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Function of the hypothalamic–hypophysial axis during the post-partum period in ewes and cows

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Summary. During pregnancy the hypothalamic–hypophysial axis is suppressed by the high concentrations of progesterone and oestradiol in the circulation. The high concentrations of these steroids appear to inhibit secretion of GnRH from the hypothalamus, resulting in inadequate stimulation of pituitary gonadotrophs to maintain synthesis of LH. This produces a depletion of LH in the anterior pituitary gland that must be restored after parturition before normal oestrous cycles can begin.

Introduction

Post-partum anoestrus refers to the absence of ovarian cycles after parturition. In domestic ruminants the factors known to affect the duration of post-partum anoestrus include pre- and post-partum level of nutrition, whether the young are allowed to suck, and season in which the young are born. Theoretically, the lack of ovarian cycles after parturition could be due to an inability of the ovaries to respond to gonadotrophins, or of the hypophysis to secrete a sufficient quantity of gonadotrophins to stimulate follicular growth and ovulation. Moreover, since the production and secretion of gonadotrophins is dependent on hypothalamic input, i.e. gonadotrophin-releasing hormone (GnRH), a lack of sufficient gonadotrophin may be due to inadequate production and/or secretion of GnRH. Early studies indicated that ovulation could be induced soon after parturition in cows by injection of urine from pregnant women and a hypophysial emulsion (Zawadowsky *et al.*, 1935) or by injecting pituitary gonadotrophins (Casida *et al.*, 1943). The ovary therefore appears to be capable of responding to gonadotrophic stimulation very early in the post-partum period. From these observations, one can infer that the lack of ovarian activity following parturition is due to the lack of gonadotrophic stimulation. The remainder of this review will address factors that are perhaps responsible for the suppression of gonadotrophin secretion during the post-partum period in cattle and sheep.

Circulating concentrations of luteinizing hormone (LH) are low during the early post-partum period in beef cows (Arije *et al.*, 1974), dairy cows (Edgerton & Hafs, 1973) and ewes (Restall & Starr, 1977), apparently due to a decreased frequency of pulses of LH. The secretion of LH must return to a level similar to that observed in cyclic animals before normal ovarian cycles are initiated. Although the circulating concentration of LH is lower in post-partum animals than in cyclic animals, the circulating concentrations of follicle-stimulating hormone (FSH) are not suppressed (Walters *et al.*, 1982a; Moss *et al.*, 1985). This may be due to the relative lack of follicular development during late gestation, and without the negative feedback of folliculostatin, secretion of FSH remains normal (Miller *et al.*, 1982).

What component of the hypothalamic–hypophysial axis causes a deficiency in the secretion of LH during the post-partum period?

There are several components of the hypothalamic–hypophysial axis that, if their function was suppressed, could lead to a reduction in the secretion of LH, probably manifested as a decrease in

frequency and/or amplitude of LH pulses. These include the amount of GnRH synthesized and stored in the hypothalamus, the amount of GnRH secreted into the hypophysial portal circulation, the number of receptors for GnRH in the anterior pituitary gland and the amount of LH synthesized and stored in the anterior pituitary gland. If the quantity of GnRH synthesized in the hypothalamus and stored in the median eminence were reduced to very low levels, then it is possible that the amount of GnRH available for release would be insufficient to stimulate normal function of the anterior pituitary gland. Likewise, direct inhibition of the secretion of GnRH could occur without a concomitant reduction in the content of GnRH in the hypothalamus. If either of these scenarios occurred, one would anticipate a reduction in function of the gonadotrophs due to a lack of trophic support. It is also possible that the anterior pituitary gland could become insensitive to trophic stimulation due to an inadequate number of receptors for GnRH or to a post-receptor defect. If this occurred, then the synthesis and secretion of GnRH could be normal but the gonadotrophs could not respond appropriately. This, in turn, would result in insufficient secretion of LH to stimulate ovarian cyclicity. Finally, it is possible that each portion of the hypothalamic–hypophysial axis described above is functionally competent, but that the anterior pituitary gland contains too little LH to elevate circulating concentrations of LH into the normal range after GnRH stimulation.

Hypothalamic content of GnRH

To date, only a few investigators have examined the content of GnRH in the hypothalamus in the periparturient animal. Moss *et al.* (1980) did not observe a significant change in content of GnRH in the preoptic area, medial basal hypothalamus or median eminence of ewes from 1 day after parturition until the ewes began cycling, about Day 40 after delivery (Fig. 1). Likewise, there was no significant change in the content of GnRH in the same areas of the hypothalamus of beef cows between Days 5 and 30 after parturition (Moss *et al.*, 1985). Similar observations for beef heifers have been reported by Cermak *et al.* (1983), but the hypothalamic content of GnRH was less in cyclic cows (Braden *et al.*, 1983) than in post-partum cows, suggesting that release of GnRH may have been inhibited during the post-partum period leading to increased stores of GnRH. Carruthers *et al.* (1980) did not find a difference in the content of GnRH between suckling and non-suckling dairy cows. From these observations, one can conclude that during the post-partum period the hypothalamus contains sufficient quantities of GnRH to stimulate the anterior pituitary gland.

