HORMONAL PROFILE IN PROLONGED LACTATION

THESIS
SUBMITTED IN PARTIAL FULFILMENT OF M.D. DEGREE IN OBSTETRICS AND GYNAECOLOGY

BY

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M. B.B. Ch. and M. Sc. (Obstetrics and Gynaecology)

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INTRODUCTION
AND
AIM OF WORK
INTRODUCTION AND AIM OF WORK

Breast feeding is a highly emotive subject, the advocates of which have often depended more on personal beliefs and anecdotal evidence than on subtle scientific data. In recent years, there has been a phenomenal increase in the use of cliches such as "breast is best", both as regards to its nutritional and contraceptive values. Survival and multiplication of man throughout aeons of breast feeding may support such somewhat simplistic naturopathic view that "breast is best".

For centuries women have viewed breast feeding as a major contraception. It has been used as the primary means of child spacing in many traditional cultures (Salber et al., 1965; Potter et al., 1965; Berman et al., 1972). Moreover, the pronounced urban-rural difference in birth intervals in all areas of the world may support such breast-feeding practice impact (Delgado et al., 1978; Prema et al., 1979; Howie and McNeilly, 1983). Today, there is a growing tendency to promote the habit of breast feeding for 1-2 years in an attempt to postpone a new pregnancy.

The details of how fertility is inhibited during maintained lactation are still open to speculation. This entails the study of the extent of hypothalamic-pituitary ovarian impairment during maintained breast feeding.

Several investigators attempted to study such impairment using endometrial biopsy (Topkins, 1943; Sharman, 1951), basal body temperature (Lyon and Stamm, 1946) or cervical mucus (Perez et al., 1972). However, all these methods failed to give adequate explanation.

With the introduction of hormonal assay procedures, direct steps have been taken towards the correct clarification of hypothalamic-pituitary ovarian function during lactation. However, most of these studies have
missed to great extent to investigate the endocrine milieu during prolonged breast feeding.

The present work aims at studying various reproductive hormones profiles in prolonged lactation (one an half year or more), with or without menstruation, and comparing the hormonal profile with matching groups lactating for 6 and 12 months.

**Specific Aims:**

1 - The study of serum levels of FSH, LH and progesterone in lactating menstruating and amenorrhoic females.

2 - Evaluation of the difference in serum hormone levels between menstruating and amenorrhoic females.

3 - The study of corpus luteum function in prolonged lactation.

4 - The study of the degree of hyperprolactinemia in prolonged lactation and any possible relationship between it and corpus luteum function.
REVIEW
OF
LITERATURE
PHYSIOLOGY OF LACTATION

Lactation comprises two main phases:

First, milk is secreted and stored within the mammary gland (the phase of milk secretion).

Second, the stored milk must be made available to suckling as required (the phase of milk removal).

The recognition of these phases is necessary for the proper understanding of the physiological processes involved in lactation.

MILK SECRETION

Milk secretion comprises the process by which the alveolar cells first synthesize milk constituents from precursor substances derived from the blood and then pass or excrete these substrates into the lumen of the alveolus in which the milk, so formed, is stored. There is no evidence that the activity of the mammary alveolar cell is in any way controlled by secretory nerves; indeed the mammary gland can be transplanted to another part of the body and will secrete milk normally as long as satisfactory vascular connection with the general circulation is achieved (Linzell, 1963). The secretory activity of the mammary gland is regulated by the endocrine system and in particular by the hormones of the anterior pituitary. The first evidence of the active participation of the anterior pituitary hormones in the initiation of milk secretion or lactogenesis was observed in 1928, when injections of anterior pituitary extracts induced copious milk secretion in the mammary glands of pseudopregnant rabbits.
(Stricker, 1951). In 1933, Riddle and his colleagues, showed that the lactogenic effects of pituitary extracts were associated with the active substance present in the extracts that induced growth of the crop gland when injected into pigeons. This response, the now classical "pigeon-crop" test for prolactin, greatly facilitates the preparation of prolactin from ruminant pituitaries in a highly purified form - the first of the anterior pituitary hormones to be so obtained. While prolactin gave a dramatic effect in pseudopregnant rabbits, its lactogenic effects in another species were less marked; it became evident that in other species other hormones were less involved. So it was thought of an anterior-pituitary lactogenic complex of hormones rather than a single lactogenic hormone (Cowie, 1977). The component of the complex have been studied in a few species by replacement studies in hypophysectomized animals. It is now known that the differentiation of terminal alveolar cells into active milk-secreting units requires the availability of insulin, prolactin and cortisol, as well as, oestrogen and progesterone (Speroff et al., 1983).

METHODS OF STUDY OF HORMONAL CONTROL OF MILK SECRETION

The main lines of research that have led to most of the information in this field are the in vitro culture of mammary tissue and examination of its hormonal requirements for lactogenesis, the in vivo administration of exogenous hormones, with or without removal of endocrine glands, and the measurement of endogenous concentrations of lactogenic hormones during pregnancy and lactation.
A) Studies using organ culture of mammary tissue:

The technique used in vitro is the culture of small pieces of mammary tissue containing ducts and alveoli from animals at varying stages of physiological development. The culture can be maintained for 1-2 weeks, during which time hormone stimulation can be measured either physiologically or biochemically. Tissues obtained from mice, rats, rabbits and women have been extensively studied with considerable general agreement as to the effects of hormones on the mammary development and lactogenesis (Falconer, 1980).

Mammary ducts and alveoli are hormone responsive from early fetal development. The primary mammary spout, that grows out on day 16 of fetal life in the mouse, and at 17 days mammary analagen of fetal rats respond to culture with insulin, aldosterone and prolactin by extensive development of a duct system and formation of intracellular organelles (Ceriani et al., 1970). Progesterone added to the three hormones gives rise to secretion containing casein-like proteins within 9 days of culture (Barnawell, 1965).

Mammary tissue obtained from rabbit on days 12-14 of pseudopregnancy, which has proliferated in vivo under the influence of endogenous hormones, can be stimulated to lactate in culture by prolactin alone (Forsyth et al., 1972). Results of other studies have shown lactogenic stimulation of alveolar tissue, from rabbits, cultured in the presence of placental lactogens and human growth hormone - polypeptide hormones with close similarity to prolactin (Falconer, 1980).

To summarize the results obtained from studies using organ cultures of mammary tissue, insulin and an adrenal corticosteroid appear to be
necessary for maximum growth and division of alveolar epithelial cells. In spite of this, these hormones are not lactogenic. Gonadal steroids stimulate selective duct and lobular growth in mammary explants but are not lactogenic in \textit{vitro}. Lactogenesis in \textit{vitro} appears to be dependant upon the presence of prolactin or the closely related hormones, human placental lactogen and growth hormone.

\textbf{B) Studies in surgically modified or intact animals:}

Investigations of the effects of surgical ablation of endocrine glands on the initiation of lactation have resulted in comprehensive understanding of the hormone requirements for mammary growth and lactation in \textit{vivo}. In particular, replacement of hormones in hypophysectomized animals or hypophysectomized, overiectomized and adrenalectomized animals have allowed the effects of individual hormones and combinations to be assessed (\textit{Lyons, 1958}). In the triply operated rat, the minimum hormone requirements for mammary duct growth are oestrogen, adrenal steroids and growth hormone. For lobulo-alveolar growth, progesterone and prolactin are additionally required (\textit{Falconer, 1980}).

\textbf{C) Measurements of endogenous concentrations of lactogenic hormones:}

The introduction of sensitive bioassays for lactogenic activity and later of radioimmunoassay, has provided much information on the concentrations of lactogenic hormones in the blood under a range of physiological conditions including pregnancy and lactation. Even more recently the development of receptor assays for prolactin that combined the biological relevance
of the bioassay with the sensitivity and sample capacity of radioimmunoassays is yet a further technical aid to progress in this field (Shiu et al., 1973).

HORMONAL CONTROL OF MILK SECRETION

The onset of milk secretion from the developed mammary gland occurs near the time of parturition, but the timing is difficult to determine with accuracy. Milk constituents begin to appear well before parturition but high rates of synthesis do not occur until after birth (Falconer, 1980).

The principal hormone involved in milk biosynthesis is prolactin. Without prolactin, synthesis of the primary protein, casein, will not occur and true milk secretion will be impossible. The administration of certain pharmacological agents that inhibit the release of prolactin from the pituitary, thereby lowering its concentration in the blood, rapidly inhibits milk secretion. One such agent is bromocriptine which will prevent the initiation of milk secretion after delivery and will also inhibit the established lactation (Schams et al., 1972). Moreover, the loss of prolactin secretion in intrapartum pituitary necrosis was followed by a failure to lactate (Tyson et al., 1975a). In an experimental study, casein mRNA was measured in organ culture of rat mammary gland within one hour after addition of prolactin. Casein mRNA was observed to increase above the amount observed in cultures containing insulin and hydrocortisone alone. The effect was not dependent on glucocorticoids but maximum stimulation only occurred in its presence (Matusik and Rosen, 1978). Prolactin is, therefore, an essential component of the hormonal complex involved in the initiation and maintenance of lactation.

During pregnancy prolactin is found to undergo a gradual rise until term
preparing the breast for milk production. The mean basal prolactin concentration in normal gestation rises from 24.8 ± 2.4 ng / ml beginning at about 7-8 weeks of gestation, to a mean of 207.3 ± 46.8 ng / ml at term (Tyson et al., 1972 a). The initial rise at 7-8 weeks of gestation occurs within few days after oestradiol levels start to increase markedly above the non-pregnant level (Barberia et al., 1975).

Tyson and Friesen (1973), studied prolactin levels in six women between seven and forty weeks of gestation after intravenous thyrotropin-releasing hormone (TRH) and found marked increase in prolactin levels which is more pronounced in late pregnancy. The enhanced prolactin secretion in late pregnancy is probably related to hyperplasia and hypertrophy of the pituitary pregnancy cells. Prolactin level does not change appreciably prior to the onset of labour. During labour, maternal serum prolactin levels were found to decline beginning approximately 4 hours before delivery. Immediately following delivery they rise and then decline over the next 24 hours to levels found in the normal postpartum period. Prolactin is unlikely involved in or influenced by the hormonal interplay that occurs during labour (Gregoriou et al., 1979).

The differentiation of terminal alveolar cells into active milk-secreting units requires the preparatory events induced during pregnancy, that is, the availability of prolactin, insulin and cortisol as well as oestrogen and progesterone (Kase, 1983). Increased prolactin in pregnancy is essential for the development of lobular-alveolar differentiation of the breast. Oestrogen alone can promote ductal proliferation and, synergistically, with progesterone result in the development of lobules of alveoli, which are the primary secretory structures of the breast. Some prolactin appears to be
necessary for this alveolar differentiation (Josimovich and Archer, 1978). The increased availability of free cortisol results in extensive proliferation of endoplasmic reticulum necessary for subsequent production of milk proteins. The increased level of insulin will help in alveolar cell division (Kase, 1983).

Although prolactin stimulates significant breast growth and is available for lactation, only colostrum (composed of desquamated epithelial cells and transudate) is produced during gestation. Full lactation is inhibited by oestrogen and progesterone which interfere with prolactin action at the alveolar cell prolactin receptor level (Bruce and Ramirez, 1970).

Made by the placenta and actively secreted into the maternal circulation is the placental lactogen. It interacts with prolactin binding sites and is believed to have a role in the development of mammary gland and milk production (Gorski, 1979). Research on rodents, ruminant and human placental lactogen has raised the question of their physiological roles for the mammary gland. In rats, placental lactogen shows a peak in blood concentration at about the 12th day of pregnancy, with a fall until days 17–21 when a rise is followed by a sharp fall just before parturition. On the other hand, prolactin concentrations were found to be low in the latter part of pregnancy, rising just before parturition. It is thus likely that in rat, placental lactogen has an effect during the middle of pregnancy, when major growth of the ducts and alveolar tissue of the mammary gland is occurring (Josimovich et al., 1974).

Bolander and his co-workers (1976), measured placental lactogen in the serum of dairy and beef cattle and showed that there is a substantial increase in circulating hormone in the last 100 days of pregnancy. The
higher milk producing cows had significantly higher placental lactogen concentration in the plasma. On the other hand, the ability of the rabbit to lactate after pseudopregnancy and the successful induction of lactation by exogenous steroid hormones in non-pregnant animals shows that their presence is not obligatory for mammary development and lactation (Falconer, 1980).

The human placental lactogen rises progressively during the second half of pregnancy reaching a level of approximately 6000 ng/ml at term. Though displaying less activity than prolactin, placental lactogen is produced in such large amounts that, although not conclusively demonstrated, it may exert a lactogenic effect (Speroff et al., 1983). At parturition the level of placental lactogen falls rapidly to about 100 ng/ml some 24 hours after delivery and to less than 2 ng/ml after 24 hours (Cowie, 1977).

The hormonal trigger for initiation of milk production within the alveolar cell and its secretion into the lumen of the gland is the rapid disappearance of oestrogen and progesterone from the circulation after delivery. The clearance of prolactin is much slower, requiring 7 days to reach non-pregnant levels in non-breast-feeding women. The discordant hormonal events result in removal of the progesterone and oestrogen inhibition of prolactin action on the breast (Klefski et al., 1980). Breast engorgement begins 3-4 days postpartum when steroids have been sufficiently cleared. It was considered possible that the degree of breast engorgement in non-breast-feeding women might relate to the levels of prolactin since lactation can be completely inhibited with bromergocriptine (Schams et al., 1972). However, West and McNeilly (1979), found no direct relationship between the degree of breast engorgement (and presumably the ability of the breast to secrete milk), and the actual level of circulating prolactin.
It is uncertain which of the placental steroids acts as the inhibitor, or how this inhibition is brought about. Evidences in animals suggest that the active steroid may be progesterone. In vitro studies demonstrated that progesterone inhibited lactogenesis in explants of mammary gland in culture (Turkington and Hill, 1969). Milk secretion can be induced in vivo in pseudopregnant rabbit by the injection of prolactin. When progesterone was injected simultaneously with prolactin milk secretion was suppressed (Assairi et al., 1974). Moreover, it has been widely shown that removal of the corpus luteum, or ovaries, from pregnant animals initiates lactation (Archer, 1980). The mechanism of action of progesterone is not well understood, but it appears to be acting in pleiotropic manner. Progesterone may inhibit the synthesis of prolactin receptors, prevent the nuclear translocation of glucocorticoid receptors or directly inhibit casein mRNA transcription (Matusik and Rosen, 1978). This inhibition of mRNA is dose dependent, with 30, 50, and 100% inhibition at molar concentrations that are 7-, 14-, and 70-folds greater, respectively, than the molar concentration of prolactin (Matusik and Rosen, 1978).

Oestrogen, on the other hand, presents a dual role with respect to the pituitary-mammary axis. First, the prolonged oestrogen stimulation during pregnancy results in increased prolactin secretion. Yen and co-workers (1974), have indicated a positive feedback relationship between oestrogen and prolactin at hypothalamic level where administration of 1 ug/kg/day of ethinyl oestradiol, a pharmacological dose of oestrogen, induced a significant elevation of serum prolactin levels in four hypogonadal women. Oestrogens have two effects on the pituitary resulting in such increased prolactin levels: 1) increasing the release of pituitary
prolactin, principally by increasing the number of membrane receptors, although a direct effect on biosynthesis of prolactin by the cell cannot be excluded and 2) a physiologic hyperplasia of the pituitary lactotrophes resulting in a numerical increase in these cells (Noel et al., 1974a - Tyson et al., 1975a - Archer, 1980). A strong correlation between peripheral serum prolactin levels and number of pituitary lactotrophes has been found in experimental animals but have not been reported in human to date (Djiane et al., 1977).

Next, it inhibits the effect of elevated prolactin concentrations on mammary alveolar cells (Fournier et al., 1974). Several studies indicate that the maintenance of elevated oestrogen levels after exogenous administration of oestrogen inhibits lactation by blocking the peripheral action of prolactin. Therefore, maintenance of steroidal inhibition (oestrogen and progesterone) is effective in preventing postpartum milk synthesis and secretion (Burn et al., 1974, Gezelle et al., 1979).

After delivery, if a woman does not breast-feed her baby, the prolactin levels will return to normal in 2 to 3 weeks (Fournier et al., 1974). In women who breast-feed, however, Tyson and associates (1972a), presented a detailed description of how prolactin responses to suckling. In the first postpartum week, prolactin levels in breast-feeding women decline approximately 50%. Suckling, however, leads to increments in prolactin which can be divided into three distinct patterns. The first occurs in the immediate puerperium, i.e. the first postpartum week, where there is a high basal rate with maximal postsuckling rise of 50% appearing after 30 minutes of suckling. In the next 2 to 3 months, the pattern is much more stable (approximately 40-50 ng/ml basal level) and associated with the greatest increments in postsuckling prolactin about 10-20 folds.
appearing 10 to 30 minutes of breast feeding. After 3–4 months, basal levels are normal, but suckling still produces an increase that is essential for continuing milk production. Despite the diminished response of prolactin release to suckling, and the return to near basal levels in the blood, milk production may be abundant suggesting that prolactin concentrations in the blood and milk production bear no direct relationship to each other (Rolland et al., 1975). Nevertheless, there is clear evidence that a minimum level of prolactin in blood must be maintained for initiation and maintainance of milk production (Speroff et al., 1983). Such transient increase above the normal basal level of prolactin is only maintained by continuing suckling.

Suckling supresses the formation of a hypothalamic substance, prolactin-inhibiting factor (PIF). This intrahypothalamic effect is either mediated by dopamine, or in contrast to the peptide nature of other hypothalamic hormones, PIF is dopamine itself (Macleod and Lehmeyer, 1974). Dopamine is secreted by the basal hypothalamus into the portal system and conducted to the anterior pituitary to act on the prolactin cell to inhibit its secretion. Dopamine supresses secretion of prolactin into the general circulation; in its absence prolactin is secreted. The secretion of PIF is controlled by hypothalamic catecholamine levels. Conditions leading to a depletion of catecholamines cause a decrease of PIF secretion, leading to an increase in prolactin release, whereas the opposite effect is observed with an increase in dopamine levels in the hypothalamus (Fournier et al., 1974). Pharmacological agents which increase prolactin secretion by decreasing effective catecholamine levels include reserpine, phenothiazines, methylidopa, etc. L. dopamine have the opposite effect (Kase, 1983).

Prolactin may also be influenced by a positive hypothalamic factor;
prolactin releasing factor (PRF). PRF identity has not been elucidated. It is possible that thyrotropin-releasing hormone (TRH) can be as active as a PRF (Noel et al., 1974b). In patients in the postpartum period, TRH administration leads to a prompt and exaggerated release of prolactin which is followed several hours later by breast engorgement and milk leakage (Jacobs et al., 1971; Noel et al., 1974b; Rabello et al., 1974). However, the stability of plasma thyroid-stimulating hormone (TSH) levels in nursing mothers provides evidence for a neurogenic stimulus for prolactin release which is independent of TRH (Frantz, 1981).

With use of TRH, chlorpromazine and L-dopamine considerable information can be obtained about the integrity of the hypothalamic-pituitary prolactin axis (Tyson et al., 1975a). A response to TRH indicates the presence of functional pituitary tissue. If the TRH response is normal, failure to respond to chlorpromazine would indicate a hypothalamic disorder. Again, should the pituitary be functioning autonomously, L-dopamine would fail to depress the prolactin level.

Growth hormone has been implicated in the maintenance of milk yield in dairy cows, whether this is a direct hormone effect or an indirect one through increasing the availability of nutrients, is not clear. There is no increase in growth hormone levels in the blood in response to breast-feeding (Noel et al., 1974a). Moreover, the fact that ateliotic dwarfs who are essentially completely lacking in growth hormone develop breasts and lactate normally postpartum, suggest that growth hormone is not important for lactation in humans (Frantz 1981).

MACHANISMS OF LACTOGENESIS

All polypeptide hormones initiate their action by binding to specific
reports on the plasma membrane of cells. Specific binding sites have been identified by showing that $^{125}$I-labeled hormone bound to a membrane preparation can be displaced by unlabeled biologically active hormone (Posner et al., 1974).

Prolactin, as previously indicated, is the chief hormone involved in milk synthesis. Prolactin appears to initiate its action by interacting with a specific receptor on the cell surface of the mammary epithelial cell. This mode of action was first suggested by Turkington (1970), who showed that prolactin covalently linked to sepharous beads is biologically active in mouse mammary epithelial cells. The direct interaction of prolactin with membrane receptors was also shown with autoradiographic techniques in rabbit mammary glands by Falconer (1972). Whether the labeled prolactin was administered intravenously or intraductally, the bound hormone was localized only on the cell membrane adjacent to capillaries and never on the luminal surface. This suggests that the binding sites are not uniformly distributed on the cell surface. Because prolactin-sepharous complexes are many times larger than a mammary cell, it is very unlikely that prolactin can enter the cell. Fournier et al. (1974), concluded that prolactin initiates its effect by an action on the cell membrane. In the last few years, much information on prolactin receptors has accumulated.

Prolactin receptor, by analogy with many other membrane proteins, appears to be a glycoprotein with its hydrophobic portion embedded in the lipid bilayer of the plasma membrane and its hydrophilic portion, which contains the carbohydrates moiety, protruding outwards (Shiu and Friesen, 1980). The membrane receptor sites for prolactin exhibit very high affinity for the hormone. Binding of prolactin to the receptors occurs readily at
physiological concentrations of the hormone. Binding of prolactin to the receptor sites occurs at a hormone concentration of about 7 ng ml$^{-1}$ (Shiu et al., 1973).

In order to demonstrate unequivocally that the membrane receptor sites mediate the lactogenic response of prolactin in the mammary gland and that the binding of prolactin to membrane receptor represent the first step in the action of hormone, anti-receptor antibodies were raised in guinea-pig. These antibodies blocked binding of prolactin to its receptors but showed no similar effect on insulin. Also they prevent prolactin-mediated incorporation of leucine into casein in explants of rabbit mammary gland (Shiu and Friesen, 1976). These findings provide direct evidence for an obligatory functional role of a membrane receptor in mediating the action of prolactin.

Since prolactin receptors are obligatory for mediating some, if not all, of the actions of the hormone, the receptors may be important in determining the sensitivity of the tissue to the hormone. If this is the case, then biological factors that affect tissue sensitivity to prolactin may also influence the activity of the receptor where receptor number varies with the physiological condition of the tissue (Shiu and Friesen, 1980). Frantz et al. (1974), demonstrated that prolactin binding activity in lactating rat mammary gland is three times that in pregnant tissue. In rat mammary slices, prolactin binding remains low until parturition when a large and sustained twenty folds - increase occurs. Moreover, the lactational increase in receptor content is abolished when suckling by pups is prevented. These findings suggest that prolactin release caused by suckling increase prolactin receptor levels. Self induction of receptors by prolactin is observed in the rat liver (Posner, 1976). Some of the increase in prolactin receptor
occurring naturally at parturition and in early lactation is probably due to an increase in the number of secretory epithelial cells, since prolactin is demonstrably mitogenic for the mammary epithelium (Bourne et al., 1974). So, it is clear that not only do fluctuations affect the mammary tissue directly but the numbers of hormone receptors in mammary tissue also vary. The increase in prolactin receptors caused by prolactin, appears to be a positive feedback system for accelerating lactogenesis, probably acting through an increase in receptors per cell, and an increase in cell number (Falconer, 1980). It is believed that progesterone inhibits prolactin lactogenesis function during pregnancy by decreasing prolactin receptors (Matusik and Rosen, 1978).

As prolactin exerts biological effects while bound to receptors on the plasma membrane of the cell, it is necessary to postulate a "second messenger" system, whereby nuclear function can be modulated. Alternatively, if intact prolactin membrane or biologically active fragments are taken into the cells together with their receptor, and then the hormone or its fragments interact with the nucleus to stimulate lactogenesis, then the prolactin receptors are simply a system for internalizing prolactin in alveolar cells (Goldstein et al., 1979). Although internalization of polypeptides does occur and is a major degenerative pathway, there is no evidence at present that prolactin is internalized in order to function or that hormone fragments are biologically active. The postulated second messenger for prolactin has, therefore, been assiduously sought (Falconer, 1980).

What then is the second messenger system, if any, for prolactin? Unlike that of most polypeptide hormones, prolactin binding to the cell membrane receptor does not lead to activation of membrane-bound
enzyme adenylate cyclase. So cyclic 3,5 - adenosine mono - phosphate has no stimulatory effect. Furthermore, cyclic 3,5- guanosine monophosphate together with spermidine have no effect alone (Majumder and Turkington, 1971). Rillema and his colleagues (1977) suggest activation by prolactin of the membrane - associated enzyme, phospholipase A. Since the activation of phospholipase is a key rate - limiting step in prostaglandin synthesis, this suggests that some of the effect of prolactin are mediated by prostaglandins. This notion is supported by studies showing that prostaglandins B2, E2 and F2 α stimulate RNA synthesis in mammary explants of mice in a prolactin - like manner (Rillema, 1975). Moreover, indomethacin, an inhibitor of prostaglandin synthesis, blocks the stimulatory effect of prolactin. Despite these facts, prostaglandins cannot mimic the effect of prolactin on casein synthesis (Rillema et al., 1977). Additional factors are, therefore, required. Increased tissue polyamines are widely associated with hormone responses, and the key enzyme for their synthesis, ornithine decarboxylase, rapidly increases in activity upon stimulation of many tissues (Thomson and Richards, 1978). This effect is, however, highly non - specific either for prolactin or the mammary gland. Spermidine alone stimulates RNA synthesis in perfused lactating mammary glands (Mepham and Peters, 1978). This indicates an effect at nuclear level. A combination of polyamines (spermidine) and prostaglandins exhibit a prolactin - like effect (Shiu and Friesen, 1980). It is clear that these biologically active compounds have a potential role in normal hormonal stimulation but their implications for the initiation of milk synthesis await further research.

Protein phosphorylation has been implicated as an intermediate step of prolactin action in the mammary gland (Turkington, 1973). In mouse
RNA synthesis, such as actinomycin-D. Therefore, induction of milk protein is dependent upon the preceding increase in RNA formation (Shiv and Friesen, 1980). Prolactin stimulates synthesis of milk proteins at the transcriptional level through accumulation of messenger RNA for casein and α-lactalbumin (Matusik and Rosen, 1978). Hydrocortisone potentiates while progesterone inhibits the prolactin response on the accumulation of casein mRNA and therefore the stimulation of casein synthesis and secretion (Turkington, 1973). Progesterone results in low ratio of bound/free polyribosomes and this might be due to the absence of developed endoplasmic reticulum in the mammary gland. Moreover, with progesterone there is complete absence of large aggregates of polyribosomes from the mammary gland cells which might be simply due to the lack of mRNA coding for milk protein synthesis in these ribosomes (Assairi et al., 1974).

