immunoactive LH [Serón-Ferré M, unpublished results], when compared to other women who sustain amenorrhea for longer periods. These differences are associated with an early recovery of ovarian function and fertility in spite of frequent suckling and high PRL levels. Several sources of variability can be proposed:

a) Features of the suckling stimulus, other than frequency or duration, such as intensity, distribution along 24 hours, lengths of the longer intervals between nursing episodes, interference by the use of pacifiers or alternation of breasts between episodes have not been studied.
b) The good correlation between the length of lactational infertility among successive pregnancies in the same women suggests genetic components [Gross B, unpublished results]. For example it may be related to the enzymatic pathways for steroid metabolism that show ethnic variations [39]. Different rates of estradiol clearance may affect the feedback mechanisms in which estradiol is involved. An alternative explanation could be repetitive environmental or other factors that may affect the same women in similar ways in successive postpartum intervals.
c) Diet may play a role in many ways. Malnourished women have longer periods of amenorrhea. Low fat intake changes estrogen concentration and metabolism [27,35] and the ratio lean/fat mass affects the peripheral aromatization of androgens [35]. Diet may also incorporate compounds, either natural or environmental pollutants like DDT, that have estrogenic activity.
d) The impact of physical or psychological stress may play a role in the different duration of lactational amenorrhea between populations.

RESEARCH QUESTIONS THAT NEED TO BE ADDRESSED

The participants in the Symposium identified the topics listed below. In all the suggested studies, both time postpartum, type of breast feeding, suckling pattern and stage of ovarian function need to be controlled or determined. The results need to be compared to what occurs in the follicular phase of non nursing, normally cycling women.

1. Gonadotropin profile associated with follicle growth and PRL and steroid secretion need to be studied during early and late stages of lactational amenorrhea and at the time of impending recovery of ovulation. The purpose is to determine what triggers follicular development beyond the antral stage (> 8 mm).
2. Sensitivity to the negative feedback of exogenous estradiol and progesterone and the effect of antiestrogens on the same should be studied at different times postpartum in which different endogenous levels of steroids occur spontaneously. The purpose is to determine the levels of estradiol and progesterone at which the negative feedback of estradiol operates and conversely, the conditions in which it fails.
3. LH and FSH pulsatile patterns should be studied at early and late stages of lactational amenorrhea and at the time of impending ovulation as assessed by ovarian ultrasound. Immunoactive and bioactive gonadotropins and urinary steroid levels should be assayed. The purpose is to determine changes in gonadotropin secretion over postpartum time and their relationship to follicular development.
4. Quantitation of the LH releasable pool and the response of the pituitary to estradiol priming should be done at various stages during lactation. The study should use a dose of GnRH capable of maximally stimulating LH release. The purpose is to assess the contribution of the pituitary to decreased LH secretion and how it is affected by estradiol.
5. The responsiveness of the ovary to administration of exogenous gonadotropin in terms of follicular growth and estradiol and inhibin production should be assessed at different times postpartum using pulsatile administration of either FSH or LH alone, or

FSH/LH (pituitary clamp) or GnRH. The purpose is to determine how postpartum time and breast feeding pattern affect the responsiveness of the ovary to gonadotropins.

6. The pituitary LH and FSH secretion in response to pulsatile administration of different doses of GnRH using an infusion pump. GnRH should be given at different postpartum times before and following estradiol priming. The purpose is to assess pituitary contribution to decreased LH secretion and how it is affected by estrogen levels.

7. The role of the suckling stimulus on the direct suppression of the GnRH signal needs to be studied. It seems important to develop means to evaluate the characteristics of suckling (e.g.: suckling pressure and frequency) and to correlate these characteristics to the degree of hypothalamic-pituitary-ovarian inhibition.

8. The presence and role of PRL receptors in the ovary during lactation needs to be studied in human and primate models to assess if PRL may play a role in ovarian function.

9. The need to study the pituitary sensitivity to GnRH in terms of a dose-response curve, as well as the influence of GnRH antagonist in different doses at different postpartum times and breast feeding stages, was considered.

10. Other issues suggested were steroid metabolism in women who experience different lengths of lactational amenorrhea, the role of breast secretions that may act as inhibitors of the hypothalamic-pituitary-ovarian system, collection of information on induced lactation and ovarian function, the morphology of the ovary at the end of pregnancy, the importance of corpus luteum and endometrial tissue as source of inhibiting factors and the effect of suckling in hypogonadal women.