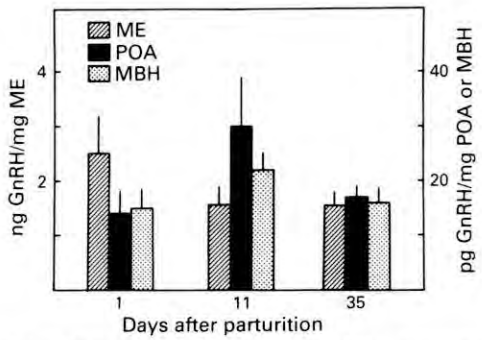

Fig. 1. Concentration of GnRH in the median eminence (ME), preoptic area (POA) and medial basal hypothalamus (MBH) of ewes at various times after parturition. Each of the ewes in the Day 35 group had ovulated and established a functional corpus luteum. Values represent mean ± s.e., $n = 4$–8 per group. (Adapted from Moss *et al.*, 1980.)

Secretion of GnRH

Although there is sufficient evidence to conclude that adequate amounts of GnRH are present in the hypothalamus during the early post-partum period, the question that must be answered is, 'Is the GnRH secreted in a manner that will stimulate reproductive activity?' Unfortunately, to date no one has examined the concentrations of GnRH in hypophysial portal or cavernous sinus blood during the post-partum period of domestic ruminants. However, by utilizing the relatively new technique for collecting portal blood developed by Clarke & Cummins (1982) or the push–pull technique for sampling secretions from the median eminence of sheep (Levine *et al.*, 1982), it should now be possible to obtain such information. Considering the reported insensitivity of the anterior pituitary to exogenous GnRH during the early post-partum period in cattle (Carter *et al.*, 1980) and sheep (Jenkin *et al.*, 1977), such information is essential for elucidating the mechanisms responsible for restoring sensitivity of the anterior pituitary to GnRH.

Sensitivity of the anterior pituitary: receptors for GnRH

The amount of LH released in response to GnRH decreases as gestation progresses and remains low during the early post-partum period in cattle (Carter *et al.*, 1980) and sheep (Rippel *et al.*, 1974; Chamley *et al.*, 1974; Jenkin *et al.*, 1977; Crowder *et al.*, 1982). This has led some investigators to conclude that the pituitary was 'insensitive' to the effects of GnRH (Jenkin & Heap, 1974; Chamley *et al.*, 1974). A reduction in sensitivity of the anterior pituitary to GnRH could be interpreted to mean that there was a decrease in the number of receptors for GnRH. With this in mind, we undertook two series of experiments to examine the sensitivity of the anterior pituitary to GnRH and to quantify the number of hypophysial receptors for GnRH during the post-partum period of the ewes. In the first series of experiments, ewes were induced to ovulate and were mated during anoestrus so that lambing would occur during the breeding season. The anterior pituitary glands of these ewes were collected at various times during the post-partum period and dissociated into single cells. The cells were cultured overnight, incubated with various doses of GnRH and the amount of LH released into the incubation medium was measured.

Release of LH from cultured cells increased progressively with increasing doses of GnRH. Further, there was a progressive increase in the amount of LH released from cells in response to all doses of GnRH as time from parturition increased from Day 1 to Day 35. However, the dose of GnRH ($0{\cdot}5 \times 10^{-9}$ M) that caused half-maximal release of LH from the pituitary cells was similar at each of the times examined (Moss *et al.*, 1980). These results have been interpreted to mean that the anterior pituitary cells contain similar numbers of receptors for GnRH at each of the times after parturition. Moreover, based on these data, there does not appear to be a post-receptor defect in the mechanism responsible for secretion of LH.

In the second series of experiments, the number of receptors for GnRH was measured during late pregnancy and on Days 1, 11, 22 and 35 after parturition. The number of GnRH receptors was higher ($P < 0{\cdot}05$) at Days 1 and 11 after parturition than during late gestation or on Days 22 and 35 after parturition (Fig. 2). However, at no time during late gestation or after parturition did the number of GnRH receptors fall below that seen in ovariectomized ewes (Crowder *et al.*, 1982) in which secretion of LH is always high. In a similar study in post-partum beef cows, we observed a transient increase in the number of hypophysial receptors for GnRH between Days 1 and 15 *post partum*. The number of receptors then returned to the lower level by Days 30 and 45 after parturition (Cermak *et al.*, 1983). Moss *et al.* (1985) quantified hypophysial GnRH receptors on Days 5, 10, 20 and 30 after parturition in beef cows. They also found the number of receptors for GnRH to be reduced at 20 and 30 days after parturition when compared to Days 5 and 10 after parturition.

