THE ROLE OF PROLACTIN IN THE CONTROL OF OVINE LACTOGENESIS

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ABSTRACT

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A series of trials was carried out to examine the role of prolactin (PRL) in the control of lactogenesis in New Zealand Romney x Border Leicester ewes. In addition, a study was made of differences in milk yields and plasma PRL concentrations between spring- and autumn-lambing ewes.

Daily subcutaneous injections of 2 mg CB154 inhibited PRL secretion and delayed lactogenesis. There were no consistent effects on plasma progesterone or insulin concentrations. CB154 treatment was more effective in reducing milk yield in twin-bearing than in single-bearing ewes when used for 20 days than for 9 days prepartum. The differential effects on milk yield cannot be explained by corresponding effects on plasma PRL or insulin concentrations. Circulating PRL during the period 20 to 10 days prepartum may have an important effect on milk yield in twin- but not single-bearing ewes.

Subcutaneous injections of 0.5 mg/kg live weight oPRL, administered on 2 consecutive days peripartum, to ewes treated with CB154 for 7 days prepartum, resulted in milk yields similar to those in control ewes and significantly ($P<0.01$) greater than those in ewes treated with CB154 alone. This indicated that oPRL prevented the CB154-induced reduction of milk yields and has established that the effect of CB154 on lactogenesis is mediated through suppression of PRL secretion and not by effects on some other hormone.

Injection of 10 mg oPRL directly into one mammary gland (via the teat duct) increased milk yields relative to the contralateral, bicarbonate-treated gland in CB154-treated ewes. The intramammary oPRL injection did not raise circulating PRL concentrations. Furthermore, the milk yields of bicarbonate-treated glands in ewes treated with bicarbonate only, did not differ from those of bicarbonate-treated glands in ewes treated with oPRL in the contralateral gland, demonstrating that there were no effects of oPRL, transferred via the circulation from the treated gland, on the contralateral gland. Glands treated with oPRL produced 15% ($P<0.05$) more milk than the bicarbonate-treated glands during the first 8 days of lactation and the difference was maintained throughout the 8-week lactation period, indicating that the oPRL had effected a permanent change in the ability of the gland to produce milk. It is concluded that PRL acts directly on the mammary gland without the need for a putative intermediate hormone, and that intramammary PRL concentrations during lactogenesis may have long-lasting effects on lactation.

The possibility was examined that dietary differences were responsible for seasonal differences in plasma PRL concentrations, milk yields, milk composition, lamb birthweight and lamb growth rate, observed in earlier trials. Mean plasma PRL levels were significantly ($P<0.01$) higher in spring- (192±38 ng/ml) than in autumn- (71±17 ng/ml) lambing ewes housed indoors under constant photoperiod (18L:6D) and fed the same diet. Milk yields were also significantly ($P<0.05$) higher in the spring- (2041±114 g/d) than in the autumn- (1563±109 g/d) lambing ewes over the 8 day lactation. Lamb growth rates (adjusted for birthweight, birthrank and sex of lamb) from birth to 8 weeks of age were significantly ($P<0.001$) higher in spring (282±12 g/d) than in autumn (225±15 g/d). The seasonal differences were confounded with corresponding differences in ewe live weight and it was not possible to determine whether dietary differences contributed significantly to the differences observed.

Two routes of oPRL supplementation were used to test the effectiveness of elevating peripheral or local levels of PRL in autumn-lambing ewes which, based on previous results, were expected to have low plasma PRL concentrations and milk yields relative to spring-lambing ewes. Administration of 10 mg supplementary oPRL directly into the gland or subcutaneous injection of 0.5 mg/kg oPRL did not increase the milk yields, or change the composition of milk, compared to controls. These results suggest that the circulating level of PRL, and the intramammary concentration of PRL, in autumn-lambing ewes are not limiting lactogenesis. Because the plasma prolactin concentration in the ewes was unexpectedly high, it was not possible to reach firm conclusions regarding possible effects of supplementary oPRL in ewes with naturally low plasma PRL concentrations. Nevertheless, the results indicate that raising the intramammary concentration of PRL around the time of parturition, in ewes with circulating PRL levels characteristic of normal spring-lambing ewes, does not enhance lactogenesis.

It is concluded that PRL is important to the complete initiation of lactogenesis in ewes, that it acts directly on the gland and that it is necessary for establishing the maximum potential of the gland to secrete milk.

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Ultimately, it is the pursuit of knowledge that has lead to this research programme. Often the search is tedious but I have found these two thoughts inspiring:

"There are things that are known and things that are unknown, and in between are the doors."
William Blake (1757-1827)

"If the doors of perception were cleansed, then all things would appear infinite."
Aldous Huxley (1894-1963)

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18L:6D	18 hours of light and 6 hours of darkness per day
BIC	sodium bicarbonate
bPL	bovine placental lactogen
bPRL	bovine prolactin
CB+BIC	group treated with CB154 plus bicarbonate
CB+PRL	group treated with CB154 plus ovine prolactin
CB154	2-bromo-α–ergocriptine mesylate
CB20	group treated with CB154 for a mean period of 20 days
CB9	group treated with CB154 for a mean period of 9 days
CIDR	controlled internal drug release (device)
Cl	chlorine
CV	coefficient of variation
cAMP	cyclic adenosine monophosphate
cDNA	complementary deoxyribonucleic acid
cRNA	complementary ribonucleic acid
DM	dry matter
d	day(s)
d.f.	degrees of freedom
E/S	group treated with subcutaneous ethanol/saline injections
EDTA	disodium ethylene diaminetetraacetic acid
GH	growth hormone
GRF	growth hormone-releasing factor
g	gram(s) or acceleration due to gravity
h	hour(s)
hGH	human growth hormone
hPL	human placental lactogen
IgG	immunoglobulin G
i.mam.	intramammary administration of oPRL via the intraductal route
i.u.	international units
i.v.	intravenous
K	potassium
kg	kilogram(s)
kPa	kilopascals
LPL	lipoprotein lipase
LWT	live weight
M	molar
ME	metabolisable energy

MJ	megajoules
mg	milligram(s)
min	minute(s)
ml	millilitre(s)
mRNA	messenger ribonucleic acid
Na	sodium
NIADDK	National Institute of Diabetes and Digestive and Kidney Diseases
ng	nanogram(s)
^{o}C	degrees Celsius
oGH	ovine growth hormone
oPL	ovine placental lactogen
oPRL	ovine prolactin
PGE2	prostaglandin $E_{2\alpha}$
PGF2α	prostaglandin $F_{2\alpha}$
PGFM	13,14-dihydro-15-keto-PGF, the main metabolite of $PGF_{2\alpha}$
PIF	prolactin release inhibiting factors(s)
PL	placental lactogen
PMSG	pregnant mares serum gonadotrophin
PRF	prolactin-releasing factor
PRL	prolactin
PRLsc	group treated with oPRL by subcutaneous injection
pg	picogram(s)
pH	hydrogen ion potential
RIA	radioimmunoassay
RPM	revolutions per minute
RRA	radioreceptorassay
r	correlation coefficient
rbST	recombinantly derived bovine somatotropin
rT_3	reverse triiodothyronine
SAM	S-adenosyl-methionine
SE	standard error of the mean
s.c.	subcutaneously
T_3	triiodothyronine
T_4	thyroxine
TSH	thyroid stimulating hormone
w/v	weight/volume

CHAPTER 1
REVIEW OF LITERATURE

CONTROL OF LACTOGENESIS IN SHEEP

1.1 INTRODUCTION: OBJECTIVE, DEFINITION OF TERMS AND SCOPE OF REVIEW

Ruminants may exhibit marked secretory activity during the second half of pregnancy and produce considerable quantities of milk, especially if milked prepartum. Conversely rats, mice and sows show minimal secretion until very late in gestation (Cowie and Tindal 1971) and primates do not exhibit lactogenesis until 1-2 days after the loss of the placenta at parturition (Kuhn 1977). These differences are a warning against unconditional extrapolation of results from other species to the sheep. Nevertheless the insight provided from studies across species is very useful in suggesting possible mechanisms for the control of lactogenesis in the sheep. It is the purpose of this thesis to investigate the mechanisms by which sheep initiate lactogenesis, with a view to identifying means of manipulating subsequent lactational yields by altering the hormonal environment in late pregnancy.

Lactogenesis is defined as the initiation of milk secretion (Cowie *et al.* 1980). Folley (1969) stated that lactogenesis marks the change from a developed gland composed mainly of functionally competent but quiescent cells to one capable of secreting large amounts of protein, fat and carbohydrate. Secretory activity may be first detected at various stages from mid-pregnancy onward, but milk synthesis increases markedly at about the time of parturition. Fleet *et al.* (1975) distinguished between lactogenesis type I, the gradual appearance of precolostrum in the gland, and lactogenesis type II, the onset of copious milk secretion. It is the latter event which is the subject of this study and henceforth the term "lactogenesis" will be used synonymously with lactogenesis type II. Thus, the phrase "initiation of lactogenesis" is equivalent to "initiation of the onset of copious milk secretion". Cowie *et al.* (1980) recognised that the existing definitions may not be adequate to deal with all situations. It is evident that the above definition of lactogenesis involves a quantitative aspect as well as a temporal component. Although the time of initiation may not alter, lactogenesis may be said to be delayed if the quantity of milk produced is slow to increase to "copious" volumes.

The meaning of the term "lactogenic" is obviously important to the understanding of this topic, yet many authors use the term very loosely to indicate any factors which promote lactation, while others select a very specific meaning. Thordarson and Talamantes (1987) used the term

"lactogenic hormone" to designate a group of protein hormones of pituitary and placental origin that are structurally similar to, and/or show activity in radioreceptor assays (RRA) for, prolactin-like and or growth hormone-like hormones. This lactogenic activity has been demonstrated in bioassays (Thordarson and Talamantes 1987) such as the pigeon crop sac assay and mammary gland organ culture (Cowie *et al.* 1980). Kuhn (1977) recognised this problem of terminology and chose to use the term "prolactational". In this thesis the term "lactogenic" will be used specifically to describe factors which promote lactogenesis. The term "galactopoietic" will be used to imply an augmenting action on established lactation as defined by Lyons (1958).

According to Campbell and Lasley (1985), hormones are the only stimulators of lactation. The validity of this statement may be questioned depending upon how one defines the term "stimulators". If the removal of inhibitory factors is considered stimulatory then the statement may indeed be false, since the removal of the hormone progesterone, as well as the removal of non-hormonal factors, such as milk and inhibitory substances in milk, are thought to be stimulatory to lactogenesis.

Cowie (1969) reviewed the early attempts of researchers to identify the hormone responsible for lactogenesis. When it became apparent that no one hormone would satisfy the criteria, Folley and Young (1938 and 1941) proposed the concept of a lactogenic complex consisting of two or more lactogenic hormones. As details of the actions of many hormones have been elucidated the "black box" approach to the lactogenic complex is no longer favoured. It is now recognised that many hormones contribute to lactogenesis in varying degrees. The period around parturition is characterised by declining concentrations of progesterone and placental lactogen and by increases in circulating levels of prolactin (PRL), oestrogens, prostaglandin F2α, oxytocin and adrenal corticoids (Cowie *et al.* 1980). This review will consider the effect of each of these hormones on lactogenesis with emphasis on PRL, which will be considered last, since this hormone is the subject of the studies reported here. The effect of milking and milk removal will also be briefly considered. Wherever possible information provided will relate to sheep, but other species (in particular goats and cattle) will be considered where information on sheep is absent or requires support. Statements which do not identify a particular species refer to eutherian (placental) mammals in general.

Growth Hormone (GH) will not be considered in depth in this review since there is little evidence that it is involved in lactogenesis, other than in a permissive role. Plasma levels of GH are low in pregnant ewes (Bassett *et al.* 1970) and, although they increase in late pregnancy, this is thought to reflect metabolic adaptation (Blom *et al.* 1976) rather than a role in lactogenesis. The possibility that GH is involved in lactogenesis was apparently not considered worthy of mention by several pre-eminent researchers reviewing the subject (Cowie and Tindal 1971; Cowie *et al.* 1980; Vonderhaar 1987). On the other hand, Convey (1974) reviewed the possible evidence for a lactogenic role of GH in ruminants and concluded that a surge of GH at parturition would benefit

lactogenesis. Recently however, Stelwagen *et al.* (1990) reported the prepartum treatment of nulliparous heifers with recombinantly derived bovine somatotropin (rbST) at 2 dose rates. The low dose increased colostrum and milk yields while the high dose depressed yields. It was concluded that rbST treatment had stimulated prepartum milk accumulation in the gland. Although Stelwagen *et al.* (1990) attributed this result to a galactopoietic action of rbST, it is more likely to have been due to effects on mammogenesis and lactogenesis. In sows treatment with growth hormone-releasing factor (GRF) from d 100 of gestation failed to alter subsequent lactational parameters (Farmer *et al.* 1992). It is apparent that the prepartum actions of GH require further elucidation, at least in the ruminant.

1.2 PROGESTERONE

Progesterone has a major role in the maintenance of pregnancy and a role in the co-ordinated growth and development of the mammary gland could be anticipated. Thus, the concentration of progesterone in the blood and its actions, particularly towards the end of pregnancy, are of interest in relation to the onset of lactation.

1.2.1 PLASMA CONCENTRATIONS OF PROGESTERONE

There is wide variation between individual pregnant ewes in plasma progesterone concentrations (Slotin *et al.* 1971) and wide variation in the values reported by different workers. Nevertheless, the published profiles of progesterone concentrations are similar in shape. The corpus luteum is the principal source of progesterone for about the first 50 days of gestation after which the placenta assumes an increasingly important role (Bedford *et al.* 1973; Moore *et al.* 1972). Plasma progesterone concentrations remain low, fluctuating between 2-8 ng/ml for the first 50-80 days of gestation (Bassett *et al.* 1969; Bedford *et al.* 1973; Kelly *et al.* 1974). Thereafter levels rise steadily to peak in the last trimester (Bassett *et al.* 1969; Bedford *et al.* 1973). Progesterone concentrations in plasma peaked about 10 days prepartum and were generally higher in twin-bearing than in single-bearing ewes in several reports (Bassett *et al.* 1969; Chamley *et al.* 1973; Kelly *et al.* 1974). Earlier peak plasma levels were recorded, between days 25 and 18 prepartum, by Mellor *et al.* (1987). The peak concentrations (42-52 ng/ml) did not differ significantly between groups of twin-bearing ewes on different planes of nutrition. Late in pregnancy, plasma concentrations decline again to about 10 ng/ml by one or two days prepartum and reach negligible values by 18 h after birth (Mellor *et al.* 1987). However, reports differ with respect to the time at which the decline begins. According to Bedford *et al.* (1973) this decline occurred within 2 days prior to parturition, but Cowie *et al.* (1980) contended that it may begin 2 weeks prepartum or not occur until the last day of gestation. Hartmann *et al.* (1973) reported that the fall in plasma progesterone concentrations occurred in the last 1-4 days while Chamley *et al.* (1973) detected it 4 or 5 days prepartum. The data of Kelly *et al.* (1974) indicated a precipitous fall in the

last 3-7 days prepartum. Boulfekhar and Brudieux (1980) found, in Tadmit ewes, that mean progesterone concentrations declined during the 17 days preceding lambing, decreasing more rapidly during the last 3 days. Moore *et al.* (1972) reported that progesterone concentrations in both the uterine and ovarian veins fell after day 120 and by day 142 were markedly lower (but failed to explain why jugular plasma concentrations peaked (at 12 ng/ml) on day 142). Bassett *et al.* (1969) reported that the mean plasma progesterone concentration fell from 12 ng/ml 7 days before lambing to approximately 1 ng/ml on the day of delivery. However, they presented data showing that the decline in plasma progesterone before parturition was not consistent between animals. Indeed there was considerable variation between ewes and the decrease did not occur in some ewes until less than 24 h before parturition. Much of the variation in the reported time at which plasma progesterone concentrations begin to decline may be due to nutritional differences. The concentration began to decline 18 days prepartum in ewes on a high plane, 5 days prepartum in ewes on a rising plane and 3 days prepartum in those on a low plane of nutrition . Furthermore, the presence of higher concentrations of progesterone in the low plane group than in the better fed ewes, several hours after the expulsion of the placenta, suggests that undernutrition reduced the rates of excretion and/or metabolism of progesterone (Mellor *et al.* 1987). The reported differences in the progesterone decline are important in light of the hypothesis that there are two types of ewe with respect to plasma profiles of progesterone and PRL (Kann *et al.* 1978). The nature and significance of these two postulated types will be discussed in detail in Chapter 3.

Progesterone concentrations in mammary secretion followed the trends reported above, falling linearly from a peak of 5.8 ± 0.8 ng/ml 2 days prepartum to reach baseline values by 2 days postpartum (Heap *et al.* 1986).

1.2.2 ROLE OF PROGESTERONE IN LACTOGENESIS

Progesterone appears to be the major factor inhibiting the onset of lactation during pregnancy in the sheep (Hartmann *et al.* 1973) as in all eutherian mammals (Cowie *et al.* 1980). Kuhn (1971 and 1977) concluded that progesterone withdrawal constituted the most promising candidate for a lactogenic trigger in almost all species.

Most of the evidence for the role of progesterone in lactogenesis in the sheep is indirect and relies on associations between various indicators of lactogenesis and changes in plasma progesterone concentrations during late pregnancy and around parturition. Thus, falls in circulating progesterone concentrations recorded 1-4 days prepartum were closely related to rapid increases in the lactose concentration of the mammary secretion (Hartmann *et al.* 1973). Delayed declines in plasma progesterone, brought about by low levels of nutrition, were associated with reduced prenatal colostrum accumulation and a slower onset of lactogenesis (Mellor *et al.* 1987), and there were significant negative correlations between progesterone

concentration one hour postpartum and colostrum production or milk production for the remainder of the first day of lactation (Hall *et al.* 1990). Lactogenesis occurs when progesterone concentrations have fallen to about 1 ng/ml in both plasma (Hartmann *et al.* 1973) and in milk (Heap *et al.* 1986).

Direct evidence, obtained by manipulating plasma progesterone concentrations in ewes, is limited. Administration of progesterone inhibited the increase in milk yield and lactose concentration after caesarian section which suggests that progesterone withdrawal initiates lactogenesis in the ewe (Hartmann *et al.* 1973). Furthermore, after caesarian section of 2 pregnant ewes (on day 110 of gestation) progesterone concentrations declined abruptly, very high concentrations (greater than 1000 ng/ml) of PRL were recorded (commencing 6 h later and lasting 24-36 h) and initiation of milk secretion followed. A further 8 ewes were treated in the same way but received progesterone replacement therapy which prevented the PRL surge until the artificially maintained progesterone concentrations dropped. This is strong evidence for the role of progesterone withdrawal as the lactogenic signal. However, it should be noted that the milk yields obtained were only 25-30% of control values, so it would appear that progesterone withdrawal (at least following caesarian section) is not sufficient to trigger complete, normal lactogenesis. Alternatively this may reflect less than complete mammary gland development in the 110-day pregnant ewes. Furthermore, the possible importance of the PRL surge, although it followed progesterone removal by 6 h, should not be overlooked.

1.2.3 MECHANISM OF PROGESTERONE ACTION

When evaluating evidence for the possible mechanisms by which progesterone may inhibit lactogenesis it is useful to remember that this hormone does not inhibit established lactation (Kuhn 1977; Cowie *et al.* 1980) and that some species can lactate while pregnant. This indicates either, that progesterone does not act by preventing normal lactational processes from operating (as opposed to preventing them from commencing) or, that changes after parturition prevent progesterone from inhibiting those lactational processes.

Several mechanisms by which progesterone inhibits lactogenesis have been proposed, on the basis of evidence collected in species other than sheep. Progesterone may increase the sensitivity of cells to other hormones, it may initiate cell maturation, or it may stimulate nutrient uptake by secretory epithelial cells.

Progesterone inhibits the action of PRL on the induction of milk protein synthesis in two stages. First, it blocks the ability of PRL to induce synthesis of PRL receptors (Tucker 1981) and so decreases the number of PRL binding sites (Vonderhaar 1987). Second, progesterone selectively inhibits the formation of α-lactalbumin, the B protein subunit of lactose synthetase. The fall in circulating progesterone concentrations at the end of pregnancy thus permits the

stimulation of α-lactalbumin synthesis by PRL, completing the lactose synthetase unit, and so the catalysis of the final step in lactose synthesis (Cowie and Tindal 1971).

Interestingly, the possibility that declining prepartum progesterone concentrations may directly stimulate PRL release appears not to have been addressed by reviewers, although the injection of progesterone has been reported to decrease plasma PRL levels in ovariectomised ewes (Davis and Borger 1974), and progesterone replacement therapy after caesarian section inhibited the PRL surge (Hartmann *et al.* 1973). To the contrary, it has been suggested that PRL is involved in the control of progesterone secretion in the ewe (Denamur *et al.* 1973).

Indirect effects of progesterone on circulating PRL concentrations or on lactogenesis may be mediated through actions of other hormones. Progesterone inhibited the stimulatory effect of oestradiol on PRL release in ovariectomised rats (Chen and Meites 1970) and the lactogenic action of prostaglandin $F_{2\alpha}$ (PGF2α) in pregnant rats (Vermouth and Deis 1975).

Progesterone binds to progesterone receptors in mammary tissue, but it also competes with glucocorticoids for binding on the glucocorticoid receptors (Tucker 1981). Progesterone withdrawal would thus allow the lactogenic action of glucocorticoids. Furthermore, studies in mice indicate that there are no progesterone receptors in the lactating mammary gland, possibly due to lack of oestrogenic stimulation of receptors (Shyamala and McBlain 1979; Haslam 1987). In ewes, progesterone receptor numbers in the mammary gland increased during pregnancy to peak at day 115, but had declined to low numbers by day 140 (Smith *et al.* 1987). Similar changes in progesterone receptor numbers have been reported in the rat, the rabbit, and the cow (see Haslam 1987). The disappearance of progesterone receptors from the mammary gland at the end of gestation could explain the failure of progesterone to inhibit lactogenesis. It could also explain the failure of progesterone to inhibit established lactation in some species, including the sheep, which can lactate while cycling or pregnant.

In addition to lactose and milk proteins (mentioned above), initiation of synthesis of milk fat is required for normal lactogenesis. Progesterone withdrawal may affect lactogenesis by stimulating triglyceride withdrawal from circulating plasma to be used for milk fat synthesis. Progesterone replacement during PGF2α treatment in pregnant rats prevented the increase in lipoprotein lipase (LPL) suggesting that increased LPL activity is normally brought about by falling progesterone concentrations (Thompson 1991). Furthermore, progesterone withdrawal is a necessary prerequisite to the prenatal increase in the ewes mammary blood flow (Burd, Takahashi *et al.* 1978). However, results from cows and goats, from which mammary secretion had been regularly removed before parturition, show that triglyceride extraction from plasma began while progesterone concentrations were still high (Thompson 1991) indicating either, that some factor other than progesterone withdrawal initiated LPL activity in the rats or, that there are species differences in the effect of progesterone on LPL activity. It is worth noting that the result

in cows and goats is consistent with the theory that removal of secretion removes a local inhibitor of lactogenesis from the gland (see section 1.8). Progesterone itself is not a likely candidate for the inhibitor since the gland is refractory to progesterone during lactation. Nevertheless, one might speculate that the inhibitor acts in a similar manner to progesterone and, may even be structurally related to progesterone, although several closely related steroids were not effective in inhibiting lactogenesis in the rat (Kuhn 1969).

1.3 PLACENTAL LACTOGEN

Placental hormones that demonstrate both bioassayable GH- and PRL-like activities have been observed in a number of mammalian species, including humans and sheep (Gluckman *et al.* 1979). This description coincides with the definition of a lactogenic hormone (see section 1.1), so it is important to consider the circulating concentrations, and the effects, of placental lactogen (PL), in relation to lactogenesis.

1.3.1 PLASMA CONCENTRATIONS OF PLACENTAL LACTOGEN

Although plasma concentrations of PRL in pregnant ewes are low until just before parturition (see section 1.9) total lactogenic hormone activity during much of gestation is high, due to the high plasma concentrations of ovine placental lactogen (oPL) which has been measured using both RRA and specific radioimmunoassay (RIA) methods (Cowie *et al.* 1980). In the uterine vein, oPL was detectable (greater than 1 ng/ml) after day 40 of pregnancy, and in peripheral blood, after day 48 (Chan *et al.* 1978b). In jugular serum, oPL concentrations were 159 ng/ml at 90-95 days and 487 ng/ml at 110-120 days of gestation (Martal and Lacroix 1978). Reported values for the size and time of oPL peak concentrations differ. Chan *et al.* (1978b) found peak values of about 600-700 ng/ml between days 131-141 followed by a decline commencing about 5 days before parturition, while Kelly *et al.* (1974) described peak concentrations of 1000-2000 ng/ml on days 95-114 of gestation after which there was a decline followed by one or more peaks before parturition. Such differences are not surprising when differences in experimental technique are taken into account. Chan's group analysed samples from 11 ewes (6 single and 5 twin gestations) by RIA, while Kelly *et al.* sampled 3 ewes (pregnancy status not given) for analysis by RRA. The values determined by RIA are, as might be expected, lower than those determined by RRA, since the former is specific for oPL and does not cross react with oPRL (ovine prolactin), while the latter detects both. Nutrition of the ewes was not described and, since both diet and foetal number influence oPL concentration (Oddy and Jenkin 1981), the results of the two studies may not be comparable. Oddy and Jenkin (1981) reported that ewes bearing twins had significantly higher oPL concentrations than those bearing singles, and that poorer diets resulted in higher oPL concentrations. Peak values ranged from about 800-4000 ng/ml (RIA) at days 130-140. Gluckman *et al.* (1979) reported similar values to those of Chan *et al.* (1978b) and confirmed

that oPL concentrations are lower in single- than in twin-pregnant ewes. Mellor *et al.* (1987) recorded oPL concentrations (determined by RIA) in 24 ewes between day 113 of gestation and 18 h postpartum, in groups subjected to low, rising or high planes of nutrition. Mean oPL concentrations (350-450 ng/ml occurring between days 120-140) did not differ between groups, but the decline occurred significantly later in the low plane group (2 days prepartum) than in the better fed groups (5 days prepartum). A further reason for the variation in circulating oPL levels reported by different reseachers is suggested by the recent finding that plasma oPL concentrations fluctuated by up to ±30% in successive hourly blood samples collected from ewes during late gestation (Butler *et al.* 1987). The fluctuations appeared unrelated to feeding, general activity, or time of day, and did not alter with fasting or in response to acute alterations in plasma glucose and FFA concentrations. The results concerning the effect of plasma glucose levels are in agreement with those of Brinsmead *et al.* (1981) but differ in relation to the effect of fasting. Brinsmead *et al.* (1981) reported that fasting increased plasma PL levels in ewes, but this conclusion may be less reliable than that of Butler *et al.* (1987) since the former group collected blood samples at 12-h intervals, while the latter did so at 15-min intervals. The cause of the fluctuations in plasma oPL concentrations remains unknown.

Following parturition, plasma oPL concentrations declined rapidly to the lower limits of assay detection (Chan *et al.* 1978b; Kelly *et al.* 1974; Mellor *et al.* 1987). The half-life of oPL was less than 20 min (Kelly *et al.* 1974) and there was no circadian variation in serum oPL concentrations.

1.3.2 ROLE OF OVINE PLACENTAL LACTOGEN IN LACTOGENESIS

From its name we expect homology with the first placental lactogens described (which happen to be lactogenic), but it is yet to be clearly established, *in vivo,* that oPL is lactogenic. The term "lactogenic", in this context, is quite specific and indicates functional similarity to prolactin-like and/or growth hormone-like hormones (as discussed in section 1.1).

