

Progesterone and Corticosteroids in the Initiation of Lactation in the Sow

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Abstract

The concentration of lactose in the mammary secretion from individual glands of two sows increased significantly ($P < 0.01$) between 0 and 24 h after parturition. In six sows studied during the perinatal period there was a negative correlation ($r = -0.80$; $P < 0.02$) at parturition between the concentration of progesterone in the blood and the concentration of lactose in the mammary secretion. Furthermore, the increase in concentration of lactose in the mammary secretion after parturition was related to the timing of the decline of plasma progesterone to low levels. The results indicate that the initiation of lactation occurs within 24 h of parturition in most sows, and the results are consistent with the hypothesis that progesterone withdrawal acts as the 'trigger'. Neither the changes in corticosteroid binding globulin nor the changes in total corticosteroids were temporally related to the initiation of lactation. However, a circadian rhythm was observed for total corticosteroids in the blood of three out of nine lactating and pregnant sows, whereas no circadian rhythm was observed in progesterone of the four pregnant sows. The results are discussed in relation to the disease complex mastitis-metritis-agalactia.

Introduction

Most lactation failures in sows occur within 2 days of parturition and mastitis-metritis-agalactia (MMA) has been found to be the predominant cause of these failures (Ringarp 1960; Penny 1970). The complex aetiology of MMA is evident from the observations of Martin and McDowell (1975) that over 30 different causal factors have been proposed.

Hormonal studies on MMA have implicated the corticosteroid hormones, prolactin, oxytocin and thyroxin (see review by Martin and McDowell 1975) which are known to be required for the initiation and maintenance of lactation in a number of eutherian mammals (Cowie *et al.* 1969). However, the hormonal control of the initiation of lactation in the sow has not been investigated.

This study describes the changes in the concentrations of progesterone, corticosteroids and corticosteroid binding globulin (CBG) in peripheral plasma, and of lactose, protein and fat in the mammary secretion of sows during late pregnancy and early lactation. These changes are discussed in relation of the initiation of lactation and the disease complex MMA.

Material and Methods

Animals

Sows (Landrace, Large White and Landrace \times Large White) were housed under intensive management in a commercial piggery (Baconfield Piggery, Bullsbrook, W.A.). All were tethered during pregnancy, parturition and lactation and given free access to water. During late pregnancy

and for the first day after parturition they were fed sow pellets (W. H. Milne and Co., Perth) at 0.9 kg/day. On days 2, 3, 4, 5, 6 and 7 after parturition they were fed 1.8, 2.7, 3.6, 4.5, 5.4 and 5.4 kg/day respectively. From day 8 to weaning they were fed 4.5–5.4 kg/day depending on age and weight. Piglets were weaned at 3 weeks of age.

Sampling

Blood samples (1–2 ml) were obtained by venipuncture from the ear vein of tethered but otherwise unrestrained sows. Mammary secretion (1–2 ml) was obtained by hand-milking; except at parturition and after weaning, 1 i.u. of oxytocin (Pitocin, Parke Davis and Co.) was administered into the ear vein before milking. Unless otherwise stated, samples were collected between 1000 and 1230 h. Plasma and mammary secretion were stored frozen at -15°C until analysed. Milk samples, other than those shown from individual glands, were obtained from a composite sample of milk collected from four glands which were observed to have been actively suckled—usually the anterior four glands.

Plasma Analysis

(i) Progesterone

Progesterone was determined by a modification of the protein binding assay described by Martin *et al.* (1977). As there was negligible interference from cross-reacting steroids in sow plasma, purification of the progesterone extract was not required. The recovery of [^3H]progesterone from plasma samples was estimated at various times with different batches of hexane fraction (Unilab, Ajax Chemicals Ltd, Sydney). No differences were observed and the recovery was $96.8 \pm 1.8\%$ (mean \pm s.e.m., $n = 20$).