The lactose synthetase system is unique to the mammary gland. This enzyme catalyzes the formation of lactose. It contains two subunits, A and B. The A-protein is a galactosyl transferase, the catalytic subunit; the B-protein is α-lactalbumin, the regulatory subunit of lactose synthetase (Shiv and Friesen, 1980). This enzyme is under hormonal control. In the intact mouse, the activity of A-protein increases throughout the second half of gestation and remains high during lactation. The activity of the B-protein (α-lactalbumin), on the other hand, remains low throughout pregnancy but increases dramatically with the initiation of lactation (Turkington et al., 1968). The lactose synthetase activity of the mammary gland of pseudopregnant rabbits increased after injecting low concentrations of prolactin. This effect occurred earlier (after 48 hours)
and was more marked than was the increase in galactosyl transferase activity (after 48 hours and 96 hours). This suggests that the stimulation of lactose synthetase activity which occurs during the first two days of prolactin treatment without modification of galactosyl transferase activity corresponds to the induction or stimulation of a low initial rate of synthesis of \( \alpha \)-lactalalbumin. The considerable increase in the two enzymes activities 48 and 96 hours after prolactin treatment might be the result of a stimulated synthesis of the two components. The induction of lactose synthetase was completely suppressed by simultaneous injection of progesterone during the first two days of treatment with prolactin and this corresponds to the absence of lactose synthetase (Assairi et al., 1974). Tri-iodothyronine (T\(_3\)) potentiates while progesterone blocks the stimulatory effect of prolactin on lactose synthetase (Turkington et al., 1968). The decreased lactose synthetase activity caused by progesterone is probably the result of an inhibition of \( \alpha \)-lactalalbumin synthesis (Assairi et al., 1974).

The consistent increase in human milk fat concentration after TRH suggests a direct effect of endogenous prolactin on mammary lipogenesis (Tyson et al., 1975a). Prolactin alone or in combination with insulin and hydrocortisone, stimulates the synthesis of milk fat (Shiu and Friesen, 1980). Fatty acids derived from dietary fat can be used by lactating mammary gland for synthesis of milk triglycerides and lipoprotein lipase regulates their uptake by the mammary gland. Prolactin controls lipoprotein lipase activity (Bishop et al., 1969). The molecular mechanisms by which prolactin, acting in concert with other hormones such as insulin and cortisol, stimulates enzymes that participate in the metabolism of lipid in the
mammary gland remain to be elucidated (Shiu and Friesens, 1980).

During milk production, the mammary gland transports large volumes of isotonic fluid. The major ions found in milk are Na$^+$, K$^+$ and Cl$^-$. The concentration of Na$^+$ and Cl$^-$ in milk are low, that of K$^+$ are high, relative to plasma. Na$^+$ and Cl$^-$ enter the cell by positive diffusion but prolactin promotes the active transport of these ions out of tissues by activation of Na$^+$, K$^+$ ATP-ase (Shiu and Friesens, 1980).
Milk Removal

Milk when secreted, is stored in the alveoli, ducts and sinuses or cisterns. Only that portion in the large ducts, sinuses or cisterns, is immediately available to the suckling, escaping to the nipple and may be removed by passive withdrawal. The milk in the alveoli and ducts, which may be the greater portion must be actively transferred in the larger ducts and sinuses before it is available for suckling. This transfer is brought about by contraction of the myoepithelial cells enveloping the alveoli, a mechanism known as milk ejection.

Since Ott and Scott (1911) demonstrated that the posterior pituitary extracts have the property of inducing contractions of the mammary gland, many authors have concentrated their efforts on the study of the physiology of milk ejection. This was first described as galactogogue action of the posterior pituitary extracts where it was believed that the effect was due to temporary increase in the rate of milk secretion (Folley, 1969). It is pertinent at this point to mention a classical paper by Gaines (1915), who was one of the first to draw the attention to the distinction between the physiological processes involved respectively in the secretion of milk and its removal from the mammary gland. The author further showed that milk ejection could be induced by injection of posterior pituitary extracts. By connecting a chloroform manometer with a cannulated teat duct in the lactating goat, Gaines showed that intravenous injection of the extract caused an abrupt rise of pressure with a latent period of only 15-20 seconds. However, he assumed this to be a pharmacological response and that the milk-ejection reflex was a typical neural reflex (Bisset, 1974). The modern concept of the milk ejection reflex as neurohormonal arc, the terminal phase
of which involves the release of the neurohypophysial hormone from the posterior pituitary was first postulated by Ely and Peterson in 1941 (Cowie, 1977). Richardson in 1949, using silver impregnation in histological sections of the mammary gland of the goat, established for the first time that the effector contractile tissue of the mammary gland consists of stellate myoepithelial cells situated in the outer surface of the alveoli and ducts between the basement membrane and the epithelium forming a network. Linzell in 1952, showed that these myoepithelial cells in the mammary gland were not innervated (Bisset, 1974). Cross and Harris (1952), demonstrated that lesions of the supraoptic hypophyseal tract abolished milk ejection reflex which, however, could be replaced by injection of oxytocin or by electrical stimulation of this tract. The electric stimulation of the supraoptic hypophyseal tract was observed to result in rise in intramammary pressure but not free flow of milk to the exterior, possibly because of some sphincter-like mechanism at the nipple (Harris and Pickles, 1953). However, the discovery of the structure of oxytocin as the effective galactokinetic hormone, and its subsequent synthesis was initially demonstrated by Vigneaud in 1955 (Bisset, 1974).

The milk ejection reflex is a neuroendocrine reflex having an afferent nervous arc and hormonal efferent arc. Tactile sensors concentrated in the areola and nipple activated, via thoracic nerve roots 4,5 and 6, an afferent sensory neural arc which stimulates the paraventricular and supraoptic nuclei of the hypothalamus to synthesize and transport oxytocin to the posterior pituitary and to release oxytocin from the posterior pituitary. The efferent arc (oxytocin) is blood-borne to the breast alveolus-ductal system to contract myoepithelial cells present in their walls. This contraction expels
towards the nipple the stored, preformed milk, which passes first of all to the large ducts and to the sinuses or cisterns, if present, whence it can be removed by suckling. There is, therefore, a short latent period varying from a few seconds to about one minute from the start of suckling to the appearance of a flow of milk from the lactating gland (Speroff et al., 1983). This milk ejection is very important for the efficient removal of milk from the mammary gland. Failure of the reflex means that little milk can be removed from the gland and repeated failure would lead to the rapid inhibition of milk secretion (Folley, 1969).

In lactating woman, the reflex can occur in response to stimuli other than suckling, such as the sound of the baby crying at the scheduled feeding time or even to habitual stimuli such as drinking water or smoking a cigarette before nursing. Also changes in the intramammary pressure have been reported in lactating woman in response to the mother seeing her baby or hearing his cry in a nearby room (Newton and Newton, 1967). The ejection of milk from one nipple is observed in response to suckling of the other. Moreover, suckling at one breast evokes rhythmic changes in intramammary pressure in the other breast (Tindal, 1974). These facts suggest a powerful overriding central regulatory mechanism controlling milk ejection.

The occurrence of the milk ejection reflex can be prevented by activation of the sympathetho-adrenal system by stressful stimuli and the subsequent vasoconstriction of the vascular bed of the mammary gland. Thus even if oxytocin is released in response to suckling, it may not be able to gain access to the myoepithelium to exert its milk ejection action. Similary, the sympathetho-adrenal system is capable of acting directly on the myoepithelium to render it temporarily insensitive to oxytocin (Tindal,
1974). However, Cross (1955), in a study on emotional inhibition of milk ejection considered the cause of milk ejection failure, is a second type of inhibition being a response to emotional factors affecting a central blockage of the release of oxytocin itself. In addition to emotional and stressful stimuli, anaesthetics and ethanol can also prevent the milk ejection reflex (Cobo and Quintero, 1969).

After this brief presentation of the occurrence of milk ejection reflex, it is time now to consider the various aspects of the release of oxytocin in response to suckling. Based on direct and indirect observations, it has been generally accepted that milk ejection reflex is caused by oxytocin, which acts on the myoepithelial cells of the mammary gland. The indirect observations were based on the changes in the mammary gland intraductal pressure or on milk ejection activity seen in animals and in postpartum women injected intermittently with oxytocin. Measuring intramammary pressure during an 8.5 min. suckling period, showed three distinct rises and falls of pressure. These changes were mimicked closely by subsequent intravenous injection of three separate doses of 30,20 and 20 mIU of oxytocin (Cobo et al., 1967). They also showed that a very clear response in milk ejection activity is obtained with as little as 0.1 - 0.2 mIU exogenous oxytocin.

The threshold dose for response to oxytocin of the mammary gland decreases rapidly during pregnancy reaching myoepithelial maximal sensitivity at 20 weeks gestation. However, the intensity of the contractions which can be developed by the myoepithelium evolves in a different way. The slope of the dose-response relationship increases uninterruptedly until the end of gestation. There is increasing sensitivity of the mammary gland to
oxytocin as lactation is established to reach maximum sensitivity on the fifth to seventh days postpartum where doses of 10 mIU of oxytocin produced maximum intramammary pressure with an average of about 20 mm Hg (Sala, 1964).

Direct observations are based on measurements of plasma oxytocin levels during suckling. Plasma oxytocin concentrations were measured during late pregnancy, parturition, and lactation in miniature pig and they were found to be low or undetectable in late pregnancy, reaching the highest levels at the time of delivery of the fetuses and at expulsion of the placenta. Suckling caused increases in the level of oxytocin which were greater during the first two weeks of lactation than later (Forstner et al., 1979). Dawood and his co-workers (1981), presented a report of maternal plasma oxytocin levels measured at 2 min. and then at 5 min. intervals throughout a 30 min breast-feeding episode on the third through the fifth postpartum days. There was a significant increase in plasma oxytocin in response to suckling with the increase being significant and more evident within 2 min. of initiating suckling (22.4 pg/ml). There is a bimodal type of response in the increase in plasma oxytocin levels during the breast feedings, with peak levels attained at 15 min and a smaller secondary peak at 25 min. This secondary oxytocin peak was significantly higher than baseline presuckling levels (10.8 pg/ml). The pattern and magnitude of the response in plasma oxytocin could be due to (1) the burping period in between suckling of the two breasts and therefore, removal of the suckling stimulus at that, (2) depletion of the oxytocin stores in the neurohypophysis resulting in a smaller secondary rise in plasma oxytocin when the contralateral breast is suckled, and (3) an increase in the threshold of the neural reflex to
suckling or a reduction in the vigor of the suckling by an infant that is partially satiated by the first breast. Thus, there is increasing sensitivity of mammary gland to oxytocin as lactation is established to reach maximum sensitivity on the fifth to seventh days.

The pattern of release of oxytocin may vary with the species. In the rat, the young of which remain attached to the nipples for many hours, milk ejection does not occur until some 10 min, after the onset of suckling and then there is intermittent release of oxytocin every 5-15 min., milk ejection occurring some 50-80 times in the 24 hours. There is thus in the rat no precise correlation between the suckling stimulus and oxytocin released (Cowie, 1977). In man, the work of Cross (1955) suggested an "all or nothing" type of oxytocin release in response to suckling. However, more recent studies revealed both the transient appearance as a spurt-like release and the extremely low levels of circulating oxytocin in normal milk ejection (Fox and Knaggs, 1969).

Stimulation of the reproductive tract can bring about the release of oxytocin with the occurrence of milk ejection. Cross-circulation experiments were carried out with pairs of unanaesthetized ewes in which jugular veins were crossed so that each number of a pair received jugular blood only from its partner. Distention of the vagina in one partner caused a milk response in the other but not in the stimulated ewe, and the roles of the two animals could be reversed. This is a decisive proof that the agent causing milk ejection was bloodborne and the absence of a milk ejection response in the stimulated animal provided additional evidence for the presence of oxytocin, since the hormone has a short half life in vivo and would not be expected to
survive the passage through the bodies of both animals (Debakere et al., 1961). The occurrence of milk ejection in a lactating woman during coitus (Harris and Pickles, 1953) and the release of oxytocin in the blood of a woman after orgasm is well documented (Fox and Knaggs, 1969).
MAINTAINANCE OF LACTATION

Maintainance of lactation is controlled by two hormones; prolactin which stimulates and maintains secretory activity of the mammary gland and oxytocin which is responsible for ejection of milk. While oxytocin is a release phenomenon acting on secreted and stored milk, prolactin must be available for continued secretory replacement of the ejected milk (Cowie, 1977).

The regular application of suckling stimulus with efficient removal of milk from the gland are the most important factors in maintainance of lactation. The suckling stimulus regulates mammary function in two main ways: first, by ensuring the secretion of prolactin and perhaps other hormones of the anterior pituitary; second, by releasing oxytocin from the posterior pituitary thereby eliciting milk ejection (Kase, 1983). Suckling ensures adequate prolactin availability until the 4th postpartum month, after which elevated basal levels of prolactin are no longer necessary but only transient increase associated with suckling i.e. mammary gland functions autonomously (vide supra).

The afferent pathway from the mammary gland for both prolactin and oxytocin release is common for much of its length. It starts at the sensory nerve end organs at the tip of the nipple together with sensory nerve fibres loosely surrounding the galactophores. In the midbrain, the path is common and lies in the lateral tegmentum and involves the spinothalamic system, thereafter there is some divergence. The oxytocin pathway ultimately relays within the paraventricular nucleus from which neurosecretory axons pass to the posterior pituitary. The prolactin pathway continues forward to spread out in preoptic area and to pass to the medial anterior hypothalamic area close to the third ventricle, where they abolish diencephalic inhibition.
through interacting or inhibiting a prolactin-inhibiting factor (PIF) (Cowie, 1977). A descending prolactin-releasing pathway has also been traced from the orbitofrontal cortex to the preoptic area and its presence suggests that cerebral cortical control regulates prolactin secretion and so carries the speculative implication that in man the psychiatric state may influence lactation (Noel et al., 1974a).

The release of prolactin, like the release of oxytocin can occur in response to conditioned stimuli but conditioned stimulus that releases one need not release the other. Noel et al. (1974a) have shown that when nursing women played with their infants milk ejection occurred within 5 minutes but there was no release of prolactin until breast feeding had taken place.

Tyson et al. (1978), observed no correlation between the duration of nursing and milk yield as evidenced by the standard increment in infant weight obtained over varying time period of nursing. It seems that the intensity of nursing stimulus brings about the prolactin increment and that the yield of milk is a result of oxytocin release. Furthermore, the prolactin increment during a specific period of feeding is probably responsible for the VOLUME and composition of milk in a subsequent feeding (Delvoye et al., 1976).
ENDOGENOUS OPIOIDS AND LACTATION

The demonstration of stereoselective opiate receptors in the brain (Pert and Snyder, 1973–Hughes et al., 1975), led to search for endogenous opiate-like substances. Most research centered on the role of such endogenous opiate-like peptides in the modulation of pain and their possible medication in disease state such as schizophrenia and therapies such as acupuncture. With the discovery that administration of such peptides may have significant endocrine and metabolic effects (Von Graffenried et al., 1978–Stubb et al., 1978–Imura and Nakai, 1981), progress has been made in the investigation of the role of endogenous opioid substances in the regulation of human neuroendocrine function.

The most common approach to the investigation of the role of opioids in neuroendocrinology has been to challenge the hypothalamo–pituitary axis with exogenous opiate alkaloids, opioid peptides or the opiate antagonist; naloxone.

It has been well established that lactation can be initiated by daily injection of morphine (Meites, 1966). With the advent of opioid peptides, many studies demonstrated their prolactin secretagogues in vivo. Peripheral or tuberoinfundibular injection of opioid peptides results in a striking and rapid increase of plasma prolactin (Tolis et al., 1975–Rivier et al., 1977). Infusion of a long–acting analogue of met–enkephalin DAMME, D–ALa²–Me Phe⁴–Met–enkephalin–0–ol, caused a clear elevation of prolactin with an enhanced response of prolactin to TRH (Grossman et al., 1981). Dermorphine, a recently discovered opiate-like peptide isolated from extracts from the skin of the South American frogs, induced a significant
increase in the levels of prolactin when administered by iv infusion (Degli Uberti et al., 1983).

Studies reporting the effects of opiate receptor blocker naloxone on basal and stimulated prolactin release led to inclusive results. Naloxone had been shown to suppress basal and stress stimulated prolactin in rodents and non-human primates (Gold et al., 1979). In man, naloxone at a dose of 0.2 mg/kg was reported to reduce prolactin release significantly (Rubin et al., 1979), whereas dose ranging from 0.4 - 20 mg have not been shown to affect basal prolactin secretion (Morely et al., 1980). Grossman and co-workers (1981), using high dose naloxone demonstrated no effect on basal or hypoglycaemic-stimulated release of prolactin. Delitala et al. (1982), showed that 24 hour naloxone infusion had no effect on basal prolactin secretion, moreover, no effect on nocturnal rise in serum prolactin. Serri et al. (1981), on the other hand, described a decreased response to insulin hypoglycaemia in naloxone pretreated subjects. Poitiroli et al. (1982), also observed a naloxone reversible increase in prolactin accompanying laparotomy. Moretti et al. (1983), were able to antagonize the rise of prolactin induced by heavy exercise by infusion of 18 mg/hour naloxone.

Investigation of the role of endogenous opioids in modulating prolactin response to breast stimulation led to contradictory results. Several reports have shown that prolactin response to suckling in rats can be blunted by administration of the opiate antagonist naloxone (Grossman, 1983). Genazzani and his colleagues (1982), measured B-lipoprotein, B-endorphin and prolactin in the first five days of puerperium in 7 healthy lactating women. With the exception of prolactin, all other parameters declined to
a basal normal level from the second postpartum day and remained unmodified during suckling. The effect of naloxone infusion on plasma prolactin response to suckling was investigated in lactating women. Neither baseline nor stimulated prolactin values were significantly changed implying that, in contrast to rats, endogenous opioid peptides are not major modulators of prolactin secretion during lactation (Lodico et al., 1983; Chio et al., 1984).

β-endorphin is considered to be the major modulator of serum prolactin (Grossman, 1983). Preliminary studies were unable to demonstrate an effect of parenteral β-endorphin antiserum on basal or stress-released prolactin (Ragavan and Frantz, 1980); however, when the antiserum was administered intraventricularly, prolactin clearly fell, implicating β-endorphin as the endogenous modulator (Ragavan and Frantz, 1980).

Although, there is some evidence for opioid modulation of prolactin via brainstem serotonin pathway (Spampinato et al., 1979; Johnson, 1982), most data implicate opioids as controlling serum prolactin via inhibition of dopamine release from tuberoinfundibular dopaminergic neurones in the hypothalamus. This was shown by the decrease of dopamine in stalk blood after peripheral or hypothalamic injection of opiates (Haskins et al., 1981; Grossman, 1983). Moreover, increases in prolactin induced by opioids are exaggerated by dopamine antagonists such as metoclopramide or domperidone and blocked by dopamine or a dopamine agonist such as bromocriptine (Delitala, 1981). However, by monitoring both prolactin and tuberoinfundibular dopamine release, Arita and Porter (1984), had provided convincing evidence that a second neurotransmitter system additionally participates in mediating opioid actions on prolactin. Opiate receptor mediated action at the anterior pituitary level were reported for
HYPOTHALAMIC - PITUITARY - OVARIAN
INTERRELATIONSHIP DURING EARLY LACTATION

Lactation in the presence of efficient nursing is known to be associated with protracted period of natural infertility. Such natural infertility may be related to a temporary alteration in hypothalamic-pituitary and ovarian function. It is not clear whether the anovulation and amenorrhea, which normally occur during lactation, are due to hypothalamic, pituitary and/or ovarian dysfunction. Data on the endocrine events preceding the re-establishment of normal cyclic hypothalamic - pituitary - ovarian function during lactation are cumulating.

The need for a reliable simple method to predict ovulation during lactation have urged many investigators to explore various endocrinological aspects during this period. Earlier studies depended on basal body temperature, vaginal cytology, cervical mucus, and endometrial pathology. Later studies relied mainly on interpretation of hormonal assays at different time intervals after delivery. This was achieved, either directly by determining hormonal levels in sera and urine or indirectly by testing the functional capacity of the pituitary and/or ovary using synthetic releasing hormones and human menopausal gonadotropins respectively.

Published reports, over the last years, concerning the dynamic interrelationship of the hypothalamic - pituitary - ovarian axis, have revealed uncertainty in many areas. Therefore, in order to present various reports in a classified manner of direct relevance to this study, concentration on the changing pattern of the key hormones directly implicated in recovery of the pituitary gonadotropic production, as well as, the subsequent
ovarian response, will be considered. The assumed role of prolactin in this period, will be clarified. The influence of lactation on the patterns of different hormones as demonstrated by various authors will also be mentioned.

**PITUITARY GONADOTROPIC FUNCTION**

Assessment of restoration of function of gonadotropic production during early lactation has been reported by many investigators who employed different approaches. Direct determination of circulating FSH and LH at various time intervals from delivery reflect acting tissue levels and indirectly the functional capacity of the producing cells. Pituitary challenge test using synthetic releasing factors or their recently introduced agonists will demonstrate not only the baseline levels of pituitary gonadotropins but also the magnitude and pattern of reaction of the producing cells at different time intervals after delivery.

Earlier studies by Keetel and Bradbury (1961), using a rat ovarian weight assay, showed variable amounts of gonadotropins in the urine of lactating women who had amenorrhea and relative vaginal atrophy. The amount of gonadotropins excreted, in their reports, varied widely, with one third of the subjects appeared to excrete gonadotropins intermittently. They concluded that at least in some instances, the ovary of the lactating women was refractory to pituitary gonadotropins. It was not possible for these investigators using techniques available to them at that time to discern differences in LH and FSH secretion between these lactating women with amenorrhea and ovulatory women. Moreover, they did not detect qualitative difference in gonadotropin secretory pattern.
Low or non-detectable FSH values were found during the first two weeks postpartum. Human chorionic gonadotropins (HCG) was detected in the samples obtained during the first two weeks after delivery. During the remaining period of sampling, both FSH and LH concentrations were within the range found in the normal menstrual cycle excluding midcyclic peak. No LH values in the range of the midcyclic peak could be detected in any of the samples obtained during the three periods. It was also found that there is a significantly low value of FSH during lactation than during non-lactation. However, this significant difference was found to be due to low FSH values during the first two weeks after delivery. When these values were excluded, no significant difference was found between them. Similar results were observed by Falsetti and co-workers (1982), who found that FSH levels were lower in puerperal women than in menstruating ones, and LH/HCG were very high due to persistent HCG levels.

Studying the gonadotropin function in non-lactating mothers during puerperium, revealed low basal levels of serum FSH during the first 2 weeks postpartum (Nader et al., 1975). In confirmation with the above findings, Keye and Jaffe (1976), in a group of 7 non-lactating mothers studied serum levels of FSH and LH at weekly intervals after delivery for a period of 8 weeks. Baseline concentrations of FSH were observed to be below normal follicular phase levels during the first 2 weeks. By the third postpartum week, elevation in serum FSH levels was noted to reach similar values seen in the follicular phase of the cycle and remained at these levels throughout the remainder of the study. Baseline concentrations of LH, on the other hand, were very high during the first 2 postpartum weeks due to cross-reactivity with human chorionic gonadotropin in the assay system. Thereafter, LH
levels remained similar to levels reported in the follicular phase of the menstrual cycle for the subsequent 6 weeks of the study. They concluded that there is no difference between lactating and non-lactating mothers during early postpartum period indicating a uniform alteration in hypothalamic − pituitary function. Similarly, Bonner et al. (1975), found no difference in the basal levels of FSH between nursing and non-nursing mothers during early postpartum period.

In contrast to the previous reports, Jaffe et al. (1969), failed to demonstrate any rise in FSH or LH levels in postpartum patients who were studied daily for 22 days after normal delivery. Handson and Associates (1970), failed to show detectable FSH in daily 24 hour urine samples collected from 2 postpartum patients until the 5 th postpartum week. Likewise, Crystle and associates (1970), measured plasma FSH and LH levels in 12 postpartum patients using radioimmunoassay methods and reported that plasma FSH and LH levels were detectable but considerably lower than follicular phase levels. Similarly, Nakano et al. (1975), detected low plasma FSH and LH levels compared to the normal cycle levels.

In an experimental study, Taya and Sasamoto (1981), measured plasma and pituitary LH and FSH levels in pseudopregnant and lactating rats. They found that plasma concentrations of FSH and pituitary FSH contents were significantly higher in lactating than in pseudopregnant rats. However, pituitary LH content was significantly lower in lactating than pseudopregnant rats, whereas plasma concentrations of LH were almost similar. On gonadotropin-releasing hormone (GnRH) stimulation, there followed more FSH and less LH release in lactating rats in comparison to pseudopregnant ones. They, thus, concluded that FSH secretion is not
suppressed by suckling whereas the suckling stimulus is a potent inhibitor of LH.

In another experimental study performed on beef cows (Williams et al., 1983), the effect of suckling on secretory characteristics of FSH and LH in the peripheral plasma, FSH/LH pulse frequencies, pulse amplitudes and synchrony of coincident release were studied during the first 2 weeks postpartum. Suckled cows were found to have lower mean concentrations of FSH and LH in plasma, lower FSH and LH pulse frequencies and lower pulse synchrony than non-suckled cows. Both FSH and LH pulse amplitude were not affected by suckling. It was concluded that the neuroendocrine block associated with suckling is, in addition to being associated with depressed LH release, comprised characteristic anomalies in FSH secretion, FSH/LH pulse synchrony.

In a recent study, Battin et al. (1985), attempted to trace changes in serum gonadotropins throughout the first 180 days postpartum. They studied mean serum FSH and LH in 8 nursing subjects on 5 separate occasions, on postpartum days 10, 40, 80, 120, and 180. The mean serum FSH level at 10 days postpartum was 2.8 mIU/ml then rose but remained relatively constant from 40 to 180 days postpartum with levels of 8.2 mIU/ml. Mean LH levels were constant during the study period (10.9 mIU/ml at 10 days postpartum and 11.1 mIU/ml at 180 days postpartum). Both FSH and LH levels showed no changes in response to suckling. Moreover, the mean FSH and LH levels showed no statistical difference between the amenorrhoeic and spontaneously menstruating groups.

B) Investigations Based on Pituitary-Challenge Test:

1) Using Oestradiol:
Crystle et al. (1973), based on the observation that at certain concentrations oestradiol can modulate the pituitary response to endogenous gonadotropin - releasing hormone (GnRH), demonstrated elevation in plasma FSH and LH levels on days 10 and 14 postpartum when their subjects were administered with oestrogen in low doses. However, only one showed a subsequent ovarian response in the form of increasing urinary oestradiol. The authors hypothesized that the ovaries are the most refractory component of the axis.

