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# Sensory and behavioural control of gonadotrophin secretion during suckling-mediated anovulation in cows

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A major limiting factor in the resumption of postpartum ovarian cycles in cattle is the inhibitory influence of the suckling calf on central regulatory elements controlling the release of GnRH from the median eminence. This inhibitory influence occurs only as a consequence of specifically defined behavioural interactions between the cow and calf in the presence of a maternal bond, cannot be simulated experimentally using thermal, electrical or mechanical stimuli, and is not dependent upon sensory ennervation within the udder. Indeed, the identity of the calf, either own or unrelated, appears to define the neuroendocrine events that attend the maintenance of the anovulatory state under controlled experimental conditions. Hence, new hypotheses that focus upon the relationship between physiological correlates of maternal behaviour and hypothalamic regulation of LH secretion are currently being tested. Specific aspects of these relationships remain conjectural, but are postulated to include the regulation of opioid tone, intracerebral oxytocin, and modulation of synthetic or excitatory activity of GnRH secretory neurones. Defining the role of the special senses in transduction of signals from calves that influence these neuronal processes may provide insight for developing practical intervention strategies of the future.

## Introduction

The primary endocrine deficit resulting in the establishment of postpartum anovulation in suckled beef cows is the failure to resume a pattern of LH secretion that will support the development and final maturation of an oestrogen-active, preovulatory follicle. There is firm evidence that this deficit depends upon central mechanisms associated with control of GnRH-secreting neurones (Williams *et al.*, 1983; Wise, 1990). Although depleted at parturition, anterior pituitary stores of LH are substantially replenished within the first 2–3 weeks of the puerperium (Williams *et al.*, 1982; Nett *et al.*, 1988). Therefore, it would seem reasonable to expect a near circhoral pattern of LH secretion to occur coincident with this repletion, with the onset of follicular maturation and ovulatory competence following soon thereafter. In suckled cows, this does not occur at a consistent pace, and anovulatory periods in some females persist for 100 days or more after calving (Casida, 1971).

A number of recent reviews have summarized the physiological basis of anovulation and infertility during the puerperium in several species, including cattle, with a major focus on suckling and its neuroendocrine sequelae (Short *et al.*, 1990; Williams, 1990). However, new concepts regarding the role of the suckling calf in attenuating function of the hypothalamo–hypophyseal–ovarian axis have continued to emerge (Silveira *et al.*, 1993; Viker *et al.*, 1993; Williams *et al.*, 1993). This has resulted in a re-evaluation of working hypotheses that address the role of calf-related cues in this process, with

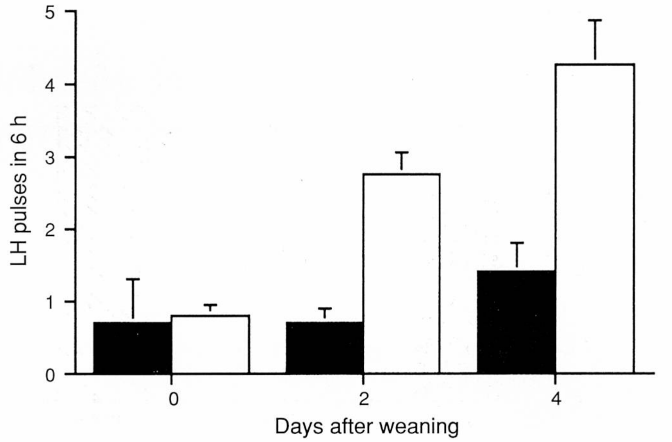


**Fig. 1.** Weaning-induced increase in the frequency of LH pulses between experimental days 0 (days 17–21 after calving) and 4 after weaning in beef cows. Frequency of LH pulses was significantly greater in weaned (□) than in suckled (■) cows on both day 2 and day 4. (Reproduced with permission from Williams *et al.*, 1993.)

special emphasis on the contribution of maternal identification of and selectivity for the cow's own calf. The purpose of this review is to bring into focus those events that must occur between cow and calf for the inhibitory influence of suckling on gonadotrophin secretion to be manifest, and to support and extend our view that the maternal–offspring bond is a critical element in the ontogeny of suckling-mediated anovulation

## Hypophysiotrophic Signalling during Suckling-Induced Anovulation

The inhibited pattern of pulsatile LH secretion observed during the anovulatory state and the corresponding increase in the frequency of LH pulses that occurs 2–6 days after removal of the suckling stimulus are two of the most widely recognized phenomena associated with postpartum beef cows (Walters *et al.*, 1982; Smith *et al.*, 1983; Williams *et al.*, 1987; Fig. 1). A number of hypotheses have been proposed to account for this physiological dichotomy, including the release of antigonadotrophic hormones during lactation. For example, prolactin, which is released in large quantities in response to suckling and milking, was proposed as a major antigonadotrophic factor over 20 years ago. However, studies using either a potent dopamine antagonist or infusions of homologous hormone have discounted the hypothesis that prolactin is an antigonadotrophic agent in suckled beef cows (Williams and Ray, 1980) and ovariectomized heifers (Forrest *et al.*, 1980).