(ii) Total Corticosteroids

Total corticosteroids were determined by the method of Martin *et al.* (1977) except that free and bound corticosteroids were separated with 100 μl of Dextran-coated charcoal (3.75 g activated charcoal and 0.375 g Dextran T500 in 100 ml of 50 mM phosphate buffer, pH 7.4). Samples were mixed and allowed to stand in ice for 10 min and they were then centrifuged at 3000 g for 10 min at 4°C . A 100- μl aliquot of the supernatant was taken to determine CBG-bound radioactivity. The recovery for [^3H]cortisol from plasma was $94.3 \pm 2.1\%$ (mean \pm s.e.m., $n = 15$).

(iii) Cortisol

The concentration of plasma cortisol was determined by radioimmunoassay using antibodies raised in rabbits against cortisol 21-hemisuccinate bovine serum albumin. The assay technique was similar to that described for oestrone determination by Rowe *et al.* (1973) except that bovine serum albumin was replaced with gelatin. Cross-reaction of the antibody with corticosterone, cortisone, 21-deoxycortisol and 11-deoxycorticosterone was 8, 7, 7 and 2% respectively. Samples were extracted with ethanol and assayed in duplicate. The concentration of total corticosteroids and cortisol estimated in six samples of plasma was 19.3 ± 8.9 and 19.8 ± 7.5 $\mu\text{g/l}$ (mean \pm s.e.m.) respectively. This finding indicates the circulating corticosteroids in the sow are almost exclusively cortisol.

(iv) CBG

CBG was measured by the method of Martin *et al.* (1977) except that plasma samples were diluted 1:6 (v/v) in 50 mM phosphate buffer, pH 7.4, and [^3H]corticosterone was replaced with [^3H]cortisol.

Milk Analysis

Lactose, in a 1:100 (v/v) dilution of mammary secretion in water, was determined by the method of Kuhn and Lowenstein (1967). Milk protein was calculated as total N \times 6.38 (Hartmann 1973). Milk fat was estimated as total esterified fatty acids (Hartmann 1973) assuming a mean molecular weight of milk fatty acids of the sow to be 266 (DeMan and Bowland 1963).

Results

Sampling Technique

A comparison of the concentration of lactose between individual mammary glands of two sows during the first week of lactation is shown in Fig. 1. The mean concentration of lactose in the mammary secretion increased significantly ($P < 0.01$) in the