On the other hand, Vemer and Rolland (1982), compared FSH and LH response to 25 micrograms LHRH in a group of lactating women and in a similar group in whom lactation was suppressed by administration of bromocriptine shortly after delivery. The response was also measured in both groups after "challenge" with oestradiol benzoate. In lactating women the FSH response to LHRH was diminished following the oestradiol challenge, as compared with that in unchallenged women. At the same time, the LH responses were low in both circumstances, indicating a negative feedback effect of oestradiol. However, the oestradiol challenge resulted in a significantly increased response of FSH and LH to LHRH in bromocriptine - treated puerperal women, i.e. a positive feedback effect of oestradiol. They concluded that the lactational amenorrhoea is caused by hypothalamic changes due to a predominance of the negative feedback system. Similar findings were observed by Acosta and co-workers (1983), in an experimental study performed on 20 crossbred beef cows, with or without 17 beta - oestradiol implants. They demonstrated that on nursing, oestradiol suppressed serum LH below that of non-oestrogen treated cows, while after weaning, oestradiol stimulated LH release above that of non-oestrogen
treated cows. They, consequently, concluded that nursing enhances the negative effect of oestrogen on LH release.

2) Using Gonadotropin - Releasing Hormone:

Tolis et al. (1973), in short reports demonstrated absence of pituitary response to injected bolus of GnRH in the first 2 weeks postpartum, followed by a normal response at the completion of 6 weeks after delivery. The obtained responses were not influenced by breast feeding.

Perhaps the first classified report on the determination of pituitary reaction to an administered dose of GnRH in early lactation was that of Jeppsson et al. (1974). Their study was performed on 3 women at 1, 2 and 4 weeks after normal delivery. Two of these women were fully lactating throughout the study, while the third was only partially lactating. For accurate comparative evaluation, they performed the same test in 6 regularly menstruating women in the late follicular phase of the menstrual cycle. All participants were administered with synthetic GnRH intravenously. Plasma levels of FSH and LH were determined by RIA procedures. They found an absent gonadotropin response during the first 8 to 10 days postpartum indicating a pituitary refractoriness. Then the pituitary responsiveness increased gradually from the second week postpartum onwards. One month from delivery, they recorded significant response in both hormones assayed which was, however, significantly less than that reported in the latter follicular phase of the cycle of the control subjects. A differential return of pituitary function was observed, the FSH response being greater than that of the LH. They concluded that pituitary gonadotropic recovery in the puerperium takes place after 2 weeks and
increased thereafter but remain below the normal parameters irrespective of lactation.

Nakano et al. (1974), showed an absent GnRH - induced gonadotropin secretion in the first postpartum month. Thereafter gonadotropin response to GnRH was greater in nursing when compared with non-nursing mothers, implying that neither synthesis, storage, nor release of gonadotropin is altered provided that GnRH is present. Similarly, Tyson et al. (1976), failed to demonstrate any pituitary reaction to administered GnRH.

In another study involving 16 normal women, classified as nursing and non-nursing, Andreassen and Tyson (1976), observed no gonadotropin response following GnRH injection in the early postpartum period. The absence of such an early response was attributed to either an insensitivity of the pituitary to the dose of GnRH used or absent gonadotropin stores. They observed a similar response among nursing women during early postpartum period and this was attributed to a uniform alteration in hypothalamic pituitary function. Beyond 5 postpartum weeks, a significant greater response following GnRH was observed in nursing compared with non-nursing women. Similar to that observed by Jeppson and associates (1974), a differential return of pituitary function was observed in terms of marked FSH response and small LH response. Nakano (1976), administering synthetic GnRH intravenously to lactating puerperal women reached similar results and concluded that gonadotropin reserve function, which is suppressed throughout pregnancy and early few weeks postpartum, recovers completely around the 5th week postpartum.

On top of that, another evidence of the differential return of gonadotrophic function was demonstrated by Lewis and Bolt (1983). They studied GnRH induced release of LH and FSH in suckled and non-suckled
The ability of pituitaries from lactating animals to secrete LH and FSH in response to GnRH was studied in vitro using a pituitary incubation system by Smith (1982). LH response by anterior pituitary from postpartum nursing rats was significantly less than that released by anterior pituitaries from diestrous females, whereas the females deprived from their litters for 48 hours showed a greater response than diestrous females. The total amounts of FSH released in vitro in response to GnRH from lactating and cycling females did not differ significantly. Pituitary LH and FSH concentrations were similar among both suckling and non-suckling ewes. So, there was no correlation between the amounts of LH or FSH released by the pituitaries in response to GnRH and pituitary gonadotropin contents. He suggested that suckling stimulus indirectly suppresses pituitary responsiveness to GnRH.

3) Using Luteinizing – Hormone Releasing Hormone Agonist:

It was postulated that long-term deprivation of endogenous luteinizing hormone – releasing hormone (LRH) during the course of pregnancy might account for the lack of gonadotropin in early lactation. Definitive proof of this hypothesis requires measurement of LRF in the portal circulation which is clearly impossible. Sheehan and Yen (1979), explored indirectly the pituitary reaction of 6 postpartum women in the first 10 days of puerperium using 50 ug of LRF agonist. They administered the drug subcutaneously every 48 hours for 4 doses starting from the first day of puerperium. Prior to treatment, each subject was tested with two pulses of LRF and again at the end of LRF agonist treatment. Pulses of LRF induced no significant elevation of FSH. However, during treatment a significant increase
in basal FSH was observed together with a remarkable gonadotropin release after LRF stimulation. Consequently, they concluded that lack of gonadotropic activity during the first 3 postpartum weeks can be partially attributed to insufficiency of endogenous LRF secretion.

**PATTERN OF GONADOTROPIN RELEASE DURING EARLY LACTATION**

In the light of such investigations, it is clear that pituitary refractoriness is evident during the first 2 weeks postpartum (Tolis et al., 1973 - Jeppsson et al., 1974 - Andreassen and Tyson, 1976 - Nakano, 1976). FSH values are low or non-detectable (Reyes et al., 1972 - Said and Wide, 1973 - Nader et al., 1975 - Falsetti et al., 1982). This suppressed FSH release in the early postpartum period is the result of the persistence of suppressive effect of pregnancy, presumably oestrogen mediated (Reyes et al., 1972). Meanwhile, baseline concentrations of LH are very high due to cross-reactivity with HCG in the assay system. However, with clearance of chorionic gonadotropin from maternal circulation, LH levels showed gradual drop to very low values with the completion of 2 weeks (Reyes et al., 1972 - Said and Wide, 1973 - Falsetti et al., 1982 - Howie and McNeilly, 1983). There was no difference between nursing and non-nursing mothers during early postpartum period indicating uniform alteration of hypothalamic-pituitary function (Bonner et al., 1975 - Nader et al., 1975 - Keye and Jaffe, 1976). This is mediated through suppression of gonadotropin reserve function (Reyes et al., 1972 - Yen et al., 1974).

From the second postpartum week onwards, the gonadotropin function recovers gradually but remains below the normal parameters (Jeppsson et al., 1974 - Andreassen and Tyson, 1976 - Howie and McNeilly, 1983).
A differential return of pituitary function was observed in terms of marked FSH response and small LH response (Jeppson et al., 1974 - Andreassen and Tyson, 1976 - Nakano, 1976 - Lewis and Bolt, 1983).

Both FSH and LH rise to reach values equivalent to those of low normal follicular phase of the cycle (Keye and Jaffe, 1976 - Howie and McNeilly, 1983). By comparison with normal cycling non-nursing women, FSH appears higher and LH appears lower during lactation (Said and Wide, 1973 - Taya and Sasamoto, 1981). Changes in FSH and LH values in relation to the menstrual condition of the mother remain uncertain. Delvoye et al. (1978), demonstrated higher FSH levels in amenorrhoeic than in menstruating nursing mothers. Mean LH, on the other hand, was not statistically different in amenorrhoeic mothers from that in menstruating ones. Battin et al. (1985), on the contrary, observed no statistical difference between FSH and LH levels in amenorrhoeic and spontaneously menstruating groups.

In an attempt to identify the mechanism responsible for the initiation of gonadotropin secretion after pregnancy termination, Marrs et al. (1981), studied eight women up to 55 days after pregnancy termination. They found that as long as serum oestradiol and progesterone levels were elevated, serum FSH remained low. With clearance of oestradiol and progesterone to levels seen in early follicular phase, initial increase in FSH occurred while serum LH remained undetectable. Thus, the clearance of oestradiol compared with advent of FSH; the two events appeared to be directly related. To demonstrate this, the administration of ethinyl oestradiol was found to induce a selective inhibition of FSH (Yen and Isai, 1971). Therefore, the high oestradiol levels during pregnancy appear to selectively inhibit the
secretion of FSH. This conclusion varies from the hypothesis of Rolland et al. (1975), in that FSH secretion may be suppressed due to elevated levels of HCG. Nevertheless, this theory is unlikely since Marrs et al. (1981) showed that FSH secretion occurs in the presence of elevated HCG. The resumption of LH secretion as measured by its B-Subunit appears to be directly influenced by prolactin levels (Marrs et al., 1981).
OVARIAN FUNCTION DURING EARLY LACTATION

In nonpregnant women, the ovaries are the main source of oestrogen and progesterone. During pregnancy, the fetoplacental unit becomes the dominant source of these hormones which reach high levels in the blood. The progressive increase in oestrogen and progesterone during gestation gives the optimum impetus for the acinar and ductal elements of the breast to organize into mature, functional lobular - alveolar - ductal units (Riddick, 1983). Moreover, the rising oestrogen exerts a stimulatory effect on the synthesis and release of prolactin (Labrie et al., 1978). This stimulatory effect of antepartum oestrogen was also demonstrated by Martin and Oakey (1982). They observed low concentration of prolactin in peripheral plasma at delivery and in puerperium among 5 women with severe antepartum oestrogen deficiency; 4 being due to placental steroid sulphate deficiency and one due to an unknown cause. Those women were unable to establish lactogenesis as assessed from plasma alpha - lactalbumin concentrations on day 4 postpartum. Inspite of this stimulatory effect on prolactin synthesis and release, oestrogen and progesterone interfere with prolactin action at alveolar cell prolactin receptor level preventing lactation during pregnancy (Bruce and Ramirez, 1970).

Immediately after delivery of the placenta, there is a rapid drop in oestrogen and progesterone (Turnbull et al., 1974). This is considered to be the hormonal trigger for initiation of milk production within the alveolar cell and its secretion into the lumen of the glands (Archer, 1980).

Hormonal parameters of ovarian origin, oestriol and progesterone, have been determined during lactation by many investigators either directly
one non-lactating) showed ovulatory oestradiol and gonadotropin peaks of lower magnitude compared with the normal menstrual cycle. However, the subsequent progesterone rise was below the levels encountered in the normal luteal phase of the cycle.

In a study of 22 postpartum women at weekly interval until return of menstruation, Said et al. (1973), found serum oestradiol levels at or below range for the early follicular phase of the normal menstrual cycle. After weaning the baby, the mother's serum oestrogen rises gradually reaching those levels of the follicular phase at one to two weeks before return of menstruation.

In one patient appropriately studied, oestradiol 17-B was found in smaller amounts than oestrone during lactation suggesting that though follicles may develop at this stage, they do not achieve maturity (Said et al., 1973). This is because oestradiol level is the best indicator for follicular maturation (Thorneycroft and associates, 1971).

Tracing of serum progesterone values throughout lactation in the previous study performed by Said and associates (1973), demonstrated that they were generally low. Occasional slightly elevated serum progesterone values were found and were attributed to luteinization of a follicle in the absence of ovulation. On weaning, they rose gradually within the range of the luteal maximum of the normal menstrual cycle which was sometimes reached at one to two weeks before return of menstruation.

Battin et al. (1985), studied changes in serum oestradiol up to 180 days postpartum and found that mean oestradiol levels were low at 10 days postpartum (7.2 pg / ml), then gradually rose to a mean level of 47.3 pg / ml at 180 days postpartum. In subjects, who were amenorrhoeic during the study period, lower oestradiol levels were seen in comparison to
spontaneously menstruating ones. The difference between mean oestradiol levels in the amenorrhoeic and spontaneously menstruating groups reached its summit at 180 days postpartum (4.2 pg / ml versus 90.3 pg / ml). Such observation was contradictory to the previous finding of Delvoye et al. (1978), where no significant difference was traced between mean oestradiol level in menstruating and amenorrhoeic mothers during the same period of lactation.

In an attempt to detect changes in oestrone, oestradiol and progesterone in relation to suckling, serial blood samples were examined in 12 women before and during 30 minutes breast-feeding. Serum oestradiol remained unchanged throughout breast feeding and so did plasma progesterone (Dawood et al., 1981).

B) Investigations Based on Direct Estimation of Urinary Steroids:

In an attempt to trace urinary steroid profile during starting lactation, Acevedo et al. (1969), measured urinary steroid excretion in 17 postpartum women. They found a sharp drop in the excretion of pregnandiol, pregnanolone, pregnantriol as early as the first 24 hours after delivery. The urinary levels remained at values found in the luteal phase of the menstrual cycle. As regards urinary excretion of total oestrogens, they found that up to the third day of puerperium, total oestrogen values were well above those found during the follicular phase of the menstrual cycle.

In another study, Said et al. (1973), studied the 24 hour urinary excretion of total oestrogens during the first 15 postpartum weeks and reported increase in total oestrogen levels only after stoppage of lactation.
However, 3 mothers from their subjects showed a rise in total oestrogens to 90-136 pg/ml in the fourth postpartum week. This goes with a previous report (Brown, 1956) which showed that urinary excretions of oestrogens in lactating women were low and within the same range normally found during the early part of a menstrual cycle. After cessation of lactation, the oestrogen levels began to increase slowly until the regular fluctuations found during a menstrual cycle were re-established. This restoration of ovarian activity was found to be preceded by full restoration of serum LH levels (Howie et al., 1982b).

C) Investigations Based on Ovarian Challenge Test:

The evaluation of ovarian response to administered LHRH or human menopausal gonadotropin (HMG) at various intervals from delivery have shown controversial results. Zarate et al. (1972), had stimulated six lactating women with exogenous HMG for 5 consecutive days starting 6 days after delivery. Ovarian responsiveness was assessed by estimation of urinary steroid output and by endometrial biopsy taken 10 and 30 days after stimulation. No significant increase in urinary steroid output was observed and the endometrium remained inactive in all patients. They suggested ovarian refractoriness during that part of the postpartum interval. Similarly, Del Pozo et al. (1975), observed no response in urinary oestrogen output following HMG stimulation in nursing and non-nursing mothers in the immediate postpartum period. Friedman et al. (1976), in addition, reported no correlation between the urinary response to LHR and the plasma levels of oestradiol 17-B nor progesterone at 1, 3 and 36 days postpartum.

In contradiction with the above findings Zanartu et al. (1974),
observed follicular development at the time of laparotomy for sterilization in a group of nursing women between the third and 14th postpartum week, when administered with LHRH before hand.

Nakano et al. (1975), studied a group of 18 puerperal lactating women, who were given a combined administration of HMG and HCG. The ovarian response to these human gonadotropins was evaluated by daily estimation of the 24 hour urinary excretion of total oestrogens. Fourteen out of the 18 subjects were responsive as evidenced by a rise in the urinary oestrogen excretion. This was associated with low plasma levels of FSH and LH estimated during the period of the study (4, 9 and 24th postpartum days). They concluded that puerperal anovulation and amenorrhoea during early lactation might be due to pituitary gonadotropin dysfunction rather than to ovarian refractoriness.

Andreassen and Tyson (1976), explored the situation at a later date of the puerperium. They reported basal oestradiol and progesterone levels comparable to those of the follicular phase of the menstrual cycle in 11 mothers, half of them were nursing, at day 24 postpartum. One non-nursing mother, in the group studied, showed elevated basal oestradiol (127 pg/ml) and progesterone (3.2 ng/ml) levels which suggests return of ovarian activity. Upon administration of HMG injection to all subjects, a significant rise in plasma oestradiol in 10 of 11 tests were observed. The levels returned to their base values by the third day. Progesterone levels, on the other hand, showed an initial slight drop then gradual increase to reach maximal elevation between 24 and 72 hours, significantly higher than baseline values.
PATTERN OF OVARIAN STEROIDS DURING EARLY LACTATION

Clearance of ovarian steroids starts one hour after delivery (Yannone et al., 1968 - Llauro et al., 1968 - Munson et al., 1968). Oestrogen levels rapidly fall to bottom values by the seventh day postpartum, then gradually rise to follicular phase values (Reyes et al., 1972). Serum progesterone levels fall rapidly postpartum and become within the follicular phase range by the third week after delivery (Llauro et al., 1968 - Yannone et al., 1968 - Reyes et al., 1972). Serum oestrogen and progesterone are maintained at or below the range for the early follicular phase of the cycle (Said et al., 1973). On milk let-down, no alteration in serum oestrogen or progesterone was observed (Dawood et al., 1981). As lactation is stopped, both oestrogen and progesterone gradually rise to reach luteal phase values (Brown, 1956 - Said et al., 1973 - Howie et al., 1982b).
PROLACTION DURING EARLY LACTATION

Prolactin is the critical hormone required for successful lactation in human subjects. It has only recently been identified as a separate human pituitary hormone. Prior to its isolation, researchers encountered the problem of producing a preparation which was free of human growth hormone (HGH). True, it was possible to measure prolactin activity with any of several bioassay systems; yet, all these failed to distinguish prolactin from growth hormone which itself has lactogenic activity. Notwithstanding, it was possible to demonstrate elevated serum concentrations in many clinical situations where HGH could not be detected by radioimmunonassay and vice versa. These reports challenged the view that in primates growth hormone and prolactin were one and the same molecule. By 1971, a series of clinical and experimental studies culminated in the isolation of a human prolactin preparation free of growth hormone. The development of a homologous radioimmunonassay for human prolactin followed this achievement. This has allowed considerable insight into the physiologic mechanisms regulating the secretion of prolactin and evaluation of the hypothalamic-pituitary-prolactin axis (Fournier et al., 1974).

Elevated serum levels of prolactin first appear in pregnancy at about 8 weeks of gestation. This continues to rise steadily to term reaching a mean level of 200 ng/ml (Tyson et al., 1972a). Cellular hypertrophy of anterior pituitary acidophils, during gestation, is most likely responsible for this increase in prolactin biosynthesis (Tyson et al., 1975a). Moreover, an enhanced prolactin response to intravenous thyrotropin-releasing hormone (TRH) in the latter half of gestation suggests increased secretory reserve of lactotrophic cells (Tyson and Friesen, 1973).
After delivery, if a woman does not breast-feed her baby, prolactin level will return to normal in 2 to 3 weeks (Fournier et al., 1974). However, with breast-feeding, prolactin levels decline approximately 50% in the first postpartum week and suckling elicits transient increases in prolactin. Until 2-3 months postpartum, basal levels are approximately 40-50 ng/ml and there are large surges about (10-20 folds) after suckling. Beyond 3-4 months, the basal levels are normal and suckling continues to elicit a smaller but significant increase in prolactin secretion. The attained baseline levels continue to be higher in breast-feeders when compared with levels in those who are not breast feeders (Tyson et al., 1972 a - Archer, 1980). Dawood et al. (1981), also demonstrated such postsuckling prolactin peak while tracing serial prolactin levels by RIA in 12 women before and during a 30 minute breast-feeding. Similarly, Martin (1983), showed that as lactation continues, the importance of absolute values of prolactin diminishes while the capability to secrete prolactin in response to suckling stimulus is maintained.

In contrast to the previous findings, Battin et al. (1985), demonstrated that baseline prolactin levels remain elevated throughout the first six months postpartum in women who nurse their infants at regular intervals. Baseline prolactin levels were high at 10 days postpartum (90.1 ng/ml) then slowly declined but remained elevated at 180 days postpartum (44.3 ng/ml), with the stimulus of suckling being able to double the baseline prolactin value throughout the study period. Differences between these findings and previous reports may be in the frequency with which subjects nursed as well as the fact that in the study by Battin and associates (1985), serial samples were obtained before the initiation of suckling and during the next 120 minutes.
HYPOTHALAMIC - PITUITARY - OVARIAN
INTERRELATIONSHIP DURING PROLONGED LACTATION

While several studies have reported the endocrine changes associated with short-term lactation, only few have investigated those associated with prolonged lactation. Several authors have attempted to correlate clinical details of lactational and menstrual history in long-lasting lactation with measurements of serum prolactin, luteinizing hormone (LH), follicle-stimulating hormone (FSH), oestradiol and progesterone totally or separately.

PITUITARY GONADOTROPIC FUNCTION

As indicated before, baseline FSH concentrations are low or non-detectable during the immediate postpartum period (Reyes et al., 1972 - Said and Wide, 1973 - Nader et al., 1973 - Falsetti et al., 1982). Meanwhile, baseline concentrations of LH are very high due to cross reactivity with HCG (Reyes et al., 1972 - Said and Wide, 1973 - Falsetti et al., 1982). With clearance of HCG, by end of the second postpartum week, LH drops to low normal follicular phase values (Keye and Jaffe, 1976 - Speroff et al., 1983 - Battin et al., 1985). As regards to FSH, it undergoes gradual rise from the second postpartum week onwards to reach values equivalent to those of the follicular phase of the cycle (Keye and Jaffe, 1976). Debate has arose about the timing for FSH to reach such level. Some investigators consider this to occur by the third postpartum week (Keye and Jaffe, 1970 - Speroff et al., 1983), while others consider it to occur latter, by the 8th week (Howie and McNeilly, 1983) or by the 40th postpartum day (Battin et al., 1985).
However, owing to the more frequent sampling Keye and Jaffe (1976) still appear to be the most accurate.

Delvoye et al. (1978), in a cross-sectional study reported serum gonadotropin patterns during the first 2 postpartum years. They found that serum FSH remained unchanged between 3 months and 18 months postpartum with a mean value significantly higher in amenorrheic nursing mothers (15.4 mIU/ml) than in menstruating nursing mothers (12.6 mIU/ml). Mean while, the mean serum LH was not statistically different in amenorrheic mothers (8.3 mIU/ml) from that in menstruating mothers (9.1 mIU/ml). During the second postpartum year, basal serum FSH and LH concentrations were in the normal range observed during the follicular phase of the cycle. Mean serum LH concentration was higher in menstruating (12.9 mIU/ml) than in amenorrheic mothers (6.4 mIU/ml). Mean serum FSH concentrations were about the same in amenorrheic (15.5 mIU/ml) and in menstruating (14.6 mIU/ml) mothers. They concluded that hypogonadotropinemia is an endocrinologic correlates of late physiologic lactation particularly during the period of lactation amenorrhoea.

In an evaluation of gonadotropin profile in the sera of 15 full breast-feeding mothers between 10 and 410 days postpartum, Tyson and associates (1978), found that tonic gonadotropin secretion was low assuming a more episodic secretory pattern either when the frequency of breast feeding was reduced or when weaning took place. Significant increments in peripheral concentrations of luteinizing hormone was seen in response to weaning.

In another study, twenty-four hour secretory patterns of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were obtained on two
separate occasions from a woman with late physiologic lactation, before and after resumption of regular cycles. The resumption of menses was associated with an increase in the plasma concentrations of LH and FSH and, in case of LH aquisition of an apparently qualitively more normal secretory pattern (Madden et al., 1978). Similarly, Duchen and McNeilly (1980), found that during lactational amenorrhoea mean levels of serum LH were significantly lower than normal while FSH were in the normal follicular range. However, in menstruating breast-feeding mothers levels of LH increase.

Several investigators tried to speculate that the extent of suckling, hyperprolactinemia and hypogonadotropinemia are causally related. Smith (1978a), demonstrated in lactating rats that the LH concentration in serum is inversely related to the number of pups suckling the mother. Moreover, a similar relationship was seen between the hypothalamic content of luteinizing hormone - releasing hormone of lactating rat and the number of suckling pups, indicating that the reduction in LH secretion involves hypothalamus as well as the pituitary. The same author in another report (1978b), demonstrated a very much smaller oestrogen - induced LH surge during lactation than spontaneous proestrous surges. However, normalization of such LH surges where detected after treating nursing rats with CB-145 indicating that prolactin is responsible for such suppressed hypothalamic - pituitary responsivness.

Such suggestion is consistent with the findings of Madden and associates (1978), who though detected no direct relationship between suckling and plasma LH and FSH concentrations, they propose that the hyperprolactinemia was a consequence of suckling and that hypogonadotropinemia was the resultant of increased prolactin secretion. Similar findings were detected by
Duchen and McNeilly, (1980), who found that with declining prolactin levels during menstruation in nursing mothers levels of LH increase and when serum levels of prolactin drop near or within the normal non-lactating range, normal cyclic gonadotropin secretion occurred. Contrary to the above speculations several investigators reported no change in FSH and LH levels in response to suckling (Delvoye et al., 1976-, 1978 - Madden et al., 1978). In agreement with that, Tyson et al. (1975 b) found that tonic gonadotropin secretion were unaffected while cyclic LH surges were inhibited by hyperprolactinemia indicating that subjects with higher prolactin levels may have remained amenorrhoeic due to inhibition of LH surges.

The exact mechanism whereby elevated prolactin secretion might lead to suppression of gonadotropin secretion is unknown. Fuxe et al. (1969), suggested that the elevation of basal prolactin observed in nursing mothers would alter hypothalamic catecholamines metabolism since suckling is known to deplete hypothalamic catecholamines and this in turn influence the release of prolactin and cyclic gonadotropins. Nakano et al. (1974), suggested an effect of prolactin on the secretion of GnRH. Rolland et al. (1975), theorized that high levels of prolactin inhibit ovarian oestradiol productions, which by a positive feed-back effect is responsible for the stimulation of GnRH secretion and, thus, LH secretion. The fact that resumption of FSH secretion occurred before LH secretion indicated that increasing oestradiol levels are not necessary for initiation of LH secretion (Marrs et al., 1981).

As regards the mechanism by which the strength of suckling stimulus can affect gonadotropin values, Baird et al. (1979), suggested that it might render the hypothalamic-pituitary axis more sensitive to the negative
feedback and less sensitive to the positive feedback effects of ovarian steroids. Others suggested that suckling stimulus could indirectly suppress pituitary responsiveness to GnRH. A suppression which differentially affects basal LH secretion but not FSH and may be the direct result of inadequate GnRH (Taya and Sosamoto, 1981 - Smith, 1982).

In view of what is mentioned above, hypogonadotropinemia appears as an endocrinologic correlate of prolonged lactation, particularly lactation amenorrhoea (Delvoye et al., 1976-1978 - Tyson et al., 1978 - Madden et al., 1978). The resumption of menses is associated with an increased LH levels (Delvoye et al., 1978 - Madden et al., 1978 - Duchen and McNeilly, 1980). Tonic gonadohropin secretion assumes a more episodic secretory pattern when the frequency of breast-feeding is reduced suggesting that hyperprolactinemia is responsible for such hypogonadotropinemia (Tyson et al., 1978 - Duchen and McNeilly, 1980). After lactation is stopped, there is a sharp rise in follicular phase of LH levels assuming normal gonadotropin secretion (Tyson et al., 1978 - Howie et al., 1982 b).
OVARIAN FUNCTION

Ovarian quiescence during prolonged lactation has attracted the attention of several investigators. After clearance of oestrogen and progesterone postpartum, both become maintained at or below the range for early follicular phase of the cycle by the third postpartum week (Reyes et al., 1972 - Said et al., 1973).