Other factors that have been considered to modulate the hypothalamo–hypophyseal–ovarian axis during the puerperium include increased negative feedback sensitivity to oestradiol and the biological activity of secreted LH. Negative feedback sensitivity is potentiated by suckling and has been confirmed as a major factor regulating the resumption of normal LH secretion (Acosta *et al.*, 1983); however, oestradiol antagonists have not been consistently successful in shortening the postpartum interval (Schramm *et al.*, 1990). In dairy cows, the biopotency of secreted LH increases from parturition until about 12 days after calving and may play an important role in the sequence of events leading to first ovulation (Weesner *et al.*, 1987).

### *Synthesis, accumulation and release of hypothalamic GnRH and pituitary LH during the puerperium*

Although measurement of GnRH in the hypophyseal portal system of cattle has not been reported, several studies in sheep have demonstrated a clear and predictable relationship between GnRH

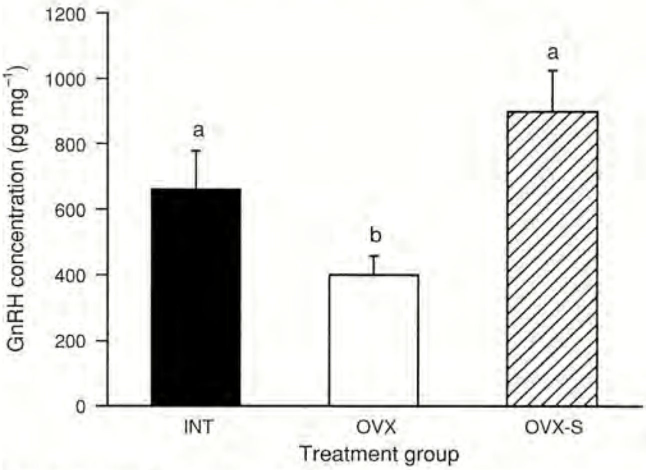


**Fig. 2.** Mean ( ± SEM) content of GnRH in total median eminence tissue collected from intact, nonsuckled (INT), ovariectomized, nonsuckled (OVX) and ovariectomized, suckled (OVX-S) cows on days 31–37 after calving. Means without common letters are significantly different. (Reproduced with permission from Zalesky *et al.*, 1990.)

secretion and LH pulses during different physiological conditions, including the postpartum period (Wise, 1990). Whether concentrations of GnRH and LH change in the hypothalamus and anterior pituitary, respectively, during late gestation or the postpartum period has also been examined. For GnRH, results have been somewhat controversial, and appear to depend to a large degree upon the choice of controls, time of examination and whether the effects of weaning were chronic or acute. No differences were observed in hypothalamic content of GnRH in studies involving suckled postpartum cows (Nett, 1987), or suckled and nonsuckled dairy cows within 14 days of parturition (Carruthers *et al.*, 1980). However, when cyclic cows were compared with postpartum anoestrous cows (Nett *et al.*, 1988), or ovariectomized cows whose calves were weaned at birth were contrasted with ovariectomized, suckled cows (Zalesky *et al.*, 1990), marked accumulations of GnRH within the stalk median eminence were found to be potentiated by chronic suckling (Fig. 2). Anterior pituitary concentrations of LH are low at parturition owing to the inhibitory effect of placental-derived oestradiol on the synthesis of the $\alpha$ and $\beta$ subunits of LH (Nett, 1987). This inhibition ends at parturition and, within approximately 2 weeks after calving, a progressive pattern of LH repletion is evident (Williams *et al.*, 1982). Correspondingly, if calves are weaned at birth, a resumption of pulsatile LH secretion is observed within 7–14 days postpartum, followed by the initiation of ovarian cyclic activity (Williams *et al.*, 1983). On the contrary, the pattern of secretion in suckled, anoestrous cows remains suppressed. As the postpartum period progresses, these two divergent patterns of secretion give rise to additional contrasting features within the hypothalamus and pituitary, including noted differences in quantities of GnRH in the median eminence (Zalesky *et al.*, 1990) and greater accumulations of releasable gonadotrophin pools in suckled, compared with nonsuckled, cows (Williams *et al.*, 1982).