Collectively, these data regarding the number of GnRH receptors and the in-vitro responsiveness of anterior pituitary cells to GnRH indicate that the sensitivity of the pituitary gland to GnRH is not reduced during the early post-partum period. Therefore, this portion of the regulatory system

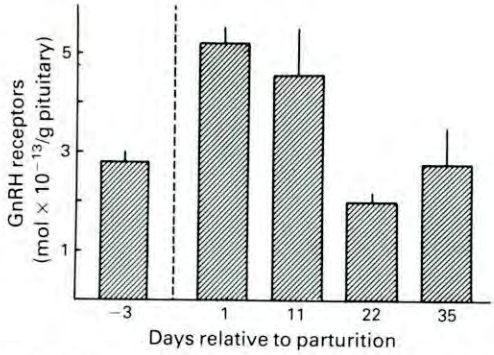

Fig. 2. Concentration of GnRH receptors during late pregnancy and after parturition in ewes. Values represent mean ± s.e., $n = 3$ or 4 per group. (Adapted from Crowder *et al.*, 1982.)

for secretion of LH does not appear to be responsible for the anoestrus that occurs after parturition.

Pituitary content of LH

The content of LH in the anterior pituitary glands of cows (Nalbandov & Casida, 1940) and ewes (Chamley *et al.*, 1976; Jenkin *et al.*, 1977) is reduced by as much as 95% during gestation. In both species there is a gradual increase in the pituitary content of LH after parturition. In suckling beef cows the content of LH in the anterior pituitary increased from very low levels at parturition to levels similar to those present in cyclic animals by Day 30 *post partum* (Saiddudin *et al.*, 1968; Cermak *et al.*, 1983; Moss *et al.*, 1985). Likewise, the pituitary content of LH in ewes gradually increased during the post-partum period, reaching levels similar to those observed in intact ewes between 3 and 10 weeks *post partum* (Fig. 3). The length of time for complete restoration of pituitary LH depends on whether lambing occurred during anoestrus or during the breeding season, and whether the ewes were suckling (Jenkin *et al.*, 1977; Moss *et al.*, 1980).

During the post-partum period in ewes, the pituitary content of LH was highly correlated ($r > 0{\cdot}9$) with the amount of LH released in response to a half-maximal dose of GnRH (Jenkin *et al.*, 1977; Crowder *et al.*, 1982). Therefore, during the early post-partum period in cattle and sheep, it seems likely that if pulses of GnRH of normal amplitude were released into the hypophysial portal circulation, the resulting pulses of LH would be lower than those observed in cyclic animals. From this information, I suggest that a lack of stores of LH in the anterior pituitary gland, rather than reduced sensitivity to GnRH, is one of the initial limitations to the resumption of normal oestrous cycles in post-partum animals.

What causes depletion of LH in the anterior pituitary gland during gestation?

From the information presented above, it is clear that in cattle and sheep the amount of LH in the anterior pituitary gland dramatically decreases during gestation, and is slowly replenished during the post-partum period. It therefore appears that some factor(s) secreted during pregnancy might inhibit synthesis of LH, thereby resulting in depletion of LH in the anterior pituitary. Since progesterone and oestradiol are present in the circulation for most of gestation and are particularly high during the last third (Stabenfeldt *et al.*, 1972; Burd *et al.*, 1976; Carnegie & Robertson, 1978),

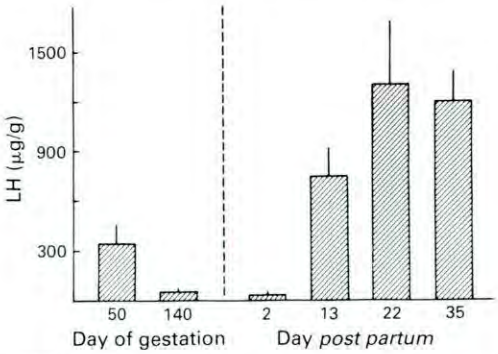

Fig. 3. Concentration of LH in the anterior pituitary glands removed from ewes during pregnancy and at various times after parturition. Values represent mean ± s.e., $n = 4$ or 5 per group. (Adapted from Wise *et al.*, 1986b.)