Most available *in vivo* evidence supports a role for oPL in mammogenesis rather than in lactogenesis. The appearance and rise of oPL in ewe plasma coincided with the rapid increase in mammary growth during pregnancy (Gluckman *et al.* 1979). Inhibition of PRL secretion with bromocriptine (Schams *et al.* 1984), or by hypophysectomy (Denamur and Martinet 1961), failed to prevent mammary development in ewes (which was presumably stimulated by oPL, since neither bromocriptine (Forsyth *et al.* 1985) nor hypophysectomy are known to affect plasma oPL levels). Furthermore, twin-pregnant ewes had higher concentrations of oPL (Gluckman *et al.* 1979; Oddy and Jenkin 1981), and had markedly higher mammary gland weights at day 140 of gestation, than single-pregnant ewes (Rattray *et al.* 1974). In both sheep and goats, postpartum milk yield is positively correlated with plasma PL concentrations in late pregnancy (Forsyth 1986). These results strongly support a mammogenic role for oPL.

Purified oPL was as effective as oPRL in displacing ^{125}I-oPRL from rabbit mammary gland receptors. It was active in stimulating both casein synthesis in rabbit mammary gland explant culture (Chan *et al.* 1976) and secretory activity in the rabbit mammary gland when administered intraductally (Thordarson and Talamantes 1987). It also stimulated synthesis of α-lactalbumin (Thordarson and Talamantes 1987) and ß-casein (Servely *et al.* 1983) in mammary explants from late-pregnant ewes, but was less potent than oPRL. These *in vitro* results might lead one to assume that oPL is involved in the process of lactogenesis (at least stage I). However, bovine placental lactogen (bPL) exhibited lactogenic activity in pregnant rabbit, but not pregnant heifer, mammary gland explants (Byatt and Bremel 1986), indicating the danger of testing the biological activity of placental lactogens in a heterologous system. This should be considered when interpreting the *in vitro* studies with oPL in rabbit mammary gland cultures described above.

There is no *in vivo* evidence that oPL is lactogenic in ewes. Forsyth *et al.* (1985) found indirect evidence of a lactogenic role for PL in goats given long term bromocriptine treatment during pregnancy. Accumulation of precolostrum in the udder was not affected by the bromocriptine treatment in goats carrying twin foetuses, but in single-bearing does it was delayed about 4-6 weeks. Presumably the higher concentrations of PL in the twin-pregnant does compensated for the low PRL concentrations but PL concentrations in the single-pregnant does were not sufficient to do so.

Research in cows provides no supporting *in vivo* evidence for lactogenic actions of PL. Initial attempts to detect bPL by RIA failed (Cowie *et al.* 1980; Houdebine *et al.* 1985), but more recently it was detected in assays sensitive to pg concentrations (Bremel and Schuler 1987). The physiological significance of these concentrations is unknown. Subsequently the amino acid sequence of bPL was determined and found to have a high degree of homology with bovine PRL (bPRL) (50%) but a relatively low homology with bGH (25%) (Byatt *et al.* 1988).

There is no evidence that oPL is involved in the normal initiation of lactogenesis in sheep. Since plasma oPL concentrations decline rapidly at the end of gestation, the only mechanism by which it could act would be through removal of inhibition. Indeed, it has been postulated that this is the case, but the idea was refuted by Neville (1983) based on the reported lactogenic effects of PL in *in vitro* systems. Servely *et al.* (1983) concluded that the contribution of the declining oPL to lactogenesis was most likely negligible, since very high doses were necessary to trigger milk synthesis in mammary gland explants. Nevertheless there is evidence that, in the absence of PRL, oPL is able to initiate lactogenesis (see next section).

Like human placental lactogen (hPL), which is thought to act to spare glucose for foetal metabolism and growth, oPL is thought to play a role in redirecting nutrients from mother to foetus (Handwerger *et al.* 1976; Brinsmead *et al.* 1981; Oddy and Jenkin 1981; Thordarson *et al.* 1983).

1.3.3 MECHANISM OF PLACENTAL LACTOGEN ACTION

oPL is similar in amino acid composition to oPRL and oGH (ovine GH) (Chan *et al.* 1986). It binds to receptors for oPL, oPRL (Emane *et al.* 1986), rabbit PRL and rabbit GH (Chan *et al.* 1976). Chan *et al.* (1976) also found that oPL and human GH (hGH) were equipotent in competing for hGH receptor sites and had equal growth promoting (rabbit RRA-GH) and lactogenic (rabbit RRA-PRL) activity. It is contended that when PRL is absent or suppressed in sheep or goats, PL can fulfil a role as a stimulator of mammary epithelial cell differentiation. Hence, lactogenesis still occurs, although it may be delayed (Forsyth 1986; Servely *et al.* 1983). However, since Chan *et al.* (1978a) and Servely *et al.* (1983) found very low specific binding of ^{125}I-oPL to ovine mammary tissue, such lactogenic activity must be mediated by another receptor. Specific oPL binding sites have been identified in foetal and maternal livers (Byatt *et al.* 1992), but have not been reported in the ovine mammary gland. Mediation of the lactogenic activity of oPL apparently involves the PRL receptor (Houdebine *et al.* 1985) since it is inhibited by antiprolactin antibodies (Chan *et al.* 1986) and totally suppressed by antiprolactin-receptor antibodies (Servely *et al.* 1983).

Servely *et al.* (1983) showed that oPL, like PRL, stimulated accumulation of ß-casein mRNA in ovine mammary gland explants, although its potency was much less than that of oPRL. They suggested that the PRL-like activity of oPL may have little physiological significance compared to its GH-like properties. It may thus have a role in stimulation of increased cell numbers in the gland. It is not known whether this hyperplastic effect is mediated directly through GH or PRL receptors in the gland, or indirectly through receptors elsewhere such as the liver (Forsyth 1986). Servely *et al.* (1983) postulated that a large part of the growth-promoting activity of oPL is mediated by its GH-like activity via liver cells. oPL binds specifically to sites in liver and adipose tissue (Emane *et al.* 1986; Servely *et al.* 1983). oPL receptor numbers were higher in liver than in any other tissues tested and remained high even when the ewes were not pregnant, suggesting that liver could be the main target for oPL in the ewe (Emane *et al.* 1986). Whether these receptors mediate metabolic and/or growth activity is as yet unknown, but it is likely that they mediate the role of oPL in partitioning nutrients between mother and foetus (see section 1.3.2.). It has also been suggested that PL promotes the appearance of PRL receptors in the rat mammary gland during pregnancy (Holcomb *et al.* 1976).

1.4 OESTROGENS

Plasma oestrogen concentrations increase markedly during the peripartum period and so could be the trigger for the initiation of lactogenesis.

1.4.1 PLASMA CONCENTRATIONS OF OESTROGEN

There is considerable variation in the plasma concentrations of oestrogen reported in the literature, but general agreement regarding the changes which occur. Cowie *et al.* (1980) reported that, in the first month of pregnancy, oestrogen concentrations in sheep plasma were lower than 5 pg/ml and rose gradually to 20-40 pg/ml by 5 days prepartum. One day before, or on the day of parturition, oestrogen concentrations rose rapidly to peak at more than 400 pg/ml, then declined to barely detectable levels by 1 day postpartum. Chamley *et al.* (1973) reported lower mean periparturient peak concentrations (and ranges) as follows: oestradiol 141.8 pg/ml (62-320) and oestrone 228.0 pg/ml (75-597). Mellor *et al.* (1987) found no gradual rise, as low levels (about 10 pg/ml oestradiol-17ß) were maintained throughout the last month of gestation, before beginning to rise 1 day prepartum to peak at 40 pg/ml at parturition, then declining to negligible values by 18 h postpartum. Similarly, Thorburn *et al.* (1977) maintained that oestrogen concentrations remained low throughout pregnancy, increasing only during the last 24 h before parturition. Oestrone concentrations were approximately double those of oestradiol-17ß in the peripartum period.

Challis *et al.* (1973) stated that the ovary secretes mainly oestradiol-17ß and a little oestrone while the adrenal secretes very low amounts of oestrone and no oestradiol-17ß. The rapid increase in total oestrogen during the last 24 h of pregnancy was attributed to increased secretion by the gravid uterus (presumably of both oestrone and oestradiol-17ß) which also metabolised oestradiol-17ß to oestrone. Production rates of both oestrone and oestradiol-17ß increased markedly in the 24 h preceding parturition. At the onset of dexamethasone-induced labour, a substantial rise in circulating oestradiol-17ß was observed in most ewes at 130 days or more of pregnancy, but this was not detected in ewes induced prior to 130 days of gestation (Liggins *et al.* 1972). A tissue undergoing developmental changes, such as the uterus, is likely to produce these differing responses at different times. The placenta is the major source of oestrogens in the late pregnant ewe, and it has been suggested that glucocorticoids may promote the synthesis of oestrogen from progesterone in the placenta (Cowie *et al.* 1980).

Oestrone sulphate concentrations in mammary secretion of ewes also exhibited a peripartum increase, doubling on the day before parturition and continuing to rise to peak 1 day postpartum. Concentrations then declined sharply over the next 2 days and reached baseline values by 5 days postpartum (Heap *et al.* 1986). Mammary tissue from goats at days 148-149 of pregnancy exhibited higher synthesis of oestradiol-17ß than earlier in pregnancy or in lactation. This corresponded with the time of peak output of oestradiol-17ß by the gland into the mammary venous blood (Peaker and Taylor 1990).

1.4.2 ROLE OF OESTROGENS IN LACTOGENESIS

Oestrogen has been used to induce lactation in ewes. Satisfactory lactation was observed 9-15 days after implantation of stilboestrol in the udder of virgin ewes (Peeters and Massart 1947), and some ewes produced twice as much milk as required to feed a lamb after only 7 days of treatment (McPherson 1955). Hexoestrol (0.25 mg/d) alone not only promoted udder growth in goats but also initiated the secretory process, while progesterone administration prevented this latter action of oestrogen (Cowie *et al.* 1952). The combination of hexoestrol with progesterone did not produce higher total milk yields than hexoestrol alone. However, hexoestrol treatment alone resulted in abnormally large or cystic alveoli, although, when it was combined with progesterone, lobuloalveolar development was normal (Cowie *et al.* 1952). Fulkerson and McDowell (1974a) primed nulliparous ewes with progesterone and oestradiol benzoate treatment over 60 days. They then compared dexamethasone alone, with oestradiol benzoate plus progesterone, and found both treatments equally effective in stimulating milk secretion, at a rate approaching that of normal lactation. A combination of oestradiol-17ß and progesterone is able to stimulate development of the mammary gland in nulliparous ewes, but only in the presence of pituitary PRL (Schams *et al.* 1984) which is similar to the situation in laboratory species (Lyons 1958).

These results indicate that oestrogens are certainly active in mammogenesis, and may be involved in lactogenesis. In goats, the first significant change in arterial plasma hormones, during the period 7-9 days prepartum, was a rise it the concentration of total unconjugated oestrogens 3 days prepartum (Davis *et al.* 1979). The increase in oestrogens occurred prior to changes in the plasma levels of progesterone, PRL, oestradiol 17ß, and PGFM[1]. However, there is no evidence that the peripartum surge of oestrogen is itself directly involved in lactogenesis. In fact excessively high doses (1 mg/d) of hexoestrol considerably delayed, or prevented, lactogenesis in goats (Cowie *et al.* 1952), and (5000 µg/d) oestradiol benzoate markedly reduced milk yields of ewes during established lactation (Fulkerson and McDowell 1974b). Prior to the clinical use of bromocriptine, oestrogens were used to suppress puerperal milk secretion in women (Neville 1983). Furthermore, removal of the ovaries and/or conceptus during human pregnancy initiates lactation (Kuhn 1977). Haslam (1987) summarised the main effects of oestrogen in the gland as stimulation of ductal growth and an increase in progesterone receptor concentration. There is evidence, however, that oestrogen has indirect effects through other hormones (see next section). In this way it may have an indirect effect on initiation of lactogenesis.

1.4.3 MECHANISM OF OESTROGEN ACTION

Oestrogen has mitogenic effects on the mammary gland that may be mediated via specific receptor molecules situated in epithelial and stromal cells (Haslam 1987). The mitogenic effects

[1] PGFM is 13,14-dihydro-15-keto-PGF, the main metabolite of $PGF_{2\alpha}$

are probably elicited indirectly, in both the epithelium and the stroma, via the induction and release of paracrine or autocrine growth factors from stromal cells (Haslam 1987). Peaker and Taylor (1990) could find no correlation between aromatase activity (oestradiol-17ß synthesis) and the activities of key enzymes involved in cellular differentiation in goat mammary tissue at day 135 of gestation. Thus, while oestrogen is mitogenic, it is apparently not involved in mammary cell differentiation during late pregnancy.

The ability of oestrogen to regulate progesterone receptors appears to be a direct consequence of the hormone acting on the epithelial cell oestrogen receptors (Haslam 1987). Oestrogen control of progesterone receptors, and the lack of oestrogenic stimulation during lactation, may explain the absence of progesterone receptors in lactating mouse mammary glands (Shyamala and McBlain 1979; Haslam 1987), and the inability of progesterone to inhibit established lactation in most species (see section 1.2.3). Mouse mammary progesterone receptor numbers cannot be restored by oestrogen treatment during lactation. Following unilateral nipple removal (thelectomy), the thelectomized non-secretory glands maintained basal progesterone receptor levels and responsiveness to oestrogen. In contrast, the nipple-intact secretory glands lacked progesterone receptors and were refractory to oestrogen. These results indicate that refractoriness to oestrogen during lactation is not due to the hormonal milieu but is related to the secretory state of the gland (Haslam 1987). Perhaps a component of the milk regulates refractoriness to oestrogen. Further speculation leads to the suggestion that the local inhibitor (see section 1.8) may perform this role.

It is not clear why progesterone receptor concentrations decline in late pregnancy (Smith, Capuco and Akers 1987)(see section 1.2.3), whilst oestrogen concentrations are low or increasing (Chamley *et al.* 1973; Thorburn *et al.* 1977). If oestrogen is responsible for increasing progesterone receptor concentrations (Shyamala and McBlain 1979; Cowie *et al.* 1980; Haslam 1987), then one might expect the peripartum oestrogen surge to be associated with increased progesterone receptor numbers. Therefore, it would seem that progesterone receptor numbers are no longer responsive to oestrogens at this time, but this issue appears not to have been addressed. A further result which may be relevant at this juncture, is the observation that the efficiency of milk production during the first 2 weeks of lactation, measured as the amount of milk produced per gram of tissue, was correlated (r=0.75) with oestrone sulphate concentration in ewe's milk on the day of parturition (Heap *et al.* 1986). Whether these two correlated events are related as cause and effect is not known. Although lactating mouse mammary glands contain relatively large numbers of oestrogen receptors (Shyamala and McBlain 1979), their purpose is not clear. One would have expected the lactating gland to be refractive to oestrogens (as it is to progesterone) otherwise oestrous cycles (if they occurred) could adversely affect lactation. This suggests that oestrogen does not directly affect milk secretion and that oestrogen must serve some other purpose in the lactating mouse mammary gland.

Oestrogen may exert indirect effects on lactogenesis through influences on other hormones. There is evidence that oestrogen stimulates PGF2α release from the sheep uterus (Kuhn 1977; Liggins *et al.* 1972) and that PGF2α stimulates oestrogen secretion (Liggins *et al.* 1972). The initial stimulus to PGF2α synthesis, provided by the foetal adrenal, followed by an explosive release of free oestrogen and PGF2α as each stimulates production of the other, could explain the reason for the peripartum oestrogen surge, i.e. the oestrogen surge may only be required to produce the PGF2α surge. A possible role for PGF2α in lactogenesis is discussed in section 1.6.2. Liggins *et al.* (1972) stated that oestradiol injections led to marked elevations in blood concentrations of oxytocin during vaginal distension in the cycling ewe, and proposed that an enhanced release of oxytocin during labour could be an important consequence of the oestrogen surge. (See also sections 1.6.2 and 1.7.2 for discussion of a positive feedback cascade between PGF2α and oxytocin). Thus, the oestrogen surge may be primarily of importance to labour and may only have indirect effects on lactogenesis through its effects on other hormones.

Oestrogen may exert its effects indirectly, through the actions of PRL. Oestrogen stimulated PRL secretion in the rat (Kuhn 1977) and may do so in the ewe (Schams *et al.* 1984). A combination of oestradiol benzoate and progesterone, injected daily into virgin goats, increased plasma PRL concentrations and promoted considerable mammary growth, but no increase in udder size was observed when the steroid-induced increase in plasma PRL was inhibited by simultaneous injection of bromocriptine (Hart 1976; Hart and Morant 1980). (The role of PRL in the initiation of lactogenesis is discussed in section 1.9.2.)

1.5 ADRENAL CORTICOIDS

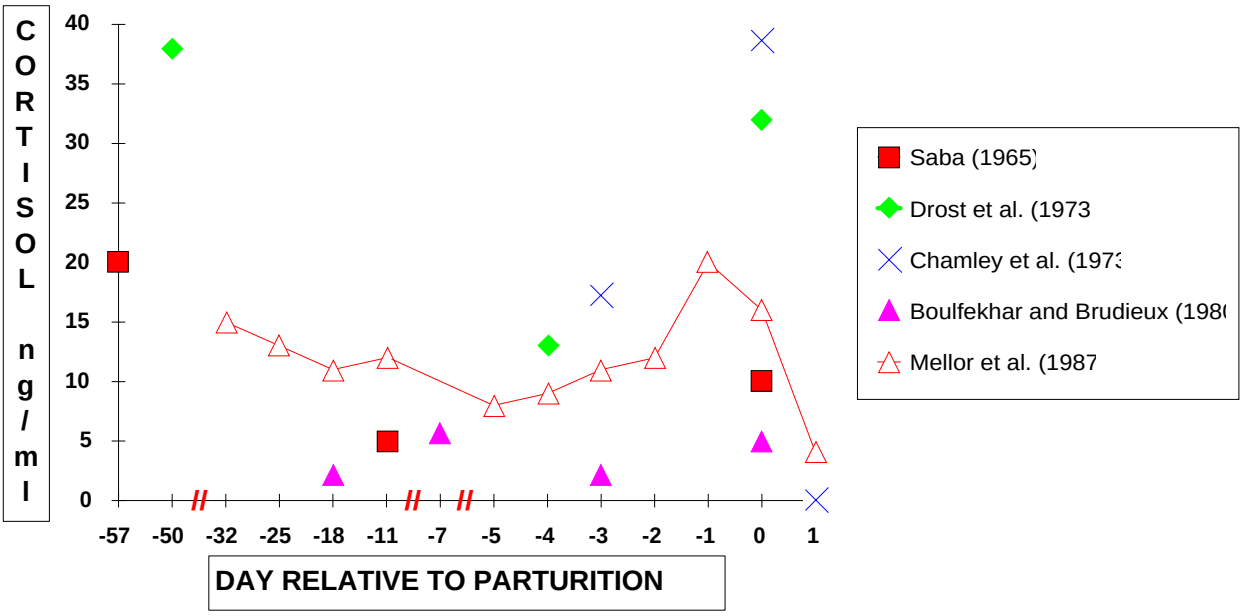
It has long been known that hypophysectomised animals require ACTH or cortisol, in addition to PRL, for lactogenesis and that lactation can be induced with exogenous glucocorticoids. It is therefore necessary to examine the possibility that circulating adrenal corticoids may be natural initiators of lactogenesis.

1.5.1 PLASMA CONCENTRATIONS OF CORTICOSTEROIDS

There are few reports of corticosteroid levels in pregnant ewes (Cowie *et al.* 1980). The most complete set of data is that of Mellor *et al.* (1987) from 24 twin-bearing ewes. Plasma concentrations reported in the literature are summarised in Figure 1.1. They indicate that cortisol concentrations decline from relatively high values at the end of the second trimester, remain low for about 40 days, then rise a day or so prepartum to reach peak concentrations at parturition, before declining to low levels by 18-30 h postpartum. The low range of values reported by Boulfekhar and Brudieux (1980) may be due to breed (they used Tadmit ewes) or assay differences. Corticosteroid concentrations were generally higher in twin-bearing ewes than in those with single foetuses (Chamley *et al.* 1973).

Trenkle (1978) described daily rhythms in plasma concentrations of corticosteroids with the lowest concentrations occurring during the dark period and highest during the early part of the light period for sheep and cattle. The circadian rhythm disappeared if sheep were moved, until they adjusted to their new quarters.

FIG 1.1 PLASMA CORTISOL CONCENTRATIONS (ng/ml) IN PREGNANT AND PARTURIENT EWES PUBLISHED IN FIVE REPORTS



1.5.2 ROLE OF CORTICOSTEROIDS IN LACTOGENESIS

It has been known since the 1940's that hypophysectomised guinea pigs require ACTH or cortisol, in addition to PRL, for lactogenesis (Cowie 1969). Folley and Young (1938 and 1941) proposed the concept of the lactogenic complex, two members of which appeared to be PRL and ACTH. Adrenal corticoids subsequently proved to be required for lactogenesis in the hypophysectomised rat, mouse, and goat, but were not essential in the rabbit (Cowie 1969). In the rat and the mouse, glucocorticoids have been shown to be an absolute requirement for lactogenesis, although they may be replaced *in vitro* by a mineralocorticoid (Kuhn 1977).

Exogenous glucocorticoids administered to the mother induce parturition in the sheep, goat and cow (Thorburn *et al.* 1977) and large doses have been used to induce lactation in pregnant rabbits, rats, cows (Cowie 1969; Kuhn 1977), and sheep (Delouis and Denamur 1967). Fulkerson and McDowell (1974a) induced lactation in nulliparous ewes, primed with progesterone and oestradiol benzoate treatment over 60 days, using subcutaneous injections of 10 mg dexamethasone daily for 6 days. Subsequent milk production was similar to that of normal control ewes. It is quite apparent that exogenous glucocorticoids are very lactogenic, but it is not clear

whether there is any direct effect on lactogenesis, or whether the induced lactogenesis is simply a result of the induced parturient process (in pregnant animals) or stimulation of the release of other hormones.

Kuhn (1977) concluded that since induced premature delivery is preceded by lactogenesis and a premature decline in progesterone concentrations, it is probable that the lactogenic effect of glucocorticoids is secondary to an induced withdrawal of progesterone. It is evident, however, that Kuhn misread the results of Chamley *et al.* (1973) in relation to the timing of the corticosteroid peak in naturally lambing sheep, which occurred before parturition (in 5 of the 12 ewes) rather than after parturition, as stated by Kuhn in his review. Nevertheless, despite this error, Kuhn may still be correct in his conclusion. Neville (1983) argued (similarly, yet conversely) that since the cortisol peak in women precedes stage II lactogenesis by 2-3 days, it is not a lactogenic trigger. Neville and Berga (1983) concluded that glucocorticoids were not major lactogenic triggers, since the doses used to initiate milk secretion are in the pharmacological range, and also because the high dose steroids given during pregnancy, as therapy for inflammatory disease, do not initiate premature lactogenesis in women.

1.5.3 MECHANISM OF CORTICOSTEROID ACTION

Although glucocorticoids are recognised as essential hormones for mammary secretory activity, their precise role is not understood and results for different species are conflicting (Houdebine *et al.* 1985). There is little reported research on the action of glucocorticoids in sheep mammary cells.

Glucocorticoids bind to specific receptors within mammary cells. In mice the number of cortisol receptors in the mammary gland increased threefold during the second half of gestation. In alveolar cells, cortisol induced differentiation of the rough endoplasmic reticulum and golgi apparatus. Adrenalectomy inhibited, whilst cortisol stimulated, synthesis of casein mRNA in mouse mammary gland (Tucker 1981). Glucocorticoids potentiated PRL induction of milk protein mRNA and milk synthesis, while being inactive alone (Houdebine *et al.* 1985). Mouse mammary gland, developed *in vitro* in the absence of glucocorticoids, was insensitive to PRL, i.e. there was an absolute requirement for glucocorticoids for casein gene expression in the presence of PRL (Ganguly *et al.* 1980). In contrast, PRL was able to induce ß-casein synthesis in the absence of glucocorticoids in ewe and rabbit mammary gland explants (Servely *et al.* 1983; Houdebine *et al.* 1985). Glucocorticoids, however, were potent amplifiers of PRL action in the sheep gland (Houdebine *et al.* 1985).

In cows, progesterone blocked binding of cortisol at the cortisol receptor. This may explain, in part, how progesterone is able to inhibit lactogenesis (Tucker 1981). If this were true in sheep, progesterone withdrawal would eliminate the competitive displacement of glucocorticoid from its

receptor and allow glucocorticoids to potentiate the PRL activation of the expression of the milk protein genes.

1.6 PROSTAGLANDIN $F_{2\alpha}$

Prostaglandin $F_{2\alpha}$ (PGF2α) is the primary luteolysin in sheep (Rice *et al.* 1984), and its active involvement in the normal termination of pregnancy points to a possible role in the initiation of lactogenesis.

1.6.1 PLASMA CONCENTRATIONS OF PROSTAGLANDIN $F_{2\alpha}$

PGF2α was not detected in the jugular plasma of pregnant sheep by Liggins *et al.* (1972) at any stage, even during labour, although low concentrations of PGFM are found in peripheral plasma throughout pregnancy (Rice *et al.* 1984). Release of PGF2α is inhibited throughout pregnancy (Rice *et al.* 1984) since its release, in sufficient amounts, would terminate pregnancy. Liggins *et al.* (1972) did not detect (less than 2 ng/ml) PGF2α in the uterine vein before labour but Rice *et al.* (1984) stated that low concentrations are found in the uterine vein throughout pregnancy. The classic studies of Liggins *et al.* (1972) demonstrated that elevated foetal corticosteroid levels stimulated the synthesis and release of PGF2α. The PGF2α is released by the endometrium into the uterine vein where it is thought to diffuse into the ovarian artery via a countercurrent mechanism (Rice *et al.* 1984). During labour, induced prematurely by administration of dexamethasone to the foetus, PGF2α was detected in the uterine vein in nine out of ten ewes at concentrations up to 37 ng/ml (Liggins *et al.* 1972). According to Thorburn *et al.* (1977) some of the massive release of PGF2α during labour escapes metabolism and significant concentrations appear in the arterial plasma.

In goats, arterial plasma PGFM concentrations exhibited a marked rise at 0.5-1 day prepartum (Davis *et al.* 1979). Since PGFM is the major primary metabolite of PGF2α appearing in the peripheral circulation, the concentration of PGFM reflects the total production of PGF2α. Maximum values were attained near parturition, after which the concentration fell.

1.6.2 ROLE OF PROSTAGLANDIN $F_{2\alpha}$ IN LACTOGENESIS

PGF2α is said to aid the onset of lactation in ewes after induced mammogenesis (Cowie *et al.* 1980) and to initiate lactogenesis in pregnant humans and rats (Neville and Berga 1983).

Injections of an analogue of PGF2α initiated secretion of copious amounts of fluid resembling normal milk, when given to non-pregnant ewes with mammary glands developed by exogenous ovarian steroids (Field *et al.* 1977; Fulkerson *et al.* 1977). The injections given by both groups of researchers elicited large peaks in plasma PRL concentrations, similar to those normally seen at parturition. Three of the six ewes injected with PGF2α by Field and co-workers also received

bromocriptine and exhibited peak milk yields less than half those of the other three ewes. The results of Field and co-workers were similar for intact and ovariectomised ewes. The progesterone status of the ewes in both reports was unclear since the PGF2α injections were given three days after the last administration of ovarian steroids, and plasma progesterone concentrations were not determined.

Intraperitoneal injection of PGF2α into pregnant rats on the day of unilateral ovariectomy advanced lactogenesis by 12 h, and caused abortion, whereas rats which were unilaterally ovariectomised without PGF2α treatment did not abort (Vermouth and Deis 1975). The reduced delay between treatment and lactogenesis indicated that the combined treatments were more effective in reducing progesterone secretion than unilateral ovariectomy alone, or that PGF2α may induce PRL release. Progesterone treatment delayed lactogenesis and prevented abortion. PRL treatment delayed only abortion. The authors concluded that the abortive and lactogenic action of PGF2α may be dependent on the uterine and plasma concentration of progesterone.