If weaning is delayed until at least 2 weeks after calving, a different response is observed than that seen immediately after parturition. Unfortunately, reports have often confused the interpretation of events that attend these two contrasting scenarios. Unlike the transient changes observed following calf removal at birth, acute weaning of the chronically suckled, anoestrous cow is characterized by a rapid rise in the frequency of LH pulses within 48–96 h (Smith *et al.*, 1983; Shively and Williams, 1989). This occurs as a result of abrupt changes in hypothalamic signalling that are similar, if not identical, to those occurring during the preovulatory period of normal ovarian cycles (Wise, 1990). These changes

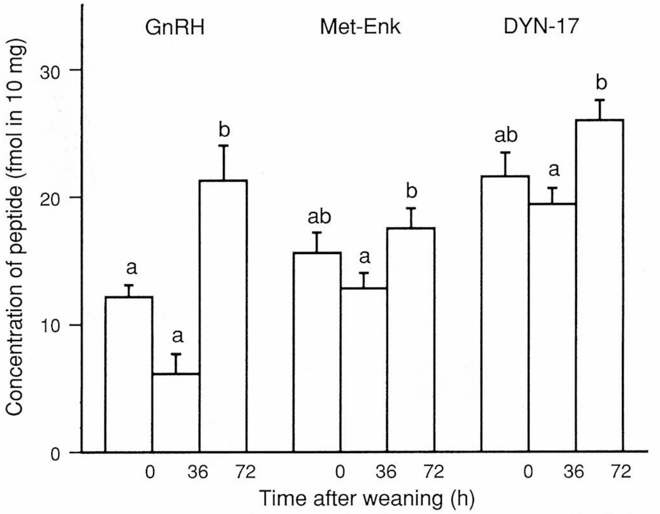


**Fig. 3.** Concentrations of GnRH (fmol in 10 mg), met-enkephalin (Met-Enk) (fmol in 20 μg) and dynorphin A, 1–17 (DYN-17) (fmol $mg^{-1}$) in combined preoptic area plus hypothalamic tissues of cows at 0, 36 and 72 h after weaning. Bars (± SEM) that do not have a common superscript are significantly different. (Reproduced with permission from Malven *et al.*, 1986.)

are accompanied initially by a rapid decline (secretion > synthesis) followed by an increase (synthesis > secretion) in content of GnRH within the hypothalamus (Malven *et al.*, 1986), increased responsiveness of the anterior pituitary to GnRH, and increased release of LH into the peripheral circulation (Walters *et al.*, 1982). After the resumption of ovarian cycles, the characteristics associated with content and secretion of both hypothalamic GnRH and pituitary LH revert to those described previously for cows weaned at birth. However, the acute response to weaning can be markedly attenuated by the premature return of calves and it may require up to 144 h for all cows to respond to temporary weaning (Shively and Williams, 1989; Cutshaw *et al.*, 1991).

*Endogenous opioid tone*

Concentrations of endogenous opioid peptides in neural tissue are affected by suckling (Fig. 3), and short-term administration of an opioid antagonist, naloxone, increases the frequency of LH pulses in suckled anoestrous cows (Fig. 4). These results indicate that opioid tone within the hypothalamus is increased by chronic suckling. Whether such an increase is mediated by increments in the number or affinity of opioid receptors and which class of receptors (β, δ or μ) are involved have not been determined. Studies in rats indicate that both pharmacological and physiological increments in oestradiol increase opioid tone through the irreversible destruction of β-endorphin neurones and an upregulation of μ opioid binding sites (Brawer *et al.*, 1993). During late pregnancy in cows, extremely high concentrations of placental-derived oestradiol inhibit the synthesis of LH by a direct action on pituitary gonadotrophs (Nett, 1987). If the fact that both oestradiol negative feedback and opioid tone at the hypothalamus are heightened during suckling-induced anoestrus is considered, it is possible that gestational increases of oestradiol induce changes in the microenvironment of GnRH-secreting neurones that, although perhaps not irreversible, require a recovery period before normal function is restored. Hence, suckling may impede the rate of recovery of the hypothalamo–pituitary axis from a state of high opioid tone that is established initially during late gestation. Recovery to a more normal opioid tone occurs because the physiological effects of suckling are eventually reduced and most cows return to normal ovarian cyclicity.

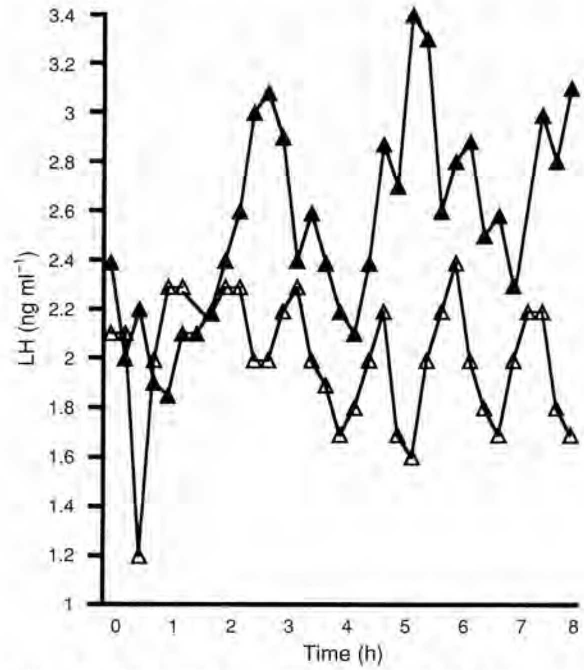


**Fig. 4.** Mean serum concentrations of LH in postpartum suckled beef cows infused with naloxone (▲) or saline (△) during the early postpartum period (day 39 ± 2.1). The effect of naloxone was significant. (Reproduced with permission from Whisnant *et al.*, 1986.)