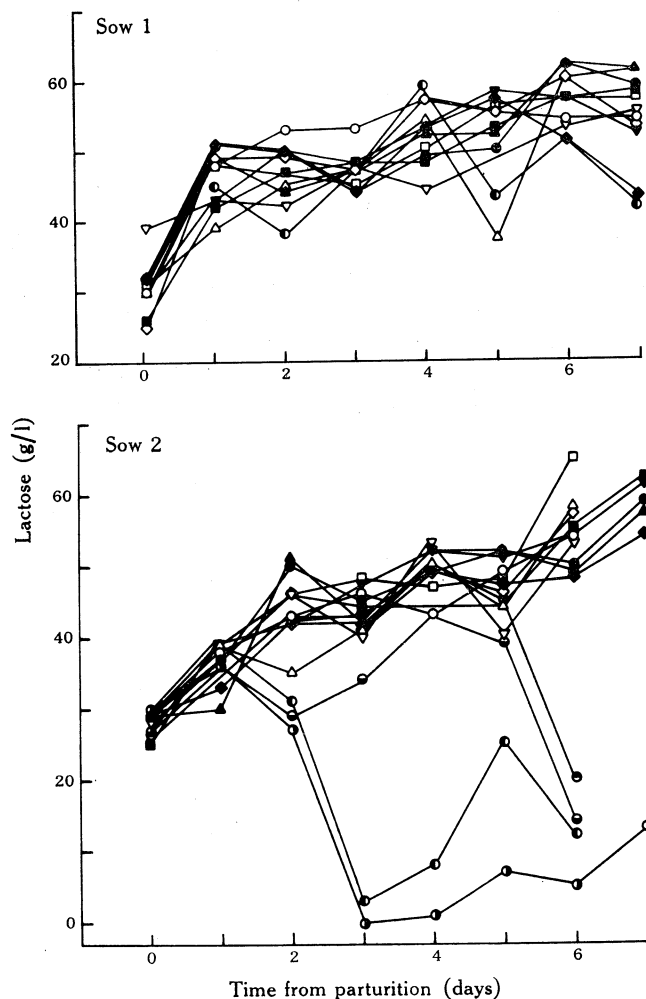


Fig. 1. Lactose in the secretion from mammary glands of sows 1 and 2 during the first 7 days after parturition. Sows 1 and 2 had 11 and 14 glands respectively. Mammary glands are numbered anterior to posterior (R = right, L = left); 1R (●), 1L (○), 2R (▲), 2L (△), 3R (▼), 3L (▽), 4R (◆), 4L (◇), 5R (⊖), 5L (⊕), 6R (⊙), 6L (⊚), 7R (■), 7L (□).

two sows from 30.6 ± 1.2 and 28.1 ± 0.4 g/l at parturition to 46.2 ± 1.2 and 36.4 ± 0.9 g/l respectively 24 h after parturition. There was no significant change between 1 and 2 days *post partum*. Whereas in sow 1 (suckling 13 piglets) the con-

centration of lactose remained relatively constant in all mammary glands from 2 to 7 days *post partum*, in sow 2 (suckling six piglets) the concentration of lactose in the secretion from glands 5R, 5L, 6R and 6L (R = right, L = left) decreased to low levels by 3, 3, 6 and 6 days respectively *post partum* (Fig. 1). In this sow, as in many other sows observed at Baconfield Piggery, the mammary glands which involuted in early lactation were the posterior rather than the anterior glands.

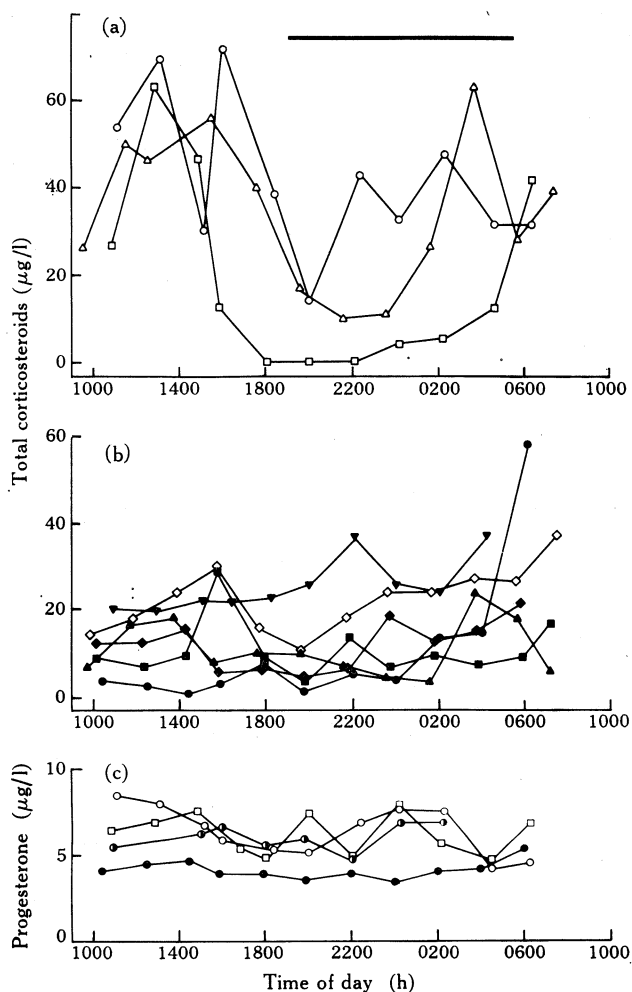


Fig. 2. Total corticosteroids in the plasma of (a) sows 9 (\square), 10 (\triangle), and 11 (\circ); (b) sows 3 (\bullet), 4 (\blacksquare), 5 (\blacklozenge), 6 (\blacktriangle), 7 (\blacktriangledown) and 8 (\diamond), and (c) progesterone in the plasma of sows 3, 9, 11 and 12 (\bullet) over a 24-h period. Sows 3, 9, 11 and 12 are pregnant and the remainder are lactating. The period of darkness is represented by the bar.