Complete suppression of uterine activity with progesterone did not prevent increased concentrations of PGF2α in maternal cotyledons and myometrium (Liggins *et al.* 1972). However, high circulating concentrations of progesterone appeared to inhibit the release of PGF2α into the uterine vein of ewes, and the decrease in progesterone concentrations before parturition probably facilitated the synthesis and release of PGF2α (Thorburn *et al.* 1977). Thorburn *et al.* (1977) also promoted the existence of a positive feedback cascade between PGF2α and oxytocin which could explain the sharp rises in both hormones during labour (see sections 1.7.2 and 1.4.2). The timing of the PGF2α peak would be ideally suited to enhance lactogenesis either, by indirect effects mediated by other hormones or, by direct effects on the mammary gland. However, the latter is unlikely, for reasons discussed below.

Prostaglandins are produced in large quantities by the mammary gland, both *in vitro* and *in vivo* (Neville and Berga 1983). Prior to parturition, the goat mammary gland synthesises and secretes large quantities (1 ng/min) of PGF2α, some of which enters the milk, reaching concentrations of about 100 ng/ml (Maule Walker and Peaker 1980; Maule Walker 1984a), while most enters the venous circulation. Just before parturition the gland ceases to secrete PGF2α into the venous circulation and begins to catabolise PGF2α to PGFM. The intraductal administration of cloprostenol[2] around this time delayed lactogenesis. After parturition there was a 100-fold increase in PGF2α production by the gland but almost all was immediately metabolised and milk levels fell to less than 1 ng/ml. The production of PGF2α within the gland, during this particular period, indicates a specific function for the hormone in the gland, unrelated to its actions outside the gland. Could the intramammary PGF2α be especially produced to initiate lactogenesis? Neville and Berga (1983) argued that, despite the large amounts produced in the gland, the lactogenic effects of prostaglandins were probably related to the stimulation of the complex

[2] Cloprostenol, ICI 80,996 is a stable analogue of $PGF_{2\alpha}$

hormonal changes that ultimately lead to parturition and lactogenesis, rather than to a direct action on the mammary gland itself. Maule Walker and Peaker (1980) suggested that PGF2α in the gland acts as a prepartum inhibitor of milk secretion and that after parturition its metabolism prevented further inhibitory action. These explanations are not mutually exclusive and both may be correct.

1.6.3 MECHANISM OF PROSTAGLANDIN $F_{2\alpha}$ ACTION

The association between PGF2α release and lactogenesis has stimulated the search for a mechanism whereby PGF2α could initiate milk secretion. An initial suggestion that prostaglandins are the intracellular mediators of PRL action in a variety of tissues (Horrobin *et al.* 1978) including the mammary gland (Rillema 1980), was discounted by Neville and Berga (1983) since it could not be confirmed by later investigators (Matusik and Rosen 1980) using more sensitive and specific assays. Matusik and Rosen (1980) measured casein mRNA by molecular hybridisation using a full length selective cDNA probe, and found that neither PGF2α nor PGE2, vasopressin or oxytocin could duplicate the effect of PRL on casein gene expression in rat mammary gland cultures. Similarly, prostaglandins were unable to replace PRL in inducing lactogenesis in cultured rabbit mammary tissue (see Vonderhaar 1987). Furthermore, indomethacin[3] did not modify the induction of casein synthesis (see Houdebine *et al.* 1985). These results indicate that prostaglandins are not involved in PRL action on casein gene expression.

Recent reviews (Houdebine *et al.* 1985; Vonderhaar 1987) provide little information regarding the mechanism of prostaglandin action in the mammary gland, other than that prostaglandins stimulated cyclic nucleotide synthesis in cultured mammary cells (see Vonderhaar 1987). It may be concluded from the limited evidence that prostaglandins have no direct effect on lactogenesis. However, Tucker (1981) stated that PGF2α stimulated rapid release of several lactogenic hormones (citing one reference to studies in heifers) including PRL, GH and glucocorticoids, and expressed the view that PGF2α should be considered part of the lactogenic complex which initiates lactation (because of its indirect effects).

1.7 OXYTOCIN

The role of oxytocin in stimulating uterine contractions during parturition is well established (Campbell and Lasley 1985) as is its role in the milk-ejection reflex (see review by Cowie *et al.* 1980). Galactopoietic responses to oxytocin have been observed, which are not likely to have been responses to increased milk removal (see Cowie and Tindal 1971). The release of this

[3] Indomethacin is an inhibitor of prostaglandin biosynthesis

hormone during parturition, combined with evidence of galactopoietic activity, warrants consideration of the possibility that oxytocin has a role in the initiation of lactogenesis.

1.7.1 PLASMA CONCENTRATIONS OF OXYTOCIN

Oxytocin is secreted into the blood in response to the suckling or milking stimulus in all species so far studied (Cowie *et al.* 1980) including the ewe (Cowie and Tindal 1971). A transient release of oxytocin was detected during suckling in the ewe. Peak activity observed was 114 µU/ml plasma which fell to a very low level within 2 min after the start of suckling (Fitzpatrick 1961). However, prior to the development of RIA's for oxytocin (first publications in 1970) the bioassays employed were slow and suffered problems of poor specificity, sensitivity, and precision (Cowie and Tindal 1971). Thus, such reports of oxytocin concentrations are unreliable, but they are included here due to the lack of other reported values in sheep. Similar values were reported in machine-milked cows and hand-milked goats, but it is interesting to note that no release was detected in a high proportion of individuals (Cowie and Tindal 1971). The half-life of oxytocin in the ewe is less than 1 min according to Schmidt (1971), but Wachs *et al.* (1984), using a highly specific RIA, reported a "rapid" half-life of 3.87 min and a "slow" half-life of 25.5 min (representing the initial phase and terminal phase of elimination, respectively). These values indicate the need for speed and accuracy during blood sampling in order to measure, or indeed to detect, oxytocin release.

There appear to be no reported measurements of oxytocin concentrations in relation to lactogenesis in sheep, but Rice *et al.* (1984) stated that levels are elevated in the latter stage of delivery. Oxytocin is also released during second stage labour in the goat (Fitzpatrick and Walmsley 1965). According to Thorburn *et al.* (1977) both PGF2α and oxytocin were elevated in response to vaginal distension in goats. Since each is believed to stimulate the release of the other, a positive feedback cascade may exist between these substances (and also with oestrogen; see section 1.4.3). Release of oxytocin caused by vaginal distension was enhanced by oestrogen (confirmed by Liggins *et al.* 1972) and inhibited by progesterone. Thorburn *et al.* (1977) suggested that PGF2α was a prerequisite to parturition and that, in normal circumstances, oxytocin has an important role in augmenting second stage labour.

Thus, the available evidence, although limited, indicates that plasma oxytocin concentrations are elevated at a time when they could contribute to the initiation of lactogenesis.

1.7.2 ROLE OF OXYTOCIN IN LACTOGENESIS

There appear to have been no direct studies of the effect of endogenous oxytocin on lactogenesis, probably because there are no known means of specifically inhibiting or stimulating the release of oxytocin. Lowering plasma oxytocin levels using antibodies to oxytocin does not appear to have been attempted. Removal of the sources of endogenous oxytocin would

necessitate ablation of not only the posterior pituitary gland, but also the corpus luteum since the latter is also a potential source of oxytocin (at least during the oestrous cycle) (Rice *et al.* 1984). Removal of the former could make interpretation of results difficult since, in addition to the well established hormones it secretes, it also contains (at least in the rat) a potent, unidentified PRL-releasing factor (PRF) (Hyde *et al.* 1987). Removal of the corpus luteum by ovariectomy would necessitate replacement with ovarian steroids, which could themselves affect lactogenesis. Enucleation of the corpus luteum, leaving the ovary, should be possible and would not require progesterone replacement if performed during late pregnancy. However, this would still leave the pituitary source.

Clinical use of oxytocin provides limited information. Although obstetricians frequently use oxytocin to induce and assist labour (Campbell and Lasley 1985), initiation of lactation in women did not appear to coincide with the release of oxytocin (Peterson and Bowes 1983). The presence in the plasma of pregnant women of an oxytocinase, which remains until very shortly after parturition, may provide circumstantial evidence for a lactogenic role of oxytocin in women, but the presence of this enzyme in other species has not been clearly demonstrated (Campbell and Lasley 1985).

Indirect evidence for a role in lactogenesis might arise from reported galactopoietic responses to oxytocin. However, results indicating that oxytocin stimulated increased milk production during established lactation are equivocal. Delouis and Denamur (1967) showed that injection of oxytocin into ewes milked during late pregnancy led to the secretion of copious quantities of milk. Recently, Nostrand *et al.* (1991) reported that cows which received 20 i.u. of oxytocin at each milking produced significantly more milk during the declining phase of lactation than control cows. Such differences were not detected in early lactation. These observations must be interpreted with care because they do not necessarily indicate direct effects of oxytocin. The galactopoietic effects may have been due to increased milk removal, or were perhaps mediated through the effects of other hormones such as PRL or prostaglandin. It is likely that these were not direct effects of oxytocin, since Linzell and Peaker (1971a) found, in the goat, that hourly massage of the gland without milk removal had no effect, while increasing the frequency of milking increased the rate of milk secretion in that gland (see also Wilde and Peaker 1990).

1.7.3 MECHANISM OF OXYTOCIN ACTION

Intravenous injection of oxytocin caused sharp increases in jugular plasma concentrations of PRL in the goat (Cowie 1969), but doses of 5-80 i.u. produced no PRL response in cows (Karg and Schams 1974). The suggestion that oxytocin may stimulate the release of anterior pituitary hormones via retrograde flow in the pituitary stalk cannot be dismissed (Cowie *et al.* 1980). However, the specific oxytocin receptor antagonist ornithine vasotocin completely abolished the PRL-releasing effect of oxytocin in perifused anterior pituitary cells, yet failed to reduce the PRF

activity of posterior pituitary extracts, indicating that oxytocin is not the PRF (Hyde *et al.* 1987). Furthermore, since removal of the neuro-intermediate lobe of rats did not alter plasma PRL levels during late pregnancy or lactation, it is probable that the neuro-intermediate lobe is not involved in control of PRL release at these times (Grattan and Averill 1991).

Oxytocin binding to receptors in mammary tissues has been observed in the rat (Cowie *et al.* 1980; Tucker 1981). Binding is principally to myoepithelial cells but effects have also been reported on secretory epithelial cells. There is evidence from the lactating rat and rabbit that binding is to the plasma membrane of the mammary gland cells. Furthermore oxytocin has been reported to have effects on milk protein secretion from epithelial cells (see Cowie *et al.* 1980) and it has been suggested that oxytocin may increase membrane permeability, thereby increasing the supply of nutrients to the alveolar cells (see Cowie *et al.* 1980). Thus, there is very limited evidence that oxytocin has a direct effect on mammary epithelial cell secretory activity.

1.8 MILKING AND MILK REMOVAL

The removal of milk is essential for the continued synthesis and secretion of milk, since milk secretion quickly stops following the cessation of milk removal. While the importance of milk removal to continued lactation is obvious, a role in the normal initiation of lactogenesis is less likely since in most species lactogenesis is initiated before parturition, to ensure the immediate availability of food for the neonate. Primates differ in this respect and, since lactogenesis occurs 2-7 days postpartum, milk removal may be a primary lactogenic stimulus in that order. Although prepartum milk removal in ruminants results in lactogenesis, milk production is considerably less than that observed following parturition, indicating that milk removal *per se* is not sufficient for complete lactogenesis. Nevertheless, it has been suggested (Kuhn 1977) that milk removal may be a necessary component of complete lactogenesis.

1.8.1 PREPARTUM MILK REMOVAL

The ruminant mammary gland can be induced to substantial secretion by milking prepartum (Cowie and Tindal 1971) and the secretion obtained acquires all the characteristics of mature milk (Maule Walker 1984a). Lactogenesis in response to prepartum milking has been observed in cows, goats and sheep (Maule Walker 1984a). Prepartum milk removal, in itself, accelerates mammary differentiation, acting by local mechanisms independent of systemic influences, and stimulates secretory cell differentiation (see Cowie and Tindal 1971). Since individual glands responded to milking independently, Linzell and Peaker (1974) suggested that prepartum mammary secretion contains a locally active inhibitor of mammary secretion. Prepartum milking could stimulate lactogenesis by removal of the inhibitor.

1.8.2 POSTPARTUM MILK REMOVAL

An increase in milk yield in response to an increased milking frequency is well recognised (McMeekan 1959; Campbell and Lasley 1985), but the reasons for this have only recently been elucidated. In goats, increasing the frequency of milking of one of the two glands from twice a day, to either hourly or three times a day, increases the rate of milk secretion only in that gland (Linzell and Peaker 1971a) indicating that systemic factors are not responsible. Thrice-daily milking increased goat milk yields by approximately 8% relative to the contralateral gland which was milked twice daily, irrespective of the stage of lactation (Henderson *et al.* 1983). Additional evidence that hormonal factors are not involved was obtained by milking autotransplanted glands (Peaker and Wilde 1987). Autotransplanted glands are denervated, and there is no release of hormones when they are milked. Milk secretion was increased in autotransplanted glands milked hourly, but not in the contralateral gland *in situ* that was not milked as frequently (Peaker and Wilde 1987). Furthermore, the increased rate of secretion was maintained even when the milk removed was replaced with an equal volume of isotonic sucrose (Henderson and Peaker 1984), thus establishing that physical distension does not cause the reduction in milk secretion rate that normally takes place when milk accumulates in the gland. Instead it was concluded that a locally-active chemical inhibitor reduces milk secretion by negative feedback.

Wilde and Peaker (1990) reviewed the evidence for the existence of the inhibitor. They described its isolation in their laboratory, its characterisation as a heat-labile whey protein, and adopted the term autocrine control to describe its action in the gland. Since that report the chemical identity of the inhibitor has been identified, but this information has not been revealed and has been patented (Wilde 1991). The mechanism by which the inhibitor acts is being investigated. It is already apparent that autocrine modulation of the secretory rate depends not only upon the concentration of the inhibitor in milk, but also upon the anatomy of each mammary gland and the efficiency of milk ejection and removal. The local inhibitor affects both the activity and number of cells as well as the number of PRL receptors on cells (Wilde and Peaker 1990).

While milk removal (and hence removal of the local inhibitor) is important to continued lactation, it has not been established that milk removal is an essential requirement for lactogenesis. Since the changes in the mammary secretion of non-breast-feeding women during the first 3 days postpartum were similar to those observed in breast-feeding women, Kulski and Hartmann (1981) concluded that breast-feeding is not a major factor in the initiation of lactation.

1.9 PROLACTIN

PRL has for many years been implicated as an important lactogenic hormone (Bauman and McCutcheon 1986). It is therefore necessary to examine the available data thoroughly to determine whether it may have a role in the initiation of lactogenesis in sheep.

1.9.1 PLASMA CONCENTRATIONS OF PROLACTIN

Plasma PRL concentrations are subject to seasonal and diurnal variation and are sensitive to various environmental influences, factors which must be considered when comparing reported values. To further complicate matters PRL seems to be the most influenced, of all the metabolic hormones, by the stress of handling and venipuncture (Trenkle 1978). Lamming *et al.* (1974) reported that the stress of blood sampling via indwelling jugular catheter elevated plasma PRL concentrations, even though the sheep were accustomed to handling and to the sampling procedure for a period of weeks, and seemed unafraid. Indeed these sheep were apparently even aware of extra movements made by the operator to inject 1 ml of saline into the catheter. This produced a release of PRL which did not occur when the saline was administered by remotely controlled infusion pumps. The observations made by Lamming and co-workers indicate the care needed when conducting such trials and interpreting short term changes in plasma PRL concentrations.

Longer photoperiod has been shown to increase PRL levels greatly in wethers (Trenkle 1978; Eisemann *et al.* 1984a and 1984b) and in both pregnant and lactating ewes (Munro *et al.* 1980; Perier *et al.* 1986). Other ruminant species have provided similar results. Longer photoperiod increased PRL levels during milking greatly in goats (Hart 1975) and lactating dairy cows (Peters *et al.* 1978 and 1981), and recently it was demonstrated that both basal and periparturient peak plasma PRL concentrations in pregnant dairy heifers were increased by exposure to longer photoperiod (Newbold *et al.* 1991).

There is considerable disparity in reported attempts to measure diurnal variation in circulating PRL concentrations. Plasma PRL levels in ovariectomised ewes were highest between 1800-2200 h (Davis and Borger 1974; Trenkle 1978). Similar results have been reported in intact lactating cows (Trenkle 1978). No diurnal variation was found in 4 hourly samples taken from pregnant and cycling ewes (Davis *et al.* 1971), but this may have been masked by the high variation in PRL concentrations, perhaps resulting from the stress of venipuncture. Brown and Forbes (1980) observed a surge in PRL levels at dusk in both intact and pinealectomised animals but Eisemann *et al.* (1984a and 1984b) reported that plasma PRL levels peaked in early afternoon and reached a nadir between 0100 and 0800 h. Munro *et al.* (1980) found no diurnal rhythm in New Zealand Romney ewes (which were apparently in the first month of gestation and grazed at pasture).

Circulating PRL concentration has also been shown to increase with ambient temperature in three-month heifers (Wettemann and Tucker 1974), after feeding in goats and cattle, and was higher in wethers fed *ad lib.* than those on restricted feed (Trenkle 1978). However, the data of Annison *et al.* (1982) showed no consistent effect of DM intake differences on PRL levels in dry, pregnant or lactating ewes.

Convey (1974) noted that changes in PRL during pregnancy in sheep were difficult to assess from the literature. This would seem to be due to differences in the absolute levels reported by various workers as indicated in Table 1.1.

TABLE 1.1 SUMMARY OF REPORTED PROLACTIN CONCENTRATIONS (ng/ml) IN PREGNANT, PARTURIENT AND LACTATING EWES

REFERENCE	n	PREGNANCY	PARTURITION	LACTATION	
				EARLY	LATE
Davis *et al.* 1971	3	2.4-6.0	402	100-350	
Fell *et al.* 1972	6		>400	decline	
Chamley *et al.* 1973	2-4	2.0-29.0	100-640	decline	
Kelly *et al.* 1974	6	<50	300-600	decline	
Lamming *et al.* 1974	6	10-15	800	100-150	5-20
Kann *et al.* 1978			400-1600		
Cowie *et al.* 1980	?	<50	200-400	decline	
Annison *et al.* 1982	4	27±18.1		94±28.9	73±22.4
Gow *et al.* 1983	10	c.50	1000	100-200	
Mellor *et al.* 1987	24	<20 rising to 100	800-1000	decline	

These differences are probably due in part to assay differences and in part to the many factors mentioned above. However, the results are remarkably consistent as to the direction of the changes, if not in their magnitude. PRL concentrations are low throughout pregnancy. The very low levels characteristic of months 3 and 4 of gestation correspond with the winter photoperiod. However, PRL concentrations at that time may also be affected by progesterone stimulation of PRL release inhibiting factor (PIF) release (Davis *et al.* 1971).

All workers are in agreement that a rapid rise occurs in the last 2-3 days of pregnancy to peak around the time of parturition, followed by a decline during early lactation to levels higher than those characteristic of late pregnancy (before the surge). Reports differ with respect to the time at which increased PRL levels occurred. Lamming *et al.* (1974) detected increased PRL levels as early as 6 weeks prepartum, with wide variation (100-500 ng/ml) between sheep. Davis *et al.* (1971) reported a gradual increase 3-5 weeks prepartum (in 2 out of 3 ewes) while Cowie *et al.* (1980) reported no change until the last few days before parturition. Gow *et al.* (1983) reported that the rise did not occur until the last 1-2 days prepartum, but only sampled the ewes over the last month of gestation and so would have missed any earlier rise. Mellor *et al.* (1987) reported that a significant gradual increase occurred between days 32-3 prepartum before rising rapidly to peak either the day before, or on the day of, parturition. Kann *et al.* (1978) proposed that there were two distinct types of sheep with respect to progesterone and PRL profiles in late pregnancy. Type I ewes exhibited a gradual rise in PRL beginning about 40 days prepartum, and an early

decline in progesterone beginning about 20 days prepartum. Type II ewes maintained progesterone levels until days 130-135 after which levels fell precipitously immediately prepartum. PRL concentrations remained relatively low until a few hours prepartum when they rose to very high values.

The cause of the prepartum PRL surge is yet to be identified. The prepartum surge in PRL levels could result from stress associated with parturition or be a response to increasing levels of PGF or oestrogen, or to declining progesterone levels. However, the rise in PRL occurs before the onset of uterine activity, which begins 12-24 h prepartum (Rice *et al.* 1984), indicating that the PRL rise is most likely to be related to the hormonal changes of late pregnancy. The biological significance of the rapid decline in PRL concentrations following the peak is unknown.

Following parturition, the initiation of suckling evokes the release of PRL, a process which may be reinforced by visual, olfactory and auditory stimuli (Cowie 1969). Massage of the udder, washing of the teats, and a short premilking before actual milking, all increased PRL concentration in most cows, which Karg and Schams (1974) concluded was not elicited via oxytocin, since injections of 5-80 i.u. did not evoke PRL release. Plasma PRL concentrations began to rise before the commencement of milking in some cows, but in most cows and goats, levels increased markedly 1-2 min after milking began and reached peak values (26-545 ng/ml) near the end of milking (4-15 min) (Johke 1969). Circulating PRL concentrations increased at the time of milking in sheep, beginning just before, or soon after, teat stimulation had commenced (Fell *et al.* 1972).

1.9.2 ROLE OF PROLACTIN IN LACTOGENESIS

The role of PRL in lactogenesis has been studied in many species (see reviews by Cowie 1969; Cowie and Tindal 1971; Kuhn 1971 and 1977; Cowie *et al.* 1980; Akers 1985; Forsyth 1986; Vonderhaar 1987). One of the more convincing results demonstrating that PRL plays a major role in lactogenesis came from a study by Cowie (1969) in an hypophysectomised goat. Milk production was reduced by 95 % immediately after hypophysectomy and remained at low levels for 2 months despite corticosteroid replacement therapy. Administration of the glucocorticoid, triiodothyronine, and GH restored lactation to approximately 28% of normal, but when oPRL was added to the combination, milk yields were restored completely. Earlier trials, in which insulin was included in the replacement therapy for 6 hypophysectomised does, produced similar results (Cowie, Knaggs *et al.* 1964). Work in laboratory species also indicates a lactogenic role for PRL, the following being a salient example. When PRL was introduced into the teat ducts of pseudopregnant rabbits the treated ducts and associated alveoli developed the ability to secrete milk, and showed increased LPL activity (Falconer and Fiddler 1970). The rabbit, however, may not be a good model for lactation in the sheep. In rabbits, but not in other species, PRL alone will restore milk yields to normal following hypophysectomy (Cowie *et al.* 1969) indicating that PRL is

necessary for the maintenance of established lactation (not just for lactogenesis). The ensuing discussion will consider the evidence for a lactogenic role of PRL in domestic ruminants, in the ewe in particular, although several key studies have been carried out in does and cows.

Experimental studies of the role of PRL in lactogenesis require the manipulation of plasma levels of the hormone by administration of exogenous PRL, enhancement of endogenous secretion, or by inhibition of endogenous secretion. Evidence from experiments utilising each of these methods will be considered here, while the advantages and constraints associated with each method will be discussed in Chapter 7.

Administration of exogenous PRL *in vivo* has not frequently been reported due to the large amount of the hormone required. There are also few reports in which PRL release has been stimulated by pharmacological means. Since these methods have almost invariably been used in association with methods of suppressing endogenous PRL secretion, they will be considered in that context. Other methods which have been used to enhance circulating PRL levels are pituitary stalk section and exposure to long photoperiod.

Pituitary stalk section of pregnant goats resulted in abortion (9-17 days after operation) followed by lactogenesis (Cowie, Daniel *et al.* 1964). Restoration of a substantial degree of lactation was obtained by replacement therapy including glucocorticoid, triiodothyronine, insulin, and GH, but not PRL. Since it was known that lactation could be restored in hypophysectomised goats by giving PRL in addition to these four hormones (Cowie, Knaggs *et al.* 1964), it was concluded that the anterior lobe continued to secrete PRL, although deprived of direct hypothalamic stimuli. It is now generally accepted that the hypothalamic stimuli consist, at least in part, of the inhibitory actions of the hypothalamic prolactin-release inhibiting factors (PIF) dopamine (McNeilly 1987) and noradrenaline (Dailey *et al.* 1987). Furthermore, the rat posterior pituitary has recently been discovered to contain a potent PRF (Hyde *et al.* 1987). Thus, lactotrophs in the anterior pituitary of Cowie's goats were deprived of PIF and subjected to PRF activity, which may have elevated circulating PRL levels (depending upon how may cells atrophied following stalk section). Nevertheless, it is apparent that the conclusions of Cowie, Knaggs *et al.* (1964), that the stalk sectioned pituitary continued to secrete sufficient PRL to induce lactogenesis, were indeed correct. In lactating sheep, unlike the goat, stalk section resulted in a rapid decline, and eventual cessation, of milk secretion (Denamur and Martinet 1961), suggesting that complete pituitary function is more important for lactation in the ewe than in the doe.

Exposure to long photoperiod is well known to increase plasma PRL concentrations in sheep (see discussion above and in Chapter 7). However, there are apparently no reports in which lactogenesis has been studied under conditions of differing, controlled photoperiod. Although long photoperiod increases PRL levels and milk yields in lactating ewes (Bocquier *et al.* 1986; Perier *et al.* 1986) and cows (Peters *et al.* 1978 and 1981), in the case of the ewes plasma GH

was also elevated (Perier *et al.* 1986). Thus, altering photoperiod as a means to study specific effects of elevated plasma PRL concentration in sheep may not be effective.

In order to examine the importance of PRL in lactogenesis, many workers have employed bromocriptine to suppress natural prepartum PRL secretion. Forsyth *et al.* (1985) found that accumulation of precolostrum in goats' udders was delayed about 4-6 weeks in singleton gestation by long-term bromocriptine treatment during pregnancy. However, in goats carrying twin foetuses precolostrum accumulation was not affected, presumably due to the action of PL (see section 1.3.2). Maule Walker (1984b) treated goats with CB154[4] from 4 days prepartum until 4 days postpartum, which markedly delayed the onset of lactogenesis. However, when the CB154 treatment ended, milk production improved rapidly, and the total lactation milk yield exceeded their production in the previous year by 38%. This was perhaps due to a rebound effect of PRL, but the possibility of uncontrolled between-year effects cannot be ignored.

In cows, prepartum CB154 treatment depressed the onset of lactation, reduced the concentration of lactose in milk, increased protein concentration, and altered the casein composition. During established lactation, CB154 treatment had only a minor effect on milk yield and no effects on milk composition (Schams *et al.* 1972; Karg and Schams 1974). CB154 injections during the period 2 weeks prepartum suppressed PRL concentrations to less than 2 ng/ml, and reduced mean milk yield during the first week postpartum to 56% of that of the previous lactation, while concentrations of α-lactalbumin and lactose in the colostrum decreased markedly but ß-lactoglobulin and IgG concentrations increased (Johke and Hodate 1983). CB154 administration from 12 days prepartum until 10 days postpartum markedly reduced milk yields but PRL infused for 6 days prepartum prevented any decline in yields (Akers, Bauman, Capuco *et al.* 1981). This experiment is of particular importance because it is the only one in which CB154 treatment of prepartum ruminants has been combined with PRL administration, thus establishing that the effect of CB154 in delaying lactogenesis is due to the depression of PRL concentrations. The inhibitory effects of CB154 on plasma PRL concentration and milk yields were also reversed by oPRL administration in mice (Knight *et al.* 1986).