In a study performed on 465 nursing mothers in Central Africa (Kivu, Zaire) during the first 2 postpartum years, Delvoye et al. (1978), found that between postpartum months 3 and 12, there was no significant difference between mean serum oestradiol level in menstruating mothers (102 pg/ml) and that in amenorrhoeic mothers (83 pg/ml). In the second postpartum years, serum oestradiol was significantly lower in amenorrhoeic mothers (82 pg/ml) than in menstruating ones (118 pg/ml).

In a study performed on Tansekei women lactating for an average duration of 11 months Duchen and McNeilly (1980), correlated clinical details of lactational and menstrual history with measurements of oestradiol and progesterone. Of 44 amenorrhoeic women studied 29 showed no endocrine evidence of ovarian activity (oestradiol level below 90 pg/ml and progesterone levels below 0.2 ng/ml). In the remaining 15, increased levels of oestradiol (120-150 pg/ml) were found comparable to those seen in the early follicular phase of the normal menstrual cycle. It is apparent from these results that some follicular development can occur during lactation amenorrhoea. However, such conclusion is compatible with reports that the postpartum ovary can secrete oestrogen in response to gonadotropic stimulation (Andreassen and Tyson, 1974 - Zanartu et al., 1976). Of 27 women who had menstruated during lactation, only 15 had raised oestradiol
levels suggesting ovarian follicular development and only 4 (10%) had serum progesterone levels above 2 ng/ml suggesting luteal activity. They suggested that a good portion of lactating menstruating women were experiencing oestrogen withdrawal bleeding only i.e. anovulatory cycles.

In another study, Delvoye and his associates (1980), detailed the extent of luteal activity in relation to menstrual rhythm in mothers lactating for a period of 2 years. Serum progesterone was 3.2 nmol/liter, indicating corpus luteum activity in 8% of amenorrhoeic lactating mothers and in 20% of menstruating nursing mothers. In amenorrhoeic lactating mothers the mean serum progesterone value was 1.0 nmol/liter and occurrence of corpus luteum activity was found to precede return of menstruation. In menstruating nursing mothers the mean value (1.3 nmol/liter) was insignificantly higher than that of amenorrhoeic ones but significantly low when compared with that in non-lactating women (10.6 nmol/liter). This further support the conclusion of increased incidence of anovulatory cycles and/or cycles with short luteal phases among nursing mothers.

Similar findings were detected by Howie et al. (1982b), while following 27 breast-feeders by weekly 24 hour total oestrogen and pregnandiol. Follicular development (total urinary oestrogens more than 10 μg/24 hours) and corpus luteum activity (urinary pregnandiol levels of more than 1 mg/24 hours) first occurred at a mean of 36.4 weeks. Ovulation occurred in 33% of breast-feeding mothers during the phase of lactational amenorrhoea but was followed by menstruation in every case. The frequency of ovular cycles progressively increased with time being observed in 45% of the first cycles and 66% of the latter cycles during prolonged breast-feeding.
There have been few studies of luteal insufficiency in breast-feeding mothers. Reyes et al. (1972), reported defective progesterone secretion in the first postpartum cycles of the lactating women. Delvoye et al. (1980), found that the mean serum progesterone levels were lower in cycles in mothers lactating for 2 years than in the normal cycles suggesting a high number of defective luteal phases. Similarly, Gross et al. (1980), found that defective luteal function is common in Australian women returning to menstruation during long-lasting breast-feeding. McNeilly et al. (1982), followed urinary pregnandiol weekly estimations in cycles of twenty-seven breast feeding mothers. The luteal phases were classified as absent in 42% of patients (two urinary pregnandiol levels collected in the two weeks prior to menstruation below 1 mg/24 hours), deficient in 31% (either the peak luteal phase urinary pregnandiol value fell between 1.0 and 1.3 mg/24 hours or the lower luteal phase urinary pregnandiol value fell below 0.5 mg/24 hours), or normal in 27% of lactating mothers (both values above 1.0 mg/24 hours).

The return of ovarian activity is influenced by the intensity of suckling stimulus. Howie et al. (1982a), found that mothers who suppressed ovulation for more than 40 weeks postpartum breast-fed for longest and induced supplementary feeds most gradually. Hennart et al. (1985), compared nursing behaviour and its impact on return of luteinization in Sweden and Zaire. In Sweden, where low number and duration of breast-feedings was recorded, 13% of the mothers had serum progesterone level above 1.0 ng/ml, indicating return of luteinization, at 3 months of lactation. At 6 and 9 months of lactation, 24% of the mothers had signs of luteinization and the mean serum progesterone was at 6.2 ng/ml and thus
very similar to that seen during the luteal phases in regularly menstruating women. In Zaire, where regular breast feeding habit was recorded, some 10% of the mothers had serum progesterone levels above 1.0 ng/ml. In mothers with signs of luteinized follicles, the mean serum progesterone was quite low, only slightly higher than 1.0 ng/ml. During the second year of lactation, the percentage of mothers with serum progesterone levels above 1.0 ng/ml increased slightly but never exceeds 22%.

Such an influence of suckling behaviour on ovarian activity may reflect a secondary response to the extent of hyperprolactinemia existing. In a study by McNatty et al. (1974), the addition of prolactin in concentrations greater than 25 ng/ml to human granulosa cell cultures caused a dose-response inhibition of progesterone production. They suggested that prolactin could have an influence on the end organ responsiveness to gonadotropins. Delvoye and associates (1980), found that the mean serum prolactin was lower in mothers with serum progesterone above 3.2 nmol/liter than in mothers with serum progesterone levels below this limit, indicating that during prolonged lactation hyperprolactinemia is associated with anovulation and inadequate corpus luteum. Such relationship was further emphasized by Hennart et al. (1985), who found that luteinization occurred only exceptionally when serum prolactin was above 500 µU/ml, besides, when such limit was exceeded the percentage of mothers with luteinization was doubled.

**In view of what is mentioned above**, ovarian quiescence is characteristic of long-lasting lactation particularly during lactation amenorrhoea. A good portion of nursing mothers, while they are menstruating, are experiencing anovulatory cycles and/or cycles with short luteal phases. The return of ovarian activity is influenced by the intensity of suckling which in turn reflects the impact of the associated hyperprolactinemia.
PROLACTIN DURING PROLONGED LACTATION

Prolactin has been shown to be important in the initiation of lactation immediately postpartum (Tyson et al. 1972 a; Tyson et al. 1973 - Noel et al. 1974 a - Battin et al. 1985), however its role during prolonged lactation is not well defined. Many authors tried to establish a cause- and - effect relationship between hyperprolactinemia and prolonged breast-feeding.

A close relationship has been demonstrated between lactational hyperprolactinemia and prolonged lactation In studies performed on rats, continued application of suckling stimulus by litter replacement (Bruce ,1958) or injection of prolactin ( Meites and Nicoll, 1959) was found to prolong lactation considerably. In human, Delvoye and his associates in their cross-sectional studies (1976, 1977, 1978), found that the mean serum prolactin concentrations were almost the same between 9 and 15 months postpartum (851 μU/ml). This value was significantly higher than that observed between 15 , and 24 months postpartum (600 μU/ml). Serum prolactin at 15-18 months postpartum was some three times higher than in the non - pregnant and non- lactating women. However, serum prolactin decreased progressively after postpartum month 21 to be close to the values seen in the non - pregnant and non- lactating women. Gross and Eastman (1979), on the other hand, found that nursing mothers in Australia had elevated baseline serum prolactin levels twice as high as non- lactating control subjects. In rural Tanseki women, where it is customary to breast - feed for up to 2 years, elevated serum prolactin levels were detected and those with normal prolactin levels had reported difficulty of breast feeding (Duchen and McNeilly, 1980)
In a longitudinal study performed on 57 lactating Indian women between 3 weeks, and 1 year postpartum, basal prolactin values were significantly higher in all lactating women irrespective of the duration of lactation, as compared to the non-pregnant and non-lactating women. Moreover, basal prolactin levels were significantly lower in mothers whose infants were being supplemented (Shatrugna et al., 1982).

Prolactin, also, plays a key-role in maintenance of lactational amenorrhoea. Several studies have reported that the duration of lactational amenorrhoea is directly associated with the degree of hyperprolactinemia. Maneckjee et al. (1976), using the lactating monkeys, observed that a single injection of prolactin can extend the anoestrous period significantly. Studies in human demonstrated that thyrotropin-releasing hormone (TRH) through releasing prolactin extended the lactational amenorrhoeic period (Jewelewicz et al., 1974).

Delvoye and co-workers (1978), measured serum prolactin in 465 nursing mothers between the first and twenty-seventh months postpartum in Central Africa. During the second year, the mean serum prolactin level was lower in menstruating (631 μU/ml) than in amenorrhoeic nursing mothers (649 μU/ml). The difference in serum prolactin levels between menstruating and amenorrhoeic mothers was more apparent during the first postpartum year than during the second postpartum year. They concluded that plasma prolactin level is one of the critical determinants of the return of menstruation. An average circulating prolactin level around 560 μU/ml seemed to be associated with a 50% incidence of amenorrhoea and an average value of 860 μU/ml or more with almost 100% incidence of amenorrhoea.
Madden and associates (1978), compared twenty-four hour secretory pattern of prolactin on two separate occasions in a woman with more prolonged physiologic lactation, 26 and 34 months after her child's birth. At the 26th month the patient was amenorrhoeic, while at 34th month regular menses were resumed. The average concentrations of prolactin was significantly greater during the period of amenorrhoea than after resumption of menses, 40 ± 1.0 ng/ml and 31 ± 1.4 ng/ml, respectively. Many other investigators also confirmed such impact of hyperprolactinemia on lactational amenorrhoea (Gross and Eastman, 1979; Duchen and McNeilly, 1980).

The above findings are contradictory to those of Sharugna et al. (1982), who through calculation of mean basal and peak plasma prolactin levels in relation to return of menstruation showed that though both were lower in menstruating women as compared to amenorrhoeic ones irrespective of the duration of lactation, the differences were not statistically significant. However, they failed to give an obvious explanation for this. In another study, the percentage of menstruating mothers increased faster with time in the urban than in the rural Zairean populations irrespective of the higher serum levels of prolactin in the urban populations (Hennart et al., 1985).

Suckling frequency and duration of breast feeding are important determinant of plasma prolactin levels and hence duration of lactation (Delvoye et al., 1978). Serum prolactin levels in full breast feeding Australian women were significantly higher than in women who are partially breast-feeding (Gross and Eastman, 1979).

Howie et al. (1982a) recorded suckling duration and frequency throughout lactation in twenty-seven mothers. Reduced suckling frequency
to less than 6 times / day and reduced suckling to less than 60 min. / day were associated with fall of mean prolactin levels to the non-pregnant range.

In a cross-sectional study, nursing behaviour and serum prolactin levels were compared in 1036 nursing mothers: 61 in Sweden, 457 in an urban area of Zaire (Bukava, Kivu) and 815 in rural area of the same region (Kabara, Kivu). A close association between the prolactin levels and the number of feeds per day was concluded but not with the duration of breast-feeding per day (Hennart et al., 1985). However, if the number of breast stimulations is a key factor in the maintainance of lactational hyperprolactinemia, it is not the only one. Indeed, at three months of lactation the frequency of feeds was already much lower in Sweden than in Zairean; yet, the levels of serum prolactin in the European mothers were very similar to those of the Africans (Hennart et al., 1985). A tentative explanation is that the pituitary gland, hypertropied during pregnancy under the influence of massive oestrogenic stimulation (Labrie et al., 1976), is most sensitive to breast stimulation soon after delivery; thus, a rather low frequency of feedings may still achieve maximum continuous release of the hormone. After three months, such sensitivity of lactotrophs regresses (Tyson et al., 1972a) and a high frequency of breast-feeding then becomes of decisive importance for maintaining the lactational hyperprolactinemia (Hennart et al., 1985).

In spite of the association between nursing frequency and hyperprolactinemia, suckling does not acutely stimulate prolactin release in long-term lactation (Delvoye et al., 1976 - 1978 - Madden et al., 1978 - Gross and Eastman, 1979). Shatrugna et al. (1982), found that while the
high basal prolactin levels were maintained through the first 12 months of lactation, beyond 8 months postpartum suckling induced no longer a significant prolactin release.

The lower magnitude of prolactin response to suckling late in lactation was attributed to much briefer period of vigorous suckling which occurs during this period. Also it was said that older babies suckled efficiently for about 10 minutes and then tended to stop (Noel et al., 1974a). Now it is clear that the reduced plasma concentration of prolactin in response to suckling in late lactation is the result of an impairment within the prolactin secretory mechanism. The depressed plasma prolactin concentration in the late lactation appears to result from reduced rate of secretion of the hormone into the circulation i.e. low secretion rate (Grosvenor and Whitworth, 1975). The lower secretion rate could possibly be the result of decreased prolactin stores within the pituitary gland. However, the resting pituitary prolactin concentrations at early and late lactation were found to be similar (Subramanian and Reece, 1975) and the amount of prolactin depleted by suckling is the same during various stages of lactation, provided that similar non-suckling intervals are used (Grosvenor and Mena, 1971).

During the first few minutes of suckling there is an acute, precipitate drop in pituitary prolactin concentration and relatively little depletion occurs thereafter, even though suckling continues. In contrast, the secretion of prolactin into the plasma is characteristically steady, minute by minute secretion of small amounts which is not influenced by the amount of prolactin which has been depleted in the pituitary glands (Grosvenor et al., 1975). These results suggest that depletion of pituitary prolactin may not reflect direct secretion into the circulation, but rather indicate that
particularly in urban areas, mothers are turning away from the traditional patterns of prolonged breast-feeding to milk substitutes, often in the belief that what is good for Western mothers is good for themselves. Such a trend will have serious consequences for infant health and, in the absence of a major expansion in family planning services, for population growth as well (McCann et al., 1981). So, it is quite evident that lactation has been having a major impact on contraception.

Here, we are going to discuss breast feeding as a natural and effective means of child spacing. Also the effect of various hormonal contraceptives on milk flow and constituents will be discussed.

**Breast Feeding as a Means of Birth Control**

The concept that breast feeding might prevent more pregnancies than all other methods of contraception is a difficult one for those who are accustomed to consider fertility control exclusively in terms of modern family-planning methods. Nevertheless, there is now increasing awareness of the importance of breast-feeding as a method of birth control in developing countries.

**Fertility During Breast-feeding**

The effect of breast-feeding on fertility can be illustrated by comparing the time to next conception in lactating and non-lactating mothers who use no artificial contraception. The conception rates after childbirth for lactating and non-lactating mothers were studied in Eskimo (Berman et al., 1972),
and rural India (Potter et al., 1965) populations, neither of whom used contraception. Despite the widely different cultural settings, the results were broadly similar. In the non-lactating mothers, 50% were pregnant at about 8 months postpartum, but in the lactating mothers it took between 18 and 24 months before 50% of the mothers had conceived again. The difference would mean that non-lactating women would have approximately double the number of babies of lactating women over the same period of time.

Rosa (1975), attempted to qualify the contraceptive effect of breast-feeding in the non-communist countries of the developing world. He estimated that breast feeding contributed 31 million couple years of fertility protection per anum compared with 24 million couple years of all family-planning programmes. Whether the details of Rosa's calculations are precisely accurate or not, there can be no doubt of the importance of the impact of breast-feeding upon fertility.

**Effect of Breast-Feeding on Postpartum Menstruation and Pregnancy Rate**

Breast-feeding increases the duration of postpartum amenorrhoea and this provides indirect evidence that breast-feeding inhibits ovarian activity (Connel, 1978). The difference in pregnancy probability rates observed between nursing and non-nursing women postpartum can be accounted for by the differences in the length of the period of amenorrhoea and the number of anovulatory cycles after menstruation recurs (Berman et al., 1972).

The period of time that elapses after delivery before menses returns varies. It has been reported that menstruation is absent in practically all
women during the early months of lactation and as lactation proceeds the proportion of women menstruating increases (Kamal et al., 1969a). The longer the mother nurses the longer is the period of postpartum amenorrhoea. Tietze (1968), deduced that the mean duration of postpartum amenorrhoea could be expected to be 75% of the mean duration of nursing. Kamal and associates (1969a), noticed that by 9 months two thirds of lactating Egyptian mothers were menstruating and at the end of the tenth month, which they considered to be the average age of weaning, 87% of nursing mothers were menstruating. In a study performed on Alaskan Eskimos, the mean duration of postpartum amenorrhoea was found to be 10 months in nursing mothers and 2 months in non-nursing ones (Berman et al., 1972). In another report, Prema and co-workers (1979), found the mean duration of postpartum amenorrhoea to be 11.1 months among lactating women compared with 4.6 months for non-lactating ones. Delgado et al. (1979), deduced that prolonged lactation can extend the period of postpartum amenorrhoea about nine months beyond the usual in populations not breast feeding at all.

In a comparative cross-sectional study of nursing mothers in Africa (Zaire) and in Europe (Sweden), the return of menstruation during lactation was much slower in Zaire than in Sweden. In Sweden, 13% of mothers reported the return of the menstrual bleeding during the first three months as compared to only 3% in Zaire. Between 6 and 9 months of lactation, 60% of the Swedish mothers were menstruating as compared to 25% in the urban and 15% in the rural area of Zaire. The difference between the two Zairean groups became more marked during the second year of lactation. This discrepancy was found to be because the Zairean mothers gave the breast much more frequently than the Swedish and the
duration of breast-feedings was also longer in Zaire (140-170 min. / day) than in Sweden (70 min. / day). (Hennart et al., 1985).

Nursing women who experienced relatively long amenorrhoea in connection with one baby tended to do the same with a subsequent baby and those who experienced relatively short amenorrhoea tended to repeat the same pattern (Salber et al., 1968- EL Minawi and Foda, 1971). Such consistent postpartum amenorrhoeic pattern was labeled as "habitual postpartum lactational amenorrhoea". Prema et al. (1979), suggested that the duration of lactation is relatively fixed for each woman and that this fixed the duration of lactational amenorrhoea.

The duration of lactation, lactational amenorrhoea and interpregnancy interval are closely related variables. Kamal and co-workers (1969 a), interviewing women up to 5 years postpartum reported that 22.2 % of women became pregnant during postpartum amenorrhoea while nursing. This very high figure may be related in part to errors of recall. Berman and associates (1972), stated that no pregnancies could be expected during the first 4 months among women who nursed and thereafter the probability of becoming pregnant increases gradually to about 20 % at 12 months. On the other hand, in women who do not nurse they found that some could be expected to be pregnant in the second month, over 50 % at 7 months and occasionally 80 % within 10 months. In another study, between 1 and 10 % of nursing mothers were found to be liable for conception during lactational amenorrhoea (Buchanan, 1975). Prema et al. (1979 ), however, found this conception rate to be only 75 %. Leshaghe et al. (1981 ), have estimated that if the mean duration of lactational amenorrhoea in Pakistan fell from
the present 13 months to 6 months, the number of nursing mothers using contraception would have to rise from 6% to 30% to keep fertility rates at the same level. It is unfortunate that conception can occur before first menstruation, because it would be convenient if contraception could be postponed until it occurred.

The return of menstruation during lactation greatly increases the chances of conception, although it still remains less than it would be among non-nursing mothers. Potter and co-workers (1965), found 22% of breast-feeding women pregnant 3 months after ending postpartum amenorrhoea and 32% at 6 months, compared to 39 and 47% respectively of non-breast-feeding group. Tietze (1969), found this somewhat higher in non-nursing women, where 50% became pregnant at 3 months and 71% at 6 months first menstrual flow.

Hefnawi et al. (1977), followed the return of menstruation and ovulation and the incidence of pregnancy in a group of 148 lactating women who delivered normally at El-Galaa Hospital, Cairo. By the end of the sixth week postpartum, only 1.3% of the women had begun menstruating, the percentage gradually increased to 60.2% by the end of the first year. Ovulation had occurred in 58.1% and pregnancy in 26.1% of all cases at the end of the 12th postpartum month. Among menstruating lactating mothers, ovulation occurred in 86.5% and pregnancy in 32.6%, while in amenorrhoic lactating mothers only 6.1 percent had become pregnant at one year postpartum. In another study, Prema et al. (1979), found the conception rate to rise from a mean of 7.5% during lactational amenorrhoea to about 37.5% once menstruation returns during nursing. The lower conception rates among the nursing mothers, after menstruation have
returned, probably reflect the larger number of anovulatory cycles during lactation. However, the conception rates during menstruation are sufficiently high to make contraception essential for any woman who wishes to avoid a further pregnancy during lactation.

**Ovulation During Breast-Feeding**

The period immediately following delivery is traditionally utilized as a safe period. Pregnancy can not occur except after ovulation is resumed. Lactation is considered to postpone the resumption of ovulation and hence to prolong the infertile period. Practical application of published data concerning the occurrence of ovulation during lactation to the field of fertility control remains sketchy. No rule of thumb concept has gained wide clinical acceptance. Two distinct time factors appear to be involved in the postpartum resumption of ovulation, one concerned with ovulation "per se", the other with coordination of ovulation and menstruation.

Topkins (1943), studied 21 women during lactational amenorrhea with endometrial biopsy and detected ovulation in one case as early as the sixth week postpartum. Lyon and Stamm (1946), using basal body temperature found that the initial ovulation following parturition in non-lactating mothers occurred at an average of 10.2 weeks compared to 17.0 weeks in lactating mothers of at least 3 months. In a study of 626 endometrial biopsies for 285 women on the fifth day to the ninth month postpartum, Sharman (1957), found that the earliest appearance of secretory endometrium was on the forty-fourth day. He concluded that ovulation does not occur before the end of the sixth postpartum week. Kava et al. (1968), obtained endometrial biopsies from non-lactating women 33 to 93 days
Once menstruation begins, the percentage of subsequent menses that are ovulatory rises rapidly. Grunberger (1948), examined the endometrium in 34 postpartum patients and found that one third of the cases ovulated in the first cycle while the second cycle was ovulatory as a rule and the third cycle was always ovulatory. Davis (1949), according to the daily basal body temperature found that the first bleeding was rarely ovulatory but, the second period was associated with ovulation in approximately half of cases. Sharman (1951), noted about 29% "irregularity of menstruation" in women during continued nursing, compared with 10% irregularity in non-nursing women. Irregular menstrual bleeding after re-establishment of menstruation have been found to represent anovulatory cycles (Howie and McNeilly, 1983). Pavarti (1960), found that in lactating mothers the incidence of ovulation before the first, second and the third menstruations was 16%, 50% and 83%, respectively. Potter and associates (1965), estimated the anovulatory cycles in post-lactation amenorrhoea to be 2 months on the average, with no difference between nursing and non-nursing women. On the other hand, Berman et al. (1972), found it to be 3 months for non-nursing and 4 months for nursing women. Using serum progesterone, Said and co-workers (1974), found that the incidence of ovulation was 28.1% and 66.6% before the first and the second menstruation, respectively. They also found that a longer period of lactation increases the chance of an ovulatory first menstruation.

Vorherr (1973), after reviewing several published data, summed up the situation in respect to the return of postpartum menstruation and ovulation and this is presented in table (1):
Table (1): Return of postpartum menstruation and ovulation

<table>
<thead>
<tr>
<th></th>
<th>Return of menstruation</th>
<th>Return of ovulation</th>
<th>First ovular cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6w 12w 24w</td>
<td>6w 12w 24w</td>
<td></td>
</tr>
<tr>
<td>Nursing mothers</td>
<td>15% 45% 85%</td>
<td>5% 25% 65%</td>
<td>Preceeded by one or more anovular cycle in 80%</td>
</tr>
<tr>
<td>Amenorrheic</td>
<td></td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td>nursing mothers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-nursing mothers</td>
<td>40% 65% 90%</td>
<td>15% 40% 75%</td>
<td>the first post-partum cycle was ovular in 50%</td>
</tr>
<tr>
<td>Amenorrheic non-</td>
<td></td>
<td>20% 40%</td>
<td></td>
</tr>
<tr>
<td>nursing mothers</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This shows that the degree to which initial menstruation is ovulatory or anovulatory is quite variable. However, once menstruation begins, the percentage of subsequent menses that are ovulatory rises rapidly.

There have been few studies of luteal insufficiency in breast-feeding mothers. Reyes et al. (1972) reported defective progesterone secretion in the first postpartum cycles of two lactating women. Using a cross-sectional
design, Delvoye et al. (1980), found that the mean serum progesterone levels were lower in lactating mothers than in non-pregnant, non-lactating women suggesting a high number of defective luteal phases. Similarly, Gross et al. (1980), found that defective luteal function is common in Australian women returning to menstruation during lactation. In another study, McNeilly and associated (1982), found normal luteal phase pregnandiol levels in only 27% of the first cycles during lactation, the remainder of the luteal phases being deficient (31%) or absent (42%). The proportion of normal luteal phases rose to 77% in subsequent cycles.

The reason for the frequent failure of normal corpus luteum function during lactation is still unexplained. The finding of reduced urinary oestrogen excretion in addition to the defective pregnandiol excretion would be consistent with the possibility of an abnormal follicular development (McNeilly et al., 1982). However, the associated hyperprolactinemia which may act at the ovarian level to interfere with normal development of the pre-ovulatory follicle is a more plausible suggestion (McNatty, 1974-Delvoye et al., 1980-McNeilly et al., 1982-Hennart et al., 1985).

**DYNAMICS OF LACTATIONAL INFERTILITY**

The details of how fertility is inhibited during lactation are not fully understood, but it is likely that more than one mechanism is involved. The endocrine function of the hypothalamic-pituitary-ovarian axis during lactation remains open to speculation.

Some earlier investigators reported low hypophyseal FSH and LH levels during lactation and assumed that a suppressed gonadotrophic function of the anterior pituitary might be responsible for lactational anovulation and
amenorrhoea (Jaffe et al., 1969-Handson et al., 1970-Crystle et al., 1970-Madden et al., 1978). This was suggested to be due to failure of the pituitary to respond to endogenous GnRH resulting from absence of gonadotropin stores. Others referred such lack of an anovulatory surge of gonadotropins to absence of an oestradiol peak such as seen in the late follicular phase of the cycle (Keye and Jaffe, 1976).