## Linking Maternal Behaviour to the Puerperal Control of Gonadotrophin Secretion

Frequency and duration of suckling by single calves *ad libitum* appears unrelated to the duration of anoestrus in cattle (Day *et al.*, 1987; Williams, 1990), except under extreme circumstances. Namely, the adoption of an additional calf will extend the postpartum anoestrous interval markedly (Wettemann *et al.*, 1978), and frequency of suckling by single calves that is less than twice daily (Randel, 1981) substantially and consistently reduces the postpartum anovulatory interval (Fig. 5). Experiments in our laboratory have now clearly demonstrated that the neural input at the teat by the suckling calf is irrelevant with respect to inhibition of LH secretion. Mechanical, electrical and thermal hyperstimulation of sensory neurones in the teat failed to simulate the effects of suckling; therefore, neither the free-running pattern of LH secretion in ovariectomized, non-lactating females (Williams *et al.*, 1984), nor the postweaning rise of LH in lactating cows (Cutshaw *et al.*, 1991) was impeded. Conversely, neither mechanical masking (McVey, Jr and Williams, 1991) nor complete mammary denervation (Williams *et al.*, 1993) prevented suckling-induced inhibition of LH, and intervals from parturition to first ovulation were not affected by masking or denervation (Fig. 6).

### *Requisite features of cow–calf association*

Although mastectomized cows whose calves are removed at parturition exhibit slightly shorter postpartum anovulatory intervals than do weaned-intact cows (Short *et al.*, 1972), mastectomized cows maintained with their natural calves with opportunity for unlimited interaction exhibit anovulatory periods similar to suckled-intact cows (Viker *et al.*, 1989) and to milked cows maintained with muzzled calves that had unlimited oral access to the inguinal region (MacMillan, 1983). Calves maintained with mastectomized cows exhibited chronic 'pseudosuckling', which was defined as positioning by the calf in

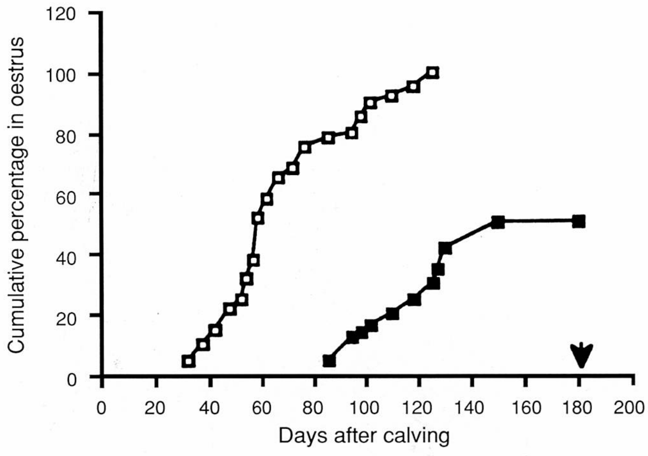


**Fig. 5.** Effect of suckling stimuli on return to oestrus in first calf heifers grazing common bermudagrass pastures. Once daily suckling (□) significantly increased the cumulative percentage in oestrus during the 180 days before weaning (▼) compared to normal suckled controls (■). (Reproduced with permission from Randel, 1981.)

the reverse parallel or perpendicular position, bunting, oral manipulation of skin of the leg or flank, and characteristic head to tail contact between cow and calf. Conversely, if cow–calf interaction is restricted mechanically (Williams *et al.*, 1987) or spatially (Viker *et al.*, 1993; Stevenson *et al.*, 1994), thus eliminating direct oral contact with the inguinal region, the anovulatory state is not maintained. Hence, the mere perception of being suckled may be sufficient for prolonging the period of anovulation.

### *Effects of suckling by own versus unrelated calves*

The majority of cows allow only their own calves to suckle under natural conditions; therefore, it is possible that only a cow's own calf can attenuate gonadotrophin release. Hence, we tested the hypothesis that exteroceptive cues responsible for the suppression of LH secretion are specifically attributable to the dam's own calf and, therefore, are a product of the maternal–offspring bond (Silveira *et al.*, 1993). Removal of natural calves from cows resulted in the expected increase in serum LH concentrations and pulse frequency within 48 h, but enforced suckling by unrelated calves every 6 h for 4 days did not prevent these increases (Fig. 7). In a third group, enforced suckling by 'own' calves at 6 h intervals maintained the suppressed pattern of LH release typical of suckled, anovulatory females. Moreover, mean postpartum intervals to onset of luteal activity in weaned and unrelated-suckled groups were nearly identical, both of which were markedly reduced compared with cows suckling their own calves (Fig. 7).