In cows artificially induced to lactate using oestradiol and progesterone, CB154 treatment during the induction phase delayed lactogenesis (Peel *et al.* 1977 and 1978). Although plasma PRL levels were significantly lower than those of control cows, the low level (approx. 13 ng/ml) in CB154-treated animals was sufficient to initiate lactogenesis and produce milk yields which were not significantly lower than those of control cows. Raising the level of PRL using reserpine[5] prevented the delay in lactogenesis but did not change the yield relative to control cows . Heifers induced to lactate in the same way provided similar results (Johke and Hodate 1983). While

[4] CB154 (Sandoz Pharma, Basle) is 2-bromo-α–ergocriptine mesylate, a dopamine agonist (PRL release from the pituitary is under inhibitory control by dopamine)

[5] Reserpine is a rauwolfia alkaloid which stimulates PRL, but not glucocorticoid or GH, release

these results have shown that PRL is important for lactogenesis, it is not important during established lactation in the cow (Schams *et al.* 1972; Karg and Schams 1974; Plaut *et al.* 1987) or doe (Hart 1973).

In the ewe, suppression of PRL with CB154 around the time of parturition does not consistently block lactogenesis. Kann (1976a and 1976b) administered CB154 to ewes for 1 week prepartum, completely abolishing milk production in 30% of ewes, and delaying or depressing it in others. Treatment with CB154 for 3-4 week periods immediately prepartum (or after 10 days postpartum) always resulted in 60-70% decreases in milk yield. Subsequent milk yield was not affected if CB154 treatment was terminated a few days prepartum (Kann 1976a).

It is apparent then, that PRL is not obligatory for lactogenesis in the sheep. This is probably because, in the absence of PRL, oPL can stimulate lactogenesis (Servely *et al.* 1983; Forsyth 1986) (see section 1.3.3). Failure of ergot alkaloids to completely suppress postpartum milk secretion appears to be limited to ruminants since treatment of nonruminants with these drugs has led invariably to complete inhibition of lactogenesis (Tucker 1981). Since PRL is not obligatory for lactogenesis in the sheep, but suppression of PRL results in delayed onset of lactation, it seems logical to assume that PRL has an important role in maximising the rate of onset and the completeness of lactogenesis. The following discussion will investigate this hypothesis.

Gow *et al.* (1983) reported that one injection of a large dose of ergocriptine (0.5 mg/kg) given 0.5-20 days prepartum, or 2 injections (given 30 and 10 days prepartum), reduced plasma PRL concentrations to less than 5 ng/ml for 4 weeks after parturition, but that all ewes secreted copious quantities of milk. Between days 3-10 of lactation the mean milk yield of ewes treated 2-5 days prepartum was significantly lower than that of control ewes, but yields of groups treated outside this period did not differ significantly. Over the first 3 weeks of lactation the overall mean milk yields of all treated groups of ewes did not differ significantly from those of the control ewes. Despite the significant effect in the group treated 2-5 days prepartum, Gow and co-workers concluded that PRL is not essential for lactogenesis. It is unclear, however, whether Gow *et al.* (1983) were statistically justified in pooling the 21 day data for treatment groups since they did not report the statistical significance of differences between these groups nor present standard errors of the means. Moreover, visual examination of the data suggests that the administration of ergocriptine resulted in depressed milk yields for an extended period in all groups, except those treated with a single injection less than 1 day prepartum. The milk yield profiles for each group (Gow *et al.* 1983, Fig. 1) indicated that groups treated at different times apparently differed in their subsequent milk yields; many mean milk yields were lower than the values for the one group which was reported to be significantly different from the controls. In particular, the milk yields of the 2 groups treated for the longest periods were approximately 25% lower than those of the control group and the group treated with ergocriptine for less than 1 day prepartum.

Thus, contrary to the conclusion of Gow and co-workers (that PRL is not essential for lactogenesis), although it may not be essential, examination of their data suggests that it had a major influence. Perhaps the infrequency of the ergocriptine injections contributed to the size of the milk yield responses in the treated ewes, although the reported PRL concentrations (less than 1-4 ng/ml throughout the first 4 weeks of lactation) do not support this. The frequency of blood sampling was not reported, so that the validity of the reported PRL concentration values over the extended period of the trial cannot be assessed. Furthermore, the students *t*-test employed by the authors may not have been adequate to distinguish differences in the parameters reported in this paper. Given the size of the apparent differences in milk yields between groups, the fact that significance was detected in only one group indicates that there must have been very large variation within the groups. This was probably due to milking without the aid of oxytocin. It may also be the reason for the low milk yields obtained (0.6-0.7 kg/d in control ewes).

In view of these comments, the conclusions of Gow *et al.* (1983) may not be valid. In the absence of complete and appropriate statistical analysis, visual examination of their data suggests an important role for PRL in the attainment of complete lactogenesis.

An alternative approach, to inhibiting PRL secretion in pregnant animals, is to study induced lactation in non-pregnant animals. This approach is important because it eliminates any effect of PL and allows PRL-like activity to be better attributed to PRL. Nevertheless, induced lactation represents an artificial situation, and extrapolation of the results to normal parturient animals must be considered with caution. Hooley *et al.* (1978) induced lactation in ovariectomised ewes by treatment with oestrogen plus progesterone (priming phase, days 0-30) and dexamethasone (trigger phase, days 31-44). They then studied the effects of CB154 administered during the priming phase, trigger phase and established lactation. Results are summarised in table 1.2.

TABLE 1.2 EFFECT OF CB154 TREATMENT AT DIFFERENT STAGES OF INDUCTION OF LACTATION ON MILK COMPOSITION AND YIELD IN EWES. DERIVED FROM HOOLEY *et al.* (1978)

STAGE OF INDUCTION	EFFECT ON MILK YIELD	EFFECT ON MILK COMPOSITION
priming phase	reduction	normal
trigger phase	unaltered	increased fat & protein %
established lactation	reduction	increased fat %

The authors concluded from the unaltered milk yield in ewes treated with CB154 during the trigger phase that PRL was not the trigger for lactogenesis. Furthermore they suggested that dexamethasone itself is lactogenic, or that it effects the release of lactogenic hormones other than PRL. However, there are several aspects of this trial which may invalidate their conclusions

with respect to normal parturient ewes. First, the milk yields achieved by their induction process were extremely low (1446 g over 13 days in ewes which received no CB154). It could be argued that lactogenesis had not in fact occurred since the yields are not "copious" as required by the definition of lactogenesis stage II (see section 1.1). Second, the use of daily injections of 10 mg dexamethasone over 4 days to initiate lactation is a massive dose compared to the 1 mg/day infused by Liggins *et al.* (1972) to induce parturition in ewes, and is considered to be a pharmacological dose (Neville and Berga 1983). Furthermore, the finding that dexamethasone initiates lactogenesis in non-pregnant ewes does not imply that PRL is inactive in normal lactogenesis. Third, results which they cited in support of their conclusions (Fulkerson *et al.* 1975) were contrary to their own, in that milk yields were reduced when ergocriptine was given to ewes during dexamethasone treatment. Fourth, they did not comment on the changes in milk composition in the ewes treated with CB154 during the trigger phase (Table 1.2) which, when compared to the effects of CB154 in cows (see discussion above) suggest a delay in lactogenesis. Nor did they comment on the fact that the milk yield of the same group of ewes appeared to fall behind that of the control group on the last 5 days of the 13-day milking period.

In a second experiment Hooley *et al.* (1978) reported that CB154 treatment (10 mg twice daily) during established lactation reduced milk yields to 73% of pretreatment levels and that these effects were reversed by concurrent infusion of PRL. However, they published no absolute values for milk yields, but expressed them as percentage changes from pretreatment levels, and presented no statistical evidence that the result was significant. Although the results of this second experiment are not strictly relevant to lactogenesis, they are included here since the authors concluded, from the sum of their results, that PRL was important for mammogenesis and galactopoiesis (maintenance of established lactation) and not for lactogenesis. They further considered it possible that in pregnant animals PL may render PRL unnecessary for mammogenesis. However, given the above comments it is evident that this study does support such a conclusion for mammogenesis, but does not unequivocally support such a case for lactogenesis and galactopoiesis.

The results of Schams *et al.* (1984) support the conclusion of Hooley *et al.* (1978) regarding a role for PRL in mammogenesis. Treatment with oestradiol and progesterone induced lobuloalveolar development in nulliparous intact ewes, in the presence of pituitary PRL. Suppression of PRL by CB154 prevented this development indicating that PRL was responsible for these mammogenic changes. However, Vonderhaar (1987) asserted that, in general, ductal growth and branching were controlled by oestrogen and progesterone while lobuloalveolar development and extensive growth of alveolar epithelial cells required PRL. Studies in cows, which involved infusion of bPRL during CB154 treatment have shown that the periparturient secretion of PRL is essential for complete structural differentiation of the alveolar epithelium (Akers, Bauman, Goodman *et al.* 1981). This has implications regarding the definitions of

"mammogenesis" and "lactogenesis", and the mechanism by which PRL affects milk yields (which will be discussed in section 1.9.3 and Chapter 7).

In primigravid ewes and heifers, CB154 treatment suppressed PRL to minimal levels, yet mammary gland development (including weight and volume) was not affected (Schams *et al.* 1984). Since oPL levels were apparently not affected by the CB154 it is probable that oPL was responsible for mammogenesis. In some lobules, however, secretory activity was reduced in the CB154-treated ewes, and the authors suggested that PRL might be essential for certain secretory functions which cannot be stimulated by PL. This may be evidence that PRL is needed for the complete activation of lactogenesis in all epithelial cells, or may be confirmation that PRL is needed for the complete structural differentiation of the alveolar epithelium (see discussion of the conclusions of Akers, Bauman, Goodman *et al.* (1981) above). In mammary glands from pregnant and lactating ewes use of a cRNA probe for the bovine α-lactalbumin gene revealed that α-lactalbumin gene expression was found in the secretory epithelium of some alveoli but not in others (Molnaar *et al.* 1991). Thus, it would seem that either α-lactalbumin gene expression can be turned on and off in specific areas, or that there is distinct compartmentalisation in the gland, and synthesis of certain products occurs in certain cells only. From these studies it appears that PRL is essential for complete structural differentiation, or for stimulating certain functions of secretory epithelial cells, or for both. Furthermore, since certain cell functions may be active in some cells but not in others, it is possible that PRL is responsible for such differential activation of secretory mechanisms. Hartmann (1992) suggested that the endocrine system sets the upper limits to lactation while autocrine mechanisms down-regulate production, so it might be that PRL sets the upper limit during lactogenesis by determining the proportion of secretory cells which express the α-lactalbumin gene. The mechanism by which this may be accomplished will be considered in Chapter 7.

1.9.3 MECHANISM OF PROLACTIN ACTION

PRL binding has been demonstrated in target tissues such as the mammary gland, ovary, testis, prostate and adrenal gland , and also in tissues such as the liver and kidney where its role, if any, is not known (Cowie *et al.* 1980). In addition to these sites, PRL receptors have also been found in the seminal vesicles, hypothalamus, choroid plexus, pancreatic islets, lymphoid tissue (Kelly *et al.* 1984) and in tumours (Vonderhaar *et al.* 1985). The receptor sites have been located in plasma membrane fractions and found to exhibit very high affinity for PRL (Shiu and Friesen 1980). Internalisation of the hormone-receptor complex occurs following binding and PRL receptors are localised in the Golgi and lysosomes (Kelly *et al.* 1984). Golgi receptors are probably only precursors of membrane receptors (Vonderhaar 1987) or receptors destined for recycling, while those in lysosomes are to be degraded (Kelly *et al.* 1984).

The PRL receptor is subject to both up- and down-regulation by PRL, and exists in cryptic and active forms (Vonderhaar *et al.* 1985; Vonderhaar 1987). Membranes of lactating mouse mammary glands contain the phospholipid-N-methyltransferase system (Vonderhaar *et al.* 1985) which transfers 3-methyl groups from the methyl donor S-adenosyl-methionine (SAM) to phosphatidyl ethanolamine, to form phosphatidyl choline (see Vonderhaar 1987). Local changes in phosphatidyl choline concentrations bring about activation of cryptic binding sites (Vonderhaar *et al.* 1985). It has been suggested (Vonderhaar 1987) that thyroid hormones act, at least in part, by unmasking cryptic PRL binding sites.

Information concerning PRL binding to ruminant tissues is extremely scarce (Gertler *et al.* 1984). PRL receptor numbers (per gram of tissue) in the ewe mammary gland increased up to day 100 and remained stable during the last trimester of pregnancy. A second increase occurred during early lactation (Emane *et al.* 1986). In cows a similar increase has been detected with the onset of lactation (Kazmer *et al.* 1986). Specific binding of bPRL and oPRL has been demonstrated in the mammary gland and liver of lactating cows, although interestingly, they had a much lower affinity for the receptor than did hGH (Gertler *et al.* 1984). In rat mammary glands PRL binding was low during pregnancy and increased shortly after birth, but it has been suggested that PRL receptor numbers are stimulated during pregnancy by PL which prevents PRL binding (and receptor detection) at this time by binding to the receptors (Holcomb *et al.* 1976). In the rabbit, which apparently does not produce a placental lactogen, specific binding of oPRL to mammary gland receptors was detected early in pregnancy and increased again following parturition (McNeilly and Friesen 1977).

The intracellular effects of binding of PRL to the membrane receptor are still unclear. Unlike most polypeptide hormones, PRL binding to the cell membrane receptor does not lead to the activation of adenylate cyclase nor stimulate cAMP production (Shiu and Friesen 1980; Vonderhaar 1987). On the assumption that there may be a second messenger analogous to those present for other peptide hormones, several groups have looked for such a second messenger. A case was made for spermidine[6] and prostaglandins as second messengers (Rillema 1980) but the involvement of prostaglandins was subsequently discounted (see section 1.6.3). Kelly *et al.* (1984) asserted that neither cAMP, nor cGMP, polyamines, calcium ions, or prostaglandins are PRL intracellular mediators for the activation of gene transcription. Since protein hormones are known to be internalised by cells, it is possible that PRL itself, or a portion of the molecule, may be its own second messenger (Cowie *et al.* 1980). Further research is required to elucidate the intracellular mechanism of PRL action. The reader is referred to Kelly *et al.* (1984), Friesen *et al.* (1985), Rillema (1985) Vonderhaar *et al.* (1985) and Vonderhaar (1987) for further information, as discussion regarding the intracellular messengers is beyond the scope of this review.

[6] Spermidine is a polyamine

Although it is also outside the scope of this review to examine in detail evidence for a mammogenic role for PRL (at least in species other than sheep) it should be noted that Friesen *et al.* (1985) and Vonderhaar (1987) discussed the mechanism by which PRL stimulated mammary cell proliferation. There is apparently no doubt that PRL is mitogenic. Furthermore, the proposal that such mitogenic actions are mediated by synergy between PRL and a PRL-induced somatomedin-like molecule (called synlactin) produced by the liver, suggests that PRL may act both directly and indirectly on target tissues (see Vonderhaar 1987). Extending this scenario, the possibility must be considered that PRL initiates lactogenesis via a second, intermediate hormone. Speculating further, the actions of a putative second hormone may explain the failure of CB154 to abolish lactogenesis in ruminants (provided that release of the second hormone is not completely reliant on PRL).

The actions of PRL on mammary gland include stimulation of synthesis of lactose, milk fat and protein as well as cell multiplication . The decline in circulating progesterone concentrations at the end of pregnancy permits the stimulation of α-lactalbumin synthesis by PRL, completing the lactose synthetase unit and so the catalysis of the final step in lactose synthesis (Cowie and Tindal 1971). Following binding of PRL to its plasma membrane receptor, effects at the nuclear level include a stimulation of mitotic activity and activation of milk protein genes. Within the cytoplasm the transcriptional products (mRNA) are stabilised and translation of mRNA is stimulated, probably by changing the activities of various enzymes (Kelly *et al.* 1984).

PRL administered into the teat ducts of pseudopregnant rabbits increased LPL activity in the gland by stimulating protein synthesis as evidenced by the inhibitory effects of actinomycin D[7] and cycloheximide[8] (Falconer and Fiddler 1970).

Servely *et al.* (1983) showed that oPRL stimulated accumulation of ß-casein mRNA in ovine mammary gland explants, and that its potency was much greater than that of oPL. oPRL induced milk protein mRNA and milk synthesis in both ewe and rabbit mammary explants (Houdebine *et al.* 1985).

In addition to these mechanisms PRL also has roles in ion transport and water balance, and is essential for the induction of synthesis and secretion of immunoglobulins (Vonderhaar 1987), all of which are essential for normal lactogenesis. It is pertinent to note that, unlike most milk solids, immunoglobulins are not synthesised by mammary secretory cells, but are transferred intact from the blood.

[7] Actinomycin D selectively inhibits DNA-directed synthesis of RNA
[8] Cycloheximide inhibits protein synthesis at a stage subsequent to the amino acid-tRNA complex

1.10 CONCLUSIONS AND RATIONALE FOR EXPERIMENTAL WORK

The term "lactogenesis", as used in this thesis, is synonymous with "lactogenesis type II", defined as the onset of copious milk secretion. This definition is not entirely adequate since it requires subjective assessment of the term "copious". Studies of lactogenesis reviewed above have reported widely differing measurements of milk yield and milk components. It is thus apparent that the onset of milk secretion is not an instantaneous, all-or-none process, but a graded response subject to many influences, especially those of certain hormones. This review has considered the roles of progesterone, placental lactogen, oestrogens, adrenal corticoids, prostaglandin F2α, oxytocin and PRL, since they were identified by Cowie *et al.* (1980) as hormones likely to be involved in lactogenesis. The effect of milk removal was also briefly considered.

It appears that progesterone withdrawal is the primary initiator of lactogenesis and that PRL is intimately involved in the completion of the process. Other hormones permit, potentiate or complement oPRL action in sheep, and some, particularly oPL, can partially, but not completely replace oPRL. Of all the hormones involved in lactogenesis, PRL appears to have the greatest potential value for manipulating lactogenesis in ewes due, in part, to its apparent central role in the process and, to the lack of specific information regarding responses to the administration of oPRL.

There is ample evidence indicating that inhibition of the peripartum PRL surge has delaying or inhibitory effects on lactation and at least one report (Maule Walker 1984b) suggesting that subsequent milk yields may be substantially improved. However, there are no published reports in which oPRL has been administered to CB154-treated ewes. This "positive control" treatment is required to conclusively establish that the effects of CB154 on milk yields are mediated through PRL, and not through effects on some other factor. Furthermore no published research has reported an attempt to increase milk yields by peripartum oPRL supplementation, and no reports have established that PRL is active when introduced directly into the mammary gland of ruminants.

No other hormone currently offers the opportunity to improve milk production by such short term manipulation as does PRL. Other possibilities, such as oPL or oGH, would require long term treatment with associated labour and expense, even allowing for the development of appropriate slow release technology. Even when these problems are surmounted, consumer resistance to perceived problems relating to exogenous hormones entering milk will present major obstacles to the adoption of these technologies in commercial farming. Administration of oPRL, or its manipulation by other means, should not be subject to these problems due to the short period of treatment envisaged to modify the PRL surge. However, it has not yet been determined whether modification of the peripartum PRL surge will improve milk yields in domestic ruminants.

Accordingly it was decided to investigate the role of PRL in the initiation of lactogenesis in sheep, with a view to:

1 determining if CB154 treatment does inhibit or delay the onset of lactation in local New Zealand ewes

2 determining the period during which the presence of PRL is essential using differing prepartum periods of CB154 treatment (if PRL proved to be required for lactogenesis in 1 above)

3 examining the possible existence of type I and type II ewes as described by Kann *et al.* (1978)

4 establishing whether PRL is essential for complete initiation of lactogenesis by comparing milk yields in ewes whose PRL secretion is inhibited by CB154 treatment with milk yields of ewes treated with CB154 plus concurrent administration of oPRL (the positive control)

5 determining whether milk yield can be increased by short term peripartum administration of oPRL to ewes with normal PRL secretion at this time

6 establishing whether oPRL acts directly on the mammary gland by administration of oPRL directly into the gland, via the teat duct, at doses which preclude a marked rise in circulating oPRL concentrations.

CHAPTER 2
MATERIALS AND METHODS

2.1 ANIMALS

Border Leicester x Romney ewes were used in all trials. They were obtained from a commercial flock on the Sheep and Beef Cattle Research Unit at Massey University. Ages ranged between 3 and 5 years, except in one trial in which a number of two-year olds were used owing to a shortage of available older ewes. Weights were generally around 60 kg with a range of 50-90 kg. Selection of ewes for use in each trial was based mainly upon mating dates (indicated by mating harness marks) in order to obtain maximum synchrony at lambing. Other factors considered were;

-number of foetuses (determined by real-time ultrasound)
-health (especially clinical evidence of facial eczema and pregnancy toxaemia)
-functional glands and teats
-freedom from clinical mastitis
-adaptation to experimental conditions (especially diet)
-successful delivery and survival of the lambs.

In most experiments it was necessary to use all of the available ewes which met these criteria, in order to have sufficient numbers. There was little opportunity to select on the basis of weight or age and no opportunity to select on suitability for milking (teat placement and anatomy, milk flow rate and yield, and temperament). Animals which developed life-threatening conditions were removed from trial, but in order to maintain numbers it was necessary to persevere with ewes which developed less severe conditions (e.g. mastitis, sore teats, foot scald). Such animals were treated and, if they responded satisfactorily, remained on trial.

2.2 HOUSING AND FEEDING

2.2.1 HOUSING

In some trials ewes were grazed at pasture (mainly ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*)) but in most cases they were housed indoors in the Animal Physiology Unit (APU) in individual pens (1.5x1.0 m) on mesh floors. Ewes entered the building approximately 3 weeks prior to the expected start of lambing and were allocated to pens at random. Whenever space allowed, ewes which gave birth to more than one lamb were given the area equivalent to two pens. The pens and surrounding area were hosed out daily. Photoperiod was set at 18L:6D (18 h light and 6 h dark). Ambient temperature was not controlled during the trials. Reported temperatures are the mean (half of the sum of daily maximum and minimum) air temperatures for the calendar month indicated. Data were collected by the New Zealand Meteorological Service at the Grasslands Division of the DSIR (about 1 km from the experimental facilities).

2.2.2 FEEDING EWES

Daily feed allowances were based upon individual energy requirements calculated using equations derived from data for pregnant and lactating ewes (Rattray 1986) and adjusted for the reduced maintenance requirements of ewes housed indoors (Coop 1961). Details of the derivation of the equations are given by Beer (1986). Typical values for the dry matter content (DM %) of each feedstuff were used to calculate feed allowances. Using these equations feed allowances were adjusted on a weekly basis according to live weigh, stage of pregnancy or lactation and number of foetuses or lambs.

Ewes were fed one of two diets:

Diet 1. Fresh cut pasture and meadow hay. Pasture provided 67% of metabolisable energy (ME) requirements and the remainder was hay.

Diet 2. Sheepnuts and meadow hay. Each component provided 50% of ME allowances. The sheepnuts were a proprietary brand (NRM Multifeed nuts, Central Feed Mills, Levin, New Zealand) of concentrate based upon lucerne and barley.

Pregnant ewes were fed once daily (at about 1600 h) until the commencement of lambing after which time all were fed following the afternoon milking (around 1800 h). By the time that they lambed, the majority of ewes were eating most of their allowance before the following morning. Once each ewe began lactation, feed bins were removed at about 0830 h to allow easy access for milking and cleaning. Thus the ewes did not have access to feed during the period over which milk yield was estimated. Since they had learned to eat their allowance before removal of the bins each morning, lack of feed during this period was not considered to be a problem. Refusals were recorded on a wet-matter basis. It was considered impractical to measure the DM % of refusals since ewes differed in their eating habits. Some left negligible amounts, having eaten all

components of the diet, while others left certain weeds or woody material and still others left a small proportion of their diet apparently unselected. Furthermore, since the DM % of the diet was not measured for each ewe, or even for the bulk supply on a daily basis, the determination of DM refusals was not appropriate. It was considered that measurements of intake on a wet-matter basis were sufficient to monitor changes in feed intake (forewarning of possible health problems) and to identify any differences in intake between treatment groups.

2.2.3 FEEDING LAMBS

In order to estimate milk production of the ewes, the lambs were separated from their dams for about 6 h each day. During the period of separation from their mothers, all lambs were bottle-fed. Most lambs adapted well to bottle feeding, but a few remained or became reluctant to feed from the bottle and were apparently satisfactorily supplied by their dam. Nevertheless every effort was made to give lambs the opportunity to drink their allowance since it was thought important to ensure that milk yield differences in ewes, brought about by experimental treatments, did not result in different demand for milk by the lambs. This was because ewes with underfed lambs might have experienced a greater demand for milk from their lambs which might, in turn, have led to increased milk production, compared to ewes with well-fed lambs. Milking the ewes completely, both before and after the 6-h measurement period, would reduce the effect on milk production of differences in the lamb's demand, but differential feeding of lambs was used to minimise demand effects during the period when lambs were present.

Each lamb was offered at least 300 g of ewes milk daily by bottle, and intake was recorded. This value was based upon the observation that normal, twin-bearing ewes produced about 2.4 kg of milk each day during the first week of lactation (i.e. 1200 g/lamb) and that in measuring each 1200 g of milk produced, we had removed 300 g. In cases where the ewe was producing less than 1200 g/lamb daily her lambs were offered 1200 g in three feeds. The value of 1200 g was also based upon the average daily production of a twin-bearing ewe during the first week of lactation. Calculation of the milk requirement of a 4.5-5 kg lamb, derived from published estimates of lamb ME requirements (ARC 1980; Rattray 1986), the ME content of ewes milk and the average composition of ewes milk (ARC 1980) resulted in a value of about 930 g/d. This indicated that the value chosen (1200 g) as the minimum acceptable milk yield, was more than adequate to ensure all lambs were well fed.

2.3 MILKING

Milk yield was estimated by the so-called "oxytocin method" described by McCance and Alexander (1959). The technique involves emptying the udder by milking with the aid of oxytocin and repeating the milking procedure a measured time later, at which time the milk yield is measured. The lambs are separated from the ewes during the intervening period. Advantages and constraints associated with this method will be discussed in Chapter 7.

2.3.1 PREPARATION AND RESTRAINT

Lambs were separated from their mothers between 0830 and 0900 h each morning and kept separate until after the afternoon milking. Ewes housed indoors were milked in their pen. Each ewe was restrained by one person while being milked by another. Ewes grazed at pasture were brought into the shed and milked on a raised platform holding 4 or 6 ewes. In this case 2 ewes could be restrained by one person while being milked. It was found necessary to have a person to restrain the ewes during milking because they were not able to be trained, as dairy animals are, to accept the milking process calmly.

2.3.2 OXYTOCIN

To ensure milk letdown it was necessary to use oxytocin. The recommended dose of 10-15 i.u. was tested and found to be excessive, resulting apparently in severe discomfort or pain in the area of the mammary gland as evidenced by the abnormal behaviour of the ewes. Successively lower doses were tried until letdown was observed to be compromised at a dose of 0.5 i.u.. It was also difficult to ensure accuracy of delivery of such a low volume (0.05 ml) with a 1 ml syringe. Accordingly 1 i.u. (0.1 ml) was adopted as the effective dose. This was diluted with 0.9 ml of physiological saline to facilitate injection. Injections were administered into the jugular vein 0.5-1 min before milking commenced.

2.3.3 THE MILKING MACHINE

The milking machine was a small, portable, electrically powered unit, designed to milk one cow or two sheep or goats, manufactured by OTENZ, (Otorohanga, New Zealand). The pulsator frequency was set at 60 pulses/min and the vacuum at -40 kPa. Milk lines were adapted to collect the milk from each ewe, and in some trials, from each gland, in separate containers. The machine and milk receptacles were mounted on a trolley for mobility. After each milking the machine was rinsed with cold water and, following the afternoon milking, hot water and detergent followed by a hot water rinse were used.