Lactational gonadotrophic dysfunction also was attributed to failure of the hypothalamus to secrete endogenous GnRH in quantities large enough to cause an ovulatory surge of gonadotropins (Jeppson et al., 1974). This was also demonstrated through the daily administration of LRF - agonist to postpartum mothers where augmentation of LH response to LRH was observed similar to the observation noted in hypogonadotropic hypogonadism patients (Smith et al., 1979-Sheehan and Yen, 1979, Smith, 1981). Some other investigators, on the other hand, referred such lactational anovulation to an abnormality in the ratio FSH/LH (Smith, 1982-Williams et al., 1983).

Several investigators, however, have opposed this hypothesis and assumed that lactational anovulation and amenorrhoea might be due to ovarian refractoriness. Faiman et al. (1968), using radioimmunoassay methods, found normal follicular levels of serum FSH and LH after 6 weeks postpartum. According to Seddon (1970), 3,000 IU of HMG were injected into 3 women on postpartum day 14 and the response of the ovary was determined by assaying urinary total oestrogens. Two of these three women showed no evidence of a significant increase in steroidogenesis. Marshall et al. (1970), observed also that patients with secondary anovulation during lactation required a substantially greater amount of
gonadotropin to achieve ovulatory response. However, they failed to reveal the underlying mechanisms of such ovarian refractoriness.

Recent observations suggested that the ovaries as well as the pituitary are responsive to adequate tropic hormones stimulation (Tyson et al., 1978; Polan and Behrman, 1981). It was suggested that it is the high concentration of prolactin that works at both central and ovarian sites to produce lactational amenorrhoea and anovulation (Kase, 1983).

The suppressive effect of elevated circulatory prolactin levels on ovarian steriodogenesis is worthy to mention. Prolactin appears to affect granulosa cell function in vitro by inhibiting synthesis of progesterone (McNatty et al., 1974). It also may change the testosterone: dihydrotestosterone ratios thereby reducing aromatizable substrate and increasing local anti-oestrogen concentrations (Polan and Behrman, 1981). Bonner et al. (1975), suggested that prolactin interfere with ovarian steriodogenesis via competitive inhibition of FSH activity. However, they depended on isolated values of FSH and with frequent sampling it appears that the most important determinant in ovarian function may be the integrated FSH and LH levels.

In experimental animals, Fluckiger and Del Pozo (1978), demonstrated that prolactin is necessary for the maintenance of corpus luteum. Direct evidence in respect to the interference of progesteone synthesis by high prolactin levels was demonstrated by the prompt restoration of fertility by bromocriptine treatment in women with regular cycles and inadequate luteal function (Del Pozo et al., 1979). Furthermore, the reported restoration of ovarian function in amenorrhoea - galactorrheic women with bromocriptine and not HMG suggests that elevated prolactin levels inhibit ovarian sensitivity to gonadotropins (Mroueh and Siler-Khodr, 1976). Such an
effect of hyperprolactinemia on ovarian activity was also demonstrated by Delvoye et al. (1980), and Hennart and associates (1985), who demonstrated that luteinization occurred with lowering of serum prolactin.

The effect of elevated prolactin levels on pituitary gonadotropins during lactation remains largely debatable. In nursing rats, normalization of LH surge was observed after treatment with CB 154 indicating that prolactin is responsible for such suppressed hypothalamic-pituitary responsiveness (Smith, 1978b). Similarly, a rise of gonadotropins occurred upon the decrease in prolactin levels by bromocriptine in galactorrhea-amenorrhoea patients (Tyson et al., 1975a). During lactation, Delvoye et al. (1976), reported a significant positive correlation between a return to normoprolactinemia and the onset of postovulatory uterine bleeding implying that a decline in prolactin secretion and a rise in LH secretion might be inherently linked to the nursing stimulus. Similarly, with declining prolactin levels in nursing mothers, levels of LH were found to increase and when serum levels of prolactin drop near or within the normal non-lactating range, normal cyclic gonadotropin secretion occurred (Tyson et al., 1978; Madden et al., 1978; Duchen and McNeilly, 1980; Marrs et al., 1981). Such a suppressive effect of elevated prolactin on gonadotropin secretion may be through alteration of hypothalamic catecholamines metabolism (Fuxe et al., 1969), impairment of GnRH secretion (Nakano et al., 1974; Smith, 1981), or through inhibition of ovarian oestradiol production which by a positive feedback effect is responsible for the stimulation of GnRH secretion and, thus, LH secretion (Rolland et al., 1975). However, the exact mechanism is still unknown.
The relationship between ovarian steroids, gonadotropins and prolactin may be demonstrated as follows: Immediately after delivery, there is a complete ovarian suppression as shown by the rapid fall in serum oestradiol and progesterone to bottom values (Yannone et al., 1968 - Llauret et al., 1968 - Reyes et al., 1972). During the first two weeks postpartum, both FSH and LH are markedly suppressed (Reyes et al., 1972 - Said and Wide, 1973 - Jeppson et al., 1974 - Andreassen and Tyson, 1976 - Falsetti et al., 1982). From the second postpartum week onwards, both FSH and LH rise to reach values equivalent to those of low normal follicular phase of the cycle (Keye and Jaffe, 1976 - Howie and McNeilly, 1983), but are still incapable of stimulating ovarian activity possibly due to a peripheral block of gonadotropin activity at the ovarian level (Howie et al., 1982c). The associated hyperprolactinemia might be responsible for such an ovarian block (Bonner et al., 1975). Meanwhile, the inappropriately low gonadotropin levels for such low ovarian steroids level was attributed to suckling stimulus which renders the hypothalamic-pituitary axis more sensitive to negative feedback and less sensitive to the positive feedback effect of ovarian steroids (Baird et al., 1979).

Such defective gonadotropin release together with reduced ovarian responsiveness are maintained throughout long-lasting lactation (Madder et al., 1978 - Delvoye et al., 1978 - Duchen and McNeilly, 1980), and are responsible for lactational anovulation. When ovarian activity returns during lactation it is not associated with a rise in gonadotropin levels but rather with a drop in basal prolactin levels as a result of reduced suckling stimulus (Delvoye et al., 1980 - Hennart et al., 1985). This would suggest that the return of ovarian activity is associated with the removal of an
ovarian block rather than a change in gonadotropin activity (Howie and McNeil, 1983). On weaning, there is a further slight fall in prolactin but a sharp rise in follicular LH levels, which is associated with more adequate cycles as shown by the higher urinary oestrogen and pregnandiol excretion (Howie et al., 1982c). This would suggest that normal ovarian function does not resume until LH levels have been fully restored (Howie and McNeil, 1983).

**FACTORS AFFECTING LACTATIONAL INFERTILITY**

Contraceptive reliability (i.e. length of birth interval) of lactation depends on the intensity of suckling, the extent to which supplemented food is added to infant diet, the level of nutrition of mothers, the socioeconomic status of the mothers and sexual abstinence.

1) **Intensity of Suckling**:

The most important factor controlling ovarian activity during lactation is the strength of the suckling stimulus. It has been well established that the duration of lactational amenorrhoea, and the assumption of ovarian suppression, is longer among full breast-feeding mothers than among partial breast-feeding mothers (Perez et al., 1972). The relatively few studies that have measured suckling frequency or duration have found that mothers with the highest suckling frequencies have the highest prolactin levels, the longest of lactational amenorrhoea and the longest ovarian suppression (Howie et al., 1982a). The contraceptive efficiency of breast feeding is much greater in developing than in developed countries and in rural than in
urban societies. High contraceptive efficiency in lactating women in developing countries may be due to the frequent suckling on demand of feeding leading to a sustained prolactin stimulus and high plasma prolactin levels (Shatrugna et al., 1982). Mothers in traditional rural communities carry their babies with them, suckle them frequently, sleep with their babies at night and maintain lactation as long as possible. It is probable that these patterns of frequent and prolonged suckling are responsible for the contraceptive efficiency of breast feeding (Howie and McNeilly, 1983).

2) Supplementary Food:

The length of postpartum infertility is associated not only with the duration of lactation but also with whether or not an infant is receiving supplementary food. It has been postulated that nutritional supplements may discourage lactation through a "substitution" effect which leads to diminished suckling and thereby shortens the period of postpartum amenorrhoea (Delgado et al., 1978). It is not just the timing of supplements, but the rate by which they are introduced that influences suckling and ovarian activity. So, those mothers who introduced supplements most abruptly, also reduced suckling most rapidly and resumed ovulation most quickly (Howie and McNeilly, 1983).

Gioiosa (1955), studied 500 breast feeding mothers who used no contraception and found that, although 46 (9.2%) conceived during lactation, 40 (87.0%) of them had already well established the weaning process at the time of ovulation. Kippley and Kippley (1977), in the United States showed that the mean period of lactational amenorrhoea was 14.6 months in 22 American women using "natural mothering" (24 hours breast
feeding, no supplementation) and that the length of time was 43% longer than the mean for the whole group of breast feeding, including those with natural mothering and those breast-feeding but with early supplementation. So, although ovulation and conception do occur during full or un-supplemented lactation, they are less common than during partial lactation.

Measurement of plasma prolactin level, being one of the critical determinant of return of fertility, in lactating women showed higher levels in women whose infants were entirely breast-fed than those whose infants were receiving supplements (Shatrugna et al., 1982). It was suggested that the effect of supplementation on the duration of lactational amenorrhoea and infertility is mediated through prolactin.

It is often suggested that supplements need to be introduced to infants by the age of 3 months in order to prevent the fall in growth rate observed among infants in poorer segments of the population. The dangers of this are the early weaning diarrhoea in the child due to unhygienic enviromental conditions and for the mother, a faster return of fertility and the early advent of the next pregnancy with its attendant social, nutritional and health hazards for both mother and infant. So it would be of enormous value if the minimum suckling frequency to inhibit ovulation reliably could be established, so that nursing mothers could determine when it was necessary to take alternative procedures. There is insufficient data and no clear-cut guidlines to be given, although the more a mother suckles the less likely she is to conceive (Howie and McNeilly, 1983).
3) **Nutritional Status of the Mother:**

There has been some controversy about the role of maternal nutrition as a factor in postpartum infertility. It is postulated that malnutrition lowers the reproductive capacity of population (*Jelliffe and Jelliffe, 1978*). Studies from The Gambia have shown that the duration of lactational amenorrhoea is longer during wet season, when dietary intakes are low (*Lunn et al., 1980*). On the other hand, lower prolactin levels and increased ovarian activity have been found in Gambian mothers who are given caloric supplements during lactation, compared with controls who received no supplementation (*Lunn et al., 1984*).

Shatrugna and co-workers (*1982*), in a study on serum prolactin levels in undernourished Indian women had deduced that both basal and peak plasma prolactin levels continue to remain at a higher level in lactating groups for at least 12 months. This elevated plasma prolactin levels might be a protective mechanism that prolongs the duration of relative lactational infertility. It has been suggested that high plasma prolactin value might be one of the factors responsible for preferential transfer of nutrients into milk in undernourished women, a mechanism evolved over millenia to ensure infant survival (*Lunn et al., 1980*). On the other hand, Bongaarts (*1980*), has concluded that chronic malnutrition has a relatively small effect on the fecundity of lactating women.

In summary, different lines of evidence suggest that nutrition may be an important determinant of ovarian function during lactation. But, it is possible that malnourished mothers have to suckle their babies more frequently in order to nourish them adequately, and in this way chronic malnutrition have a secondary rather than a primary effect on postpartum infertility.
4) The Socioeconomic Status:

Unfortunately during the last decade, with increasing urbanization, education and betterment of socioeconomic status, there is a tendency towards decline in breast feeding. This has been partly attributed to the acceptance of bottle-feeding as a symbol of progress and sophistication among urban women. However, fortunately prolonged lactation is the rule among urban low-income groups. With increasing levels of education, the better off segments of the population tend to wean their babies earlier (Prema et al., 1979).

Delgado et al. (1978), compared the duration of lactation and duration of postpartum amenorrhoea in two different socioeconomic groups, Boston and Taiwan nursing mothers. They found that, for the same duration of lactation, mothers from Taiwan (low socioeconomic groups) present longer postpartum amenorrhoea than mothers from Boston (middle class).

Urban-rural differences in birth intervals are pronounced in all areas of the world, this may be attributed to increased amount of suckling with its subsequent elevation of plasma prolactin levels in the rural group (Cantc et al., 1980). Moreover, nutritional variation might be another underlying factor.

5) Sexual Abstinence:

In many societies, particularly in Africa, sexual abstinence is observed during lactation and this practice would obviously enhance the fertility-reducing effect of breast feeding (Makinwa and Nichols, 1982). Kayner and Zagar (1983), attributed such sexual abstinence to lack of vaginal lubrication and subsequent unpleasant coital experience especially
EFFECT OF CONTRACEPTIVES ON BREAST FEEDING

Most mothers who are fully breast-feeding will have a high degree of contraceptive protection in the early weeks of puerperium. With maintainance of regular breast feeding and gradual supplementation, the contraceptive effect of breast feeding will last for many months. However, the possibility of unexpected conception is present. So mothers will desire some additional contraceptive protection. An important practical question for breast-feeding mother is the most appropriate method of contraception during lactation. As well as being both effective and acceptable, the contraceptive method should not have any deleterious effect, either on the baby or on the quantity or quality of milk.

EFFECT OF HORMONAL STEROIDS

Over the past 20 years, multiple studies have been carried out to assess the impact of steroid contraceptive use during lactation. It is unfortunate that those studies varied in protocol, contraceptives examined, and the length of time postpartum. Such research findings may be divided into four areas: (1) effect on duration and quality of lactation, (2) effect on milk composition, (3) effects on the infant and (4) effects on the mother.

Combined hormonal contraceptives affect lactation adversely. Numerous papers have suggested that combined oestrogen / progesterone preparations may adversely affect lactation in terms of the quantity and quality of the milk (Connel, 1978). It has been suggested that combined oral contraceptives may decrease milk volume, change the composition of milk
and shorten the duration of lactation (Connel, 1978 - Edelman et al., 1981 - McCann et al., 1981 - Prema, 1982 - Aref et al., 1982). It appears that oestrogen is probably the agent which most adversely affect lactation as the decreased milk production is most evident in women using sequential pills and those combined pills which contain high doses of oestrogen (McCann et al., 1981). With combined preparations having a lower dose of oestrogen (e.g. Microliar 30), lactation may sometimes decrease but rarely stops completely (Connel, 1978 - Badrawi and Askalani, 1983). Wahby et al. (1980), investigated the effect of oral combined contraceptives on the morphology of the breast and found no distinctive morphological changes.

Progesterone-only contraceptives, either pills or injections do not seem to affect adversely the quantity or quality of maternal milk (Badrawi et al., 1977). The nor-progestens (nortryodral, norethindrone and quingestrol acetate) may resemble combined pills in their effect on lactation as they are metabolized in vivo to oestrogen. They have been reported both to inhibit the volume of milk and to shorten their duration of lactation (Vorherr, 1983). Kamal et al. (1969 b), studied the clinical effects of different combination of gestagens 6 to 10 weeks postpartum and found that the small dose lynosterol pill alone showed the best results as far as influence on lactation. Progestagens have no effect on the initiation of lactation (Badrawi et al., 1977). In a number of studies, it was found that in patients treated with medroxyprogesterone acetate (Depo-Provera), either immediately or one month postpartum, the duration of lactation was either unchanged or significantly prolonged (El-Mahgoub et al., 1972) possibly due to a stimulatory effect on prolactin (Chacery et al., 1977). One survey showed a 1 1/2 month increase in the duration of lactation over the woman's
previous experience with breast feeding when medroxyprogesterone acetate had not been used (Connel, 1978).

The content of milk has been studied by a number of investigators. A diminution was observed in milk protein and milk fat. Narrow variations were observed in milk lactose which were statistically insignificant. The milk inorganic constituents namely; sodium, potassium, calcium and magnesium were significantly decreased (Connel, 1978). It is conceivable that the change in milk composition induced by gestagens might be through a direct effect on mammary tissue or indirectly by affecting blood components. It seems more probable, however, that gestagens have a selective action on the alveolar cells which are concerned with the biosynthesis of major part of milk proteins and fat. Gestagens might also exert a selective action on the permeability of the alveolar epithelium leading to a decrease in the different milk electrolytes (Abdel-Kader et al., 1969).

The transfer of exogenous contraceptive steroids into human milk during established lactation has been also investigated. Plasma : milk ratio of contraceptive steroids have been studied and was found to be 100 : 15 for d-norgestrel, 100 : 80 for megestrol acetate, 100 : 25 for ethinyl oestradiol and 1 : 1 for medroxyprogesterone acetate (Saxena et al., 1977). This transferred steroids were also detected in milk several weeks after stoppage of pills.

In general, no adverse effects have been found on babies apart from isolated cases of gynaecomastia. This could possibly be caused by the oestrogen component of oral contraceptives. So if hormonal contraception is to be used during lactation, low dose gestagens may be preferable in order to avoid any side effects due to the oestrogens. The transfer of gestagens
derived from 19-nortestosterone could have an androgenic effect on newlyborne infants, either directly or by displacement of testosterone from sex hormone binding globulin. So far, no impairment of ovarian function or fertility have been found in human infants after breast feeding by mothers who were taking oral contraceptives during lactation. Indirect effect on the growth of babies have been reported because of decreased milk volume (Nilsson and Nygren, 1979). Experts, though not certain, account that pills and injection present no hazards if they contain 2.5 mg or less of a 19-norprogesterone and 50 µg or less of ethinyloestradiol or 100 µg or less of mestranol (Lawrence, 1982).

In addition to the nature of the hormonal contraceptive chosen, the time of its use appears to be important. When a hormonal preparation is being used, it is better to wait until lactation is established and, in most cases, 6 weeks postpartum is a convenient time. If, however, breast feeding is discontinued before then, contraception should be started immediately because ovulation may occur rapidly after complete weaning (Edelman et al., 1981). However, some clinicians initiate oral contraceptive therapy immediately postpartum feeling that this practice ensures good acceptance by the patient, minimal side effects and regular menses in addition to reliable contraception (Vorherr, 1983).

The following table shows the effect of various contraceptive steroids on lactation (Vorherr, 1983):
Table 2: The effect of various contraceptive steroids on lactation

<table>
<thead>
<tr>
<th>Agent</th>
<th>Effect on Lactation</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oestrogens</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Ethinyl oestradiol (50 µg)</td>
<td>No change</td>
<td>Vorherr, 1983</td>
</tr>
<tr>
<td>* Mestranol (80 µg)</td>
<td>No change</td>
<td>Vorherr, 1983</td>
</tr>
<tr>
<td><strong>Progestins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Medroxyprogesterone acetate</td>
<td>No change</td>
<td>El-Mahgoub, et al., 1972</td>
</tr>
<tr>
<td>150 µg (depot provera)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Medroxyprogesterone acetate</td>
<td>Significant increase in milk protein and</td>
<td>Hefnawi, et al., 1970 and 1976</td>
</tr>
<tr>
<td>300 µg (every 6 months)</td>
<td>quantity</td>
<td></td>
</tr>
<tr>
<td>* Ethinyloestrenol 0.5 mg</td>
<td>No change</td>
<td>Vorherr, 1983</td>
</tr>
<tr>
<td>* Lynestrenol 0.5 mg</td>
<td>Fat and protein are reduced</td>
<td>Abdel-kader, et al., 1969</td>
</tr>
<tr>
<td>* Norethindrone 10 mg</td>
<td>No change</td>
<td>Vorherr, 1983</td>
</tr>
<tr>
<td>* Norethindrone 200 mg</td>
<td>No change</td>
<td>Abdel-kader, et al., 1969</td>
</tr>
<tr>
<td><strong>Oestrogen-Progestin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Norethindrone 4 mg + 50 µg</td>
<td>Decrease by 40%</td>
<td>Vorherr, 1983</td>
</tr>
<tr>
<td>ethinyl oestradiol (Anovlar)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Dihydroxyprogesterone 150 mg</td>
<td>Milk fat and protein are significantly reduced</td>
<td>Abdel-Kader, et al., 1969</td>
</tr>
</tbody>
</table>
Table 2: cont.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Effect on Lactation</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>* Norethynodrel 2.5 &amp; 5 mg</td>
<td>No change</td>
<td>Vorherr, 1983</td>
</tr>
<tr>
<td>+ Mestranol 100 µg (Enovid)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Norethynodrel 10 &amp; 20 mg</td>
<td>Decrease by 40.80%</td>
<td>Vorherr, 1983</td>
</tr>
<tr>
<td>+ Mestranol 100 µg (Enovid)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Lynestrenol 1.25 mg</td>
<td>Milk fat and protein are significantly reduced</td>
<td>Abdel-Kader et al., 1969</td>
</tr>
<tr>
<td>+ Mestranol 100 µg (Lyndiol 1 &amp; 2.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Norethindrone acetate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5 mg + Ethinyl oestradiol</td>
<td>No change</td>
<td>Vorherr, 1983</td>
</tr>
<tr>
<td>50 mg (Norlestrin 2.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Norethindrone 2.5 &amp; 10 mg</td>
<td>Decrease milk supply gradually up to 58% and stop of lactation in 33%</td>
<td>Vorherr, 1983</td>
</tr>
<tr>
<td>+ Mestranol 100,60 &amp; 60 µg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Ortho-Novum 2.5 &amp; 10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Chlormadinone acetate 3 mg</td>
<td>Increase fat, lactose and total proteins</td>
<td>Ramadan et al., 1972</td>
</tr>
<tr>
<td>+ Mestranol 100 µg (Ovosiston)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Norgestrel 0.5 mg +</td>
<td>Decreased weight gain in lactated infants</td>
<td>Bersivala, 1979</td>
</tr>
<tr>
<td>Ethinyl oestradiol 50 µg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Ovral)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Ethynodiol diacetate</td>
<td>Decreased milk supply by 30-55%</td>
<td>Vorherr, 1983</td>
</tr>
<tr>
<td>1 mg + Mestranol 100 µg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Ovulen)</td>
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</table>
EFFECT OF INTRAUTERINE DEVICES ON LACTATION

The use of an intrauterine device (IUD) as a postpartum contraceptive has been of growing interest and popularity. Much discussion settled around the time of insertion, the type of device and the possible effect on lactation. However, many investigators studied the effect of common types of IUDs on lactation. Non-medicated IUDs such as Lippes loop, or safe T coil, probably do not affect breast feeding (Hefnawi et al., 1975). Copper IUDs do not appear to affect the quality or quantity of milk (Mehta et al., 1977 - Wenof et al., 1979 - Askalani et al., 1982).

On the other hand, there is a significant increase in the amount of milk yield and of its protein content in women using Progestasert devices during the first postpartum year (Badrawi et al., 1977 - 1982 - Askalani et al., 1982). In spite of such an effect on milk yield, Progestasert IUDs do not alter serum prolactin level (Badrawi et al., 1981).

The effects of IUDs on pituitary ovarian function during lactation have been investigated by Abdalla and associates (1981). Serum levels of FSH, LH and progesterone were determined in lactating and menstruating women, who were categorized into non-IUD users, CuT 200 users and Progestasert users, 6-8 months after delivery. A remarkable decrease in the mean values of FSH, LH and progesterone were evident in Progestasert users, indicating a systemic depressive effect on the hypothalamic-pituitary-ovarian axis in lactating women. However, a significant difference was noted between the non-IUD, CuT 200 groups and Progestasert group for LH only. Considering serum progesterone level as the index of corpus luteum function, the incidence of ovulation, corpus luteum insufficiency and anovulation was 50%, 17% and 33% respectively in CuT 200 group, and 17%, 33% and 50% respectively in the Progestasert group.
IUDs seem highly appropriate for the lactating women. It allows for fully uninterrupted lactation and excellent contraception. However, lactational amenorrhoea is shortened in IUD users (Badrawi et al., 1982 - Askalani et al., 1982). Problems as higher incidence of expulsion, increased request for removal and string loss may be eliminated through the use of better insertion techniques, device modifications and application at 6-8 weeks postpartum (Labbock, 1983).

**OTHER CONTRACEPTIVE METHODS**

**Barrier Methods:**

They have been considered as the best methods during lactation. Obviously, non-invasive methods of contraception as condom, vaginal diaphragm with or without spermicides do not affect neither the quality nor the quantity of breast milk (Edelman et al., 1981, Labbock, 1983).

**Natural Methods:**

Coitus interruptus, safe period based upon body temperature charts, and other natural methods have no effect on lactation (Brillings, 1970).

**Tubal Sterilization:**

Preliminary evidence indicates that the timing of postpartum sterilization may significantly affect lactation, either because of the anxiety and stress caused by the procedure or because of the use of sedation and anaesthesia (Dusitsin et al., 1980). Milk production apparently is not affected in women undergoing operation within 24 hours of delivery, but there is a significant lowering of milk volume at both 7 and 14 days
postpartum in women whose tubal ligations were done 4 to 6 days after delivery (Winikoff and Baer, 1980). The investigators hypothesize that the lactation suppressing effect of anaesthesia, heavy sedation and anxiety are most critically significant, if imposed on mothers during the time when milk production begins and lactation is being established (Winikoff and Baer, 1980). Sedatives carried in the breast milk may affect infant's suckling response leading to poor milk supply, hence more poor suckling and vicious circle begins (Hatchers et al., 1982).
MATERIALS AND METHODS
MATERIAL AND METHODS

SUBJECTS

The present study was carried out on 102 lactating women of nearly the same socioeconomic class (low socioeconomic class), with the following specifications: age 20 to 35 years, parity 1 to 5, not using any hormonal or intrauterine contraceptives or any medication known to influence hormone homeostasis and free from any apparent endocrinopathy.

Women were classified according to the duration of lactation into three groups.

Group I:

This included mothers who were lactating for approximately 6 months (N = 30). They were subclassified according to occurrence of menstruation, to menstruating (N = 17) and amenorrhoeic (N = 13).

Group II:

This included mothers who were lactating for approximately one year (N = 46) and were subclassified into 21 menstruating and 25 amenorrhoeic.

Group III:

This included mothers who were lactating for approximately 1 1/2 year or more (N = 26) and were subclassified into 16 menstruating and 10 amenorrhoeic.
**BLOOD SAMPLING**

From each woman, 10 ml of venous blood were collected in a sterile container. In menstruating females, two blood samples were collected every other day prior to the expected day of menstruation (Mo) and these coincided with day M-5 to day M-7. In amenorrhoeic females blood samples were collected twice from each woman, one day apart. Sera were separated and kept at -20 °C pending hormonal assays.

**HORMONAL ASSAYS**

Radioimmunoassay techniques were applied for the estimation of Follicle - Stimulating Hormone (FSH), Luteinizing Hormone (LH), Prolactin (PRL) and Progesterone according to WHO - RIA Matched Program using their reagents.

**I. ASSAY OF PEPTIDE HORMONES (PRL, FSH, LH)**

* Reagents :

(a) **Assay Buffer** : The following reagents were dissolved in one liter of distilled water.