### *Role of vision and olfaction*

Additional experiments have now been conducted to investigate the role of vision and olfaction in suckling-mediated inhibition of LH secretion (Griffith and Williams, 1994). Cows were allowed to calve normally, and were then control-suckled by either their own calf or an unrelated calf every 6 h for 6 days, beginning at 3 weeks after birth. Cows were maintained separately from calves except during suckling. Neither blindness nor anosmia during these suckling periods in cows nursing their own calves resulted in the disinhibition of gonadotrophin secretion. Blind, olfactory-intact cows appeared to recognize their calves by smell, and anosmic, sighted cows recognized their calves by sight. On the contrary, both blind and anosmic cows exhibited robust increases in pulse frequency for 6 days when suckling by unrelated calves was enforced, a phenomenon identical to that occurring in sham-treated,

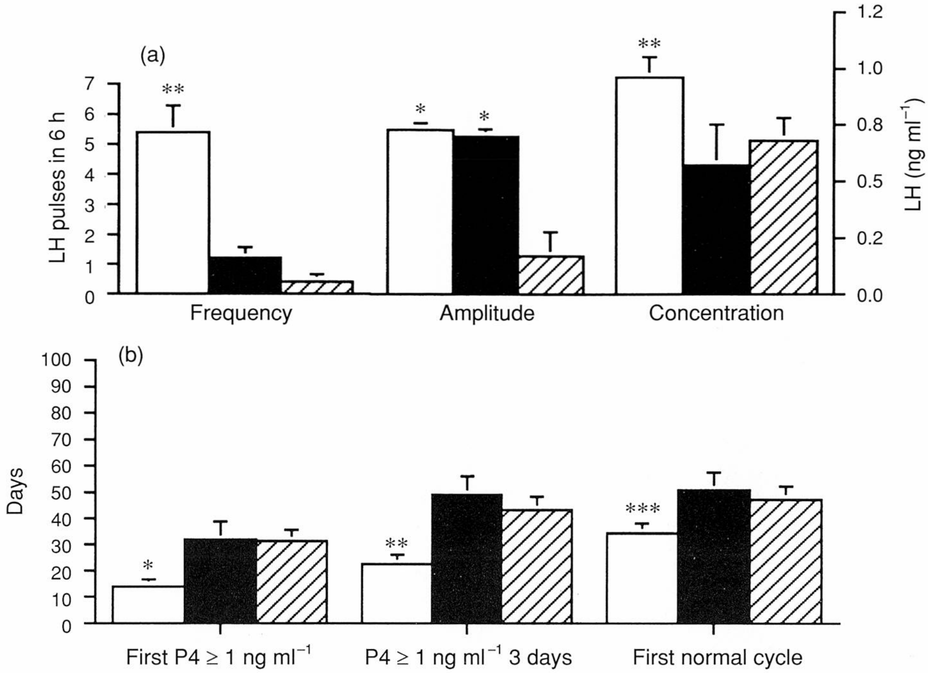


**Fig. 6.** (a) Pooled mean ( ± SEM) frequencies of LH pulses, amplitudes of LH pulses and concentrations of LH in serum pooled from day 9 to day 13 after calving in intact-weaned (□), intact-suckled (■) and denervated-suckled (▨) cows. Pulse frequency and concentration for intact-weaned differed significantly (**) from the values for intact-suckled and denervated groups, and pulse amplitude in the denervated group tended (*) to differ from the other two groups. (b) Mean ( ± SEM) intervals to resumption of partpartum ovarian activity in intact-weaned (□), intact-suckled (■), and denervated-suckled (▨) cows. Asterisks denote significant differences between the intact-weaned and the other two groups within each category (reproduced with permission from Williams *et al.*, 1993).

weaned cows. When both senses were impaired, the cows appeared to lack adequate sensory input to determine own or unrelated, but behaved as weaned from an endocrine standpoint. Thus, the typical rise in LH secretion was observed. Collectively, these results indicate that when a cow is suckled under controlled conditions, it is the ability of the cow to identify the calf as own or unrelated that determines the net effects of suckling or pseudosuckling on the central regulation of tonic LH release. Both weaning (total absence of calf-related stimuli) and suckling by an unrelated calf result in a rapid escape from the inhibitory influence that was previously maintained by the cow's own calf. From these observations, we concluded that the maternal–offspring bond is a requisite feature of suckling-induced anovulation, and that both olfactory and visual cues can be used by cows to identify own and unrelated offspring.