2.3.4 MILKING PROCEDURE

Milking was carried out only by experienced milkers, since the milking machine could not be relied upon to extract all of the available milk. Despite the use of oxytocin and the machine, differences in letdown, ewe behaviour and gland anatomy (especially teat shape and placement as well as streak canal size) led to large errors in milking if hand-stripping was not correctly done. Milkers learned the peculiarities of each ewe in order to ensure that all the milk was removed. When in doubt, completeness of milking was checked by another milker. If still in doubt at the afternoon milking, the previous day's records were consulted to ensure that the amount obtained was about the expected weight. On a few occasions, when the amount obtained was far below that expected, another dose of oxytocin was administered and the milking process repeated. Due to the potential for error, milking and oxytocin injections were done only by skilled milkers, and the number of such staff was kept to a minimum (usually 3 in each trial) to ensure consistency of technique. The milking routine was as follows;

Morning milking:

First, the lambs were separated from the ewes. About 1 minute before milking each ewe was injected intravenously *per* jugular with 1 i.u. (0.1 ml) oxytocin made up to 1 ml with physiological saline. The teat cups were then put on and the ewe machine-milked for approximately 100 seconds. During milking the cups were held on by hand and moved rhythmically in time with the pulsation to assist milk letdown, and aid blood flow to the teats. This was, in fact, a mild form of machine stripping. Following the removal of the cups each gland was hand-stripped until empty. The time at which milking ended for each ewe was recorded. Ewes housed indoors remained in their pen with access to water while their lambs were kept elsewhere. Ewes on outdoor trials were returned to pasture between milkings, while their lambs remained penned indoors.

Afternoon milking:

Following oxytocin injection, ewes were milked as above. Following the completion of milking, the milk from each gland or from each ewe, depending upon the objectives of the particular trial, was weighed. Milk weights were recorded to the nearest gram and this value was adjusted according to the time interval between milkings (recorded to the nearest minute) to obtain an estimate of the daily milk yield. The milk was subsampled for analysis of fat, protein and lactose content (see section 2.6.4). The remaining milk was saved for feeding lambs. Ewes housed indoors were then fed, and lambs were returned to them. Ewes on outdoor trials were returned to pasture with their lambs.

2.4 CANNULATION AND BLOOD SAMPLING

In most trials, blood samples (about 7 ml) were taken at 5-d intervals by jugular venipuncture using vacutainers and 100 µl of 7 mg/ml sodium EDTA as the anticoagulant. This was done as quickly as possible (usually within 30 seconds) in order to reduce any effects on plasma PRL levels due to stress (see section 1.9.2). While it is acknowledged that this method of sampling is bound to be stressful for the sheep, it was considered to be the only practical alternative. Such infrequent sampling did not warrant permanently indwelling cannulae, and maintaining such cannulae for a period of weeks would have been difficult, even in ideal conditions.

In a small number of trials, involving frequent sampling over short periods, indwelling jugular cannulae constructed from polyethylene tubing (Dural Plastics and Engineering, Auburn, NSW. Australia) were inserted using local topical anaesthetic (Xylocaine, Astra Pharmaceuticals Pty Ltd. N. Ryde, NSW, Australia). These were maintained using heparinised saline (200 i.u./ml) .

Blood samples were placed on ice immediately after collection. They were centrifuged at 3000 RPM (1800g) for 20 minutes at 4^{o} C and the plasma fraction was aliquoted into duplicate vials. Long-term refrigeration was at about -20 ^{o}C and short-term storage (during laboratory analyses) at approximately -10^{o} C.

2.5 HORMONES, DRUGS AND ANIMAL REMEDIES

The following hormones, drugs and animal remedies were used.

1. Oxytocin-TAD (Pharmazeutisches Werk GMBH, West Germany) or oxytocin-EA (Ethical Agents Ltd, Auckland) 1 i.u. in 1 ml of physiological saline (Sterile/non pyrogenic sodium chloride 0.9%. Abbott Australasia Pty LTD, Sydney, Australia.) administered i.v to induce milk letdown.

2. CB154 (2α–bromoergocriptine mesylate) kindly donated by Sandoz Pharma (Basle, Switzerland) administered s.c. at a dose of 2 mg/d dissolved in 1 ml of 60% ethanol and 40% saline to inhibit PRL release.

3. Leocillin (penethamate hydriodide, Leo Pharmaceutical Products, Ballerup, Denmark) administered at a dose of 2.5 million i.u. in 7.5 ml of sterile water by intramuscular injection in the neck region to prevent mastitis. Leocillin is a slightly soluble hydriodide of an ester of benzylpenicillin. Following injection of Leocillin, concentrations in milk are 5-10 times greater than those following injection of the same dose of procaine penicillin (Edwards 1966).

4. Ovine pituitary prolactin NIADDK-oPRL-18 (AFP 8277E, 30 i.u./mg protein), derived from sheep pituitary glands from New Zealand (Raiti 1990), purified in the laboratory of Dr A.F. Parlow, (Pituitary Hormones and Antisera Center, Harbor-UCLA Medical Center, Torrance, California) and kindly donated by NIADDK and supplied through the National Hormone and Pituitary Program, University of Maryland School of Medicine. The prolactin was dissolved in a solution of 0.15M saline and 0.03M sodium bicarbonate at pH 10.8 after which the pH was adjusted to 9.0. The PRL concentration in solutions administered to ewes was 10 mg/ml.

5. PMSG as Folligon (Intervet International B.V., Boxmeer, The Netherlands) or Consep (Heriot AgVet Pty Ltd, Victoria, Australia). PMSG was used to induce ovulation outside the normal breeding season. The doses used were 500 i.u./ewe in November and 750 i.u./ewe in December.

6. Streptopen (procaine penicillin 250,000 i.u./ml and dihydrostreptomycin sulphate 250,000 i.u./ml, Glaxo Animal Health (NZ) Ltd, Palmerston North, New Zealand) administered by s.c. injection in the neck region to prevent and to treat mastitis.

7 Ketol (propylene glycol and glycerol, batch No 7343, Veterinary Ethicals Ltd, Auckland, New Zealand) 120 ml administered orally for pregnancy toxaemia

8 Hibitane (Chlorhexidine gluconate 5% w/v. ICI PLC, Macclesfield, Cheshire, England) used topically, and on hypodermic syringes and needles, as a disinfectant.

2.6 ASSAYS

2.6.1 PROLACTIN ASSAY

The PRL assay was a homologous double-antibody competitive binding radioimmunoassay (RIA) based upon the method of van Landeghem and van de Wiel (1978). The protocol utilised was derived from Kirkwood *et al.* (1984). The assay was set up, validated for ovine plasma samples, and optimum working concentrations of antisera were determined by the author.

The following hormones and antisera were employed to assay plasma samples collected during the course of the studies described in this thesis:

1. NIADDK-oPRL-18 (AFP-82277E, 30 i.u./mg) Biological grade ovine PRL donated by NIADDK and supplied through National Hormone and Pituitary Program, University of Maryland School of Medicine. Used for preparation of standards.

2. NIADDK-oPRL-I-2 (AFP-7150B, 35 i.u./mg) Iodination grade ovine PRL donated by NIADDK and supplied through National Hormone and Pituitary Program, University of Maryland School of Medicine.

3. Rabbit anti-oPRL (rabbit 9, 7/5/76) antiserum donated by Dr D.F.M. van de Wiel, "Schoonoord" Research Institute for Animal Husbandry, Zeist, The Netherlands. (first antibody; used in assays #1-28)

4. NIADDK-anti-oPRL-1 (AFP-973269) rabbit anti-ovine PRL antiserum donated by NIADDK and supplied through National Hormone and Pituitary Program, University of Maryland School of Medicine. (first antibody; used in assays #29 onwards)

5. Goat anti-rabbit IgG (Immuno-Chemical Products Limited, Auckland, New Zealand, Lot No. 8103) (second antibody).

Iodination was based on the procedure of Greenwood *et al.* (1963) using borate instead of phosphate buffers. Iodination time was 5 seconds. Separation of bound and free iodine was carried out using a Sephadex G50 gel column (Pharmacia Fine Chemicals, Uppsala, Sweden, Lot No. 0870).

Standards ranged from 1-1200 ng/ml oPRL. The linear range was approximately 10-800 ng/ml. Optimal dilutions of antibodies were determined by factorial experiments covering a wide range of dilutions of both antibodies. The working dilution of first antibody was 1:50,000 (final dilution 1:550,000) and that of second antibody was 1:40. Assay binding was typically 45-55% and assay sensitivity about 1 ng/ml. Dilution response curves for three ovine plasma samples, assayed neat or at stepwise serial dilutions up to final dilutions of 1:128, 1:256 and 1:512, showed no evidence of non-parallelism. Further validation of the assay was not considered necessary since it is a homologous assay and since the first antibody had been subject to previous validation (Kirkwood *et al.* 1984). Plasma samples were assayed in triplicate. Those with estimated concentrations less than that of the lowest standard (1 ng/ml) were recorded as 1 ng/ml. Those samples with poor agreement between triplicate samples (CV greater than 20%) were re-assayed.

An initial problem of high variance between B_0 tubes (reaction tubes containing zero unlabeled PRL) resulted in very high intra- and inter-assay coefficients of variation (CV's). A series of 14 assays tested each component of the system, as well as the effects of factors such as temperature, time between addition of reagents, and protein concentration in the reaction tubes. Eventually the problem was traced to contamination (carry over between tubes) in the tubing of the Dilutrend (Boehringer Mannheim GMBH, Mannheim, West-Germany) used to add the reagents to the reaction tubes. This was overcome by adding the components separately and rinsing the equipment between reagents.

The performance specifications of each of the assays for which data are presented in this thesis (Table 2.1) exhibit satisfactory intra-assay CV's but relatively high inter-assay CV's. The intra-assay CV's are the actual mean CV of all the unknown plasma samples (apart from those with concentrations outside the range of standards and those with CV's greater than 20%) while intra-assay CV's were calculated from (the mean and standard error of) three bulk reference samples (pools) which were included in each assay. Closer analyses of the data revealed two possible causes of the high inter-assay CV's.

The first possible cause was the inclusion of bovine plasma pools. Assays used to analyse the plasma from trials 1 and 2 (Chapter 3) included 2 ovine and 1 bovine plasma pools. The ovine plasma pools (pools #3 and #4) exhibited a lower inter-assay CV than the bovine pool (pool #5) (see Table 2.2). Following the analyses of samples from trials 1 and 2 the ovine pools were depleted, so two new pools were created. Since the problem with bovine pools was not noticed at that time, and a source of bovine plasma was readily available, plasma used for the two new pools (pools #6 and #7) was bovine. Thus, plasma from trials 3-7 (Chapters 4-6) included only bovine plasma pools. These bovine plasma pools exhibited very high inter-assay CV's (Table 2.3).

TABLE 2.1 PERFORMANCE SPECIFICATIONS FOR PROLACTIN RADIOIMMUNOASSAYS FROM WHICH DATA ARE PRESENTED IN THIS THESIS

TRIAL No. and Chapter No.	NUMBER OF ASSAYS	FIRST ANTIBODY SOURCE	MEAN INTRA-ASSAY CV (%)	INTER-ASSAY CV (%)	MEAN SENSITIVITY (ng/ml)
1 & 2 Chapter 3	4	van de Wiel	11.2	33.9	0.32
Pilot Chapter 4	1	van de Wiel	10.8	not applicable	1.55
3 Chapter 4	2	NIADDK	11.1	27.4	0.19
4 Chapter 4	2	NIADDK	12.1	46.5	0.15
5, 6 & 7 Chapters 5 & 6	2	NIADDK	10.2	68.8	0.59

TABLE 2.2 MEAN PROLACTIN CONCENTRATIONS AND CV'S FOR REFERENCE SAMPLES (POOLS) INCLUDED IN RADIOIMMUNOASSAYS FROM WHICH DATA ARE PRESENTED IN CHAPTER 3 (TRIALS 1 & 2)

Pool Number	#3	#4	#5
Species	Ovine	Ovine	Bovine
Mean PRL concentration (ng/ml)	24	75	9
CV	36.8	19.0	45.9

TABLE 2.3 MEAN PROLACTIN CONCENTRATIONS AND CV'S FOR REFERENCE SAMPLES (POOLS) INCLUDED IN RADIOIMMUNOASSAYS FROM WHICH DATA ARE PRESENTED IN CHAPTERS 4-6 (TRIALS 3-7)

Pool Number	#5	#6	#7
Species	Bovine	Bovine	Bovine
Mean PRL concentration (ng/ml)	5	127	203
CV	48.4	143.3	69.6

Despite the poor CV's, visual examination of the data indicated that both ovine and bovine pools continued to be ranked in the correct order of PRL concentration. This was tested by regressing the concentration of each pool in each assay on the corresponding values in the subsequent assay. When two of the three pools were ovine, the correlation between reference pool PRL concentrations determined in sequential pairs of assays was high (0.995±0.005) and the value of the regression coefficient was 1±0.2 (mean±range). When all three pools were bovine, the correlation between reference pool PRL concentrations determined in sequential pairs of assays was lower (0.93-0.99) and the value of the regression coefficient more variable (1-3). The PRL assay used to analyse samples for the studies reported in this thesis has not been validated for bovine plasma samples and it would appear that bovine plasma does not perform well in the assay. Independent evidence for this suggestion was provided by examination of two assays (not part of this study) in which only bovine plasma samples were analysed. In the first assay 49%, and in the second assay 45%, of samples had CV's greater than 20%. However, despite the relatively poor performance of the bovine pools compared to the ovine pools, the ranking of the bovine pools with respect to PRL concentration was still maintained between assays. Thus, there is no reason to believe that the relative concentrations of unknown ovine plasma samples were incorrectly determined in the assays presented in this thesis.

The second factor which apparently contributed to the high inter-assay CV's reported in table 2.1 was the addition of exogenous oPRL to pools #6 and #7 to provide a wider range of PRL concentrations. These two "spiked" bovine pools exhibited markedly higher CV's (Table 2.3) than the "unspiked" plasma pools. It is likely that the problem will be rectified by replacing the reference samples with plasma collected from ewes with naturally occurring differences in PRL concentrations.

From the assay data it appears that the problem of high inter-assay CV's is related to the reference pools and not to the unknown samples. Furthermore, in most cases, all of the unknown plasma samples were analysed within the same assay, with only repeated samples being analysed in the subsequent assay. Only in trials 1, 2 and 3 were samples distributed across assays and, whenever more than one assay was required to analyse the samples from a trial, all of the samples from an animal were included within one assay, and each assay was balanced for number of ewes from each experimental group and for pregnancy status (single- or twin-bearing). Furthermore, when plasma hormone concentrations were subjected to statistical analyses, in three out of four cases no significant differences between assays were detected when "assay" was included as a main effect (first) in the analysis of variance model, and "assay" contributed only 2-15% of the variation in the total sum of squares. In one analysis, "assay" number was significant and contributed 24% of the total sum of squares. However, since the statistical analysis adjusted for preceding factors (type I sum of squares), and the assays were balanced for numbers of ewes in each treatment group and pregnancy status, it was still possible to detect significant differences in PRL concentrations between treatment groups.

Thus, if there were any fixed effects on plasma PRL concentration due to assay they were adjusted for in the analysis of variance. If, on the other hand, there were random effects due to assay, this would have increased the error sum of squares and reduced the ability to detect differences between treatment groups. Therefore, it is believed that the problem of inter-assay variation encountered in these studies is a problem of conservatism and that it did not result in the reporting of "falsely positive" significant differences in this thesis.

Since the original source of first antibody (van de Wiel) was depleted while attempting to solve the initial problem of high variance between B_0 tubes, it was necessary to obtain a replacement. The first antibody subsequently used (NIADDK) was used in the assay at the same working concentrations and was indistinguishable from the original in its performance characteristics (Table 2.4).

TABLE 2.4 PERFORMANCE SPECIFICATIONS FOR ANTIBODIES TO PROLACTIN FROM TWO SOURCES USED IN THE RADIOIMMUNOASSAYS FROM WHICH DATA ARE PRESENTED IN THIS THESIS

FIRST ANTIBODY SOURCE	NUMBER OF ASSAYS	MEAN INTRA-ASSAY CV (%)	INTER-ASSAY CV (%)	MEAN SENSITIVITY (ng/ml)
van de Wiel	5	11.2	36.3	0.57
NIADDK	6	10.5	87.1	0.31

2.6.2 INSULIN ASSAY

The insulin assay was a heterologous double-antibody competitive binding RIA based upon the method of Hales and Randle (1963).

Details of the assay have been described previously (Flux *et al.* 1984). The antisera (first antibody; guinea pig anti-insulin, GP7, 1974)(second antibody, sheep anti-guinea pig γ–globulin, "Gavin", 1984) were prepared in the Animal Science Department, Massey University. First and second antibodies were used at working dilutions of 1:25,000 and 1:40, respectively. All samples reported in this thesis were assayed in the same time (i.e. in a single assay). The performance details of the assay were: sensitivity 2 pg/ml; intra-assay CV 11.2% (the mean CV of five reference samples with a concentration range of 13-232 pg/ml).

2.6.3 PROGESTERONE ASSAY

The progesterone assays were carried out in the laboratory of Dr K. R. Lapwood. Details of the assay have been described previously (Kirkwood *et al.* 1984). The progesterone antiserum was provided by Dr John France, National Women's Hospital (Auckland, New Zealand). Intra- and inter-assay CV's were 8.39% and 16.16% respectively. The assay sensitivity was 0.15 ng/ml.

2.6.4 MILK COMPOSITION ANALYSES

Following milking, the milk from each ewe, or in many cases, from each gland, was mixed thoroughly by gentle inversion and sub-sampled for compositional analyses. Milk samples were refrigerated at approximately -10^{o} C until analyses were carried out, usually 1-2 days later.

Milk samples were analysed for fat, protein and lactose content using a Milkoscan 104 A/B (A/S N. Foss Electric, Denmark). The instrument was calibrated according to the manufacturers recommendations for normal cows milk using samples provided by the Dairy Research Institute, Palmerston North, New Zealand. Since the response of the machine is linear over a restricted range of protein and fat concentrations, it was necessary to dilute the ewes milk with an equal volume of water so that the concentration of fat and protein fell within the range of calibration. No attempt was made to verify the composition of ewes milk by independent analytical methods.

CHAPTER 3

EFFECT ON LACTOGENESIS OF INHIBITING PROLACTIN SECRETION IN EWES DURING LATE PREGNANCY

3.1 INTRODUCTION

The relative importance of various factors, including the decline in circulating progesterone concentration, the increase in PRL concentration and the removal of secretion, in initiating lactation is still unclear. Several reports indicate that PRL is essential for complete, normal lactogenesis in ewes, as evidenced by the delay which occurs when the periparturient PRL surge is abolished using CB154 (Fulkerson *et al.* 1975; Kann 1976a and 1976b; Schams *et al.* 1984). A similar delay is seen in cows (Peel *et al.* 1978; Akers, Bauman, Capuco *et al.* 1981) and does (Lee and Forsyth 1987). Other reports (Hooley *et al.* 1978; Gow *et al.* 1983) concluded that PRL was not needed for lactogenesis in ewes (see section 1.9.2). However, Hooley *et al.* (1978) used ovariectomised ewes induced to lactate (by treatment with oestrogen plus progesterone and then dexamethasone) and their conclusions may not be relevant to ewes commencing a normal lactation. Examination of the data of Gow *et al.* (1983) reveals that treatment with CB154 for certain periods reduced subsequent milk yields significantly while treatment during other periods did not. In their discussion, however, Gow *et al.* (1983) placed emphasis on the periods when ergocriptine was without effect, leading to the conclusion that PRL is not essential for lactogenesis.

Periparturient infusion of bPRL in cows treated with CB154 prevented CB154-induced reductions in milk production (Akers, Bauman, Capuco *et al.* 1981). Examination of the literature regarding the role of PRL in ovine lactogenesis reveals that there are no published reports in which oPRL has been administered to parturient CB154-treated ewes. This "positive control" treatment is required to conclusively establish that the effects of CB154 on ovine lactogenesis are mediated through PRL and not through effects on some other factor(s).

Even though progesterone withdrawal is the most likely lactogenic trigger mechanism (Kuhn 1971 and 1977) it is clear that declining progesterone levels must act in the presence of adequate levels of PRL. Other hormones permit, potentiate or complement oPRL action in sheep and some, particularly oPL, can partially (but not completely) replace oPRL (Servely *et al.* 1983; Forsyth 1986). Thus, there is ample evidence that inhibition of the peripartum PRL surge has delaying or inhibitory effects on the initiation of lactation. However, there is also the paradoxical suggestion that, following CB154 treatment, milk yield of goats over the whole lactation may

actually be increased (Maule Walker 1984b). Therefore the pattern of change in plasma PRL concentration during late pregnancy is important to lactogenesis.

Kann *et al.* (1978) stated that there are two classes of ewe with respect to their plasma profiles of PRL and progesterone in late pregnancy. "Type I" ewes exhibited a steady increase in plasma PRL concentrations from about 40 days prepartum and had achieved relatively high concentrations before the periparturient surge. Meanwhile their progesterone concentrations declined from peak levels attained at about 20 days prepartum. In contrast "type II" ewes (one third of an unspecified number of ewes) had only a small increase in PRL concentrations prior to the surge and their progesterone levels remained high until just before parturition. The existence of these two classes of ewe could provide a unique opportunity to examine the importance of relative changes in, and absolute levels of, PRL and progesterone during late pregnancy. Correlation of early lactation milk yields with prepartum hormone dynamics in the two groups of ewes would provide valuable evidence regarding the relative importance of the PRL surge and progesterone withdrawal. One might expect type I ewes to exhibit earlier lactogenesis or higher milk yields, but Kann *et al.* (1978) did not report milk yields in these ewes, nor any data or statistical evidence supporting the claim that "type I" and "type II" ewes exist.

The objectives of the studies reported here were therefore to investigate the role of PRL in the initiation of lactogenesis in sheep, with a view to:

1 determining if CB154 treatment does inhibit or delay the onset of lactation in local New Zealand ewes

2 establishing whether PRL is essential for complete initiation of lactogenesis by inhibiting endogenous PRL secretion with CB154 and restoring milk yields by concurrent administration of oPRL (the positive control) via s.c. injection.

3 determining the period during which the presence of PRL is essential using differing prepartum periods of CB154 treatment (if PRL proved to be required for lactogenesis as described in 2 above)

4 examining the possible existence of type I and type II ewes as described by Kann *et al.* (1978).

3.2 MATERIALS AND METHODS

Two experiments were carried out; trial 1 in July/August and trial 2 in the following April . Ewes in trial 1 were selected from naturally cycling ewes mated over a 10-d period while trial 2 ewes were selected from a group (synchronised with progesterone and induced to ovulate using PMSG) mated over a 36-h period. Details of animals and experimental methods are given in Chapter 2 and the experimental design is described below.

In both trials multiparous ewes aged 3-5 years were housed indoors from 3-4 weeks prepartum until 8 d postpartum, and fed calculated requirements of pasture and hay. Photoperiod was set at 18L:6D. Ewes were allocated to one of three groups at random, except that groups were balanced for age, live weight and pregnancy rank (singles versus twins, determined by ultrasound diagnosis).

TABLE 3.1 EXPERIMENTAL TREATMENTS USED IN TRIAL 1 AND TRIAL 2 AND ABBREVIATIONS USED TO IDENTIFY THEM

	TREATMENT GROUP		
TRIAL 1	CB154 20 d prepartum and 2 d postpartum	CB154 9 d prepartum and 2 d postpartum	Ethanol/saline 19 d prepartum and 2 d postpartum
abbreviation	CB20	CB9	E/S
TRIAL 2	CB154 7 d prepartum and 5 d postpartum $+HCO_3^-$	CB154 7d prepartum and 5 d postpartum + oPRL	Ethanol/saline 7 d prepartum and CB154 5 d postpartum
abbreviation	CB+BIC	CB+PRL	E/S

In Trial 1 (see Table 3.1) PRL release was inhibited using daily subcutaneous injections of 2 mg CB154 (dissolved in 60% ethanol/40% saline) administered to a group of 10 ewes for a mean period of 20±1 d prepartum and to a second group (10 ewes) for 9±1 d prepartum and continued (in both groups) until d 2 postpartum. A control group (9 ewes) received only excipient (60% ethanol/40 % saline) injections for 19±2 d prepartum and 2 d postpartum. Henceforth these groups will be referred to as CB20, CB9 and E/S respectively. Following parturition, milk yields were measured daily for 7 d until sheep were moved outdoors and grazed at pasture, after which yields were determined at weekly intervals until 4 weeks postpartum (i.e. on d 14, 21 and 28 of lactation). At 5 d intervals, from 25 d before the expected mean date of parturition until ewes were moved outdoors, blood samples were taken by jugular venipuncture at about 0830, 1630 and 2330 h. Two further blood samples were collected immediately before milking each ewe at 2 and 3 weeks postpartum. Plasma samples were analysed for oPRL, progesterone and insulin (see Chapter 2).

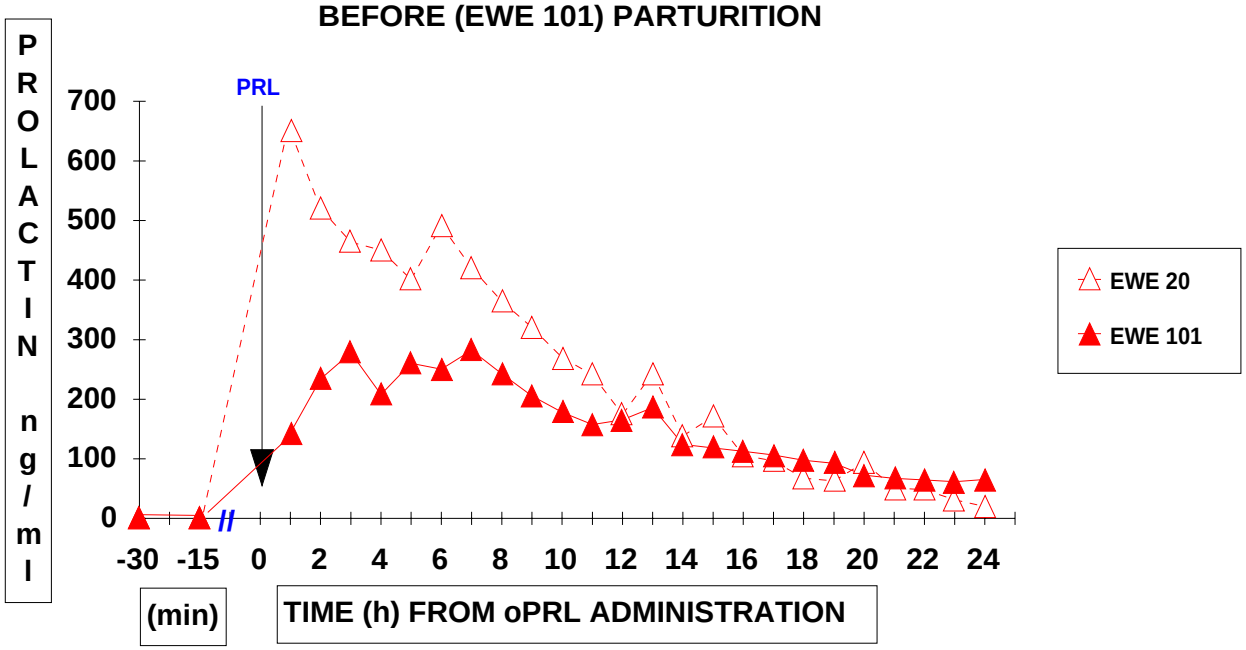
In Trial 2 (see Table 3.1), CB154 was administered (as in trial 1) for 7±0.5 d prepartum and for 5 d postpartum to 2 groups of 8 ewes. In addition to the CB154 treatment, one group (CB+PRL) also received 2 consecutive daily subcutaneous injections of 0.5 mg/kg oPRL beginning on the day that the first ewe lambed. The other group (CB+BIC) received injections of the excipient (0.15M saline, 0.03M bicarbonate) as a control to the oPRL treatment, at the same times. A third group (8 ewes) received excipient (60% ethanol/40 % saline, as a control to the CB154 treatment) for 7±0.5 d prepartum, then received CB154 for 5 d postpartum. Thus the ethanol/saline-treated (E/S) ewes received the same treatment during the postpartum period as did the CB154-treated ewes. The longer period of postpartum CB154 treatment in trial 2 (5 d compared to 2 d in Trial 1) was designed to ensure that the PRL surge was completely eliminated and not merely delayed until after parturition. Blood samples were collected at 5 d intervals from 20 d before the expected mean date of parturition until the end of the trial. Plasma samples were analysed for oPRL concentration. Following lambing the ewes were milked for 8 d.