The concentration of progesterone in the plasma of four pregnant sows (sows 3, 9, 11 and 12) and total corticosteroids in the plasma of three pregnant sows (sows 3, 9 and 11) and six lactating sows (sows 4, 5, 6, 7, 8 and 10) over a period of 24 h is

shown in Fig. 2. Sows 9, 10 and 11 showed circadian rhythms for total corticosteroids, the highest levels occurring in the morning and the lowest levels occurring at night (Fig. 2a). The concentration of total corticosteroids in the plasma of sows 3–8 was variable and tended to fluctuate around the lower levels observed for sows 9, 10 and 11 (Fig. 2b). The concentration of progesterone in the plasma from the four pregnant sows (sows 3, 9, 11 and 12) remained relatively constant over the 24 h period (Fig. 2c).

Blood Progesterone, Corticosteroids, CBG and Milk Lactose

The relationship between the changes in the concentration of progesterone and corticosteroids in the plasma and lactose in the mammary secretion of six sows during the perinatal period (i.e. between 10 days before to 7 days after parturition) is shown in Fig. 3. Although the concentration of progesterone decreased to low levels

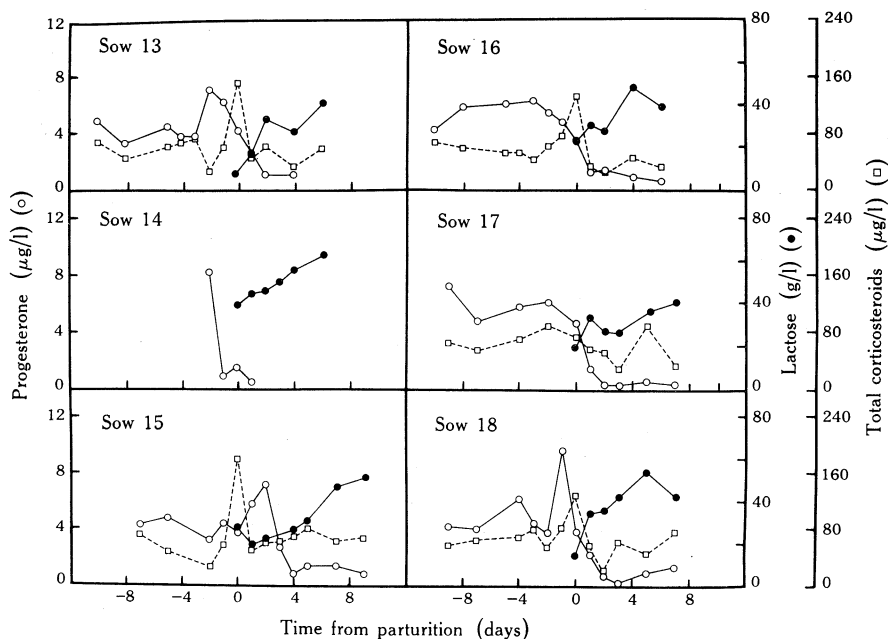


Fig. 3. Progesterone (■) and total corticosteroids (□) in the plasma, and lactose (●) in the mammary secretion of sows 13–18. Progesterone and total corticosteroids are shown during the period 10 days before to 8 days after parturition, while lactose is shown during the first 7 days after parturition.