## Physiological Regulation of Maternal Behaviour in Ungulates

The sensitive period, or period during which cows and other mammals are most responsive to the neonate, begins during the periparturient period as a consequence of the hormonal changes of late pregnancy and parturition, and reaches its maximal intensity during the first 2–6 h after parturition (Lent, 1974). This change in behaviour toward a neonate is perhaps one of the most important and dramatic phenomena to be observed in nature, rapidly transforming a female that exhibits gross disinterest, or even aggressiveness toward a neonate, to one that exhibits a complex and highly motivated sequence of maternal activities. Unfortunately, very little work has been conducted in domestic cattle that

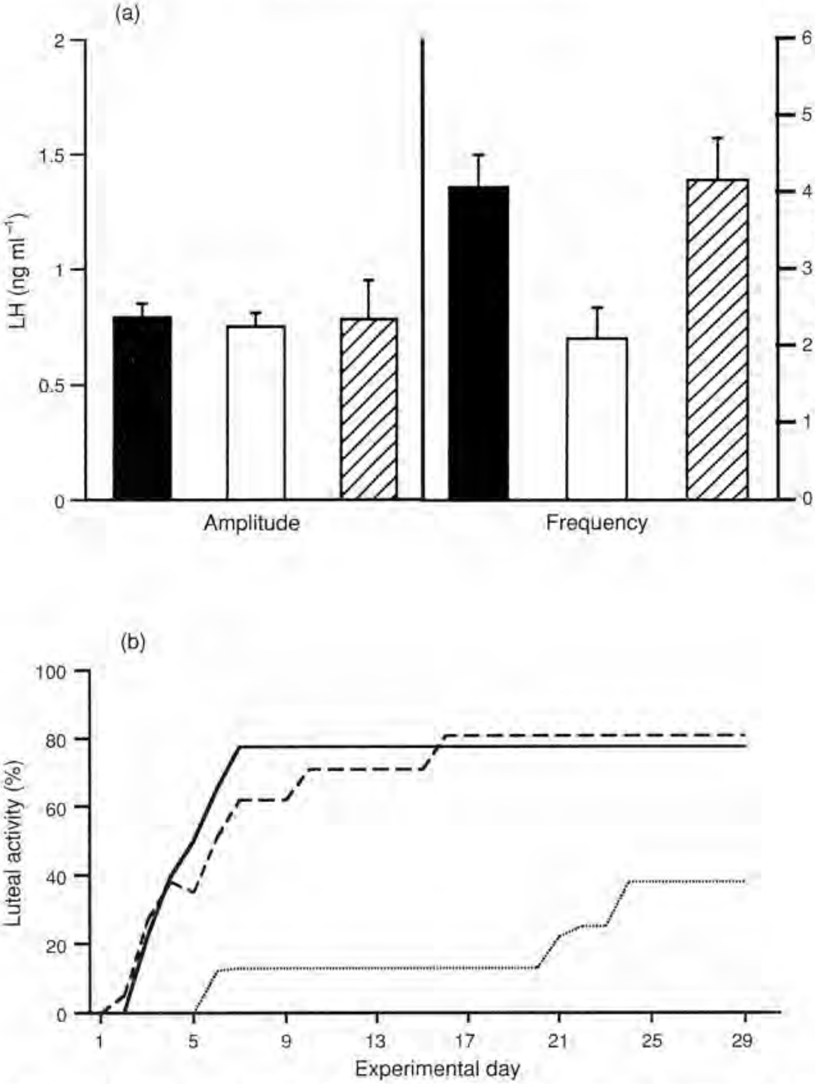


**Fig. 7.** (a) Mean (± SEM) amplitude and frequency of LH pulses on experimental days 2 and 4 combined in cows suckling unrelated calves (■) or own calves (□), or in weaned cows (▨). Day 0 of the experiment began between day 14 and day 17 after calving. (b) Cumulative percentage of cows exhibiting luteal activity in unrelated (– – –), own (······) and weaned (——) groups. Beginning on day 10, cumulative percentages of unrelated and weaned groups were similar, but differed significantly from that of cows suckling their own calves. (Reproduced with permission from Silveira *et al.*, 1993.)

describes the physiological control and development of this phenomenon. Hence, our analysis of factors regulating maternal behaviour in ungulates is restricted to reports in sheep and goats.

### *Role of hormonal steroids, olfaction and vision*

In sheep, the presence of maternal behaviour is clearly associated with increases in circulating concentrations of both progestogens and oestrogens. A degree of maternal behaviour can be induced in up to 50% of nonlactating ewes with previous maternal experience using injections of oestradiol and progesterone, either alone or in combination; however, oestradiol is the most potent (Poindron and Le Neindre, 1980). Sensory information from the lamb is critical for further development of maternal behaviour, and olfactory cue deprivation during the sensitive period disturbs maternal acceptance. Vision appears to play a minor role. A critical role for olfaction is in the development of maternal selectivity. Development of selectivity for her own lamb and rejection of an unrelated lamb requires only about 2 h (Poindron and Le Neindre, 1980). In cattle, the minimum time required for sensitization may be as little as 5 min, with the period of receptivity lasting for up to 5 h in the absence of the calf (Hudson and Mullord, 1977). Ablation of olfactory bulbs or peripherally induced anosmia during gestation in ewes leads to the absence of selectivity during lactation (Baldwin and Shillito, 1974), but does not impede maternal responsiveness *per se*. These ewes allow any lamb to suckle. Hence, olfaction is not essential for maternal responsiveness in sheep, but is essential for development of an exclusive mother–infant bond. This has been known by practical husbandmen for centuries, and the human transfer of odours from own to unrelated offspring is a well-documented fostering technique (Hudson, 1977). Ewes rendered anosmic after a selective bond has been formed can recognize their own lamb visually, but will not allow it or any other lamb to suckle (Baldwin and Shillito, 1974). This is probably not the case in cows. In our preliminary studies, cows rendered anosmic after maternal bonding recognized their calves visually, allowed them to suckle and continued to exhibit an inhibited pattern of LH secretion in a control-suckled environment (Griffith and Williams, 1994).