The aim of the oPRL injections in trial 2 was to raise circulating PRL concentrations to levels characteristic of the periparturient surge. The literature gives widely varying reports of the size of the peak PRL concentration (see Table 1.1, section 1.9.2) ranging from 100 ng/ml (Chamley *et al.* 1973) to 1600 ng/ml (Kann *et al.* 1978). Taking into account the peak plasma PRL concentrations recorded in the E/S ewes in trial 1 (see Figure 3.2), it was decided that the oPRL dose injected in trial 2 should aim to produce circulating levels of 200-400 ng/ml. Infusion of 500 mg/d was shown to produce plasma PRL concentrations of 250 ng/ml in periparturient Holstein cows (Akers, Bauman, Capuco *et al.* 1981). Since 60 kg ewes are about 10% of the weight of Holstein cows it was decided that a dose of 50 mg/d would be suitable for a ewe. If administered in a single s.c. dose, rather than by constant infusion, the resultant higher entry rate of the hormone into the circulation was expected to result in plasma concentrations at least equal to, if not higher than , the 250 ng/ml value reported by Akers, Bauman, Capuco *et al.* (1981). This relied on the assumption that distribution volumes and metabolic clearance rates of PRL were similar in cows and ewes.

In order to establish the plasma PRL response to the oPRL injection, an additional pair of ewes, diagnosed as twin-pregnant, and treated in the same manner as the CB+PRL ewes (except that only a single dose of oPRL was injected, they were not milked and their diet included concentrate (sheepnuts) rather than cut-pasture), were intensively blood sampled for 48 h following hormone administration. Indwelling jugular cannulae were inserted on the day before oPRL treatment was administered. Blood samples were collected from the jugular cannulae at 15-min intervals for 30 min before oPRL administration (0900 h), then at hourly intervals for 24 h. During the period 24-48 h after oPRL administration, samples were taken every 4 h, and subsequently at 0900 h on each day for 12 d. Plasma samples were analysed for PRL concentration in a separate assay to samples from trial 2.

Of the 2 ewes, one (Ewe 20) gave birth to a single lamb 7 h before oPRL treatment, while the other (Ewe 101) produced the predicted twins 92 h after oPRL injection. The plasma PRL concentrations during the 24 h following oPRL injection are presented in Figure 3.1.

FIG 3.1 PLASMA PROLACTIN CONCENTRATIONS (ng/ml) IN 2 EWES TREATED 7 d PREPARTUM AND 5 d POSTPARTUM WITH CB154, AND WITH 0.5 mg/kg LWT oPRL BY A SINGLE SUBCUTANEOUS INJECTION, 7 h AFTER (EWE 20) AND 92 h BEFORE (EWE 101) PARTURITION



The plasma PRL concentrations in the pretreatment blood samples, were between 1 and 2 ng/ml. Administration of oPRL via s.c. injection resulted in rapid increases in plasma PRL concentration (Figure 3.1) to levels similar to those observed in ewes lambing in Spring (trial 1, Figure 3.2). Ewe 20 exhibited a peak concentration at the first sample, 1 h after injection, while the peak value in Ewe 101 occurred after 3 h. By 24 h post-treatment, plasma PRL concentrations in both ewes had declined substantially to 20 and 65 ng/ml. After a further 24 h, PRL levels in both ewes had reached basal levels (data not shown) similar to those seen in the E/S ewes in trial 2 (Figure 3.18). Subsequently there were no substantial changes before sampling ceased on d 14. These results confirmed that the injection of oPRL, at a dose of 0.5 mg/kg LWT, did result in high plasma concentrations of PRL, similar to those seen in the periparturient surge.

In both trial 1 and trial 2, milk was subsampled and analysed for milk fat, protein and lactose content, and plasma samples were analysed for PRL in the same assays, to enable comparison between the trials.

Data were analysed using the computer statistical package REG (Gilmour 1990). Multivariate (repeated measures) analysis of variance was used to analyse all time series data. The statistical test for a delay in the initiation of lactogenesis was the interaction of group contrasts with time. Milk yield and composition data (the latter being arc sine transformed) analyses were conducted for days 1, 7, 14, 21 and 28 of lactation or for days 1 to 7 inclusive in trial 1, and for days 1 to 8 inclusive in trial 2. Hormone data (log transformed) were separated into 2 periods, corresponding as closely as possible to the period of CB154 treatment (days -14, -9, -4, +1) and the post-treatment period (days 6, 14 and 21) for analysis. In trial 1, data for the days -24 and -19 were not included in multivariate analyses since variation in lambing date resulted in some ewes (those which lambed before the expected date) that did not have samples taken on these days. These data were omitted because, in multivariate analyses, missing values result in rejection of entire records. The statistical test for differences in hormone levels was the main effect between treatment group means (rather than the interaction with time) since the objective was to identify differences between means rather than departures from parallelism.

3.3 RESULTS

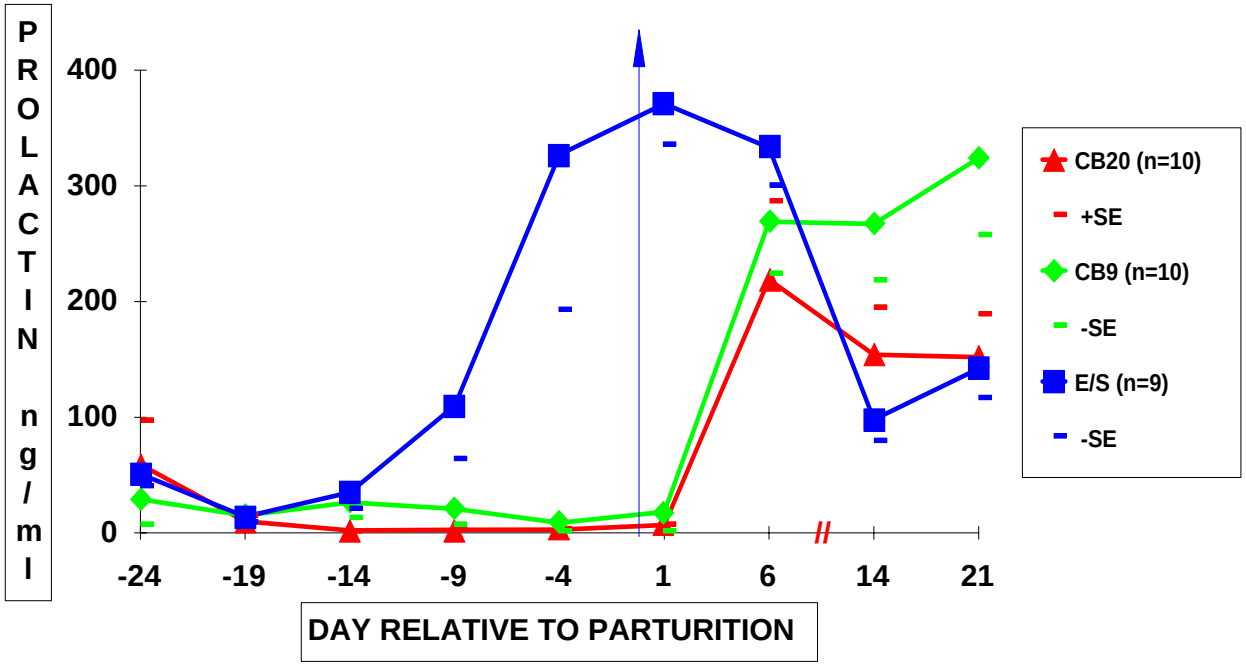
TRIAL 1:

Ewes lambed over a period of 14 d and there was no significant difference between groups in mean lambing date. The day on which blood samples were collected relative to the day of parturition varied between ewes because the actual day of lambing could not be accurately predicted when the prepartum samples were taken. Thus reported sampling days in the figures are the mean ± (SE) 0.3 d of the actual sampling days.

TREATMENT EFFECTS

CB154 treatment successfully eliminated the prepartum PRL surge, maintaining mean plasma PRL concentrations below 25 ng/ml (CB9 and CB20 ewes) while the concentration in the E/S group peaked at 371±36 ng/ml (Figure 3.2). PRL levels in CB20 ewes were significantly ($P<0.0001$) lower than those in E/S ewes over the period from 14 d prepartum until 1 d postpartum. There was a significant group x time interaction ($P<0.01$) for the contrast CB20 versus E/S, reflecting a divergence between the groups in PRL concentration as they approached parturition. Within the CB154-treated ewes, PRL levels were significantly ($P<0.05$) higher in CB9 than in CB20 ewes and there was a significant group x time interaction ($P<0.01$) for the contrast CB20 versus CB9.

FIG 3.2 MEAN±SE PLASMA PROLACTIN CONCENTRATIONS (ng/ml) IN EWES TREATED FOR 20 d (CB20) OR 9 d (CB9) PREPARTUM AND 2d POSTPARTUM WITH CB154, OR WITH ETHANOL/SALINE (E/S)



Following the cessation of CB154 treatment (d 2 postpartum), mean plasma PRL concentrations increased in both the groups previously treated with CB154. Over the period from d 6 to d 21, concentrations were not significantly different between CB20 and E/S ewes, although the group x time interaction for the contrast CB20 versus E/S was significant ($P<0.001$), reflecting the fact that PRL levels declined to a much greater extent in E/S than in CB20 ewes. In CB9 ewes, however, plasma PRL concentrations remained significantly ($P<0.001$) higher than in CB20 ewes (Figure 3.2) and the group x time interaction for the contrast CB20 versus CB9 did not reach significance ($P=0.07$).

Examination of individual plasma PRL profiles amongst the E/S ewes (Figure 3.3) revealed no evidence of the existence of "type I" and "type II" ewes.

FIG 3.3 PLASMA PROLACTIN CONCENTRATIONS (ng/ml) IN INDIVIDUAL EWES TREATED WITH ETHANOL/SALINE FOR 19±2d PREPARTUM AND 2 d POSTPARTUM

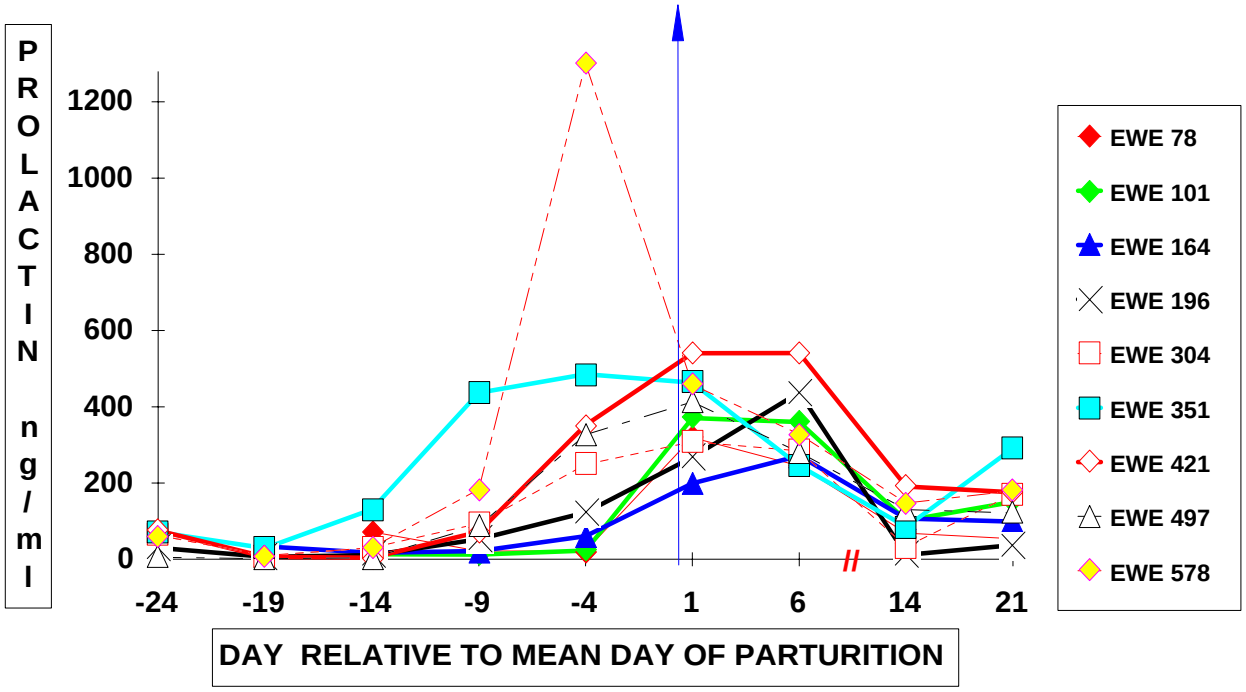
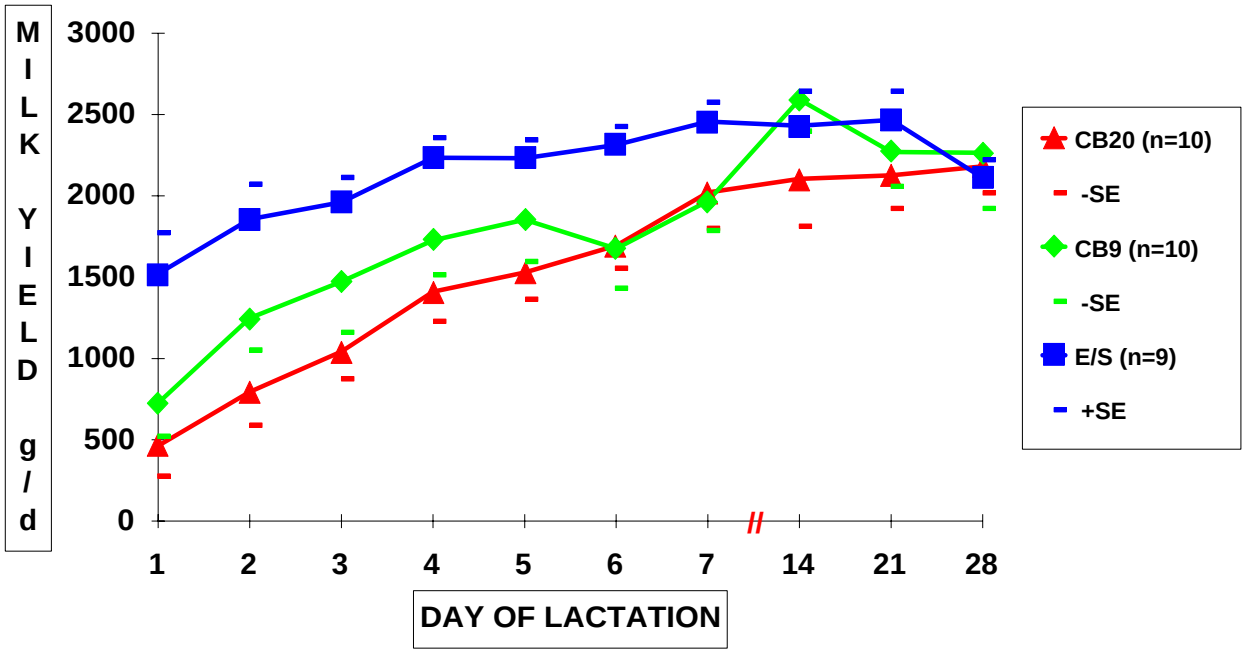


FIG 3.4 MEAN±SE MILK YIELDS (g/d) OF EWES TREATED FOR 20 d (CB20) OR 9 d (CB9) PREPARTUM AND 2 d POSTPARTUM WITH CB154, OR WITH ETHANOL/SALINE (E/S)



Milk yields in CB20 ewes were significantly (P<0.001) lower than in E/S ewes (Figure 3.4) when compared over the sampling days 1, 7, 14, 21 and 28, or over the first 7 d of lactation. However, the group x sampling day interaction was not significant for the contrast CB20 versus E/S over either period. Mean milk yields did not differ significantly between CB20 and CB9 ewes and there was no significant group x time interaction for this contrast.

Mean milk fat percentage did not differ significantly between treatment groups over the first 7 d or 4 weeks of lactation (Figure 3.5). Milk protein percentage was significantly higher in CB20 than in E/S ewes over the first 7 d (P<0.001) and over the 4 weeks (P<0.01) of lactation (Figure 3.6). CB9 ewes did not differ significantly from CB20 ewes in either milk fat or protein percentage. When analysed at weekly milking intervals, lactose percentage did not differ significantly between CB20 and E/S ewes but was generally lower in CB9 than in CB20 ewes (P<0.01) (Figure 3.7). Groups did not differ significantly when compared over the first 7d.

FIG 3.5 MEAN±SE MILK FAT PERCENTAGE IN EWES TREATED FOR 20 d (CB20) OR 9 d (CB9) PREPARTUM AND 2 d POSTPARTUM WITH CB154 , OR WITH ETHANOL/SALINE (E/S)

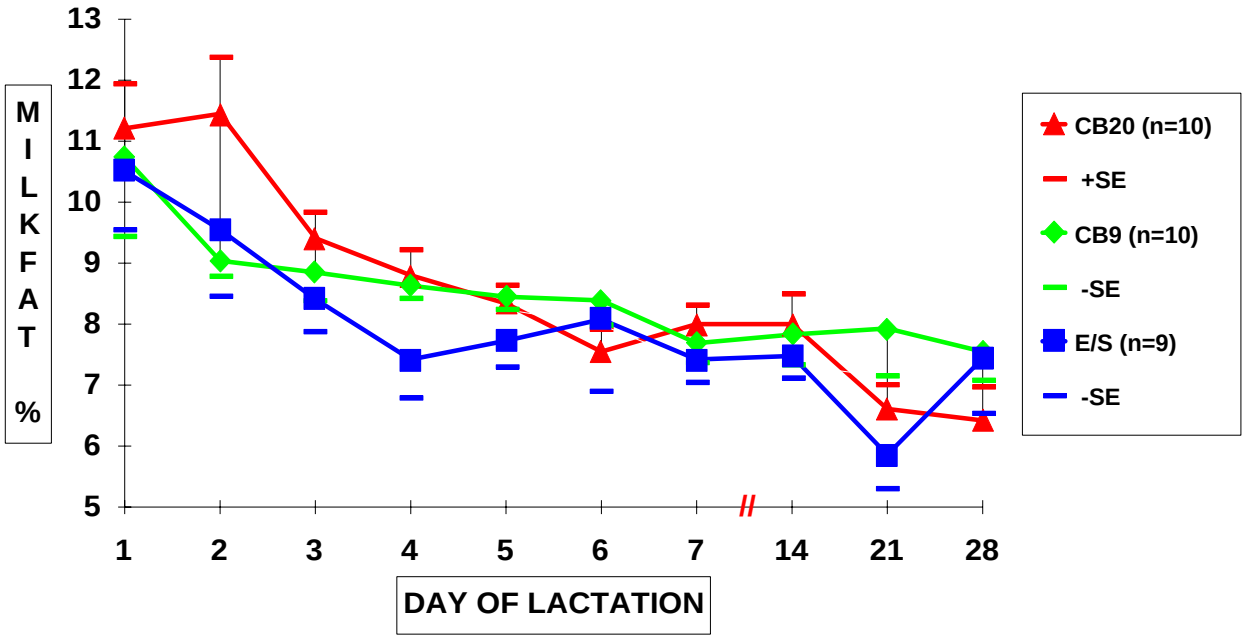


FIG 3.6 MEAN±SE MILK PROTEIN PERCENTAGE IN EWES TREATED FOR 20 d (CB20) OR 9 d (CB9) PREPARTUM AND 2 d POSTPARTUM WITH CB154 , OR WITH ETHANOL/SALINE (E/S)

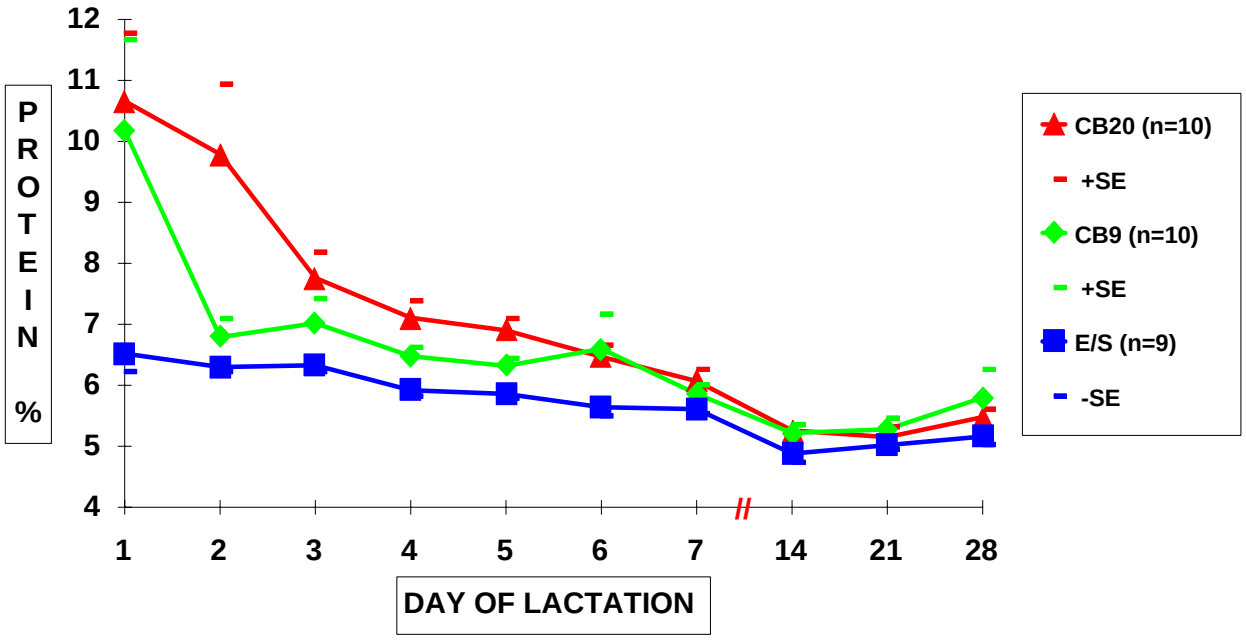


FIG 3.7 MEAN±SE MILK LACTOSE PERCENTAGE IN EWES TREATED FOR 20 d (CB20) OR 9 d (CB9) PREPARTUM AND 2 d POSTPARTUM WITH CB154 , OR WITH ETHANOL/SALINE (E/S)

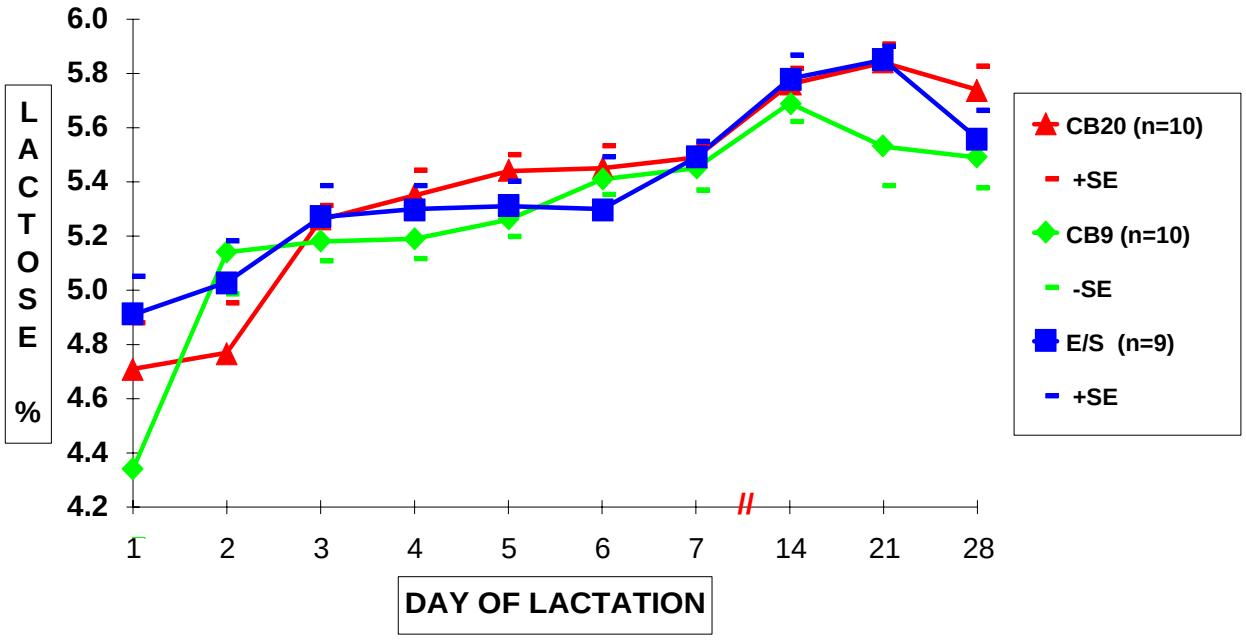
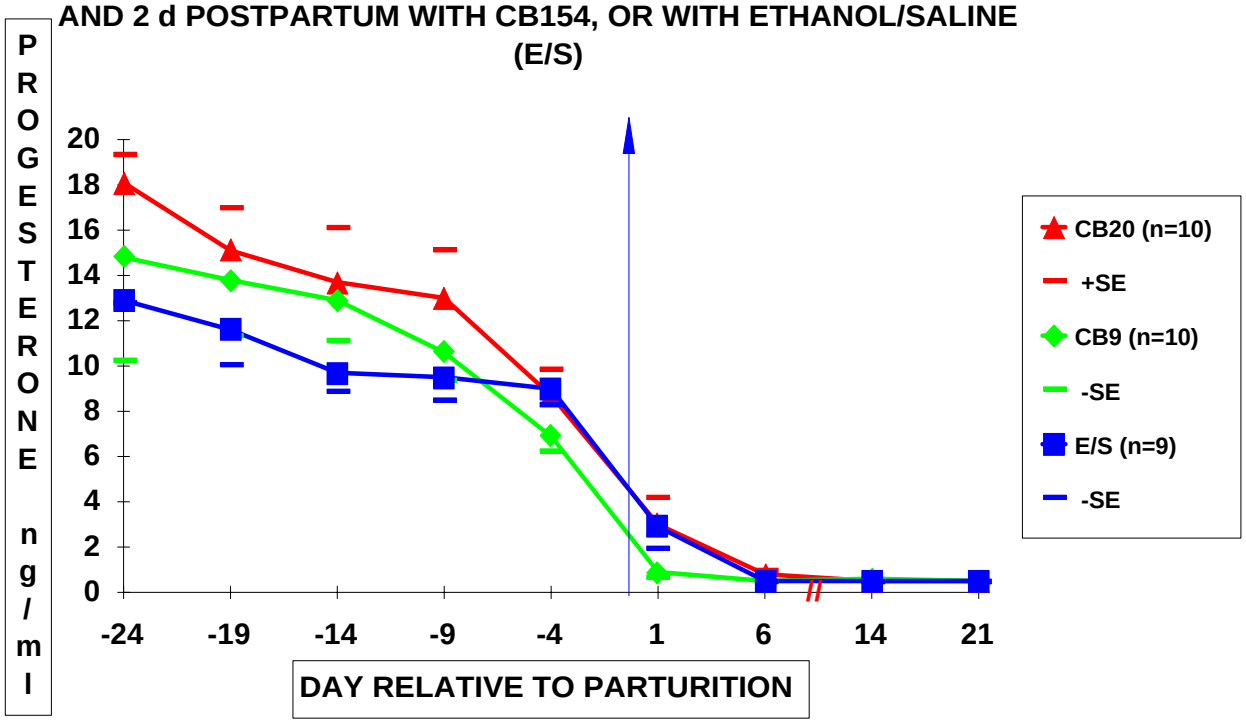
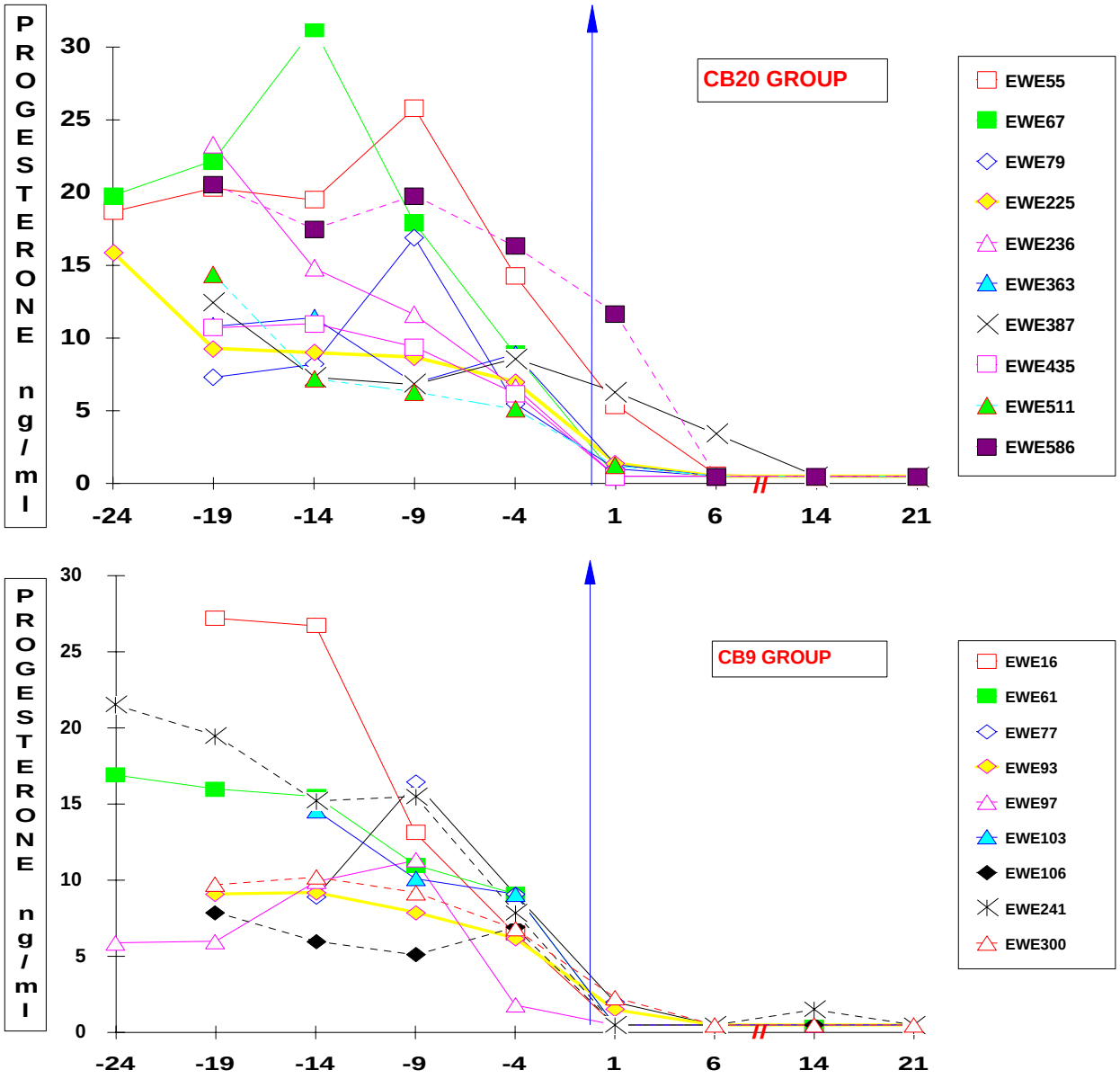