- 2.35 g sodium dihydrogen phosphate, anhydrous (NaH$_2$PO$_4$ - MW = 120).
- 11.6 g disodium hydrogen phosphate, anhydrous (Na$_2$HPO$_4$ - MW = 142).
- 8.8 g sodium chloride (NaCl).
- 0.1 g thiomersal (merthiolate).
- 5.0 g bovine serum albumin.
EDTA was added to a final concentration (in one liter) of 0.025 M. The pH was adjusted to 7.4.

This peptide buffer was used as a diluent for all reagents in the peptide assay.

(b) **Tracer Diluent**: Normal rabbit serum was added to the peptide buffer, at a rate of 0.5 ml normal rabbit serum per 100 ml peptide buffer.

(c) **Peptide Tracer**: FSH, LH and PRL iodinated tracers were provided by the WHO-RIA Matched Program in a lyophilized form. Each vial of lyophilized tracer was dissolved in 1 ml peptide buffer. The working peptide tracer was made up by diluting 0.2 ml of the redissolved tracer in 10 ml tracer diluent.

(d) **Peptide Antiserum**: Provided in lyophilized form, each bottle contains enough antiserum for one assay batch of 100 tubes. The contents of one bottle of antiserum was reconstituted with 10 ml of assay buffer. The mixture was allowed to stand for 5-10 minutes and mixed thoroughly before use. This made enough antiserum for 100 tubes.

(e) **Second Antibody**: Donkey anti-rabbit serum was provided in liquid form. It contains 0.1% sodium azide. It was diluted 1/26 in buffer, just prior to use.

(f) **Peptide standards**: Provided in lyophilized form. Before performing an assay the contents of one vial of lyophilized standard was carefully reconstituted with exactly 1 ml of assay buffer. Further dilutions was carried out using buffer.
**Assay Procedure**

- 100 µl of each of the various concentrations of the standards and unknown sera were pipetted in triplicate in the assigned assay tubes.
- To each tube, 0.1 ml of the radiotracer was added followed by the addition of 0.1 ml of the antisera.
- To all tubes, 0.4 ml of the peptide buffer were added.
- Three tubes were assigned for non-specific binding in which 0.6 ml buffer were added thus replacing the aliquots of the antisera, buffer and standard volume used in the assay tubes.
- All tubes were vortexed for 1 minute and left to stand at 4°C for 48 hours, after which 0.1 ml of diluted second antibody was added to each tube. The tubes were incubated again for another 24 hours at 4°C.
- After the second incubation period, all the tubes were centrifuged in a refrigerated centrifuge for 45 minutes at 3000 r.p.m.
- The tubes were then transferred to decantation racks and the supernatant was decanted.
- The tubes were left in the decantation racks after inverting the rack on absorbant tissue for 30 minutes to ensure complete drainage of the supernatant.
- All the tubes were placed in automatic gamma spectrometer and the radioactivity of the bound radiotracer to the antibody in the precipitate was measured. A standard curve was thus established representing the various concentrations of the standards in the assay on the x-axis and the B/Bo on the Y-axis, where B represents the percent bound of the unknown or standard and the Bo represents the radiotracer bound to hormone free tracer (buffer). The unknown values were read directly from the standard curve.
11. ASSAY OF PROGESTERONE

Reagents:

(a) Assay Buffer: The following reagents were dissolved in distilled water to 1 liter:
- 2.35 g sodium dihydrogen phosphate, anhydrous (NaH₂PO₄ - MW = 120)
- 11.6 g disodium hydrogen phosphate, anhydrous (Na₂HPO₄ - MW = 142)
- 8.8 g Sodium chloride (NaCl).
- 0.1 g thiomersal (merthiolate).
- 1.0 g gelatin.

The gelatin was dissolved in warm water first before being added to the other reagents. The pH of the buffer was adjusted to 7.4. This assay buffer was used as diluent for all reagents.

(b) Charcoal Suspension: 0.065 g dextran were dissolved in 100 ml assay buffer in stoppered flask, then 0.625 g activated charcoal were added. The slurry was shaken vigorously for 30 minutes and stored at 4°C.

(c) Steroid Standards: All steroid standards were provided by WHO in a concentrated form and further dilutions were made up by the steroid buffer.

(d) Steroid Tracers: These were supplied by WHO, each tracer (3H) - steroid tracer was supplied in amount of 250 μCi in a sealed ampoule. The contents were transferred to a 25 ml volumetric flask and made up to 25 ml with a solution of benzene : ethanol, 9 : 1 (vol / vol). This stock solution containing 10 μCi/ml was stored at 4°C. The working tracer solution was prepared for each as follows: 150 μl of the stock solution was pipetted into
a vial and the solvent was evaporated, 15 ml of the assay buffer were added to the dried tracer. This working tracer was sufficient for a test containing 100 tubes and whatever remained was discarded and not stored.

(e) Counting Solution: The scintillation fluid was made up by dissolving 7.50 g PPO (2,5-Diphenyloxazole), scintillation grade and 0.75 POPOP (1,4-bis-2,5-phenyloxazolyl benzene) in three liters toluene.

*Assay Procedures:

i- Recovery Corrections: Preliminary studies carried out in the Radioimmunology and Biochemistry Unit of the Egyptian Atomic Energy Establishment showed a constant recovery for each hormone for various recovery tests carried out on a number of the subjects at various occasions using the same reagents. Therefore, the recovery monitoring step was omitted except for occasional checks. The recovery value for each hormone was used as a constant value for correction of the extraction efficiency.

ii- Extraction and Evaporation: Diethyl ether of a highest grade was used for the extraction of steroid hormones. For progesterone 0.5 ml was extraced once with 5 ml diethyl ether. The tubes were vortexed for one minute in a suitable stoppered test tube. This was followed by allowing the tubes to stand at room temperature for half an hour for complete settlement of the two phases after which the supernatant was removed into a clean dry glass tube. The ether was then evaporated using filtered nitrogen in a 40°C water bath.

iii - Redissolving and Aliquoting: 2 ml of assay buffer were added to the dried residue in the tube and vortexed with rolling the buffer around the tube to ensure complete dissolving of the residue.
The technique used for the assay was as follows: 0.5 ml of the redissolved extract was transferred to the assay tubes and 0.5 ml of each standard connection were pipetted in the corresponding tubes. This was followed by the addition of 0.1 ml of the working tracer and 0.1 ml of the diluted antisera. Non-specific binding was included in the assay by adding 0.6 ml of assay buffer to the assigned tubes, thus replacing the sample redissolved extracted and the antisera. The contents of each tube was vortexed for few seconds to assure complete mixing. All the tubes were left for an incubation period of 24 hours at 4°C. Upon the completion of this incubation period 0.2 charcoal suspension were added to each tube using a repeated dispenseette and the tubes were shaked for few seconds. All the tubes were centrifuged immediately in a refrigerated centrifuge for 5 minutes at 3000 r.p.m. The supernatant of each tube was transferred to scintillation vial. To each vial 10 ml of the scintillation fluid were added. All the vials were placed in a water bath at 40°C for 15 minutes, then each vial was vortexed for 1 minute. The vials were then transferred to liquid scintillation counting system and left to stand there for 1 hour under refrigeration before counting.

iv. Calculation of the results: A standard curve was established, representing the standards concentration on the X-axis and B/Bo (B - percent bound of the standard; Bo - percent bound of standard 0) on the Y-axis. The steroid concentration in each of the unknown assay tube was thus obtained from direct reading from the standard curve. Appropriate corrections for the unknown values were applied to the values obtained from the standard to correct for percent recovery and the sample volume used thus expressing the values per ml serum.
**STATISTICAL ANALYSIS**

Statistical methods used for evaluation of the present results were carried out according to Armitage (1974), and can be summarized as follows:

1- The mean:

\[ \bar{x} = \frac{\sum x}{n} \]

where: \( \bar{x} \) = arithmetic mean

\[ \sum \] = summation

\( x \) = individual values

\( n \) = number of data

2- Standard deviation (S.D.):

\[ S.D. = \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}} \]

3- Standard error of the mean (S.E.M.):

\[ S.E.M. = \frac{S.D.}{\sqrt{n}} \]

4- Variance (Vr):

\[ Vr = S.D. \times S.D. \]

5- Analysis of variance (ANOVA): This was used to detect the presence of any significant variability if more than one treatment is compared. One-way classification was applied using the following equation:
RESULTS
RESULTS

SERUM LEVELS OF PITUITARY HORMONES AND PROGESTERONE IN LACTATING MENSTRUATING FEMALES

Data on individual cases regarding levels of PRL, FSH, LH and progesterone in lactating menstruating women 6 months after delivery is shown in table 3, 12 months after delivery in table 4 and 18 months after delivery in table 5.

The mean values of serum PRL, FSH, LH and progesterone at each interval of estimation in lactating menstruating females are presented in table 6. Statistical evaluation, using simple analysis of variances was carried out to test the significance of difference between the mean values of the three intervals for each hormone and a summary of such analysis are presented in tables 7, 9, 10, 12 respectively. Further statistical analysis, using unpaired "t" test was applied to the data to test which mean interval differs from which.

PROLACTIN (PRL)

A gradual decrease in serum PRL level was noted with advance of time after delivery (Table 6, Figure 1). However, remarkable decrease was noted as the stage of delivery advanced from 6 to 12 months. At 12 months the mean value decreased by 38% compared to 6th month mean value. No appreciable difference was noted between 12 and 18 months after delivery.

Statistical evaluation of the data, using simple analysis of variances indicated significant difference between the mean value of the three groups (P<0.05) (Table 7).
Further statistical analysis using unpaired "t" test, was applied to test the site of significant difference in the mean values of PRL at various time intervals after delivery. Significant difference (P<0.05) in serum PRL was obtained when the mean value of the 6 months group was compared with the mean value of any interval estimation. However, lack of such significant difference (P>0.05) was noted between samples at 12 and 18 months intervals. (Table 8).

From inspection of the individual data (Tables 3, 4, 5), wide variation was evident within each interval. Such individual variation was also apparent from the wide ranges obtained, being 8.0-70, 6.9-51.7 and 5.0-50.6 ng/ml for the 6, 12 and 18 months' intervals, respectively.

FOLLICLE-STIMULATING HORMONE (FSH)

Although there was a decrease in FSH level in the 12 months interval from the preceding or subsequent interval, the differences between the three groups were not appreciable (Table 6, Figure 2). Simple analysis of variances (Table 9) revealed insignificant difference (P>0.05) between the three interval FSH mean values. Wide individual variation was also evident within each interval of estimation (Tables 3, 4, 5).

LUTEINIZING HORMONE (LH)

As a function of advance in time after delivery, there was a gradual increase in serum LH level, reaching its peak value at the last interval of estimation i.e. at 18 months after delivery (Figure 3). At such interval, the percent increase from the 6 months interval was, on average, 164%. (Table 6).
Analysis of variances revealed significant differences between the three mean values (P<0.01) (Table 10). Unpaired "t" test was applied to the data to test which interval mean value differs from which significantly (Table 11). Significant difference (P<0.01) in serum LH was obtained when the mean value of the 18 months group was compared with the mean value of any interval of estimation. However, lack of such significant difference was noted between the 6 and 12 months intervals.

Wide individual variation was evident within the intervals (Table 3, 4, 5).

**PROGESTERONE**

No remarkable changes were noted between serum progesterone mean values of the three intervals of estimation, although, the second interval mean value was 71 and 32% higher than the corresponding mean values of the 6 and 18 months intervals (Table 6, Figure 4).

Simple analysis of variances revealed insignificant differences between the mean values of serum progesterone of the three intervals of estimation (P>0.05) (Table 12).

It is worth mentioning that wide individual variation within each interval were evident, as demonstrated from the relatively high standard deviation at each interval and the high range noted being; 0.36-3.45, 0.49-3.9, and 0.28-4.78 ng/ml for the 6, 12 and 18 months intervals, respectively.
### Table 3: Individual data of PRL, FSH, LH and progesterone in lactating menstruating females about 6 months after delivery

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Code No.</th>
<th>PRL (ng/mL)</th>
<th>FSH (mIU/mL)</th>
<th>LH (mIU/mL)</th>
<th>Progesterone (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>19.0</td>
<td>1.98</td>
<td>3.38</td>
<td>1.77</td>
</tr>
<tr>
<td>2</td>
<td>37</td>
<td>25.0</td>
<td>2.34</td>
<td>3.08</td>
<td>1.05</td>
</tr>
<tr>
<td>3</td>
<td>41</td>
<td>37.0</td>
<td>3.60</td>
<td>2.70</td>
<td>3.48</td>
</tr>
<tr>
<td>4</td>
<td>44</td>
<td>21.0</td>
<td>4.32</td>
<td>3.60</td>
<td>3.26</td>
</tr>
<tr>
<td>5</td>
<td>28</td>
<td>9.4</td>
<td>13.50</td>
<td>4.50</td>
<td>1.11</td>
</tr>
<tr>
<td>6</td>
<td>35</td>
<td>9.8</td>
<td>10.13</td>
<td>6.98</td>
<td>1.43</td>
</tr>
<tr>
<td>7</td>
<td>19</td>
<td>—</td>
<td>1.71</td>
<td>3.38</td>
<td>1.42</td>
</tr>
<tr>
<td>8</td>
<td>71</td>
<td>8.0</td>
<td>4.50</td>
<td>3.75</td>
<td>2.06</td>
</tr>
<tr>
<td>9</td>
<td>95</td>
<td>50.1</td>
<td>6.62</td>
<td>6.15</td>
<td>0.36</td>
</tr>
<tr>
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<td>99</td>
<td>13.0</td>
<td>5.40</td>
<td>4.28</td>
<td>1.85</td>
</tr>
<tr>
<td>11</td>
<td>104</td>
<td>20.0</td>
<td>8.28</td>
<td>2.96</td>
<td>1.18</td>
</tr>
<tr>
<td>12</td>
<td>105</td>
<td>39.5</td>
<td>10.26</td>
<td>4.50</td>
<td>1.21</td>
</tr>
<tr>
<td>13</td>
<td>106</td>
<td>34.0</td>
<td>10.26</td>
<td>4.43</td>
<td>—</td>
</tr>
<tr>
<td>14</td>
<td>107</td>
<td>70.0</td>
<td>9.72</td>
<td>4.20</td>
<td>0.43</td>
</tr>
<tr>
<td>15</td>
<td>108</td>
<td>28.5</td>
<td>8.64</td>
<td>5.85</td>
<td>0.78</td>
</tr>
<tr>
<td>16</td>
<td>109</td>
<td>44.6</td>
<td>8.28</td>
<td>4.05</td>
<td>1.36</td>
</tr>
<tr>
<td>17</td>
<td>110</td>
<td>70.0</td>
<td>8.10</td>
<td>4.50</td>
<td>0.86</td>
</tr>
</tbody>
</table>

| X          | 31.18    | 6.92        | 4.25        | 1.48        |
| Vr         | 367.80   | 11.62       | 1.34        | 0.72        |
| SD         | 19.18    | 3.41        | 1.16        | 0.85        |
| SE         | 4.79     | 0.83        | 0.28        | 0.21        |

Range: 8.0–70.0  1.71–13.15  2.7–6.98  0.36–3.0
Table 4: Individual data of PRL, FSH, LH and progesterone in lactating menstruating females about 12 months after delivery

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Code No.</th>
<th>PRL (ng/ml)</th>
<th>FSH (mIU/ml)</th>
<th>LH (mIU/ml)</th>
<th>Progesterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>9.0</td>
<td>1.98</td>
<td>4.69</td>
<td>0.49</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>14.0</td>
<td>4.05</td>
<td>4.69</td>
<td>1.92</td>
</tr>
<tr>
<td>3</td>
<td>92</td>
<td>14.0</td>
<td>3.06</td>
<td>6.40</td>
<td>9.58</td>
</tr>
<tr>
<td>4</td>
<td>90</td>
<td>23.0</td>
<td>9.36</td>
<td>11.10</td>
<td>1.10</td>
</tr>
<tr>
<td>5</td>
<td>82</td>
<td>10.0</td>
<td>2.52</td>
<td>4.05</td>
<td>9.58</td>
</tr>
<tr>
<td>6</td>
<td>78</td>
<td>34.9</td>
<td>2.57</td>
<td>13.05</td>
<td>0.57</td>
</tr>
<tr>
<td>7</td>
<td>77</td>
<td>9.8</td>
<td>7.36</td>
<td>7.35</td>
<td>0.86</td>
</tr>
<tr>
<td>8</td>
<td>74</td>
<td>21.3</td>
<td>0.95</td>
<td>4.80</td>
<td>_</td>
</tr>
<tr>
<td>9</td>
<td>66</td>
<td>9.4</td>
<td>8.01</td>
<td>3.45</td>
<td>0.50</td>
</tr>
<tr>
<td>10</td>
<td>59</td>
<td>14.0</td>
<td>6.21</td>
<td>4.65</td>
<td>1.86</td>
</tr>
<tr>
<td>11</td>
<td>56</td>
<td>6.9</td>
<td>6.21</td>
<td>5.40</td>
<td>0.57</td>
</tr>
<tr>
<td>12</td>
<td>55</td>
<td>14.5</td>
<td>4.32</td>
<td>3.45</td>
<td>1.70</td>
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<td>17.9</td>
<td>1.08</td>
<td>1.95</td>
<td>1.00</td>
</tr>
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<td>47</td>
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<td>8.25</td>
<td>2.70</td>
</tr>
<tr>
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<td>1.28</td>
</tr>
<tr>
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<td>1.35</td>
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<td>1.57</td>
</tr>
<tr>
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<td>13.14</td>
<td>8.25</td>
<td>2.14</td>
</tr>
<tr>
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<td>14.5</td>
<td>8.64</td>
<td>3.00</td>
<td>4.47</td>
</tr>
<tr>
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<td>27</td>
<td>28.5</td>
<td>5.04</td>
<td>4.05</td>
<td>2.55</td>
</tr>
<tr>
<td>20</td>
<td>22</td>
<td>13.2</td>
<td>7.74</td>
<td>3.00</td>
<td>3.70</td>
</tr>
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<td>21</td>
<td>111</td>
<td>22.9</td>
<td>7.92</td>
<td>3.45</td>
<td>2.50</td>
</tr>
</tbody>
</table>

X: 19.31  SE: 2.45  Range: 6.9_51.7
Vr: 125.82  SD: 11.22  1.08_13.14
SE: 2.45  Range: 1.95_13.05

Progesterone: 0.49_3.9
Table 5: Individual data of PRL, FSH, LH and progesterone in lactating menstruating females about 18 months after delivery

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Code No.</th>
<th>PRL (ng/ml)</th>
<th>FSH (mIU/ml)</th>
<th>LH (mIU/mu)</th>
<th>Progesterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>12.5</td>
<td>3.69</td>
<td>7.13</td>
<td>0.35</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>14.0</td>
<td>6.12</td>
<td>15.00</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>12.9</td>
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<td>3.75</td>
<td>3.60</td>
</tr>
<tr>
<td>4</td>
<td>23</td>
<td>17.0</td>
<td>12.24</td>
<td>24.00</td>
<td>3.14</td>
</tr>
<tr>
<td>5</td>
<td>21</td>
<td>14.0</td>
<td>—</td>
<td>4.50</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>88</td>
<td>5.0</td>
<td>2.34</td>
<td>4.13</td>
<td>0.46</td>
</tr>
<tr>
<td>7</td>
<td>87</td>
<td>12.0</td>
<td>2.61</td>
<td>19.65</td>
<td>0.99</td>
</tr>
<tr>
<td>8</td>
<td>86</td>
<td>24.4</td>
<td>13.50</td>
<td>24.00</td>
<td>2.35</td>
</tr>
<tr>
<td>9</td>
<td>85</td>
<td>25.0</td>
<td>3.33</td>
<td>19.13</td>
<td>0.28</td>
</tr>
<tr>
<td>10</td>
<td>76</td>
<td>11.5</td>
<td>3.65</td>
<td>8.25</td>
<td>0.86</td>
</tr>
<tr>
<td>11</td>
<td>72</td>
<td>21.3</td>
<td>3.38</td>
<td>3.68</td>
<td>4.78</td>
</tr>
<tr>
<td>12</td>
<td>68</td>
<td>17.0</td>
<td>5.40</td>
<td>9.72</td>
<td>1.11</td>
</tr>
<tr>
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<td>50.6</td>
<td>4.86</td>
<td>6.00</td>
<td>1.57</td>
</tr>
<tr>
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<td>12.1</td>
<td>1.22</td>
<td>0.90</td>
<td>1.28</td>
</tr>
<tr>
<td>15</td>
<td>51</td>
<td>21.7</td>
<td>9.45</td>
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<td>1.78</td>
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<td>16</td>
<td>38</td>
<td>14.9</td>
<td>9.72</td>
<td>5.80</td>
<td>4.20</td>
</tr>
</tbody>
</table>

X 17.87   6.00   11.23   1.91
Vr 103.93 14.41  64.80  1.91
SD 10.19  3.79   8.04   1.38
SE  2.55   0.98   2.01   0.37

Range 5.0_50.6 1.22_13.5  0.9_24.0  0.28_4.78
Table 6: Mean value ± SEM of serum levels of PRL, FSH, LH and progesterone in lactating menstruating females at various time intervals after delivery

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Time after delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 months</td>
</tr>
<tr>
<td>PRL (ng/ml)</td>
<td>31.18 ± 4.79</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>6.92 ± 0.83</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>4.25 ± 0.28</td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td>1.48 ± 0.21</td>
</tr>
</tbody>
</table>
Figure 1: Mean value (± SEM) of serum PRL in lactating, menstruating females at various time intervals after delivery.
Table 7: Summary of simple analysis of variance for testing the significance of difference between serum mean values of PRL in lactating menstruating females at various time intervals after delivery

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>S.S.</th>
<th>D.F.</th>
<th>M.S.</th>
<th>&quot;F&quot; value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>1762.042</td>
<td>2</td>
<td>881.021</td>
<td>4.423*</td>
</tr>
<tr>
<td>Within groups</td>
<td>9960.124</td>
<td>50</td>
<td>199.202</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11722.166</td>
<td>52</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P < 0.05

S.S. = sum of squares.

D.F. = degree of freedom.

M.S. = mean of squares.
Figure 2. Mean value (± SEM) of serum FSH in lactating, menstruating females at various time intervals after delivery.
Table 9: Summary of simple analysis of variance for testing the significance of difference between serum mean values of FSH in lactating menstruating females at various time intervals after delivery

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>S.S.</th>
<th>D.F.</th>
<th>M.S.</th>
<th>&quot;F&quot; value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>25.740</td>
<td>2</td>
<td>12.870</td>
<td>1.072*</td>
</tr>
<tr>
<td>Within groups</td>
<td>600.223</td>
<td>50</td>
<td>12.004</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>625.963</td>
<td>52</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* N.S. i.e. P > 0.05

S.S. = sum of squares.
D.F. = degree of freedom.
M.S. = mean of squares.
Figure 3: Mean value (± SEM) of serum LH in lactating, menstruating females at various time intervals after delivery.
Table 10: Summary of simple analysis of variance for testing the significance of difference between serum mean values of LH in lactating menstruating females at various time intervals after delivery

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>S.S.</th>
<th>D.F.</th>
<th>M.S.</th>
<th>&quot;F&quot; value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>454.609</td>
<td>2</td>
<td>227.305</td>
<td>9.519**</td>
</tr>
<tr>
<td>Within groups</td>
<td>1217.855</td>
<td>51</td>
<td>23.879</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1672.464</td>
<td>53</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** P < 0.01

S.S. = sum of squares.
D.F. = degree of freedom.
M.S. = mean of squares.
Table 11: Unpaired "t" test for testing the site of significant variation in the mean values of LH in lactating menstruating females at various time intervals after delivery

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Mean values mIU / ml</th>
<th>&quot;t&quot; value</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 months / 12 months</td>
<td>4.25 / 5.59</td>
<td>1.794</td>
</tr>
<tr>
<td>12 months / 18 months</td>
<td>5.59 / 11.23</td>
<td>2.816**</td>
</tr>
<tr>
<td>6 months / 18 months</td>
<td>4.25 / 11.23</td>
<td>3.181**</td>
</tr>
</tbody>
</table>

** P < 0.01
Figure 4: Mean value (± SEM) of serum Progesterone in lactating, menstruating females at various time intervals after delivery.
FOLLICLE-STIMULATING HORMONE (FSH)

A slight and gradual increase in serum FSH level was noted with the advance in time after delivery (Table 16, Figure 6). The difference between the three intervals was statistically insignificant (P > 0.05) (Table 18). Likewise, FSH individual data showed wide variation (Table 13, 14, 15).

LUTEINIZING HORMONE (LH)

While no appreciable difference was noted between serum LH mean values at the 6th and 12th months intervals, a remarkable increase was noted thereafter i.e. at the 18 months interval (Table 16, Figure 7).

Analysis of variances indicated that such differences are statistically significant (P < 0.01) (Table 19). Further statistical analysis, using unparied "t" test was applied to the data to test which interval mean value differs from which and the results of such analysis are presented in Table 20. Significant differences (P < 0.01) in serum LH was obtained when the mean value of the 18 months group was compared with the mean value of any interval of estimation.