these steroids are likely candidates. Furthermore, receptors for oestradiol and progesterone are present in both the hypothalamus and anterior pituitary gland (Clarke *et al.*, 1981; Kamel & Krey, 1982; Cermak *et al.*, 1983; Wise *et al.*, 1986a). Accordingly, we suggested that these steroids, alone or in combination, might be responsible for the reduction in pituitary stores of LH. The basis for this hypothesis is strengthened by the fact that progesterone reduces the frequency of pulsatile LH release whereas oestradiol inhibits the amplitude of LH pulses in ovariectomized ewes (Goodman & Karsch, 1980). Since pulses of LH released into the peripheral circulation are the result of pulses of GnRH in the hypophysial–portal circulation (Clarke & Cummins, 1982; Levine *et al.*, 1982), then it is reasonable to presume that there is a reduction in frequency and/or amplitude of GnRH pulses when these steroids are present in the circulation. Given the fact that GnRH is required for synthesis of LH (Fraser *et al.*, 1975), if both the amplitude and frequency of GnRH pulses are inhibited by progesterone and oestradiol, then an adequate stimulus for synthesis of LH is probably absent during gestation. A reduction in synthesis of LH would thus explain the observed decrease in pituitary content of LH. To test this hypothesis, we initiated a series of experiments to determine the effects of chronically elevated concentrations of progesterone and/or oestradiol on serum and pituitary concentrations of LH in ovariectomized ewes.

Groups of ovariectomized ewes served as controls or were treated with progesterone, oestradiol or oestradiol plus progesterone for 3 weeks at doses that produced serum concentrations of these steroids approximating those observed during the 2-week period before parturition. As expected, each steroid, alone and in combination, produced a dramatic decrease in serum concentrations of LH. Although progesterone had no effect on the hypophysial concentration of LH, oestradiol alone, or in combination with progesterone, resulted in a dramatic (>95%) reduction in the pituitary concentration of LH (Fig. 4). As in the pregnant and post-partum animals, the reduction in pituitary content of LH was not due to a lack of GnRH in the hypothalamus, or to a deficiency in the number of receptors for GnRH in the anterior pituitary gland (Moss *et al.*, 1981).

These data are consistent with previous reports for the ewe indicating that, when the anterior pituitary gland is under the influence of progesterone, the release of LH induced by GnRH is not diminished, whereas when under the influence of oestradiol the GnRH-induced release of LH is dramatically diminished (Goodman & Karsch, 1980; Tamanini *et al.*, 1986). Since there was actually an increase ($P < 0{\cdot}01$) in the number of GnRH receptors in the oestradiol-treated ewes in the study described above, it appears that oestradiol (1) directly inhibits synthesis of LH at the level of the pituitary, or (2) so completely inhibits secretion of GnRH that there is insufficient trophic stimulation for synthesis of LH.

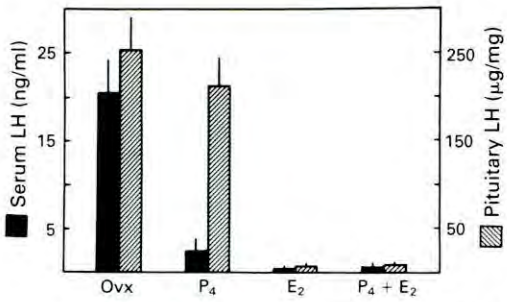

Fig. 4. Pituitary and serum concentrations of LH in ovariectomized (Ovx) ewes treated for 3 weeks with progesterone (P_4), oestradiol (E_2) or progesterone plus oestradiol. Values represent mean ± s.e., N = 5 per group. (Adapted from Moss *et al.*, 1981.)

How are the negative feedback effects of progesterone and oestradiol exerted?

Since progesterone inhibits secretion of LH by reducing the frequency of episodes of LH release without affecting the pituitary content of LH or pituitary responsiveness to GnRH, it has been postulated that progesterone suppresses secretion of GnRH from the hypothalamus (Goodman & Karsch, 1980). Indeed, progesterone will decrease the concentration of GnRH in pituitary stalk blood of ovariectomized rats (Sarkar & Fink, 1979). If progesterone decreases release of GnRH, then it is tempting to speculate that it may also suppress the synthesis of GnRH. This speculation is based on the fact that the content of GnRH in the hypothalamus does not change as a result of treatment with progesterone. If the only effect of progesterone was to inhibit secretion of LH (i.e. without affecting synthesis) then an increase in the hypophysial content of LH would be expected, unless there was an increase in degradation of LH by the gonadotroph. However, when ovariectomized ewes were treated with progesterone, there was no effect on the number of hypophysial receptors for GnRH (Moss *et al.*, 1981) nor was there a significant change in the content of mRNA for the subunits of LH (Hamernik & Nett, 1986). Therefore, there does not appear to be a direct effect of progesterone on the secretion or synthesis of LH by the anterior pituitary gland in domestic ruminants.