FIG 3.8 MEAN±SE PROGESTERONE CONCENTRATIONS (ng/ml) IN EWES TREATED FOR 20 d (CB20) OR 9 d (CB9) PREPARTUM AND 2 d POSTPARTUM WITH CB154, OR WITH ETHANOL/SALINE (E/S)



Mean plasma progesterone levels declined over the CB154-treatment period in a similar fashion in all three groups (Figure 3.8) and there were no significant differences between groups during this period or in the post-treatment period. Figure 3.9 shows the progesterone profiles of individual ewes within each group. Comparison of the distribution of values in the three groups suggested a greater range of values in CB20 and CB9 than in E/S ewes. There was no evidence of two distinct types of ewe with respect to progesterone profiles.

FIG 3.9 MEAN DAILY PLASMA PROGESTERONE CONCENTRATIONS IN INDIVIDUAL EWES IN EACH GROUP



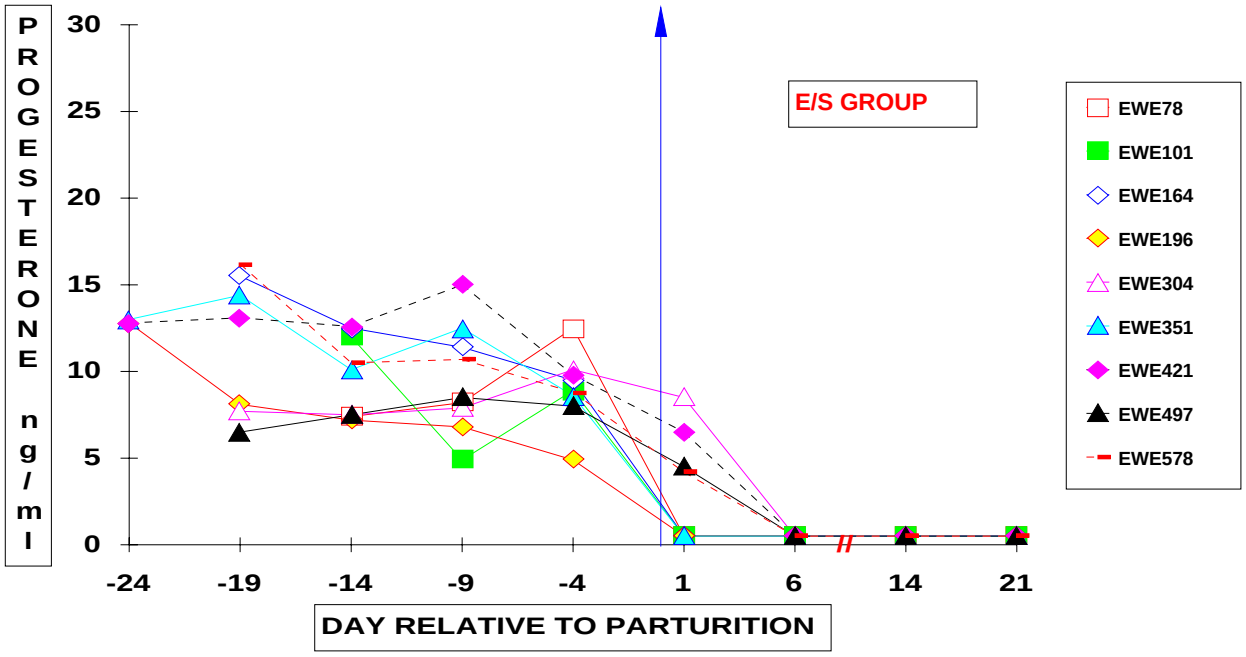
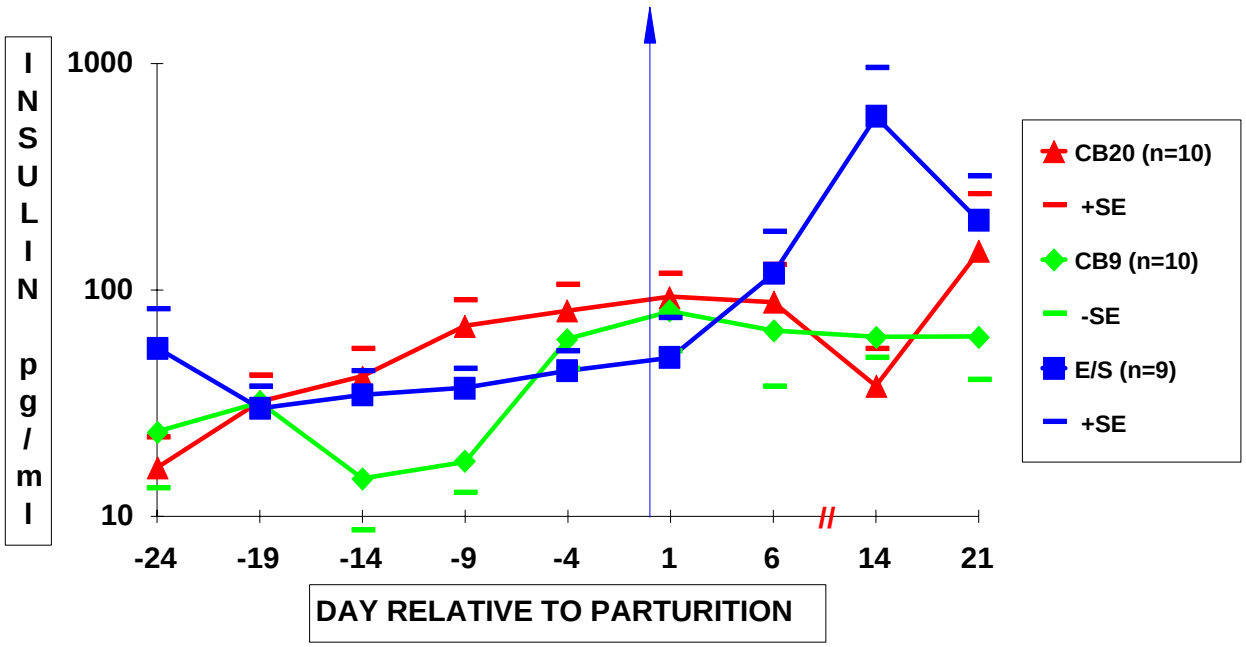

PROGESTERONE ng/ml
30
25
20
15
10
5
0
-24
-19
-14
-9
-4
1
6
14
21
DAY RELATIVE TO PARTURITION
E/S GROUP
EWE78
EWE101
EWE164
EWE196
EWE304
EWE351
EWE421
EWE497
EWE578

FIG 3.10 MEAN±SE PLASMA INSULIN CONCENTRATIONS (pg/ml) IN EWES TREATED FOR 20 d (CB20) OR 9 d (CB9) PREPARTUM AND 2 d POSTPARTUM WITH CB154, OR WITH ETHANOL/SALINE (E/S)



Mean plasma insulin concentrations were significantly ($P<0.05$) different between groups in the period from 14 d prepartum until 1 d postpartum (Figure 3.10). Concentrations were significantly ($P<0.05$) higher in CB20 than in E/S and CB9 ewes. Visual observation of the data suggests that plasma insulin concentrations in CB9 ewes also increased following the commencement of CB154 treatment. Values were in the range 2 pg/ml (the lower limit of the assay) to 94 pg/ml. There were no significant differences between groups over the period from d 6 until d 21 postpartum. There was also no significant ($P>0.05$) time effect or group by time interaction, over the period from 2 weeks prepartum until 3 weeks postpartum, indicating that the steady rise in mean insulin concentrations apparent in Figure 3.10 was not significant, and that the temporal patterns of insulin concentrations were similar in all 3 groups. The E/S group exhibited a high mean insulin concentration at d 14 postpartum due to very high levels (1690 and 3160 pg/ml) exhibited by 2 single-bearing E/S ewes. These data were not discarded as outliers since both ewes exhibited high values on 2 other days. No other ewes in the group exhibited values above 117 pg/ml on that day. The high variance contributed by these data may have influenced the sensitivity of statistical analyses.

RANK EFFECTS

Plasma PRL concentrations did not differ significantly between single- and twin- bearing ewes over the 14-d prepartum period but were significantly (P<0.001) higher in twin-bearing ewes than in single-bearing ewes over the period 6-21d postpartum (Figure 3.11). Twin-bearing ewes had significantly (P<0.0001) higher milk yields when analysed on a weekly basis and the difference approached significance when compared over the first 7 d (P<0.10) (Figure 3.12). Twin-bearing ewes also had a significantly (P<0.05) higher milk fat percentage over the 4 weekly sampling days but the difference was not significant over the first 7 d (Figure 3.13). Milk protein percentage did not differ significantly between single- and twin-bearing ewes (data not shown). Lactose percentage was higher in single-bearing ewes over the 4 weekly sampling days (P<0.06) but the difference was not significant over the first 7 d (Figure 3.14). Mean plasma progesterone concentrations were significantly (P<0.01) higher in twin-bearing than in single-bearing ewes over the period from 14 d prepartum until 1 d postpartum (Figure 3.15). Single- and twin bearing-ewes did not differ significantly in plasma insulin concentration (data not shown).

FIG 3.11 MEAN±SE PLASMA PROLACTIN CONCENTRATIONS (ng/ml) IN SINGLE- AND TWIN-BEARING EWES

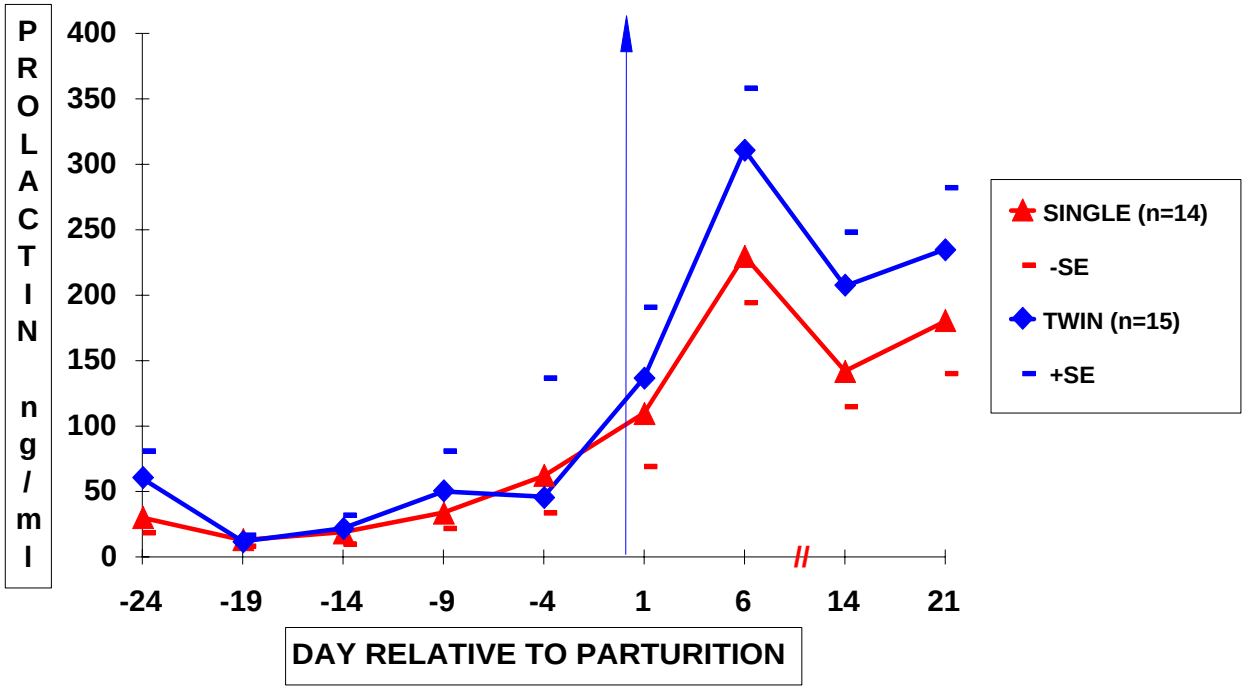


FIG 3.12 MEAN±SE MILK YIELDS (g/d) IN SINGLE- AND TWIN-BEARING EWES

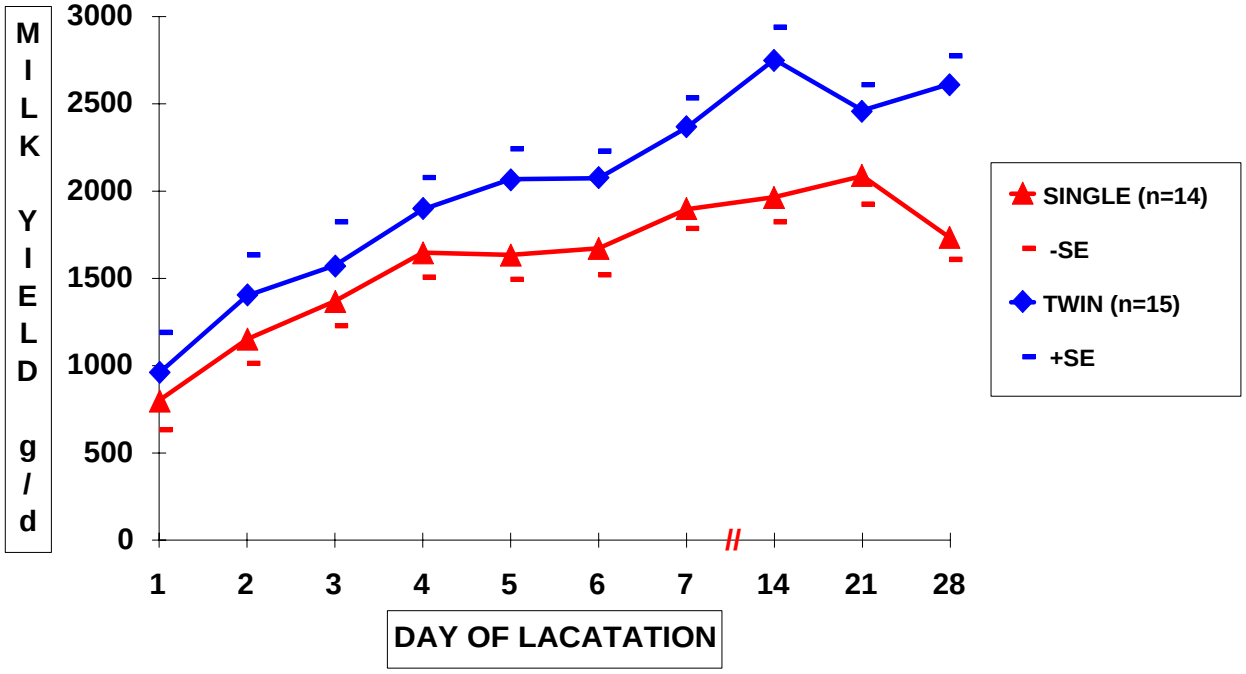


FIG 3.13 MEAN±SE MILK FAT PERCENTAGE IN SINGLE- AND TWIN-BEARING EWES

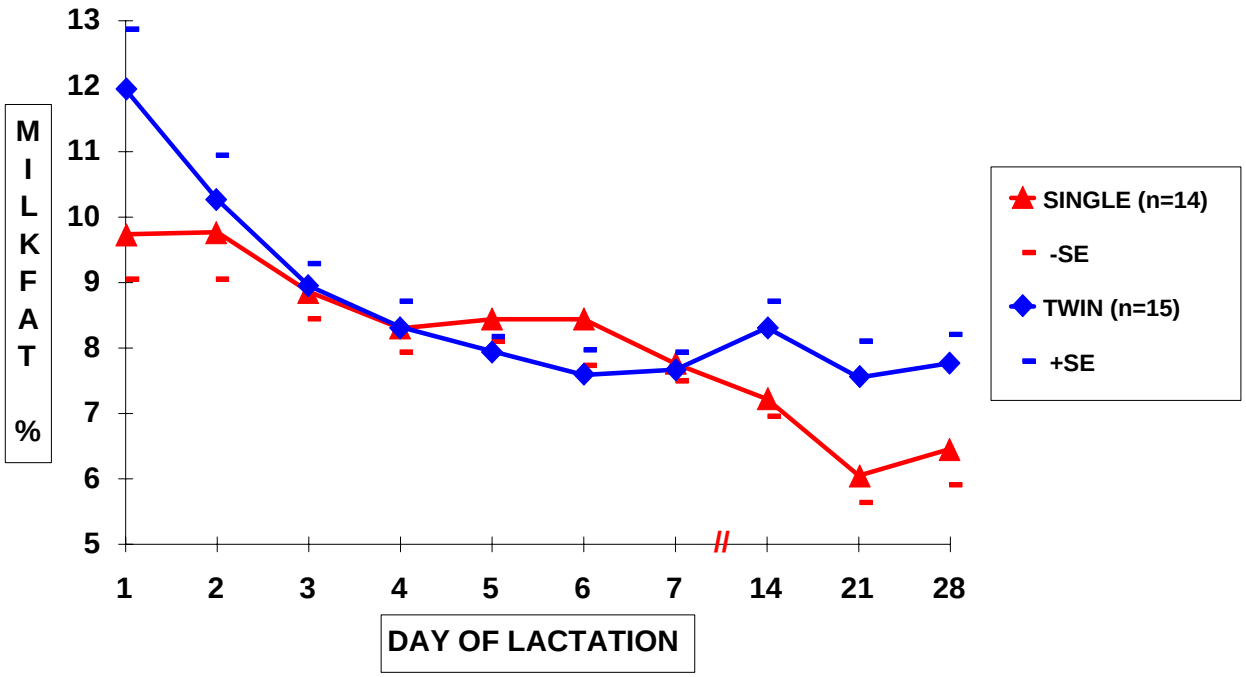


FIG 3.14 MEAN±SE MILK LACTOSE PERCENTAGE IN SINGLE- AND TWIN-BEARING EWES

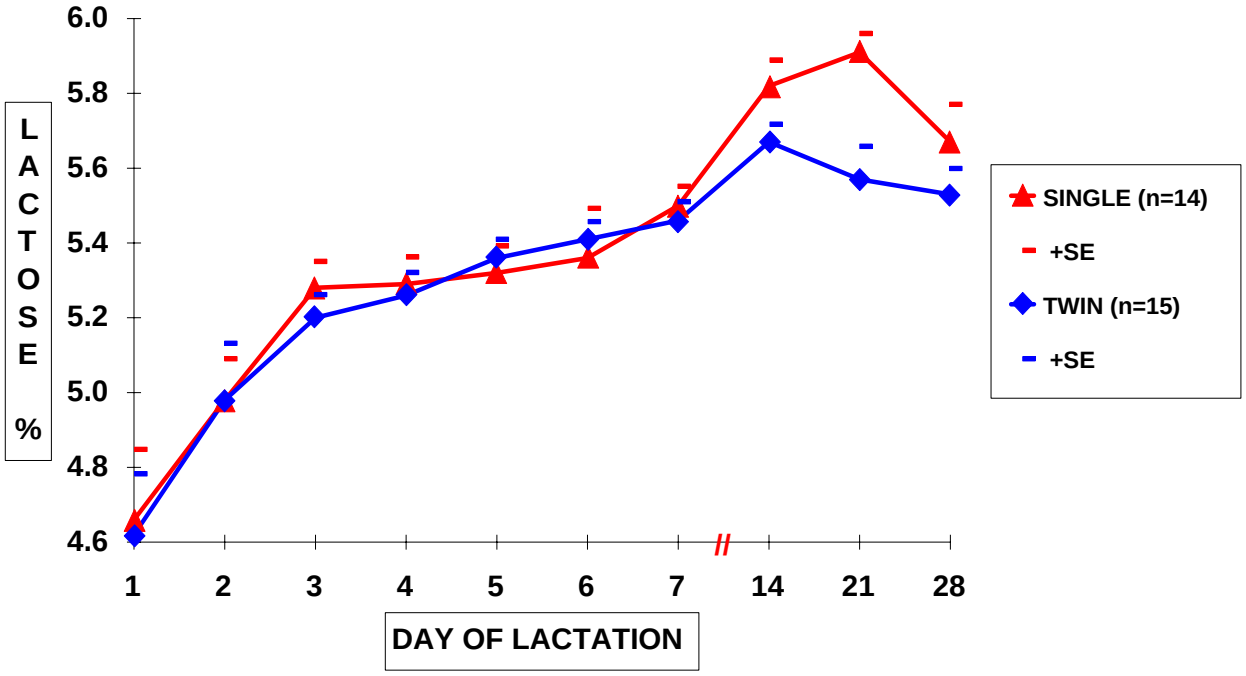
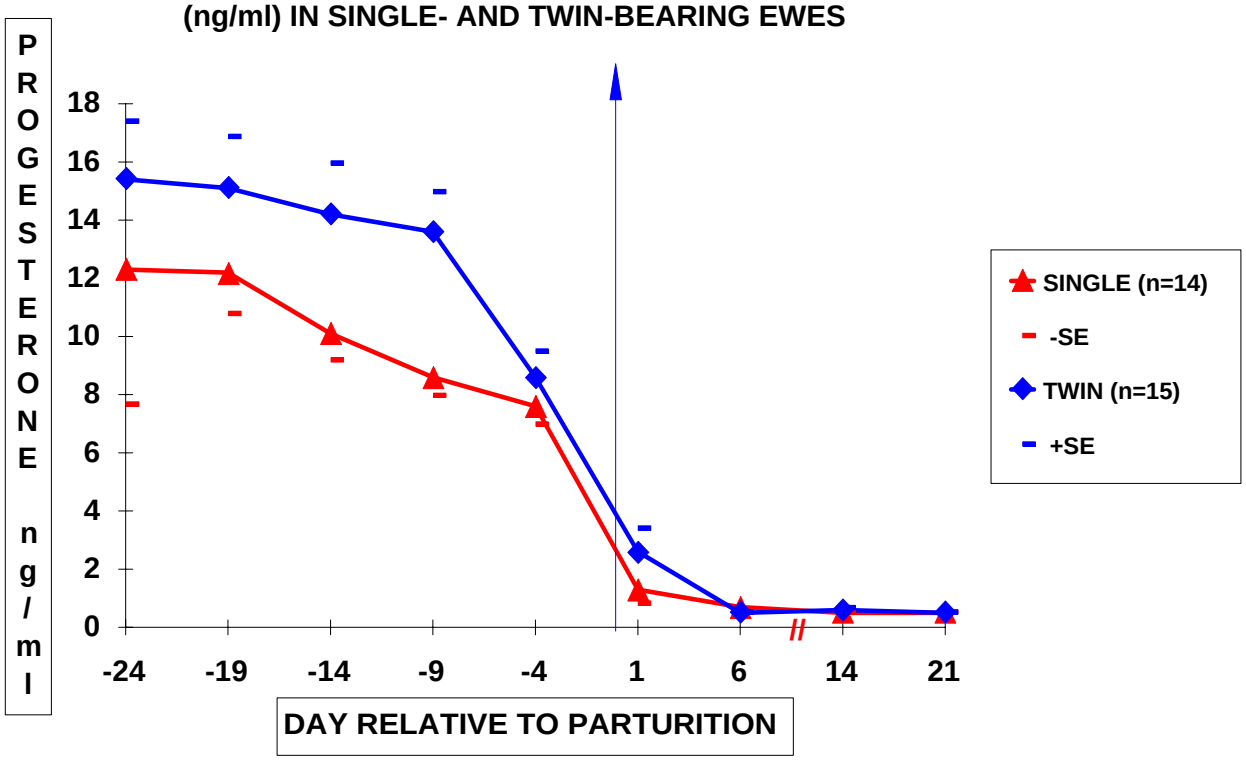