(<2 $\mu\text{g/l}$) during this period, there was considerable variation in the time at which this decrease occurred (in sow 14, progesterone decreased to low levels 1 day before parturition whereas in sow 15 progesterone did not decrease to low levels until 4 days *post partum*). At parturition the concentration of lactose in the mammary secretion was low (23 ± 4 g/l, mean \pm s.e.m.) and there was a significant negative correlation between the concentration of lactose in the mammary secretion and the concentration of progesterone in the plasma of the sows ($r = -0.80$; $P < 0.02$). With the exception of sow 17 the increase in lactose concentration in the mammary

secretion after parturition was related to the timing of the progesterone decline to low levels.

During the perinatal period the concentration of total corticosteroids in the plasma varied between 20 and 90 $\mu\text{g/l}$ except for the day of parturition when four out of the five sows studied showed elevated levels (130–180 $\mu\text{g/l}$). At parturition there was no correlation ($r = 0.15$; $P > 0.05$) between the concentration of total corticosteroids in the plasma and lactose in the mammary secretion.

The concentration of CBG in the plasma ranged from 5.5 to 11.0 μg cortisol bound per litre of plasma over the perinatal period without showing any consistent changes. The low level of CBG relative to total corticosteroids indicated that 75–90% of the total corticosteroids present in the plasma of the sow was unbound.

Milk Protein and Fat

The concentration of protein in the mammary secretion was high at parturition (189 ± 3 g/l, mean \pm s.e.m.) and declined rapidly during the first 2–3 days *post partum* to reach the lower values of normal sow's milk by 7 days *post partum* (Fig. 4).

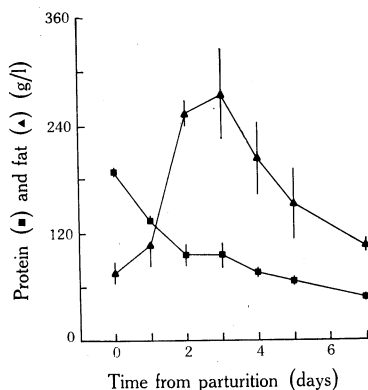


Fig. 4. Protein (■) and fat (▲) in the mammary secretion of five sows during the first 7 days after parturition. Standard errors are shown by vertical bars.

The concentration of fat in the mammary secretion from the sows was low at parturition; it increased to peak concentration between 2 and 4 days, and then declined to lower levels by 4–6 days *post partum* (Fig. 4).

Weaning

The progressive changes in the concentration of lactose and protein in the mammary secretion of four sows following the abrupt termination of suckling, at 21 days of lactation, is shown in Fig. 5. The concentration of lactose in the mammary secretion declined abruptly from normal milk levels (45 ± 4 g/l, mean \pm s.e.m.) 2 days after weaning to low levels (< 5 g/l) by 5 days after weaning. The concentration of protein in the mammary secretion increased from normal milk levels (39 ± 4 g/l, mean \pm s.e.m.) at weaning to reach a plateau (66 ± 4 g/l) by 3 days after weaning (Fig. 5).

Discussion

The changes in the concentration of lactose in the mammary secretion during late pregnancy and early lactation have been used as an indicator of initiation of

lactation in cows (Hartmann 1973), ewes (Hartmann *et al.* 1973) and women (Kulski *et al.* 1977). In the sow the histological observations of Cross *et al.* (1958) showed that the change from inactive alveoli to fully secreting tissue is very rapid and occurs predominantly within the 24-h period before parturition. These histological observations together with the changes in the concentration of lactose in the mammary secretion of two sows (Fig. 1) suggest that the initiation of lactation occurred within 24 h of parturition in all glands. It is also clear that the successful maintenance of lactation in individual mammary glands depended upon the establishment of a regular suckling pattern (Perrin 1955). With sow 2, the rapid involution of two mammary glands within 3 days of parturition suggests that these glands remained unsuckled (see Fig. 1). This feature is of particular importance in investigations of the initiation of lactation, because secretory samples from non-suckled glands (from which it is often easier to obtain secretion than from suckled glands) could bias the interpretation of results.