PROGESTERONE

The pattern of serum progesterone level with the progress of time after delivery demonstrated a gradual and consistent increase reaching its highest concentration at the last interval of estimation (1.07 ± 0.04 ng/ml) (Table 16, Figure 8). However, the difference between the three interval mean values was statistically insignificant (P > 0.05) (Table 21). It is worth noting that the individual variations within each group were relatively high.
Table 13: Individual data of PRL, FSH, LH and progesterone in lactating amenorrhoeic females about 6 months after delivery

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Code No.</th>
<th>PRL (ng/ml)</th>
<th>FSH (mIU/ml)</th>
<th>LH (mIU/ml)</th>
<th>Progesterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24</td>
<td>34.0</td>
<td>9.00</td>
<td>4.35</td>
<td>2.06</td>
</tr>
<tr>
<td>2</td>
<td>29</td>
<td>33.2</td>
<td>10.80</td>
<td>4.69</td>
<td>1.42</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>31.4</td>
<td>8.28</td>
<td>3.90</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>13.2</td>
<td>6.30</td>
<td>2.70</td>
<td>1.64</td>
</tr>
<tr>
<td>5</td>
<td>14</td>
<td>—</td>
<td>3.51</td>
<td>7.13</td>
<td>2.84</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>6.0</td>
<td>2.07</td>
<td>3.98</td>
<td>1.63</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>9.0</td>
<td>3.06</td>
<td>11.78</td>
<td>0.92</td>
</tr>
<tr>
<td>8</td>
<td>45</td>
<td>30.2</td>
<td>6.62</td>
<td>5.20</td>
<td>1.00</td>
</tr>
<tr>
<td>9</td>
<td>46</td>
<td>9.5</td>
<td>9.39</td>
<td>6.23</td>
<td>0.57</td>
</tr>
<tr>
<td>10</td>
<td>48</td>
<td>39.1</td>
<td>3.38</td>
<td>4.73</td>
<td>0.57</td>
</tr>
<tr>
<td>11</td>
<td>58</td>
<td>134.0</td>
<td>2.78</td>
<td>3.08</td>
<td>0.64</td>
</tr>
<tr>
<td>12</td>
<td>60</td>
<td>13.0</td>
<td>3.42</td>
<td>10.50</td>
<td>0.92</td>
</tr>
<tr>
<td>13</td>
<td>62</td>
<td>12.0</td>
<td>3.96</td>
<td>5.63</td>
<td>0.85</td>
</tr>
</tbody>
</table>

X: 32.05  Vr: 1138.76  SD: 33.75  SE: 9.75

Range: 6.0 - 134.0  2.07 - 10.80  2.70 - 11.78  0.57 - 2.84
Table 14: Individual data of PRL, FSH, LH and progesterone in lactating amenorrhoeic females about 12 months after delivery

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Code No.</th>
<th>PRL (ng/ml)</th>
<th>FSH (mIU/ml)</th>
<th>LH (mIU/ml)</th>
<th>Progesterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13</td>
<td>18.0</td>
<td>3.06</td>
<td>2.78</td>
<td>2.13</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>9.0</td>
<td>10.17</td>
<td>24.00</td>
<td>0.78</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>34.0</td>
<td>3.06</td>
<td>4.28</td>
<td>1.28</td>
</tr>
<tr>
<td>4</td>
<td>61</td>
<td>25.0</td>
<td>1.71</td>
<td>5.03</td>
<td>1.63</td>
</tr>
<tr>
<td>5</td>
<td>57</td>
<td>17.0</td>
<td>2.52</td>
<td>7.58</td>
<td>0.92</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>27.0</td>
<td>0.99</td>
<td>2.63</td>
<td>0.92</td>
</tr>
<tr>
<td>7</td>
<td>11</td>
<td>13.5</td>
<td>0.90</td>
<td>3.00</td>
<td>2.06</td>
</tr>
<tr>
<td>8</td>
<td>101</td>
<td>11.0</td>
<td>4.14</td>
<td>5.78</td>
<td>0.99</td>
</tr>
<tr>
<td>9</td>
<td>83</td>
<td>12.0</td>
<td>3.78</td>
<td>6.38</td>
<td>2.91</td>
</tr>
<tr>
<td>10</td>
<td>73</td>
<td>51.9</td>
<td>4.66</td>
<td>4.35</td>
<td>1.06</td>
</tr>
<tr>
<td>11</td>
<td>91</td>
<td>16.0</td>
<td>2.79</td>
<td>6.38</td>
<td>—</td>
</tr>
<tr>
<td>12</td>
<td>70</td>
<td>9.2</td>
<td>9.18</td>
<td>4.80</td>
<td>2.50</td>
</tr>
<tr>
<td>13</td>
<td>69</td>
<td>85.0</td>
<td>8.24</td>
<td>3.45</td>
<td>0.29</td>
</tr>
<tr>
<td>14</td>
<td>67</td>
<td>52.7</td>
<td>4.86</td>
<td>4.05</td>
<td>1.36</td>
</tr>
<tr>
<td>15</td>
<td>54</td>
<td>39.1</td>
<td>2.57</td>
<td>1.58</td>
<td>0.78</td>
</tr>
<tr>
<td>16</td>
<td>50</td>
<td>49.7</td>
<td>7.02</td>
<td>5.55</td>
<td>—</td>
</tr>
<tr>
<td>17</td>
<td>49</td>
<td>46.8</td>
<td>4.86</td>
<td>3.15</td>
<td>0.71</td>
</tr>
<tr>
<td>18</td>
<td>34</td>
<td>49.7</td>
<td>9.00</td>
<td>3.45</td>
<td>1.20</td>
</tr>
<tr>
<td>19</td>
<td>33</td>
<td>35.7</td>
<td>14.40</td>
<td>4.10</td>
<td>0.86</td>
</tr>
<tr>
<td>20</td>
<td>32</td>
<td>58.2</td>
<td>13.50</td>
<td>3.30</td>
<td>1.00</td>
</tr>
<tr>
<td>21</td>
<td>31</td>
<td>55.7</td>
<td>13.50</td>
<td>2.50</td>
<td>1.04</td>
</tr>
<tr>
<td>22</td>
<td>26</td>
<td>55.7</td>
<td>8.82</td>
<td>3.30</td>
<td>1.28</td>
</tr>
<tr>
<td>23</td>
<td>9</td>
<td>13.2</td>
<td>7.74</td>
<td>3.60</td>
<td>2.78</td>
</tr>
<tr>
<td>24</td>
<td>12</td>
<td>34.0</td>
<td>7.74</td>
<td>4.13</td>
<td>1.71</td>
</tr>
<tr>
<td>25</td>
<td>102</td>
<td>81.9</td>
<td>5.76</td>
<td>4.20</td>
<td>1.47</td>
</tr>
</tbody>
</table>

| X       | 36.04  | 6.20  | 4.93  | 1.38  |
| Vr     | 490.81 | 15.52 | 16.99 | 0.45  |
| SD     | 22.15  | 3.94  | 3.12  | 0.67  |
| SE     | 4.43   | 0.79  | 0.62  | 0.45  |
| Range  | 90--850 | 1.71--19.4 | 1.58--24 | 0.29--2.78 |
Table 15: Individual data of PRL, FSH, LH and progesterone in lactating amenorrhoeic females about 18 months after delivery

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Code No.</th>
<th>PRL (ng/ml)</th>
<th>FSH (mIU/ml)</th>
<th>LH (mIU/ml)</th>
<th>Progesterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36</td>
<td>36.9</td>
<td>14.40</td>
<td>4.13</td>
<td>0.86</td>
</tr>
<tr>
<td>2</td>
<td>64</td>
<td>40.8</td>
<td>—</td>
<td>10.00</td>
<td>1.16</td>
</tr>
<tr>
<td>3</td>
<td>96</td>
<td>33.0</td>
<td>5.40</td>
<td>12.15</td>
<td>0.88</td>
</tr>
<tr>
<td>4</td>
<td>97</td>
<td>12.0</td>
<td>10.26</td>
<td>23.50</td>
<td>0.99</td>
</tr>
<tr>
<td>5</td>
<td>98</td>
<td>10.0</td>
<td>11.88</td>
<td>24.50</td>
<td>2.84</td>
</tr>
<tr>
<td>6</td>
<td>94</td>
<td>7.0</td>
<td>2.25</td>
<td>4.28</td>
<td>3.26</td>
</tr>
<tr>
<td>7</td>
<td>93</td>
<td>3.4</td>
<td>5.81</td>
<td>20.00</td>
<td>—</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>14.9</td>
<td>0.95</td>
<td>—</td>
<td>1.00</td>
</tr>
<tr>
<td>9</td>
<td>75</td>
<td>16.2</td>
<td>3.65</td>
<td>10.80</td>
<td>0.36</td>
</tr>
<tr>
<td>10</td>
<td>103</td>
<td>26.8</td>
<td>11.88</td>
<td>20.00</td>
<td>3.90</td>
</tr>
</tbody>
</table>

X: 20.01  7.39  14.37  1.70
Vr: 175.82 23.25 54.75 1.44
SD: 13.26 4.82 7.40 1.20
SE: 4.19 1.61 2.47 0.40

Range: 2.4–40.8  2.25–11.88  4.13–24  0.86–3.9
Table 16: Mean value ± SEM of serum levels of PRL, FSH, LH, and progesterone in lactating amenorrheic females at various time intervals after delivery

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Time after delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 months</td>
</tr>
<tr>
<td>PRL (ng / ml)</td>
<td>32.05 ± 9.75</td>
</tr>
<tr>
<td>FSH (mIU / ml)</td>
<td>5.58 ± 0.82</td>
</tr>
<tr>
<td>LH (mIU / ml )</td>
<td>5.70 ± 0.75</td>
</tr>
<tr>
<td>Progesterone (ng / ml)</td>
<td>1.25 ± 0.19</td>
</tr>
</tbody>
</table>
Table 18: Summary of simple analysis of variance for testing the significance of difference between serum mean values of FSH in lactating amenorrhoic females at various time intervals after delivery

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>S.S.</th>
<th>D.F.</th>
<th>M.S.</th>
<th>&quot;F&quot; value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>17.488</td>
<td>2</td>
<td>8.744</td>
<td>0.58*</td>
</tr>
<tr>
<td>Within groups</td>
<td>663.771</td>
<td>44</td>
<td>15.086</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>681.259</td>
<td>46</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* N.S. i.e. $P > 0.05$

S.S. - sum of squares.
D.F. - degree of freedom.
M.S. - mean of squares.
Figure 7: Mean value (± SEM) of serum LH in lactating, amenorrhoeic females at various time intervals after delivery.
Table 19: Summary of simple analysis of variance for testing the significance of difference between serum mean values of LH in lactating amenorrhoeic females at various time intervals after delivery

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>S.S.</th>
<th>D.F.</th>
<th>M.S.</th>
<th>&quot;F&quot; value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>618.375</td>
<td>2</td>
<td>309.187</td>
<td>13.522**</td>
</tr>
<tr>
<td>Within groups</td>
<td>1006.051</td>
<td>44</td>
<td>22.865</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1624.426</td>
<td>46</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** P < 0.01

S.S. - sum of squares.
D.F. - degree of freedom.
M.S. - mean of squares.
Table 20: Unpaired "t" test for testing the site of significant variation in the mean values of LH in lactating amenorrhoeic females at various time intervals after delivery

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Mean values (mIU/ml)</th>
<th>&quot;t&quot; value</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 months / 12 months</td>
<td>5.70 / 4.93</td>
<td>0.568</td>
</tr>
<tr>
<td>12 months / 18 months</td>
<td>4.93 / 14.37</td>
<td>4.352**</td>
</tr>
<tr>
<td>6 months / 18 months</td>
<td>5.70 / 14.37</td>
<td>3.515**</td>
</tr>
</tbody>
</table>

** P < 0.01
EVALUATION OF DIFFERENCE BETWEEN MENSTRUATING AND AMENORROEIC FEMALES IN SERUM HORMONE LEVELS

Comparison between menstruating and amenorrheic nursing females was carried out for each hormone at each interval of estimation (Table 22, Figures 9, 10, 11, 12) and the difference between the two groups was evaluated statistically, using unpaired "t" test.

It was evident that at any interval of estimation serum PRL level was higher in amenorrheic than menstruating females, although the only significant difference (P<0.01) between the two groups was obtained at the 12 months interval.

FSH and LH levels, on the other hand, showed no appreciable difference between the two groups at any interval of estimation and the statistical evaluation carried out to test the apparent differences between each two mean values revealed insignificant "t" (P<0.05).

Serum progesterone level was on the average higher in menstruating than amenorrheic females and such observation was sustained at any interval of estimation. However, the only significant difference between the two groups was noted at the 12th month interval (P<0.05).
Table 22: Comparison of mean values ± SEM of serum PRL, FSH, LH and progesterone in lactating amenorrhoeic and menstruating females at various time intervals after delivery

<table>
<thead>
<tr>
<th>Time after delivery</th>
<th>Amenorrhoeic</th>
<th>Menstruating</th>
<th>&quot;t&quot; value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PRL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>32.05 ± 9.75</td>
<td>31.18 ± 4.79</td>
<td>0.083</td>
</tr>
<tr>
<td>12 months</td>
<td>36.04 ± 4.43</td>
<td>19.31 ± 2.45</td>
<td>3.070**</td>
</tr>
<tr>
<td>18 months</td>
<td>20.10 ± 4.19</td>
<td>17.87 ± 2.55</td>
<td>0.463</td>
</tr>
<tr>
<td><strong>FSH</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>5.58 ± 0.82</td>
<td>6.90 ± 0.83</td>
<td>0.415</td>
</tr>
<tr>
<td>12 months</td>
<td>6.2 ± 0.79</td>
<td>5.26 ± 0.20</td>
<td>0.161</td>
</tr>
<tr>
<td>18 months</td>
<td>7.39 ± 1.61</td>
<td>6.00 ± 0.98</td>
<td>0.160</td>
</tr>
<tr>
<td><strong>LH</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>5.70 ± 0.75</td>
<td>4.25 ± 0.28</td>
<td>1.913</td>
</tr>
<tr>
<td>12 months</td>
<td>4.93 ± 0.62</td>
<td>5.59 ± 0.62</td>
<td>0.608</td>
</tr>
<tr>
<td>18 months</td>
<td>14.37 ± 2.47</td>
<td>11.23 ± 2.01</td>
<td>0.398</td>
</tr>
<tr>
<td><strong>Progesterone</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>1.25 ± 0.19</td>
<td>1.48 ± 0.21</td>
<td>0.737</td>
</tr>
<tr>
<td>12 months</td>
<td>1.38 ± 0.45</td>
<td>5.35 ± 0.59</td>
<td>2.079*</td>
</tr>
<tr>
<td>18 months</td>
<td>1.70 ± 0.40</td>
<td>1.91 ± 0.37</td>
<td>0.358</td>
</tr>
</tbody>
</table>

* P < 0.05

**P < 0.01
Figure 9: Mean value ± SEM of serum PRL in lactating, amenorrhoeic and menstruating females at various time intervals after delivery.
Figure 10: Mean value ± SEM of serum FSH in lactating, amenorrheic and menstruating females at various time intervals after delivery.
Figure 11: Mean value ± SEM of serum LH in lactating, amenorrheic and menstruating females at various time intervals after delivery.
Corpus luteum was evaluated in all volunteers through the classification of their progesterone value according to chosen arbitrary criteria as follows:

- Progesterone level less than 3 ng/ml - Anovulation.
- Progesterone level 3 - 5 ng/ml - Corpus luteum insufficiency.
- Progesterone level more than 5 ng/ml - Normal corpus luteum.

Such classification resulted in the data presented in Table 23, for both menstruating and amenorrhoeic females at each interval of estimation. The results obtained showed a case of 100% anovulation in amenorrhoeic females at the 6th and 12th months intervals, however, corpus luteum insufficiency was noted in 22.22% of the amenorrhoeic group at 18 months after delivery (Table 23).

In the menstruating group, on the other hand, at any interval, i.e. at 6, 12 and 18 months, cases of return of corpus luteum function although with corpus luteum insufficiency and few with normal corpus luteum were noted (Table 23). The results of the menstruating group revealed the following interesting findings:

i- Percentage of females showing anovulation decreased with the progress of time after delivery.

ii- Corpus luteum insufficiency was evident in each interval of estimation.

iii- Few cases demonstrated normal corpus luteum function at 12 months after delivery. The equal percentage of subjects showing corpus luteum insufficiency and normal corpus luteum function at 12 months after delivery is an interesting observation.
Table 23: Incidence of normal ovulation and corpus luteum insufficiency in various groups

<table>
<thead>
<tr>
<th>Group</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anovulation</td>
</tr>
<tr>
<td>A. Menstruating</td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>87.50</td>
</tr>
<tr>
<td>12 months</td>
<td>80.00</td>
</tr>
<tr>
<td>18 months</td>
<td>71.42</td>
</tr>
<tr>
<td>B. Amenorrhoeic</td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>100.00</td>
</tr>
<tr>
<td>12 months</td>
<td>100.00</td>
</tr>
<tr>
<td>18 months</td>
<td>77.77</td>
</tr>
</tbody>
</table>
HYPERPROLACTINEMIA AND PROLONGED LACTATION

The individual values of serum PRL in each of the amenorrhoeic and menstruating groups at each interval of estimation were classified according to arbitrary criteria into: normoprolactinemia (PRL up to 15 ng/ml) and hyperprolactinemic (PRL > 15 ng/ml). The incidence of hyperprolactinemia in each group at each interval of estimation is presented in Table 24.

In menstruating group, there was a gradual decrease in the percentage of subjects showing hyperprolactinemia with the progress of time after delivery. However, such decline was considerably high during the first year and minimal thereafter. In the amenorrhoeic group, on the other hand, no consistent pattern was noted.

For further classification of the data the mean values of normoprolactinemia and hyperprolactinemia at each interval was evaluated for both menstruating and amenorrhoeic females (Table 25, Figure 13). At any interval of estimation the hyperprolactinemic group was significantly higher than the normoprolactinaemic one for both menstruating and amenorrhoeic group.

Such presentation showed that at any interval of estimation, the hyperprolactinemic subjects had higher mean values of PRL in the amenorrhoeic group than the menstruating group. Such difference is not apparent for the normoprolactinemic subjects.

So, a comparison was made between PRL level in amenorrhoeic and menstruating subjects at each interval of estimation for both the normoprolactinemic and hyperprolactinemic subjects (Figure 14) and the difference was evaluated statistically.
In the hyperprolactinemic subjects, the magnitude of difference in serum PRL level between amenorrheic and menstruating subjects during the first two intervals of estimation was relatively high and decreased thereafter. However, such difference was only statistically significant at 12 months interval (P < 0.05).
Table 24: Incidence of hyperprolactinemia in various groups

<table>
<thead>
<tr>
<th>Group</th>
<th>%</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normoprolactinemia</td>
<td>Hyperprolactinemia</td>
</tr>
<tr>
<td>6 months</td>
<td>25.0</td>
<td>75.0</td>
</tr>
<tr>
<td>12 months</td>
<td>52.3</td>
<td>47.6</td>
</tr>
<tr>
<td>18 months</td>
<td>56.2</td>
<td>43.8</td>
</tr>
</tbody>
</table>

**A. Menstruating**

**B. Amenorrhoeic**

| 6 months | 50.0       | 50.0     |
| 12 months | 24.0       | 76.0     |
| 18 months | 50.0       | 50.0     |
Table 25: Mean values ± SEM of serum PRL in menstruating and amenorrhoeic females classified to normoprolactinemic and hyperprolactinemic at each interval of estimation

<table>
<thead>
<tr>
<th>Group</th>
<th>Normoprolactinemic</th>
<th>Hyperprolactinemic</th>
<th>&quot;t&quot; value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Menstruating</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>10.05 ± 1.06</td>
<td>38.22 ± 5.14</td>
<td>2.958*</td>
</tr>
<tr>
<td>12 months</td>
<td>11.75 ± 0.83</td>
<td>27.54 ± 3.51</td>
<td>4.344**</td>
</tr>
<tr>
<td>18 months</td>
<td>12.10 ± 0.96</td>
<td>25.23 ± 4.39</td>
<td>3.054**</td>
</tr>
<tr>
<td><strong>B. Amenorrhoeic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>10.45 ± 1.14</td>
<td>53.65 ± 16.36</td>
<td>2.406*</td>
</tr>
<tr>
<td>12 months</td>
<td>11.32 ± 0.79</td>
<td>43.85 ± 4.50</td>
<td>3.888**</td>
</tr>
<tr>
<td>18 months</td>
<td>9.46 ± 1.99</td>
<td>30.74 ± 4.31</td>
<td>4.011**</td>
</tr>
</tbody>
</table>

* P < 0.05

** P < 0.01
**Figure 13**: Mean value ± SEM of serum prolactin in menstruating and amenorrheoic females classified to normoprolactinemic and hyperprolactinemic at each interval of estimation.
Figure 14: Mean value ± SEM of serum PRL in normoprolactinemic and hyperprolactinemic lactating females classified to menstruating and amenorrheic at each interval of estimation.
CORPUS LUTEUM FUNCTION AND HYPERPROLACTINEMIA 
IN PROLONGED LACTATION

Corpus luteum function was evaluated under the condition of hyperprolactinemia in both menstruating and amenorrheic groups. This was performed at each interval of estimation. Such evaluation is presented as mean values and the corresponding standard errors in table 26. Statistical evaluation of the difference in comparison to the corresponding mean values in the normoprolactinemic females was carried out using unpaired "t" test.

In the menstruating group, a lower mean progesterone level in hyperprolactinemic nursing females was noted at 6 and 12 months intervals. However, such difference appeared to be statistically insignificant.

In the amenorrheic group, a lower mean progesterone value is noted at the 12 and 18 months interval. However, such difference was only significant at the 12 months interval (P < 0.01).

Corpus luteum function was further evaluated under the condition of hyperprolactinemia in all volunteers through the classification of their progesterone value according to chosen arbitrary criteria as follows:

- Progesterone level less than 3 ng/ml - Anovulation.
- Progesterone level 3 - 5 ng/ml - Corpus luteum insufficiency.
- Progesterone level more than 5 ng/ml - Normal corpus luteum.

Such classification resulted in the data presented in table 27, for amenorrheic and table 28, for menstruating females at each interval of estimation.

In amenorrheic females, on the one hand, 100% anovulation was noted at the 6th and 12th months interval regardless to the presence of
hyperprolactinemic. At 18 months, the percentage of females showing anovulation was lower in the presence of hyperprolactinemic.

On the other hand, in the menstruating group normal corpus luteum activity was only evident at the 12th months interval with the return of normoprolactinaemic. However, no definite relationship of percentage of anovulation and corpus luteum insufficiency could be deduced at the various intervals of estimation owing to the wide individual variation within each interval of estimation.
Table 26: Mean values ± SEM of serum progesterone, in menstruating and amenorrhoeic females classified to normoprolactinemic and hyperprolactinemic at each interval of estimation

<table>
<thead>
<tr>
<th>Group</th>
<th>Progesterone (ng/ml)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Normoprolactinemic</td>
<td>Hyperprolactinemic</td>
<td>&quot;t&quot; value</td>
</tr>
<tr>
<td><strong>A. Menstruating</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>1.59 ± 0.19</td>
<td>1.43 ± 0.33</td>
<td>0.079</td>
<td></td>
</tr>
<tr>
<td>12 months</td>
<td>3.20 ± 1.03</td>
<td>1.71 ± 0.26</td>
<td>1.219</td>
<td></td>
</tr>
<tr>
<td>18 months</td>
<td>1.68 ± 0.92</td>
<td>2.14 ± 0.56</td>
<td>0.525</td>
<td></td>
</tr>
<tr>
<td><strong>B. Amenorrhoeic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>1.09 ± 0.18</td>
<td>1.14 ± 0.27</td>
<td>0.141</td>
<td></td>
</tr>
<tr>
<td>12 months</td>
<td>2.80 ± 0.37</td>
<td>1.15 ± 0.10</td>
<td>5.555**</td>
<td></td>
</tr>
<tr>
<td>18 months</td>
<td>2.02 ± 0.72</td>
<td>1.43 ± 0.63</td>
<td>0.588</td>
<td></td>
</tr>
</tbody>
</table>

** P < 0.01
Table 27: Incidence of normal ovulation and corpus luteum insufficiency in amenorrhoeic females classified to normoprolactinemic and hyperprolactinemic at each interval of estimation

<table>
<thead>
<tr>
<th>Group</th>
<th>%</th>
<th></th>
<th>Normal C. L.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anovulation</td>
<td>C.L. Insufficiency</td>
<td></td>
</tr>
<tr>
<td><strong>A. Hyperprolactinemic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>100.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>12 months</td>
<td>100.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>18 months</td>
<td>80.00</td>
<td>20.00</td>
<td>0.00</td>
</tr>
<tr>
<td><strong>B. Normoprolactinemic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>100.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>12 months</td>
<td>10.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>18 months</td>
<td>75.00</td>
<td>25.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Table 28: Incidence of normal ovulation and corpus luteum insufficiency in menstruating females classified to normoprolactinemic and hyperprolactinemic at each interval of estimation

<table>
<thead>
<tr>
<th>Group</th>
<th>Anovulation</th>
<th>C.L. Insufficiency</th>
<th>Normal C.L.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Hyperprolactinemic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>81.82</td>
<td>18.18</td>
<td>0.00</td>
</tr>
<tr>
<td>12 months</td>
<td>100.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>18 months</td>
<td>71.43</td>
<td>28.57</td>
<td>0.00</td>
</tr>
<tr>
<td><strong>B. Normoprolactinemic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>100.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>12 months</td>
<td>63.64</td>
<td>18.18</td>
<td>18.18</td>
</tr>
<tr>
<td>18 months</td>
<td>71.43</td>
<td>28.57</td>
<td>0.00</td>
</tr>
</tbody>
</table>
DISCUSSION
DISCUSSION

The contraceptive effect of breast feeding is of a major demographic importance in developing countries (Rosa, 1975). Although there has been a tendency to view its value with scepticism because it does not protect against pregnancy in all cases (Kamal et al., 1969 a), most mothers in the developed world dislike the currently available methods of artificial contraception and would prefer to choose breast feeding as a method of birth spacing if they had a method of defining its reliability. It is, therefore, a matter of urgency that studies should be undertaken to define the practical information upon which mothers may base their decision of when they should introduce postpartum contraception.

Several studies which have attempted to define the endocrine milieu after birth have used endometrial biopsy (Topkins, 1943; Sharman, 1951 - Kava et al., 1968 - El Minawi and Foda, 1974 - Perez et al., 1972), basal body temperature (Lyon and Stamm, 1946 - Perez et al., 1972), or cervical mucus or vaginal cytology (Perez et al., 1972 - Tyson et al., 1972). All these methods, however, present problems of interpretation.

During lactation, the histological patterns in the endometrium may show inadequate coordination of maturation signs, characteristic of the normal cycle, making it difficult to determine whether ovulation has occurred or not (El Minawi and Foda, 1971 - Perez et al., 1972).
Similarly, temperature charts during lactation may show a much shorter plateau in the premenstrual phase (Cronin, 1968), rendering precise interpretation difficult. With the introduction of hormonal assay procedure, a more direct subtle and precise means of detecting the endocrine changes associated with lactation was provided. However, most of the studies performed dealt with the endocrine changes associated with short-term lactation (less than 6 months) and only few have investigated the endocrine changes associated with long-term lactation.

The present study represent the first work which deals with the hormonal pattern associated with prolonged lactation in Egyptian women. All the same, several studies reported the endocrine changes associated with long-term lactation in nearly similar communities as Zaire (Delvoye et al., 1976 - 1977 - 1978), India (Shatrugna et al., 1982) ..........etc.

Estimation of prolactin, gonadotropins and progesterone patterns during prolonged breast feeding was done in order to detect any link between various hormonal patterns and maintainance of lactation, in particular lactation amenorrhoea. Consequently, their impact on lactational infertility can be clarified. Furthermore, any relationship between changing mean levels of each hormone in comparison to the other may become obvious.

In the present cross-sectional study, it was tried to avoid the impact of age, parity, nutritional status and socioeconomic status on
lactation. All the investigated mothers were of age group (20 - 35 years), parity (1 to 5), and of nearly similar socioeconomic class. Moreover, most of them are regular breast-feeders with no supplementation, as supplementation is recorded to be associated with shorter period of lactation amenorrhoea and a rapid return of fertility (Shatrugna et al., 1982).

However, unfortunately, precise information regarding the frequency of breast feeds was unattainable, as these women do not notice how often their babies suckle. Nevertheless, there is no doubt that the general custom is to feed many times a day on demand and freely throughout the night.