### *Genital stimulation and intracerebral oxytocin*

Maternal behaviour is facilitated by genital stimulation, particularly vaginal, uterine and cervical distension (Keverne *et al.*, 1981). Genital stimulation is effective only if the animal has been primed naturally or experimentally with oestradiol (Poindron and Le Neindre, 1980), and is most critical in the primigravid female. The action of genital stimulation appears to be mediated by oxytocin. Although neurohypophyseal release of oxytocin into the peripheral circulation occurs coincident with central release, peripheral concentrations do not appear to be important. Oxytocin does not cross the blood–brain barrier (Kendrick *et al.*, 1986) and chronic peripheral administration of oxytocin to non-seasonal ewes during the early postpartum period had no effect on anoestrous intervals (Fletcher, 1973). However, oxytocin appears to be liberated within the brain, as concentrations increased in cerebrospinal fluid collected through the lateral ventricles at parturition and following genital stimulation (Kendrick *et al.*, 1986). Moreover, oxytocinergic receptors are found throughout the central nervous system (Tribollet *et al.*, 1992). Epidural anaesthesia blocks the release of oxytocin and development of maternal behaviour (Poindron and Le Neindre, 1980), and intracerebroventricular infusions of oxytocin stimulate the expression of maternal behaviour (Kendrick *et al.*, 1987). A sequence of oestrogen priming, genital stimulation (oxytocinergic effect) and olfaction may serve as the primary communication link that drives the full establishment of maternal behavioural responses in sheep. Although no data are available, it is assumed that similar events characterize the development of maternal behaviour in cattle.

## Interrelationships Among Intracerebral Oxytocin Release, Opioid Tone, and Gonadotrophin Secretion During Postpartum Anovulation in Cattle: a Hypothesis

It is important to note that both central oxytocin and the opioid peptides act synergistically to facilitate the onset and maintenance of maternal responsiveness in sheep (McCarthy *et al.*, 1992). Although there is no experimental evidence implicating intracerebral oxytocin release with suckling-induced inhibition of gonadotrophin secretion in cattle, there is evidence for an inverse association between factors that facilitate maternal behaviour and those that modulate gonadotrophin secretion (Silveira *et al.*, 1993). Suckling stimulates peripheral oxytocin release and increases opioid tone in cattle, but when cows were forced to suckle unrelated calves, a 45–65% reduction in the frequency of peripheral oxytocin release was observed relative to normal-suckled (own) controls. This occurred coincident with a rapid resumption of pulsatile LH secretion. Hence, it is possible that suckling by an unrelated calf cannot