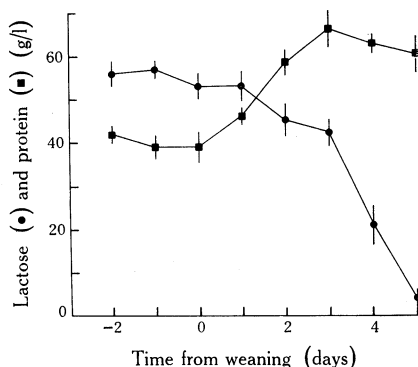


Fig. 5. Lactose (●) and protein (■) in the milk of four sows during the period 2 days before to 5 days after weaning. Standard errors are shown by vertical bars.

In intensive piggeries, piglets are routinely transferred to equalize litter numbers between sows. Since individual piglets tend to suckle from a preferred mammary gland (Fishwick 1965) and since those glands which are not suckled are most likely to involute, piglets should be transferred onto sows within 48 h of parturition, as after this time lactation would need to be re-initiated in the unsuckled mammary gland.

The hormonal signal for the initiation of lactose synthesis in the rat (Kuhn 1969) and a number of other species is considered to be a precipitous fall in the concentration of progesterone in the blood during either late pregnancy (see Hartmann 1973; Hartmann *et al.* 1973) or the first day *post partum* (Kulski *et al.* 1977). In the sow at parturition there was a negative correlation between the concentration of progesterone in the blood and the concentration of lactose in the mammary secretion. Furthermore, the increase in lactose concentration in the mammary secretion after parturition was related to the timing of the progesterone decline to low levels. These observations are consistent with the hypothesis that progesterone withdrawal also acts as a 'trigger' for the initiation of lactation in the sow.

In some species it has been postulated that either an increase in the concentration of corticosteroids (Cowie 1970) or a decrease in the level of CBG (Gala and Westphal 1967) during late pregnancy is involved in the initiation of lactation. Although the concentration of CBG in the sow remained relatively constant during the perinatal period, the concentration of corticosteroids in four sows more than doubled at

parturition and then rapidly declined to *pre partum* levels within 24 h (Fig. 3). However, these changes were neither temporally related to nor significantly correlated with the changes in the concentration of lactose in the mammary secretion and therefore do not support a positive involvement of corticosteroids in the initiation of lactation in the sow.

A circadian rhythm in the concentration of total corticosteroids in the blood of sows has been reported previously by Killian *et al.* (1973). In the present study the circadian rhythms in the concentration of total corticosteroids in the blood were evident only in three out of nine sows studied (Fig. 2). Although no reason can be given for the absence of the circadian rhythm in six of the sows, factors such as ambient temperature, crowding (Kattesh *et al.* 1976), and lighting period (five out of six lactating sows subject to continuous light in farrowing pens showed no circadian rhythm) are likely to be involved. The possibility that sows with consistently low levels of plasma corticosteroids are predisposed to metabolic or infective diseases requires further investigation.

Studies on the initiation of lactation in normal sows are of interest in relation to the MMA syndrome. Although in sow 15 progesterone remained elevated 2 days after parturition and there appeared to be a delay in the initiation of lactation (Fig. 3), this sow did not show clinical symptoms of agalactia. On the other hand, the decline in the concentration of progesterone to low levels occurred 1 day before parturition in three out of six sows studied by Ash *et al.* (1973) and in the present study in sow 14 (Fig. 3). This early decline in progesterone was associated with a high concentration of lactose in the mammary secretion at parturition (Fig. 3). Therefore it is possible that the early initiation of lactation could predispose these sows to mammary gland engorgement. A histological study by Swarbrick (1968) indicated that colostrum accumulated in the mammary glands of sow with MMA, and breast engorgement in early lactation in women results in similar clinical symptoms (Winter and Robinson 1964) to MMA in sows (Ringarp 1960). However, in the present study involution of either of one or two mammary glands after birth (Fig. 1) or involution imposed by weaning the piglets at 3 weeks (Fig. 5) did not result in clinical symptoms of MMA.

Acknowledgments

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