Our data confirm and extend those previously reported on long-lasting hyperprolactinemia during maintained lactation (Delvoye et al., 1976 - 1977 - 1978; Gross and Eastman, 1979; Duchen and McNeilly, 1980; Shatrugna et al., 1982; Hennart et al., 1985).

Follow up of mean values of serum prolactin in our work revealed that while there is inconsistent pattern in amenorrhoeic nursing females (Table 16, Figure 5), there is a gradual decline in mean prolactin level among menstruating nursing females with the progress of time after delivery (Table 6, Figure 1). Such decline was more appreciable by the end of the first year of lactation onwards (Table 7, 8).
Calculation of mean basal plasma prolactin levels in relation to return of menstruation showed that it was lower in menstruating women, irrespective of the duration of lactation. Such difference was particularly significant only at the end of the first postpartum year (Table 22, Figure 9).

Due to the wide variation of individual data within each interval of estimation, clarification of the extent of hyperprolactinemia entails further estimation of the percentage of hyperprolactinemic subjects in each interval and consequently their mean values, using a chosen arbitrary prolactin level above 15 ng/ml to diagnose hyperprolactinemia (Weil, 1983). It was observed that the percentage of subjects showing hyperprolactinemia decreased with the progress of time after delivery in menstruating mothers and such rate of decline was considerably high during the first year of lactation (Table 24). In the amenorrhoeic group, no consistent pattern was noted (Table 24). Moreover, hyperprolactinemic subjects had higher mean values in the amenorrhoeic group than the menstruating group at any interval of estimation. The magnitude of such difference was more apparent during the first year of lactation (Figure 14).

Such pattern of prolactin release further emphasises the previous observation of higher prolactin levels in lactating amenorrhoeic mothers compared to lactating menstruating ones (Delvoye et al., 1976 - 1977; Madden et al., 1978 - Gross and Eastman, 1980 - Delvoye et al., 1980 - Duchen and McNeilly, 1980). Consequently, it supports the hypothesis that
hyperprolactinemia is involved in the aetiology of lactational amenorrhoea (Delvoye et al., 1978 - Madden et al., 1978 - Gross and Eastman, 1980 - Duchen and McNeilly, 1980), and a dropping prolactin level is one of the clinical determinants of the return of menstruation (Delvoye et al., 1978 - Madden et al., 1978).

Lactation hyperprolactinemia, therefore, is of prime importance in the maintenance of lactation, particularly lactation amenorrhoea. Nursing behaviour, particularly high frequency of breast feeding, is of decisive importance for maintaining such lactation hyperprolactinemia (Hennart et al., 1985).

Estimation of serum gonadotropins revealed that FSH exhibits an indistinguishable variation between the three groups of estimation during lactation, both in amenorrhoeic (Table 18, Figure 6) and menstruating (Table 9, Figure 2), with a mean value in the range of low normal follicular phase levels (Table 6, 16). No difference was observed between amenorrhoeic and menstruating nursing mothers (Figure 10).

No appreciable difference was noted in mean LH concentration in amenorrhoeic nursing mothers during the first year of lactation, while a significant increase occurred during the second year of lactation (Tables 16, 19, 20). Similarly, LH exhibits a consistent increase in menstruating lactating mothers with progress of time after delivery reaching a significant level during the second year of lactation.
which is similar that observed during the follicular phase of the normal cycle (Table 6, 10, 11, Figure 3). Comparison of LH levels between amenorrheic and menstruating lactating females failed to demonstrate any significant variation (Figure 11).

Accordingly, the present study confirms the previous observation of an associated hypogonadotropinemia with maintained lactation during the first year of lactation (Delvoye et al., 1978 - Tyson et al., 1978 - Madden et al., 1978). However, our data failed to detect any significant difference between serum gonadotropin levels in amenorrheic and menstruating mothers postpartum at any interval of estimation. This, in fact, is contradictory to the reported observation of an increase in FSH and LH with resumption of menstruation (Delvoye et al., 1978 - Madden et al., 1978 - Duchen and McNeilly, 1980).

Hypogonadotropinemia may be attributed to failure of the pituitary to respond to endogenous GnRH resulting from absence of gonadotropin stores (Jaffe et al., 1969 - Handson et al., 1970 - Cystie et al., 1970), or failure of the hypothalamus to secrete endogenous GnRH in quantities large enough to cause an ovulatory surge of gonadotropins (Jeppson et al., 1974). However, hyperprolactinemia may have to a great extent a suppressive role on LH secretion. This may be concluded from the significant positive correlation between declining prolactin level by the end of the first year of lactation and the rising LH secretion during the second year among lactating menstruating subjects. Such
correlation was also previously mentioned by other investigators (Tyson et al., 1978 - Madden et al., 1978 - Duchen and McNeilly, 1980 - Marrs et al., 1981).

Such a suppressive effect of elevated prolactin on gonadotropin secretion may be brought about by alteration of hypothalamic catecholamines metabolism (Fuxe et al., 1969), impairment of GnRH secretion (Nakano et al., 1974), or through inhibition of ovarian oestradiol production which by a positive feedback effect is responsible for the stimulation of GnRH secretion and thus LH secretion (Rolland et al., 1975). However, the exact mechanism is still unknown.

In an attempt to further investigate the relative importance of prolonged lactation in the suppression of corpus luteum function, serum progesterone levels were measured in the studied nursing mothers. Estimation of mean values of progesterone in amenorrhoeic (Table 16) and menstruating nursing mothers (Table 6) failed to detect any significant changes among the various intervals of estimation in either of amenorrhoeic or menstruating mothers (Table 12, 21). Moreover, comparison of mean serum progesterone levels in amenorrhoeic to menstruating nursing mothers revealed significant difference only at the end of the first year of lactation and not thereafter (Table 22, Figure 12).

In the present report, evaluation of corpus luteum function was based on a chosen arbitrary criteria. Women with serum progesterone level less than 3 ng/ml are considered to be anovular, while those with
3-5 ng/ml serum progesterone are experiencing poor corpus luteum function. Levels above 5 ng/ml are considered indicative of normal corpus luteum function (Abdalla et al., 1981). We were aware, however, that it is inconclusive to consider a single progesterone value as indicative of the extent of corpus luteum activity.

Examination of mean progesterone values at the various intervals of estimation supports the previous conclusion of ovarian quiescence during long-lasting lactation (Delvoye et al., 1978 - Duchen and McNeilly, 1980 - Delvoye et al., 1980). Moreover, it also supports the conclusion that even with the resumption of menstruation in long-lasting lactation, cycles are mainly anovular (Delvoye et al., 1978 - 1980 - Duchen and McNeilly, 1980 - Howie et al., 1982b).

There have been few studies dealing with corpus luteum activity in breast feeding mothers. Using a cross-sectional study design, Delvoye et al. (1980), found that mean serum progesterone levels during a lactation period of two years, were lower in cycles during lactation than in normal cycles in non-pregnant, non-lactating women suggesting a high number of defective luteal phases. Similarly, Gross et al. (1980), found that defective luteal function is common in Australian women returning to menstruation during lactation.

The return of a normal corpus luteum activity in lactating mothers from an industrialized country has been carefully documented in a longitudinal study for twenty seven cycles by McNeilly et al. (1982).
Luteal phase progesterone levels were within normal limits in 27% of cycles, the remainder of luteal phases being defective (31%) or absent (42%). In a comparative study of nursing mothers in Zaire and in Sweden, 24% of the mothers showed normal corpus luteum progesterone at six and nine months of lactation in Sweden, While the same percentage were only observed during the second year of lactation in Zaire (Hennart et al., 1985). Such discrepancy in the evaluated corpus luteum activity observed in the various reports may be attributed to the influence of socio-cultural factors and consequently the extent of breast feeding practice.

Consequently, in our study corpus luteum function was evaluated in all volunteers to avoid the impact of the wide individual variation within each interval on the estimated mean progesterone level. Hundred percent anovulation was observed in amenorrhoeic females during the first year of lactation, however starting, albeit poor, corpus luteum activity was observed at the 18th month interval. The results at the menstruating group revealed interesting findings: i- percentage of females showing anovulation decreased with the progress of time after delivery, ii- corpus luteum insufficiency was evident in each interval of estimation, iii- few number of cases demonstrated normal corpus luteum function at 12 months after delivery with an equal percentage showing poor corpus luteum function (Table 23).

Our data throws light on the great correlation between lactation amenorrhoea and anovulation. On top of that it emphasises that
resumption of menstruation is associated with a gradual recovery of ovarian activity. Most authors agree that the longer menstruation occurs after delivery, the greater the likelihood of their being ovulatory (Sharman, 1951-Perez et al., 1972- Said et al., 1974). However, the present data shows that despite the gradual reduction of percentage of anovulatory cycles with the advance of time after delivery, there is an inconsistent pattern of return of ovulation which is only apparent at the end of the first year of lactation. These discrepant results may be related to the accuracy of the different methods used to assess ovulation but may also reflect different suckling patterns in the studied populations. Moreover, such reports missed the increasing incidence of inadequate luteal phases during lactation.

A high incidence of defective luteal phases during lactation may explain why Cronin (1968), found a short plateau phases of raised basal body temperature in menstrual cycles during lactation. Similarly, the failure of normal coordination of secretory patterns in the endometrium during lactation, may be due to subnormal ovarian progesterone secretion.

Explanation of the origin of such failed corpus luteum function is debatable. McNeilly (1980), considered the possibility that it has its origin in subnormal follicular development. A reduction of FSH/LH secretion was also blamed (McNeilly et al., 1982).
Hyperprolactinemia associated with lactation may have a suppressive effect on ovarian steroidogenesis. A close relationship between the remarkable drop in mean serum prolactin level at the end of the first year of lactation among menstruating women and the starting improvement of corpus luteum function, is observed. Furthermore, an absent corpus luteum activity appears to be relevant with the unchanged prolactin pattern among the amenorrheic nursing mothers by the same period. An evaluation of corpus luteum activity among hyperprolactinemic individuals was tried (Tables 26-28), but insignificant relation appeared due to the wide individual variation among the various intervals of estimation.

Such a suppressive effect of hyperprolactinemia on the recovery of corpus luteum activity was also reported by Mroueh and Siler-Khodr (1976), Delvoye et al. (1980), and Hennart et al. (1985). This may be via an effect on granulosa cells, inhibiting progesterone secretion (McNatty et al., 1974), or by affecting testosterone: dihydrotestosterone ratio thereby reducing aromatizable substrate and increasing anti-oestrogen concentrations (Polan and Berham, 1978).

During the second year of lactation, a remarkable increase in mean LH serum levels appears to be associated with partial recovery of corpus luteum function. The occurrence of rising LH prior to complete normalization of corpus luteum activity may confirm the previous suggestion that normal ovarian function does not resume until LH levels have been fully restored (Howie and McNeilly, 1983).
Our data suggest that during maintained lactation the endocrine balance of amenorrhoeic nursing mothers as compared to that of menstruating ones does not show remarkable difference apart from a lower serum prolactin level together with a higher serum progesterone in menstruating lactating mothers at the end of the first postpartum year. It may, therefore, be concluded from such comparison that prolactin may be the major determinant of the length of lactational amenorrhoea during prolonged lactation. Moreover, the present information suggest that lactational amenorrhoea is associated with complete gonadal inhibition. After resumption of menses, there is a continuing suppression of gonadal activity though less complete than during lactational amenorrhoea.

Delvoye et al. (1978), found that an average circulating prolactin level around 560 uU/ml seemed to be associated with 50 percent incidence of amenorrhoea and an average value of 860 uU/ml or more with almost 100 percent incidence of amenorrhoea. We failed, however, to detect an average circulating prolactin level associated with resumption of menses because postpartum amenorrhoea in one part is not maintained with the same level of circulating prolactin in all lactating mothers studied.

In contrast, Del Pozo et al. (1975), in a study performed 2 to 6 weeks postpartum, suggested that prolactin was not involved in postpartum amenorrhoea, since suppression of prolactin secretion by bromocriptine did not change the endocrine responses observed during
this period. These responses were characterized by the refractoriness of LH secretion to LRH and by the refractoriness of the ovary to react to exogenous gonadotropins by the production of oestrogen. However, it appears that the mechanism of amenorrhoea in the early postpartum period is different from that during the late lactation.

Delvoye et al. (1978), suggested that the endocrine balance of amenorrhoeic nursing mothers, as compared to that of menstruating nursing ones, changes after the first year of lactation. During the first year, serum prolactin and FSH levels were significantly higher in amenorrhoeic mothers. During the second postpartum year, on the other hand, serum prolactin declined while serum LH levels were higher in menstruating than in amenorrhoeic mothers. Madden et al. (1978), found that resumption of menses was associated with an increase in the plasma concentrations of LH and FSH, and in case of LH acquisition of an apparently qualitatively more normal secretory pattern, together with a significant decline in prolactin levels.

The difference between these reports and ours as regards to the endocrine changes associated with resumption of menstruation may be attributed to the fact that these studies are based on random blood sampling which may miss episodic variations during a 24 hour interval. Different breast feeding habits may be another cause producing different degrees of hypothalamic-pituitary ovarian block.
While there is epidemiological evidence that breast feeding prolongs the period of infertility after birth (Potter et al., 1965 - Berman et al., 1972 - Rosa, 1975), the impact of menstruation resumption on fecundity is controversial. Some reports have claimed that the reduction in fecundity associated with lactation is confined to the amenorrhoeic interval (Van Ginneken, 1977). Howie et al. (1982b), however, reported conception rates after the return of menses during lactation much lower than in non-lactating women using no contraception.

The present report suggests that lactational amenorrhoea is associated with complete gonadal inhibition particularly during the first year of lactation. Most of cycles during lactation are either anovular or exhibit poor luteal phases. Owing to the association of defective luteal phases with reduced fertility in non-lactating women (Andrews, 1979), it is likely that defective luteal phases during lactation contributes to the reduction of fertility during lactation. This reflects lower fertility rates in breast feeding mothers even those who are menstruating.

Notwithstanding, the inconsistent pattern of corpus luteum function after return of menstruation was evident from the appearance of ovulatory cycles at the end of the first year and not thereafter. This reflects the lower contraceptive benefit of breast feeding after return of menstruation in relation to lactational amenorrhoea.
The details of how fertility is inhibited during lactation may be related to more than one mechanism. Some earlier investigators assumed that a suppressed gonadotropic function of the anterior pituitary might be responsible for such lactational anovulation and amenorrhoea (Jaffe et al., 1969 - Handson et al., 1970 - Crystle et al., 1970 - Madden et al., 1978). Observation of the present data shows low gonadotropin levels and a gradual normalization of LH associated with the advance of time after resumption of menstruation. However, the absence of any significant variation in serum FSH and LH levels among menstruating nursing mothers as compared to amenorrhoeic ones may indicate that gonadotropic dysfunction is not of prime importance in maintaining lactational amenorrhoea and consequently anovulation.

Although prolactin *per se* is not yet proved to be the sole inhibitor of ovulation during lactation, it constitutes the major contributor. A high concentration of prolactin may act at both central and ovarian sites to produce lactational amenorrhoea and anovulation (Kase, 1983).

On the one hand, the suppressive effect of hyperprolactinemia on pituitary gonadotropins was obvious from normalization of LH after suppression of prolactin secretion by bromocriptine as previously reported (Tyson et al., 1975 b - Smith, 1978 b). This is further established from the positive correlation between the declining serum prolactin and the rising LH among menstruating nursing mothers in present report.
On the other, the relationship between ovarian steroids and prolactin may be demonstrated from the association of significant drop of mean prolactin level and the appearance of some ovulatory cycles among menstruating females at the end of the first year of lactation (Table 28).

Consequently, lactational hyperprolactinamia through a central block resulted in the production of insufficient FSH and LH levels, and with the advance of time a recovery of gonadotropin activity occurs but is still incapable of stimulating ovarian activity. This is possibly due to a hyperprolactinemic-induced ovarian block (Howie et al., 1982 c). Return of ovarian activity is associated with the removal of such ovarian block.

From the above findings, it is apparent that long-lasting lactation is associated with hyperprolactinemia, hypogonadotropinemia and ovarian quiescence. Hyperprolactinemia appears to play a major role in the maintainance of lactational amenorrhoea and a declining prolactin level is one of the clinical determinants of the return of menstruation. Moreover, it is obvious that while lactational amenorrhoea is associated with complete gonadal suppression, resumption of menstruation during prolonged lactation does not perclude return of ovulation. Cycles during lactation are associated with increasing incidence of poor luteal phase. Lactation anovulation appears to be closely related to the degree of hyperprolactinomia.
In conclusion, maintained breast feeds through maintainance of lactational hyperprolactinemia is essential for prolongation of lactational amenorrhoea and infertility. Such lactational amenorrhoea is in return essential for maintaining lactational infertility especially during the first year of lactation. On the other hand, returning cycles, though usually associated with anovulation and high incidence of defective luteal phases, they still carry the risk of new conception. This necessitates contraception for any woman who wishes to avoid a further pregnancy during lactation after return of menstruation.
SUMMARY
&
CONCLUSIONS
SUMMARY AND CONCLUSION

Lactation in the presence of adequate nursing is known to be associated with a protracted period of natural infertility. This effect is of major demographic importance in our country in order to reduce the rapidly expanding population and consequently its impact on the national income.

The present study was performed to clarify endocrinial changes associated with prolonged lactation for one and a half year and to compare such hormonal milieu with that of corresponding groups lactating for 6 months or one year.

One hundred and two lactating females were investigated. All were of age group (20-35y), parity (1 to 5), and of nearly similar socioeconomic status. They were classified into three groups according to the duration of lactation, whether it was 6 months, 12 months or 18 months. Each group was subclassified into amenorrhoeic and menstruating according to the return of menstruation. From each participant two venous blood samples were collected, each of which is 10 ml. The sera separated were examined by radioimmunoassay techniques for estimation of follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL) and progesterone according to WHO-RIA Matched Program.
Analysis of the results of hormonal assays revealed the following items:

1- No appreciable changes were noted in serum prolactin with the advance of time among lactating amenorrhoeic females. However, a gradual decline occurred in menstruating ones being marked at the end of the first year of lactation. Comparison of mean prolactin levels in amenorrhoeic in relation to menstruating nursing females shows a higher degree of hyperprolactinemia at any interval of estimation.

2- FSH estimation indicated no changes throughout maintained lactation regardless of return of menstruation.

3- As a function of advance in time after delivery, there was a gradual increase in mean serum LH levels both in amenorrhoeic and menstruating nursing females. Such increase was only remarkable by the onset of the second year of lactation.

4- Mean FSH and LH levels were corresponding to levels seen in the normal follicular phase of the cycle.

5- Insignificant changes in serum progesterone level were noted at various intervals of estimation, both in amenorrhoeic and menstruating lactating females.
6- The return of menstruation during lactation appeared to be associated only with declining PRL and rising progesterone levels particularly at the end of the first year.

7- Evaluation of corpus luteum activity revealed anovulation in amenorrhoeic females throughout lactation except at 18 months where 22.22% of cases experienced corpus luteum insufficiency. In menstruating mothers, the percente of females showing anovulation declined by time. The resumed ovarian function was largely inadequate with only few cases of normal ovulation at 12 months interval.

CONCLUSION

Prolonged lactation is associated with hyperprolactinemia, hypogonadotropinemia and ovarian quiescence. Such lactational hyperprolactinemia may have a suppressive effect on gonadotropin secretion. This may be apparent from the significant positive correlation between declining prolactin level by the end of the first year and rising LH secretion during the second year of lactation. Moreover, hyperprolactinemia may have a suppressive effect on ovarian steroidogenesis. This could be deduced from the starting improvement in corpus luteum function in menstruating nursing females with the declining PRL level.

Maintainance of lactation amenorrhoea appears to be relevant to the degree of hyperprolactinemia as a declining PRL level is the number
one of the clinical determinants of the return of menstruation. Moreover, it is obvious that while lactational amenorrhea is associated with complete gonadal suppression, resumption of menstruation during prolonged lactation does not preclude return of ovulation. Cycles during lactation are associated with increasing incidence of poor luteal phases.

Consequently, maintained breast-feeds through maintainance of lactational hyperprolactinemia is essential for prolongation of lactational amenorrhea and infertility, thus maximizing the contraceptive effect of breast feeding. It will, therefore, be of an optimum value to direct our attention towards full breast feeding in attempt to decrease the rapid expansion of population, particularly after other means of contraception appeared to be either insufficient or entailing diverse adverse effects.
REFERENCES
REFERENCES


ARABIC
SUMMARY
الملخص العربي

أصبحت مشكلة الانفجار السكاني من أكثر المشاكل حادة في العالم. وإما من حيث أسبابها أو من حيث طرق السيطرة عليها. ولقد اتضح من الدراسات المتعددة التي أجريت أن من أهم العوامل التي أدت إلى هذه الزيادة المفتعلة في السكان هو محافظة الرياح عن الرضاعة الطبيعية واللجوء إلى الألبان المناعية كبدائل لها. وذلك كنتيجة طبيعية لخروج المرأة إلى ميادين العمل المختلفة. أما العالم وسيلة طبيعية كان لها عظيم الأثر في تحديد النسل على مدى القرون السابقة. هذا بالإضافة إلى أن وسائل تحديد النسل المتاحة قد أظهرت كثيرًا من الأضرار الجانبية إلى جانب عدم فاعليتها كثيرة منها.

ولذا فإنها قد ظهرت خلال السنوات القليلة الماضية إتجاه عالمي إلى تشجيع الرضاعة الطبيعية، خاصة في الدول النامية، وذلك لتحل السيطرة على التعدد السكاني السريع الذي من شأنه إحداث اثار مدمرة على اقتصاديات هذه الدول خاصة وهي تعاني من وجود نقص واضح في الموازنة الطبيعية. ولذا فقد أدت كل هذه العوامل مجتمعة إلى تعدد الدراسات لمعرفة مدى فاعلية الرضاعة الطبيعية في الحد من معدل الإخصاب، وكذلك لمعرفة الفترة الزمنية التي يمكن الاعتماد عليها في الرضاعة الطبيعية أثر الولادة دون اللجوء إلى وسائل أخرى قد يكون لها بعض الآثار الجانبية إما على الطفل الرضيع أو على الأم الرضيمة. ولذا استلزام هذه الدراسات endometrial biopsy
منتظمة، والآخر سبب لديهم إنقطاع في الطمث. ولقد اعتمدت هذه الدراسة على أخذ 0.5 مل مريئ من كل سيدة مريضة في أطباء معتقلي، في حالة وجود دورات منتظمة أخذت عينتيان قبل الطمث بخمسة أيام وسبعة أيام أما في حالة عدم وجود الطمث فقد أخذت العينتان على التوالي مع مراقبة ترك يوم بينهما. ثم قمت الأحاسيس بطرق الإحصاء المركزي، واستخدمت طريقة الإحصاء الإشعاعي في هذه الأحاسيس لتحديد كمية الهرمونات الآتية:

1. هرمون التستوستيرون
2. هرمون الشتائل للجسم الأصغر
3. هرمون البرولاكتين
4. هرمون البروجستيرون

ولقد ظهرت النتائج الآتية:

1. دائما ما تكون الرضاعة المتعددة مصحوبة بإزدياد في هرمون البرولاكتين وخصول في هرمون النشط للبروجستيرون والحمر والهرمون النشط للجسم الأصغر.

2. في حالة عدم حدوث دورات طبيعية أثناء الرضاعة يظهر هرمون البرولاكتين بمعدل مرتفع مع عدم ظهور أي تغيير خلال فترات الدراسة المختلفة. ولكن مع حدوث الطمث ظهر انخفاض مثبتر في مستوى هذا الهرمون.

3. تمثل نسب هرمون النشط للبروجستيرون والهرمون النشط للجسم الأصغر في جميع حالات الدراسة مماثل لما تم錶ي في بداية الدورة المقابلة للإفراز.

4. بينما لم تظهر تباينًا معدل إفراز الهرمون النشط للبروجستيرون في تغيير ملحوظ خلال فترات الدورة المختلفة في حالة عدم وجود طمث فقد أظهرت
متابعه الهرمون النشط للجسم الأصفر إزدياداً مع مرور الوقت، خاصة مع بداية العام الثاني في السيدات اللاتي لديهن دورات طمثية منتظمة.

5- لم يظهر تغيير ملموس في هرمون البروجستيرون بمقارنة مجموعات الرضع المختلفة في حالة وجود أو إنقطاع الطمث.

6- إن عودة الطمث أثناء الرضاعة الممتدة تكون مصحوبة بإرتفاع في هرمون البرولاكتين وارتفاع في هرمون البروجستيرون وخاصة بعد السنة الأولى.

7- لم يظهر تبويض في حالة إنقطاع الطمث ولكن ظهر عدم تكافؤ في نشاط الجسم الأصفر بنسبة 26.2% من مجموع الدراس نهائياً بعد أن وصف ألم في حالة عودة الطمث فقد وجد إنخفاض في الدورات التي لا يوجد بها تبويض مع مرور الوقت بعد الولادة.

الخلاصة:

- أن نسبة ارتفاع هرمون البرولاكتين لها تأثير إيجابي على إستمرار الرضاعة وخاصة على إنقطاع الطمث أذائها.
- إن ارتفاع نسبة هرمون البرولاكتين له تأثير محدود على إنخفاض عدد النخاعي البسيط، وهذا يتضح من العلاقة الإيجابية بين إنخفاض معدلات إفراز هرمون البرولاكتين في نهاية العام الأول من الرضاعة وزيادة معدل إفراز الهرمون النشط للجسم الأصفر بعد ذلك.
- إن ارتفاع نسبة هرمون البرولاكتين له أيضاً تأثير محدود على تخليل هرمونات البيض وهذا يتضح من تحسين أداء الجسم الأصفر نسباً للصمولة بالطمث مصاحبة إنخفاض نسبة هرمون البرولاكتين.

لقد إتضح إنه في حين أن انقطاع الطمث أثناء الرضاعة له علاقة بالإحباط.
الكامل للمبيض، فإن عودة الدورات الطبيعية أثناء الرضاعة المتعددة تثبت عودة التبويض ولكن هناك زيادة في معدلات عدم تكاثر نشاط الجسم الأصفر.

وفي النهاية فقد يمكن القول إن استمرارية الرضاعة لفترات ممتدة من خلال المحافظة على ارتفاع معدلات إنزيم البرولاكتين في العامل الأساسي للإقلال من معدلات الإخصاب بعد الولادة، وبناءً على هذا فإنه من الضروري بذل جهود تشجيع الرضاعة الطبيعية الكاملة في محاولة للحد من مشكلة التضخم السكاني.
دراسة التغييرات الهرمونية المصاحبة للرضاعة لفترة طويلة

رسالة مقدمة من الطبيب / هيدي محمد فؤاد سيد

تؤتيك للحصول على درجة الدكتوراه في أمراض النساء والتوليد

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