Since the pituitary content of LH does not decrease in ewes treated with progesterone, one can conclude that infrequent pulses of GnRH (one every 6–8 h) are sufficient to maintain pituitary stores of LH. A complete absence of GnRH pulses (in hypothalamic–pituitary-disconnected ewes) results in a rapid depletion (< 48 h) in the content of mRNA for the subunits of LH and a more gradual decrease in the pituitary content of LH (Hamernik *et al.*, 1986). Therefore, it appears that, if the anterior pituitary gland is exposed to infrequent pulses of GnRH that are apparently of normal magnitude, then synthesis of LH by the anterior pituitary will be maintained. Considering this information, one would predict that administration of GnRH to pregnant animals might maintain pituitary content of LH.

The inhibitory effects of oestradiol on release of LH might be explained by decreased secretion of GnRH, similar to the actions of progesterone, but it is not clear how oestradiol acts to decrease synthesis of LH. One dramatic effect of oestradiol is to decrease the pituitary content of mRNAs for the subunits of LH (Fig. 5). In fact, after treating ovariectomized ewes with oestradiol for 3 weeks, the concentration of mRNA for alpha-subunit had decreased by approximately 85%, whereas the concentration of mRNA for the beta-subunit of LH had decreased by 98% (Nilson *et al.*, 1983). A similar decrease in the pituitary content of mRNAs for LH was observed during gestation (Wise *et al.*, 1985). Whether oestradiol directly inhibits synthesis of LH at the level of the anterior pituitary gland, or indirectly by inhibiting secretion of GnRH, remains to be determined.

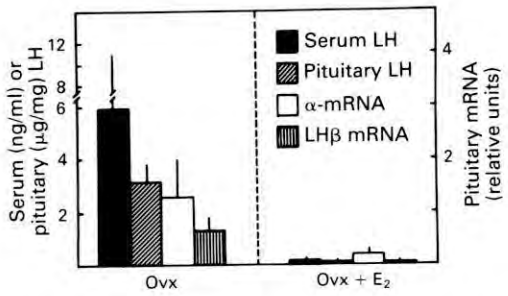

Fig. 5. Pituitary and serum concentrations of LH and pituitary concentrations of mRNA for alpha-subunit and LH-beta subunit of ovariectomized (Ovx) ewes and ovariectomized ewes treated with oestradiol (E_2) for 3 weeks. Values represent mean ± s.e., N = 4 per group. (Adapted from Nilson *et al.*, 1983.)

Since the oestradiol receptor has been shown to interact with the rat prolactin gene (Maurer, 1985), there is a precedent for a direct effect of oestradiol on the anterior pituitary gland. To date, similar studies have not been extended to gonadotrophin genes or to the prolactin gene in species other than rats. Likewise, oestradiol has direct effects on secretion of LH from ovine (Moss & Nett, 1980) and bovine (Padmanabhan *et al.*, 1978) pituitary cells cultured *in vitro*; however, the effects *in vitro* are not nearly as dramatic as those observed *in vivo*.

Oestradiol may also act at the level of the hypothalamus to decrease secretion of GnRH. Karsch *et al.* (1987) observed a nearly complete absence of GnRH pulses in the hypophysial–portal circulation of ovariectomized ewes treated with oestradiol during the anoestrous season. Since hypothalamic input is required for maintaining the pituitary content of mRNAs for the subunits of LH (Hamernik *et al.*, 1986), if oestradiol sufficiently reduces the magnitude and frequency of pulses of GnRH, this alone could account for the reduction in pituitary content of LH. Whether the inhibitory effect of oestradiol on synthesis of LH is mediated primarily at the hypothalamus or directly on the gonadotroph must be determined before a treatment regimen to prevent reduced synthesis of LH during pregnancy can be designed.

Not only is the pituitary content of LH decreased during gestation, but there are correlated changes in the morphological characteristics of gonadotrophs as well. On Day 1 *post partum* these changes include a 30% decrease in volume of individual gonadotrophs, a 50% decrease in the percentage of the gonadotroph volume occupied by secretory granules and an apparent 40–50% reduction in the percentage of gonadotrophs (identified immunocytochemically) in the anterior pituitary gland (Wise *et al.*, 1986b). The data on the percentage of gonadotrophs in the anterior pituitary gland at 1 day after parturition must be interpreted with caution since the content of LH in the pituitary decreases so dramatically. That is, the content of LH may have been so low in some gonadotrophs at the end of gestation that they could not be identified immunocytochemically. Whether an actual decrease in the number of gonadotrophs occurs during pregnancy therefore remains to be determined. This will probably require identification of gonadotrophs by a means other than immunocytochemical localization of LH. At present, it is not known whether the change in the morphology of gonadotrophs during pregnancy is due to a direct effect of oestradiol and/or progesterone on these cells or to a lack of trophic support from the hypothalamus, i.e. decreased secretion of GnRH during pregnancy.