REFERENCES

1. BAIRD DT, McNEILLY AS, SAWERS RS, SHARPE RM (1979) Failure of estrogen-induced discharge of luteinizing hormone in lactating women. J Clin Endocrinol Metab 49: 500-506
2. CAMPINO C, AMPUERO S, DIAZ S, SERON-FERRE M (1994) Prolactin bioactivity and the duration of lactational amenorrhea. J Clin Endocrinol Metab 79: 970-975
3. CANALES ES, ZARATE A, GARRIDO J, LEON C, SORIA J, SCHALLY AV (1974) Study on the recovery of pituitary FSH function during puerperium using synthetic LRH. J Clin Endocrinol Metab 38: 1140-1142
4. DIAZ S, RODRIGUEZ G, PERALTA O, MIRANDA P, CASADO ME, SALVATIERRA AM, HERREROS C, BRANDEIS A, CROXATTO HB (1988) Lactational amenorrhea and the recovery of ovulation and fertility in fully nursing Chilean women. Contraception 38: 53-67
5. DIAZ S, CARDENAS H, BRANDEIS A, MIRANDA P, SCHIAPPACASSE V, SALVATIERRA AM, HERREROS C, SERON-FERRE M, CROXATTO HB (1991) Early difference in the endocrine profile of long and short lactational amenorrhea. J Clin Endocrinol Metab 72: 196-201
6. DIAZ S, MIRANDA P, BRANDEIS A, CARDENAS H, CROXATTO HB (1991) Mechanism of action of progesterone as contraceptive for lactating women. In: SEPPALA M, HAMBERGER L (eds) Frontiers in Human Reproduction. New York: New York Academy of Sciences. pp 22-29
7. DIAZ S, SERON-FERRE M, CARDENAS H, SCHIAPPACASSE V, BRANDEIS A, CROXATTO HB (1989) Circadian variation of basal plasma prolactin, prolactin response to suckling, and length of amenorrhea in nursing women. J Clin Endocrinol Metab 68: 946-955
8. DIAZ S, CARDENAS H, BRANDEIS A, MIRANDA P, SALVATIERRA AM, CROXATTO HB (1992) Relative contributions of anovulation and luteal phase defect to the reduced pregnancy rate of breastfeeding women. Fertil Steril 58: 498-503
9. DORRINGTON JH, GORE-LANGTON RE (1982) Antigonadal action of prolactin: further studies on the mechanism of inhibition of follicle-stimulating hormone-induced aromatase activity in rat granulosa cell cultures. Endocrinology 110: 1701-1707
10. DUFAU ML, VELDHUIS JD (1987) Pathophysiological relationships between the biological and immunological activities. In: BURGER HG (ed) Bailliere's Clinical Endocrinology and Metabolism. Philadelphia: Saunders. pp 153-176
11. FOX SR, SMITH MS (1984) The suppression of pulsatile luteinizing hormone secretion during lactation in the rat. Endocrinology 115: 2045-2051

12. FRIEDMAN C, GAEKE ME, FANG V, KIM MH
(1976) Pituitary responses to LRH in the postpartum
periods. Am J Obstet Gynecol 124: 75-79

13. GLASIER A, McNEILLY AS, HOWIE PW (1983)
Fertility after childbirth: changes in serum gonado-
trophin levels in bottle and breastfeeding women. Clin
Endocrinol 19: 493-501

14. GLASIER A, McNEILLY AS, HOWIE PW (1984)
Pulsatile secretion of LH in relation to the resumption
of ovarian activity postpartum. Clin Endocrinol 20:
415-426

15. GLASIER A, McNEILLY AS, BAIRD DT (1986)
Induction of ovarian activity by pulsatile infusion of
LHRH in women with lactational amenorrhea. Clin
Endocrinol 24: 243-252

16. GORDON K, RENFREE MB, SHORT RV, CLARKE IJ
(1987) Hypothalamo-pituitary portal blood con-
centrations of ß-endorphin during suckling in the ewe. J
Reprod Fertil 79: 397-408

17. GORDON K, RICHARDSON DW (1988) Differential
opiatergic control of LH and PRL during postpartum
lactational amenorrhea. Biol Reprod 38 (Suppl 1):
SSR6, p 50

18. GOUGEON A, CHAINY GBN (1987) Morphometric
studies of small follicles in ovaries at different ages. J
Reprod Fertil 81: 433-442

19. GRAY RH, CAMPBELL OM, ZACUR HA, LABBOK
MH, MacRAE SL (1987) Postpartum return of ovarian
activity in nonbreastfeeding women monitored by
urinary assays. J Clin Endocrinol Metab 64: 645-650

20. GROSS BA, EASTMAN CJ (1985) Prolactin and the
return of ovulation in breastfeeding women. J Biosoc
Sci, Suppl 9: 25-42

21. HATTORI M, OZAWA K, WAKABAYASHI K (1985)
Sialic acid moiety is responsible for the charge
heterogeneity and the biological potency of rat
luteotropin. Biochem Biophys Res Commun 127: 501-
508

22. HOWIE PW, NcNEILLY AS, HOUSTON MJ, COOK
A, BOYLE H (1982) Fertility after childbirth: infant
feeding patterns, basal PRL levels and postpartum
ovulation. Clin Endocrinol 17: 315-322

23. JEPPSSON S, RANNEVIK G, KULLANDER S (1974)
Studies on the decreased gonadotropin response after
administration of LH/FSH-releasing hormone during
pregnancy and the puerperium. Am J Obstet Gynecol
120: 1029-1043

24. KEYE WR,JR, JAFFE RB (1976) Changing patterns
of FSH and LH response to gonadotropin releasing
hormone in the puerperium. J Clin Endocrinol Metab
42: 1133-1138