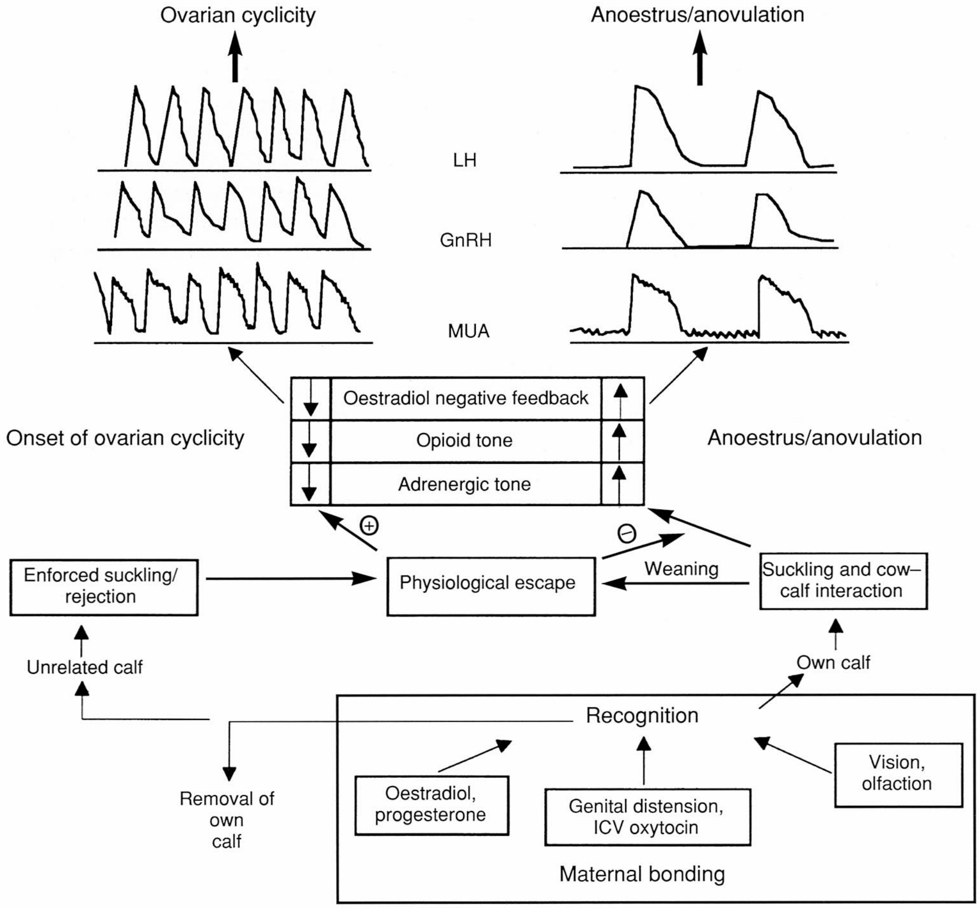


**Fig. 8.** Model describing the role of the neonate, maternal behaviour, suckling, and cow–calf interactions in neuroendocrine regulation of the hypothalamic pulse generator during the postpartum period of beef cows. The diagram shows the formation of a maternal bond with the neonate as a result of several physiological and hormonal signals associated with late gestation, parturition and the neonate itself. Thereafter, suckling by the cow's own calf results in increased adrenergic and opioid tone, increased negative feedback sensitivity to oestradiol, suppression of the hypothalamo–hypophyseal axis and maintenance of the anovulatory state. Conversely, the return to normal ovarian cyclicity occurs when requisite exteroceptive cues associated with suckling and cow–calf interaction are either absent (weaning; enforced suckling by an unrelated calf) or are no longer effective (natural physiological escape). This reflects a disinhibition of the hypothalamo–hypophyseal axis, the resumption of high frequency multiunit electrical activity (MUA), and GnRH and LH pulses. Although not measured directly in cows, MUA and GnRH activities are assumed from studies in the rhesus monkey (Knobil, 1981) and ewe (Wise, 1990), respectively.

maintain a level of opioid tone within the brain and hypothalamus that can inhibit neuronal firing of GnRH secretory neurones, just as it is unable to consistently provoke peripheral oxytocin secretion, a phenomenon widely recognized to occur simultaneously with central oxytocin release (Kendrick *et al.*, 1986). In sheep, endogenous opiates facilitate maternal behaviour by potentiating the effects of oxytocin in oestrogen-primed animals (Kendrick and Keverne, 1992). Opioidergic tone is appropriately increased at parturition, when maximum maternal responsiveness is critical. The same or a similar increment in opioidergic tone that occurs at parturition is maintained during early lactation in suckled females and suppresses LH secretion (Malven *et al.*, 1986; Whisnant *et al.*, 1986; Figs 3 and 4). The abundance of mRNA encoding pro-enkephalin in the paraventricular nucleus, bed nucleus of stria terminalis, and the medial preoptic area are correspondingly increased in sheep (Kendrick and Keverne, 1992), and Leshin *et al.* (1988) have shown that the distribution of the generic precursor of the β-endorphins, proopiomelanocortin (POMC) is found in the same neuroendocrine locations in cattle. All of these anatomical sites have been implicated in the integration of maternal behaviour (Kendrick and Keverne, 1992). Moreover, both POMC and GnRH fibres are closely intermingled at the portal capillaries and in the preoptic area, and an increase in mRNA encoding oxytocin is observed in the POA during lactation (Brooks, 1992). On the basis of these observations, we hypothesize a close linkage between physiological variables that modulate maternal behaviour, opioidergic tone, and the LH pulse generator during suckling-mediated anovulation in cows. Figure 8 is a model summarizing our current view of the interrelationships among these variables.

## Conclusion

A critical re-evaluation of hypotheses that address the neuroendocrine control of suckling-mediated anovulation in cattle is currently underway. These hypotheses suggest that exteroceptive cues responsible for inhibition of LH secretion and anovulation are specifically attributable to the dam's own calf, but mammary somatosensory stimulation is not a requisite feature. In fact, it is now clear that enforced suckling cannot produce an extended anovulatory state in the absence of a maternal–offspring bond. Hence, tests of important new hypotheses that are based upon the relationship between maternal behaviour, opioid tone, intracerebral oxytocin, and tonic LH secretion are warranted. Ongoing attempts to develop feasible intervention strategies for overcoming the biological and economic constraints imposed by the suckling calf will benefit from the successful characterization of these relationships.

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