Recovery of the anterior pituitary gland after parturition

Removal of the fetal–placental unit at parturition is accompanied by a dramatic decrease in the concentration of oestradiol and progesterone in the circulation (Burd *et al.*, 1976). This results in

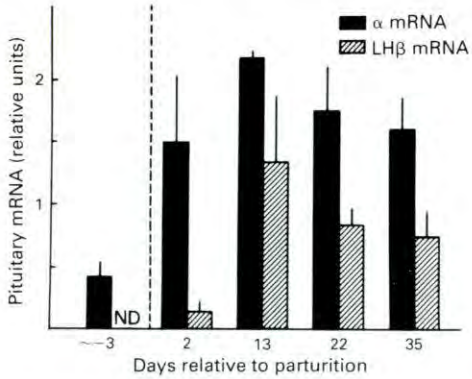

Fig. 6. Pituitary concentrations of mRNA for the alpha- and beta-subunits of LH in ewes during late pregnancy and at various times after parturition. Values represent mean ± s.e., N = 4 or 5 per group. (Adapted from Wise *et al.*, 1985.)

the removal of their negative feedback actions on the hypothalamic–hypophysial axis and permits a gradual recovery in gonadotroph function. In sheep, the first detectable change in the hypothalamic–hypophysial axis that we have observed during the post-partum period is an increase in the concentration of mRNAs for the subunits of LH (Wise *et al.*, 1985). Compared to late gestation, within 2 days after parturition there is an approximate 4-fold increase in the concentration of mRNA for the alpha-subunit of LH, and at least a 10-fold increase in the concentration of mRNA for the beta-subunit of LH (Fig. 6). The maximum concentration of mRNA for the subunits of LH in the anterior pituitary gland was noted on Day 13 *post partum* (mRNA for alpha-subunit was 5-fold greater than in late gestation, mRNA for LH beta-subunit was at least 50-fold greater than in late gestation). The concentrations of these mRNAs then decreased slightly, but remained high until normal oestrous cycles began about 35 days after parturition. A few days after the increase in mRNAs, there was an increase in the quantity of LH contained in pituitary cells (Fig. 3). As a result of these changes during the early post-partum period, there is an increase in the frequency of LH pulses observed in the peripheral circulation with time after parturition (Peters *et al.*, 1981; Walters *et al.*, 1982a).

The concentration of receptors for oestradiol in the hypothalamus and the anterior pituitary gland was very low at the end of gestation (Fig. 7). These concentrations began to increase at Day 22 *post partum* and were highest at Day 35 *post partum* (Wise *et al.*, 1986b). This was after the maximum concentration of LH was noted in the anterior pituitary. Perhaps the relatively low concentration of receptors for oestradiol renders the hypothalamic–pituitary axis less sensitive to the positive feedback effects of this hormone during the early post-partum period. If this were the case, then a higher concentration of oestradiol would probably be needed to induce an ovulatory surge of LH during the early post-partum period. Indeed, Wright *et al.* (1980) reported that 50–64% of ewes failed to show a positive feedback to a 40 μg challenge with oestradiol early in the post-partum period. This dose of oestradiol induced an LH surge in essentially 100% of anoestrous ewes (Beck & Reeves, 1973).

Concomitant with these biochemical changes, the morphology of the gonadotrophs also returns to a state similar to that observed in cycling ewes. Changes include an increase in the percentage of pituitary cells that can be identified immunocytochemically as gonadotrophs, an increase in the percentage of gonadotroph volume occupied by secretory granules and an increase in total volume of individual gonadotrophs (Table 1). Therefore, in ewes that were induced to ovulate and were mated during anoestrus so that lambing would occur during the breeding season, the function of the hypothalamic–hypophysial axis appears to have returned to normal by about 35 days *post*

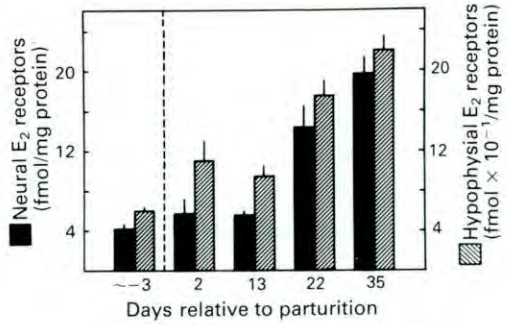

Fig. 7. Concentrations of receptors for oestradiol in the hypothalamus and anterior pituitary gland of ewes before and at various times after parturition. Hypothalamic receptors include those in the preoptic area, the medial basal hypothalamus and median eminence. Values represent mean ± s.e., N = 4 or 5 per group. (Adapted from Wise *et al.*, 1986a.)