25. KONNER M, WORTHMAN C (1980) Nursing
frequency, gonadal function, and birth spacing among
Kung Hunter-gatherers. Science 207: 788-791

26. LEE LR, PAUL SJ, SMITH MS (1989) Dose response
effects of pulsatile GnRH administration on restoration
of pituitary GnRH receptors and pulsatile LH secretion
during lactation. Neuroendocrinology 49: 664-668

27. LONGCOPE C, GORBACH S, GOLDIN B, WOODS
M, DWYER J, MORRILL A, WARRAM J (1987) The
effect of a low fat diet on estrogen metabolism. J Clin
Endocrinol Metab 64: 1246-1250

28. McNEILLY AS, HOWIE PW, HOUSTON MJ, COOK
A, BOYLE H (1982) Fertility after childbirth: adequacy
of postpartum luteal phases. Clin Endocrinol 17: 609-
615

29. McNEILLY AS (1984) Prolactin and ovarian function.
In: MULLER EE, MacLEOD RM (eds) Neuroendocrine
Perspectives. Vol 3. Amsterdam: Elsevier. pp 279-316

30. MORISHITA H, HIGUCHI K, HASHIMOTO T,
KONDO H, TOMIOKA M, KUROIWA S (1987) Study

on the hyperresponsiveness of pituitary FSH to GnRH 6
weeks after delivery. Gynecol Obstet Invest 23: 110-116

31. NUNLEY WC, URBAN RJ, EVANS WS, VELDHUIS
JD (1991) Preservation of pulsatile luteinizing hormone
release during postpartum lactational amenorrhea. J Clin
Endocrinol Metab 73: 629-636

32. PEREZ A, VELA P, MASNICK GS, POTTER RG
(1972) First ovulation after childbirth: the effect of
breastfeeding. Am J Obstet Gynecol 114: 1041-1047

33. RIVERA R, KENNEDY KI, ORTIZ E, BARRERA M,
BHIWANDIWALA PP (1988) Breastfeeding and the
return to ovulation in Durango, Mexico. Fertil Steril 49:
780-787

34. ROLLAND R, DE JONG FH, SCHELLEKENS LA,
LEQUIN RM (1975) The role of prolactin in the
restoration of ovarian function during the early
postpartum period in the human female. Clin Endocrinol
4: 27-38

35. ROSE DP, COHEN LA, BERKE B, BOYAR AP (1987)
Effect of a low-fat diet on hormone levels in women
with cystic breast disease. II. Serum radioim-
munoassayable prolactin and growth hormone and
bioactive lactogenic hormones. J Natl Cancer Inst 78:
627-631

36. SCHALLENBERGER E, RICHARDSON DW,
KNOBIL E (1981) Role of prolactin in the lactational
amenorrhea of the rhesus monkey (Macaca mulatta).
Biol Reprod 25: 370-374

37. SHAABAN MM, SAYED GH, GHANEIMAH SA
(1987) The recovery of ovarian function during breast-
feeding. J Steroid Biochem 27: 1043-1052

38. SMITH S, LEE LR (1989) Modulation of pituitary
gonadotropin- releasing hormone receptors during
lactation in the rat. Endocrinology 124: 1456-1461

39. SNOW R (1994) Body fat, fat topography and
endogenous steroid metabolism. In: SNOW R, HALL P
(eds) Steroid Contraceptives and women's response.
New York: Plenum. pp 179-186

40. TAY CCK, GLASIER AF, McNEILLY AS (1992) The
24 h pattern of pulsatile luteinizing hormone, follicle
stimulating hormone and prolactin release during the
first 8 weeks of lactational amenorrhea in breastfeeding
women. Hum Reprod 7: 951-958

41. TAY CCK, GLASIER AF, McNEILLY AS (1993)
Effect of antagonists of dopamine and opiates on the
basal and GnRH-induced secretion of luteinizing
hormone, follicle stimulating hormone and prolactin
during lactational amenorrhea in breastfeeding women.
Hum Reprod 8: 532-539

42. VAN GINNEKEN JK (1977) The chance of conception
during lactation. J Biosoc Sci, Suppl 4: 41-54

43. VELDHUIS JD, ROGOL AD, WILLIAMS FA,
JONHSON ML (1983) Do alpha-adrenergic mech-
anisms regulate spontaneous or opiate-modulated
pulsatile LH secretion in man. J Clin Endocrinol Metab
57: 1292-1296

44. WEESNER GD, NORRIS TA, FORREST DW,
HARMS PG (1987) Biological activity of luteinizing
hormone in the peripartum cow: least activity at
parturition with an increase throughout the postpartum
interval. Biol Reprod 37: 851-858

45. VELDHUIS JD, HAMMOND JM (1980) Oestrogens
regulate divergent effects of prolactin in the ovary.
Nature 284: 262-264

46. WHITWORTH NS (1988) Lactation in humans.
Psychoneuroendocrinology 13: 171-188

47. WILLIAMS RF, GORDON K (1990) Postpartum
lactational inhibition of reproductive function in primates:
the significance of ovarian components in the prolonged
suckling-induced inhibition of gonadotropin secretion.
Endocrine Society, 72nd Ann Meet. Atlanta. Abstract 664