Table 1. Changes in the characteristics of gonadotrophs of ewes at various times after parturition

Day *post partum*	Volume (μm^3)	% Staining for LH	% Gonadotroph volume occupied by granules
2	225 ± 3^a	$5{\cdot}6 \pm 0{\cdot}2^a$	$5{\cdot}5 \pm 1{\cdot}0^a$
13	277 ± 12^b	$6{\cdot}4 \pm 0{\cdot}1^a$	$6{\cdot}7 \pm 1{\cdot}2^a$
22	303 ± 20^b	$9{\cdot}5 \pm 1{\cdot}2^b$	$12{\cdot}4 \pm 1{\cdot}1^b$
35	327 ± 11^b	$10{\cdot}6 \pm 1{\cdot}1^b$	$13{\cdot}5 \pm 0{\cdot}9^b$

Values are mean ± s.e.m. for 4 or 5 ewes/group.
Values with different superscripts differ ($P < 0{\cdot}05$).

partum. This is consistent with the time of the first ovulation resulting in an oestrous cycle of normal length in our flock (Moss *et al.*, 1980; Wise *et al.*, 1986a).

Effect of suckling on gonadotrophin release

The changes described above represent a recovery of the hypothalamic–hypophysial axis from the prolonged negative feedback effects of progesterone and oestradiol to which the animal was exposed during pregnancy. From an endocrinological viewpoint, once this recovery phase is complete, the female should be ready to resume normal oestrous cycles. However, even though the function of the hypothalamic–hypophysial axis returns to normal within a relatively short period of time after parturition, frequent suckling will continue to suppress concentrations of LH in the peripheral circulation, particularly in cows (Short *et al.*, 1972; Randel *et al.*, 1976). That this effect is due to suckling, rather than lactation, is suggested by the fact that suckling will suppress pulsatile secretion of LH for a longer period after parturition than milking (Peters *et al.*, 1981), even when milking frequency is increased to 4 times per day (Carruthers & Hafs, 1980). In some cases the suckling-induced inhibition of reproductive function may persist for months. Recently, much speculation has centred on the endogenous opioid peptides as factors that might be involved in the suckling induced inhibition of LH secretion.

Evidence for this supposition is that administration of naloxone, an opioid antagonist, will stimulate secretion of LH in suckling beef cows (Gregg *et al.*, 1985), but this treatment is ineffective in non-suckling cows (Whisnant *et al.*, 1985). The fact that treatment with naloxone does not increase serum concentrations of LH in non-suckling cows provides indirect evidence that endogenous opioid peptides might be involved in the suckling-induced suppression of LH secretion.

Administration of naloxone to post-partum ewes also resulted in increased serum concentrations of LH. In contrast to the cow, however, the effect of naloxone in ewes was independent of suckling (Gregg *et al.*, 1985). The effect of suckling on length of the post-partum period in ewes is controversial. Fletcher (1973) and Moss *et al.* (1980) found no differences in time from birth to first post-partum ovulation between suckling and non-suckling ewes. Kann & Martinet (1975) reported that denervation of the udder was associated with a more rapid return to oestrus. At any rate, the effect of suckling on the duration of post-partum anoestrus appears to be less profound in the ewe than in the cow. The fact that there is an interaction between suckling and the effect of naloxone on secretion of LH in the cow (a species in which suckling reduces secretion of LH) but not in the ewe (a species in which suckling does not decrease secretion of LH), provides a further indication that suckling stimulates secretion of the endogenous opioid and this, in some way, suppresses secretion of LH.

If functional recovery of the hypothalamic–hypophysial axis is complete by 20–30 days after parturition, then by administering pulses of GnRH to suckling animals it should be possible to stimulate an increase in serum concentrations of LH and subsequent follicular development that would lead to ovulation. This appears to be the case in post-partum ewes (Wright *et al.*, 1983) and cows (Walters *et al.*, 1982b). Injections of GnRH (500 ng every 2 h for 4 days) beginning about 20 days after calving reduced the interval from parturition to first ovulation in suckling beef cows. Likewise, administration of pulses of GnRH (100 ng/h for 48 h) to ewes about 28 days after parturition induced an increase in basal circulating concentrations of LH which was followed by a surge of LH in about 85% of the animals. Inadequate secretion of GnRH therefore appears to limit the resumption of oestrous cycles during the post-partum period after the initial recovery of the hypothalamic–hypophysial axis from the negative effects of gestation.

Model for post-partum anoestrus

During pregnancy the high circulating concentrations of progesterone and oestradiol result in a prolonged negative feedback on the hypothalamic–hypophysial axis. This feedback results in an inhibition of the synthesis of LH by the anterior pituitary gland. Because synthesis of LH is inhibited for an extensive period of time, pituitary stores of this gonadotrophin become depleted and the basal release of LH is diminished. The mechanisms for releasing LH, however, appear to remain intact and functional throughout pregnancy.

During the post-partum period, a two-phase recovery of the hypothalamic–hypophysial–gonadal axis occurs. The first phase, lasting from 2 to 5 weeks after parturition, is presumed to be characterized by relatively infrequent discharges of GnRH into the hypothalamic–hypophysial portal circulation, i.e. one pulse every 4–8 h. This mode of GnRH secretion effectively stimulates the biosynthetic machinery in the gonadotroph and the rate of synthesis of LH increases. However, the pulses of GnRH are sufficiently spaced so that only a small portion of the newly synthesized LH is secreted. The increased rate of synthesis of LH coupled with the relatively slow rate of release creates a situation in which pituitary stores of LH are replenished. Since the magnitude of the LH pulse is dependent on the quantity of LH stored in the anterior pituitary gland, then during the early portion of this phase of recovery, the pulses of LH are of insufficient magnitude to induce follicular maturation.

Only after pituitary stores of LH have returned to their normal level are pulses of LH that are released into the circulation of sufficient amplitude to stimulate follicular growth. This marks the beginning of the second phase of the recovery process. During this phase the increased circulating concentrations of LH stimulate growth of ovarian follicles and the resultant secretion of oestradiol. I suggest that the first effect of oestradiol is to stimulate production of its own receptor in the hypothalamus and anterior pituitary gland, thus increasing the sensitivity of these tissues to the positive feedback effects of oestradiol. The positive feedback of oestradiol is the result of small increases in circulating concentrations for short periods in contrast to the prolonged, very high concentrations of oestradiol during late gestation that produce a powerful negative feedback effect. At this point, the frequency of discharges of GnRH increases, in turn, producing more frequent pulses of LH. These events lead to the final stages of follicular development and culminate in ovulation.

It is my supposition that the first phase of this recovery process (i.e. events leading to increased pituitary stores of LH) is relatively independent of the suckling stimulus and environmental stressors. The second phase of the recovery (i.e. events leading to an increased frequency of discharges of LH) appears to be tightly coupled to the suckling stimulus and environmental stressors in cows. Suckling and environmental stressors both appear to induce secretion of endogenous opioid peptides which, in turn, inhibit discharges of GnRH from the hypothalamus. The inhibitory effect(s) of the endogenous opioids appear to be short-lived since cows must suckle more than 4 times per day before the post-partum period is extended. Therefore, the inhibition of LH secretion (and proposed inhibition of GnRH secretion) is observed in cows whose calves are allowed to suck *ad libitum* but not in cows that are milked twice daily (Peters *et al.*, 1981). This inhibition in GnRH secretion results in a reduction in the frequency of LH pulses to such an extent that the final stages of follicular growth do not occur. Inhibition of this phase of recovery from post-partum anoestrus persists until the suckling stimulus and/or environmental stressors are reduced to the point where more frequent discharges of GnRH begin to occur. Only then are oestrous cycles initiated.

Future treatments designed to shorten the post-partum anoestrus interval should focus on two problems: (1) methods to increase the ability of the anterior pituitary to synthesize LH during late gestation, and (2) methods to prevent the suckling-induced inhibition of GnRH secretion during the post-partum period. It is possible that one treatment, namely chronic administration of GnRH, may solve each of these problems. In fact, such a treatment will induce ovulation in suckling beef cows when initiated about 20 days after parturition (Walters *et al.*, 1982b). In contrast, the ability of such a treatment to stimulate synthesis of LH during late gestation has not been examined. If the inhibitory effect of oestradiol on synthesis of LH is mediated at the anterior pituitary, then treatment with GnRH during late gestation may be without effect. If so, then one must consider other means of inhibiting the negative effects of oestradiol. Possibilities worthy of consideration include treatment with anti-oestrogens such as clomiphene and tamoxifen, or passive immunization to oestradiol. Clearly, more basic information is needed before a treatment that effectively shortens the post-partum anoestrus interval can be developed.

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