FIG 3.15 MEAN±SE PLASMA PROGESTERONE CONCENTRATIONS (ng/ml) IN SINGLE- AND TWIN-BEARING EWES

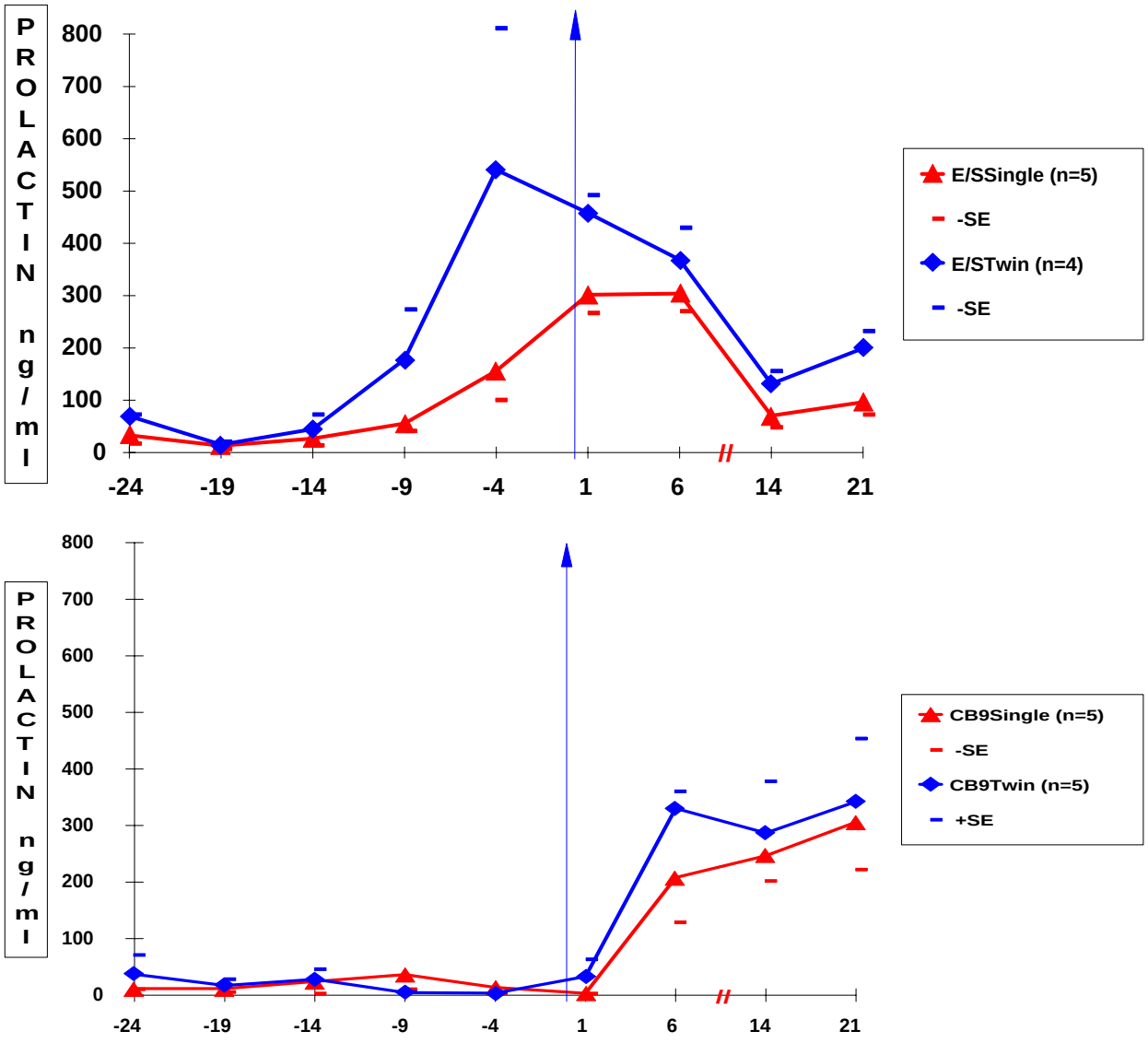


TREATMENT BY RANK INTERACTIONS

Plasma PRL concentrations in single- and twin-bearing ewes from each treatment group are shown in Figure 3.16. There was no significant interaction between the group contrast CB20 versus E/S and pregnancy rank, either during the prepartum (d -14 to d +1) or postpartum (d 6 to d 21) periods, indicating that CB154 treatment for 20 d did not differentially affect PRL concentrations in twin-bearing ewes and single-bearing ewes. Similarly there was no significant interaction between the group contrast CB20 versus CB9 and pregnancy rank, indicating that the different periods of CB154 treatment did not differentially affect PRL concentrations in twin-bearing ewes and single-bearing ewes.

Despite the lack of any such significant effects, the longer period of CB154 treatment had a greater effect on milk yield in twin-bearing ewes than in single-bearing ewes as indicated by a significant ($P<0.05$) interaction between the group contrast CB20 versus CB9 and pregnancy rank. This interaction (henceforth referred to as CB20 versus CB9xRank) was detected over both the 4 weekly samples and the first 7 d period. In ewes with twins, but not those with singles, 20 d of CB154 treatment severely delayed lactogenesis while 9 d of treatment had only a small effect (Figure 3.17).

FIG 3.16 MEAN±SE PLASMA PROLACTIN CONCENTRATION (ng/ml) OF EWES WITH SINGLE OR TWIN LAMBS TREATED FOR 20 d (CB20) OR 9 d (CB9) PREPARTUM AND 2 d POSTPARTUM WITH CB154, OR WITH ETHANOL/SALINE (E/S)



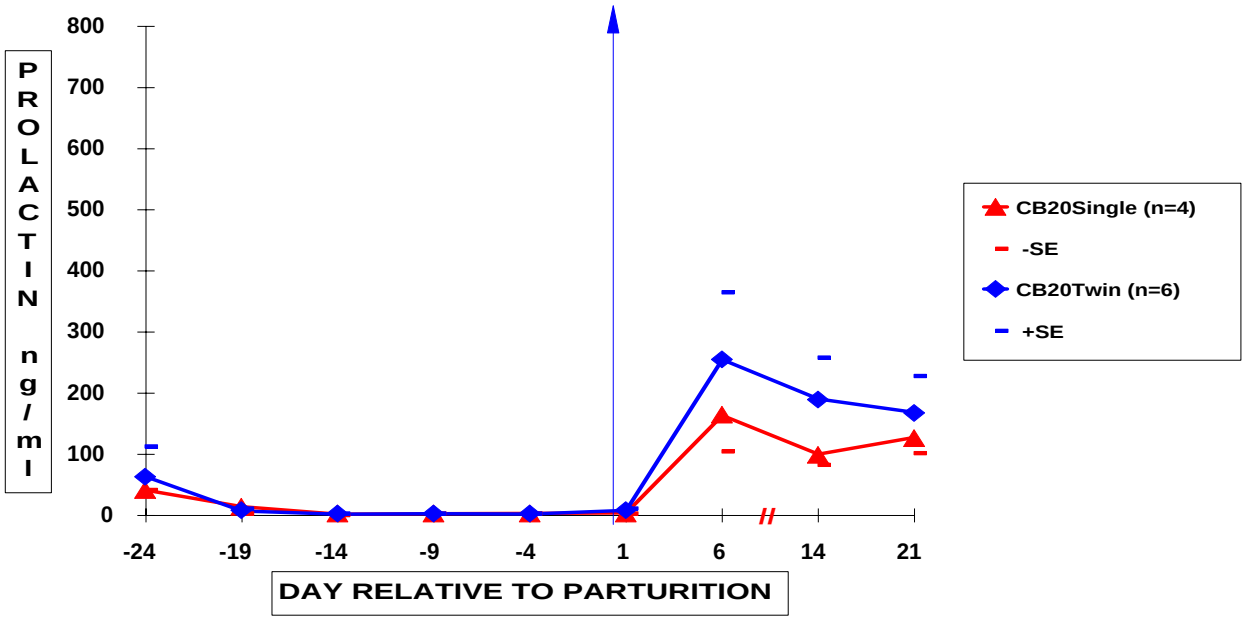
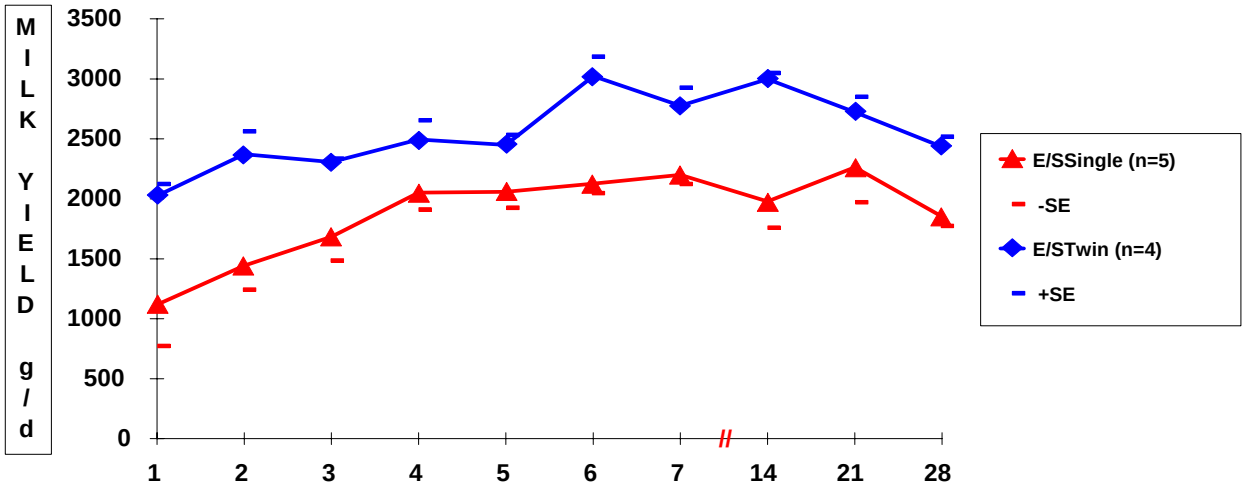
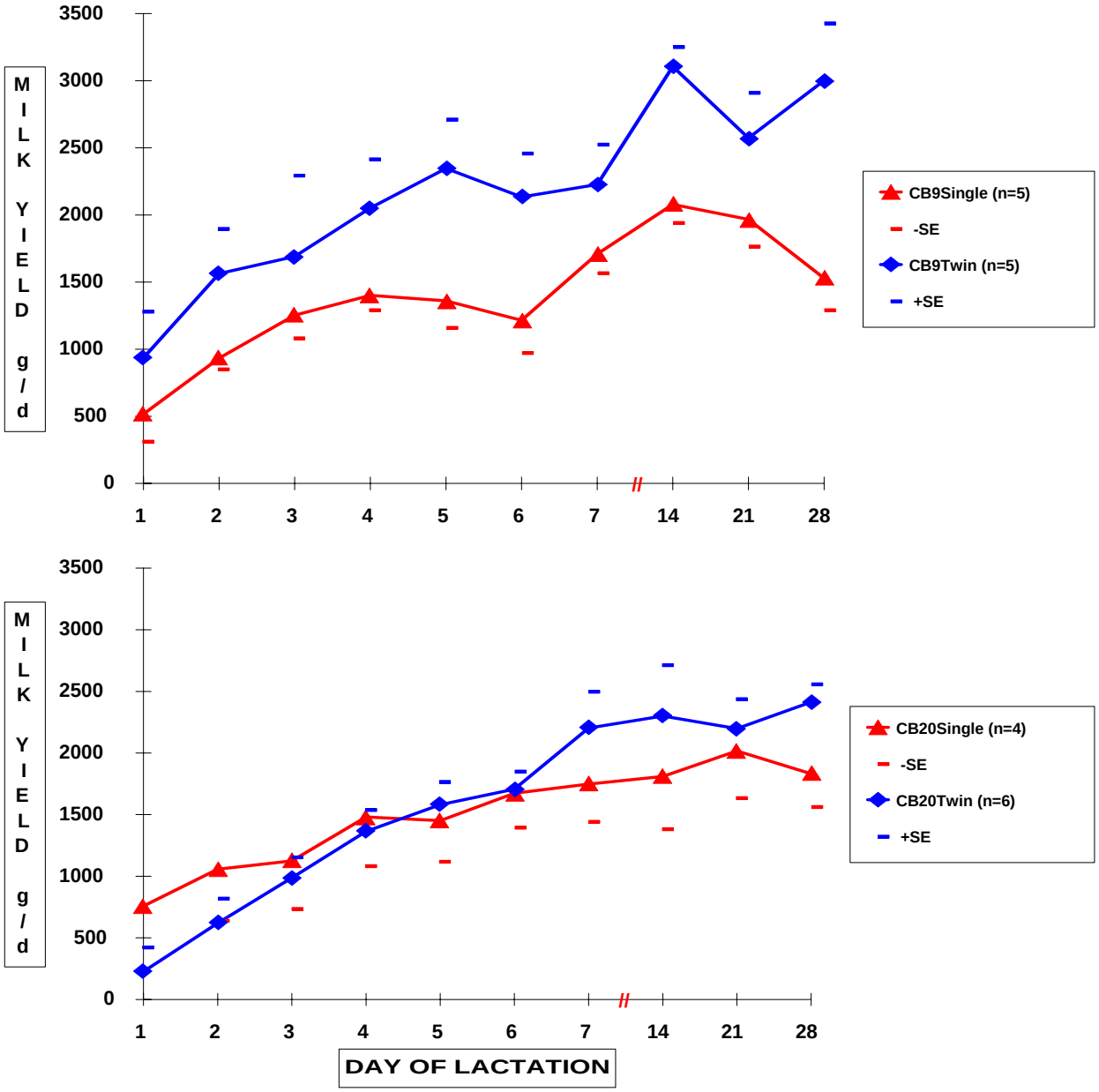


FIG 3.17 MEAN±SE MILK YIELDS (g/d) OF EWES WITH SINGLE OR TWIN LAMBS TREATED FOR 20 d (CB20) OR 9 d (CB9) PREPARTUM AND 2 d POSTPARTUM WITH CB154, OR WITH ETHANOL/SALINE (E/S)




MILK YIELD g/d
3500
3000
2500
2000
1500
1000
500
0
1
2
3
4
5
6
7
14
21
28
CB9Single (n=5)
-SE
CB9Twin (n=5)
+SE
MILK YIELD g/d
3500
3000
2500
2000
1500
1000
500
0
1
2
3
4
5
6
7
14
21
28
DAY OF LACTATION
CB20Single (n=4)
-SE
CB20Twin (n=6)
+SE

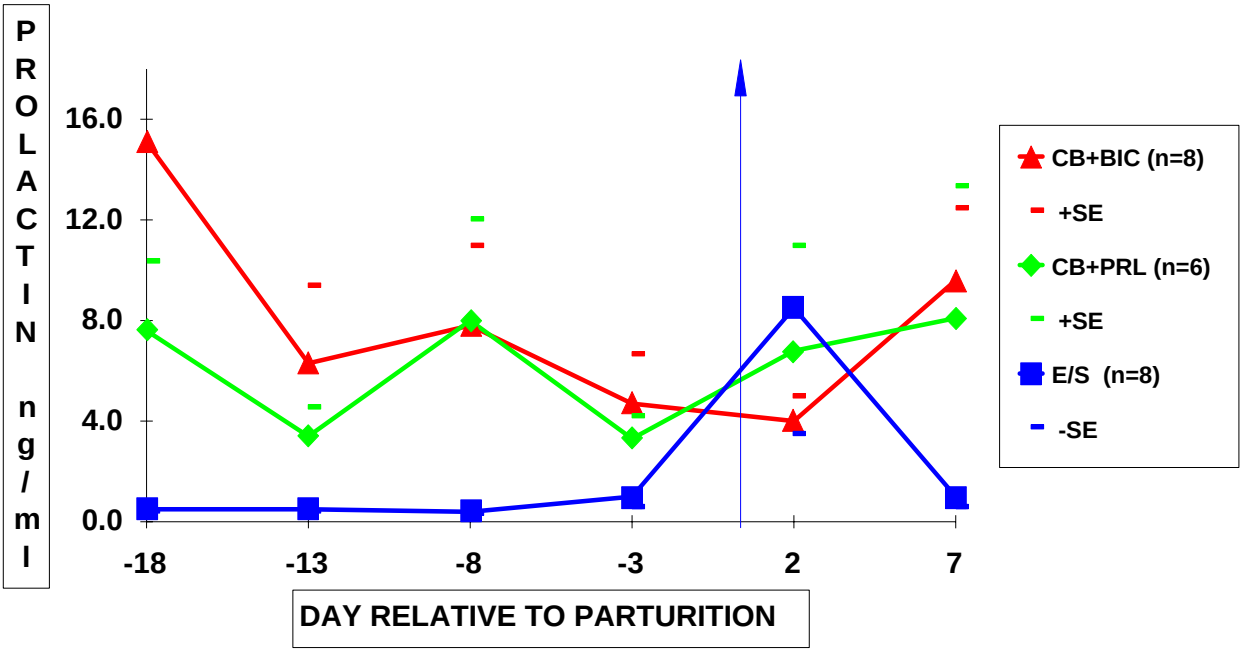
TRIAL 2:

Two ewes from the CB154+oPRL group were removed from the trial, one due to death of the lamb and the other due to lambing one oestrous cycle late. All remaining trial 2 ewes lambed within a period of 52 h and the mean date of lambing did not differ significantly between groups. One ewe in the CB+BIC group, previously diagnosed as single-bearing, gave birth to twins resulting in unbalanced numbers in that group (2 single- and 6 twin-bearing ewes).

TREATMENT EFFECTS

Plasma PRL concentrations in all ewes were very low compared to those in Trial 1. Mean plasma PRL concentration was significantly (P<0.01) lower in E/S ewes than in either of the CB154-treated groups **before** CB154-treatment started (i.e. over the period from -18 until -8 d) (Figure 3.18).

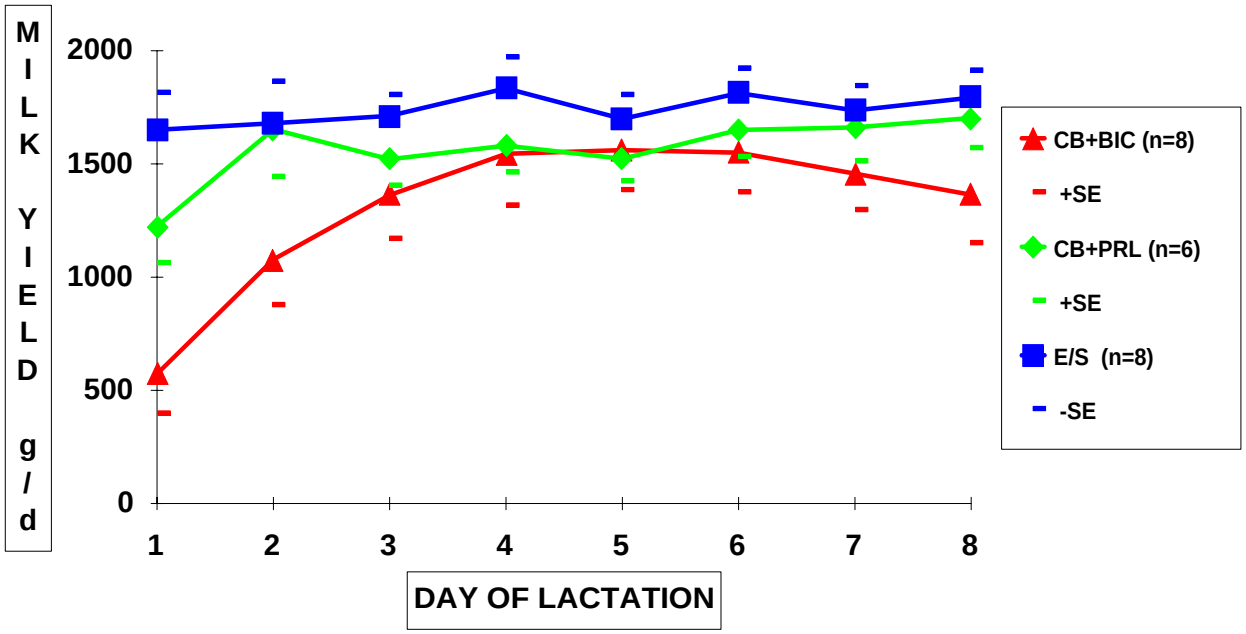
FIG 3.18 MEAN±SE PROLACTIN CONCENTRATIONS (ng/ml) IN EWES TREATED PREPARTUM WITH CB154 (7d) PLUS BICARBONATE (2d)(CB+BIC), CB154 (7d) PLUS oPRL (2d)(CB+PRL), OR WITH ETHANOL/SALINE (7d)(E/S)



The differences between E/S and both CB+PRL and CB+BIC ewes were also significant when analysed over the entire experimental period (P<0.01) and during the postpartum period alone(P<0.001). There was no statistically significant evidence of a parturient rise in PRL concentrations in any of the groups despite the apparent small increase exhibited by E/S ewes just after parturition.

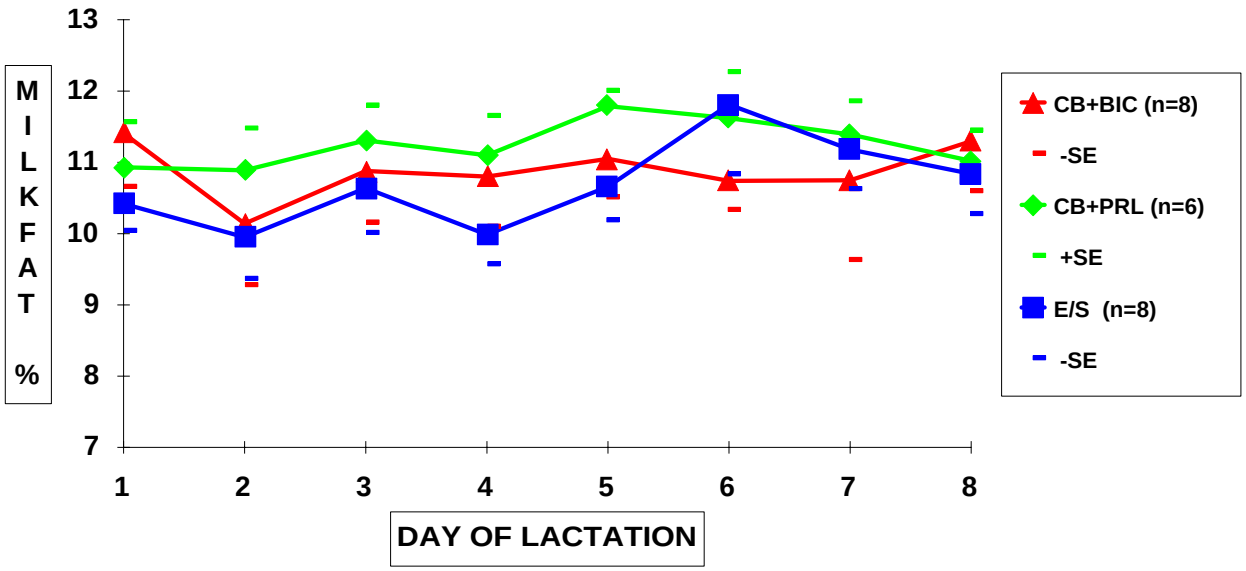
However, since the sampling interval did not coincide with the mean date of parturition, it is possible that the true size of the parturient peak in E/S ewes was much higher than the value recorded on d 2. However, the small peak evident on d 2 is the result of the expected parturient surge in PRL concentrations recorded in two ewes which lambed on that day, and of one ewe which lambed the previous evening. Individual samples within 8 h of parturition revealed plasma PRL concentrations in the range 260-515 ng/ml. In comparison, the plasma PRL concentration in a sample taken from a CB154-treated ewe at parturition was less than 1 ng/ml and in another ewe, a sample taken 12 h prepartum contained 29 ng/ml. Similarly, sampling did not coincide with elevated PRL concentrations in PRL-treated ewes (CB+PRL), but this dose of oPRL was shown previously (Figure 3.1) to result in markedly elevated plasma PRL concentrations similar to the periparturient surge seen in normal, untreated ewes (spring-lambing, trial 1).

FIG 3.19 MEAN±SE MILK YIELDS (g/d) IN EWES TREATED PREPARTUM WITH CB154 (7d) PLUS BICARBONATE (2d)(CB+BIC), CB154 (7d) PLUS oPRL (2d)(CB+PRL), OR WITH ETHANOL/SALINE (7d)(E/S)



Milk yields of CB+BIC ewes were significantly ($P<0.01$) lower than those of E/S ewes over the first 8 d of lactation (Figure 3.19). Milk yields of CB+PRL ewes did not differ significantly from those of E/S ewes over this period.

FIG 3.20 MEAN±SE MILK FAT PERCENTAGE IN EWES TREATED PREPARTUM WITH CB154 (7d) PLUS BICARBONATE (2d)(CB+BIC), CB154 (7d) PLUS oPRL (2d)(CB+PRL), OR WITH ETHANOL/SALINE (7d)(E/S)



During the 8 d of measurements, milk fat percentage did not differ significantly between treatment groups (Figure 3.20). Mean milk protein percentage was significantly ($P<0.05$) higher in CB+BIC than in E/S ewes over the 8-d lactation period (Figure 3.21) but CB+PRL ewes did not differ significantly from E/S ewes. The protein levels in CB+BIC ewes rapidly declined to reach the same levels (about 7%) as the other groups by d 3. Mean milk lactose percentage did not differ significantly between groups over the first 8 d of lactation (Figure 3.22).

FIG 3.21 MEAN±SE MILK PROTEIN PERCENTAGE IN EWES TREATED PREPARTUM WITH CB154 (7d) PLUS BICARBONATE (2d)(CB+BIC), CB154 (7d) PLUS oPRL (2d(CB+PRL), OR WITH ETHANOL/SALINE (7d)(E/S)

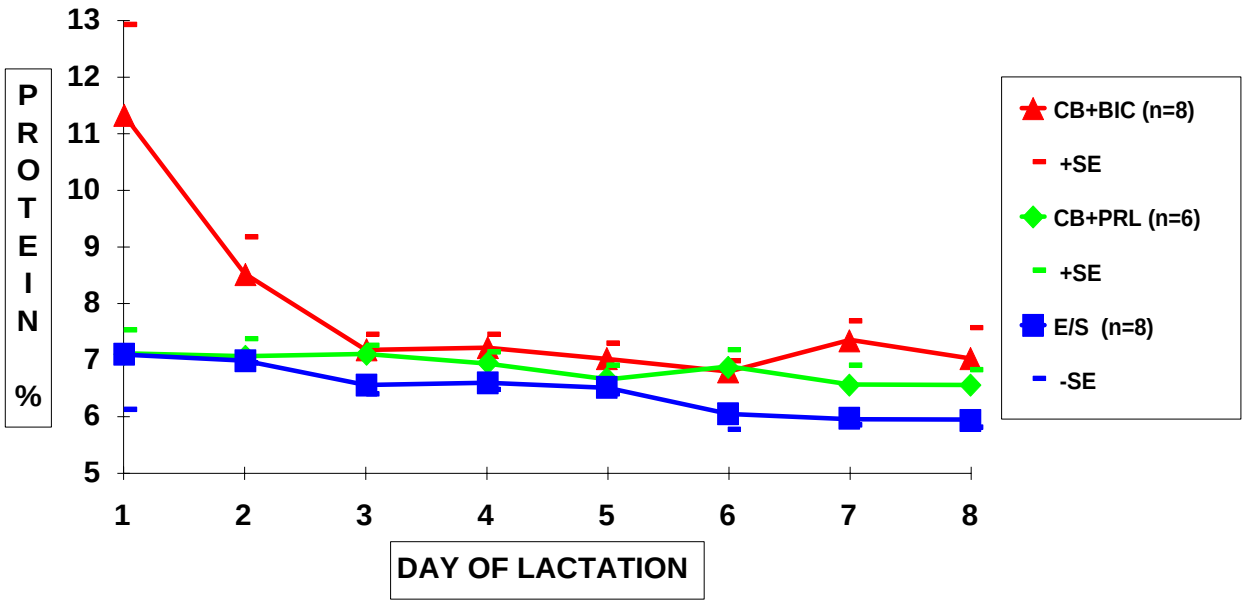
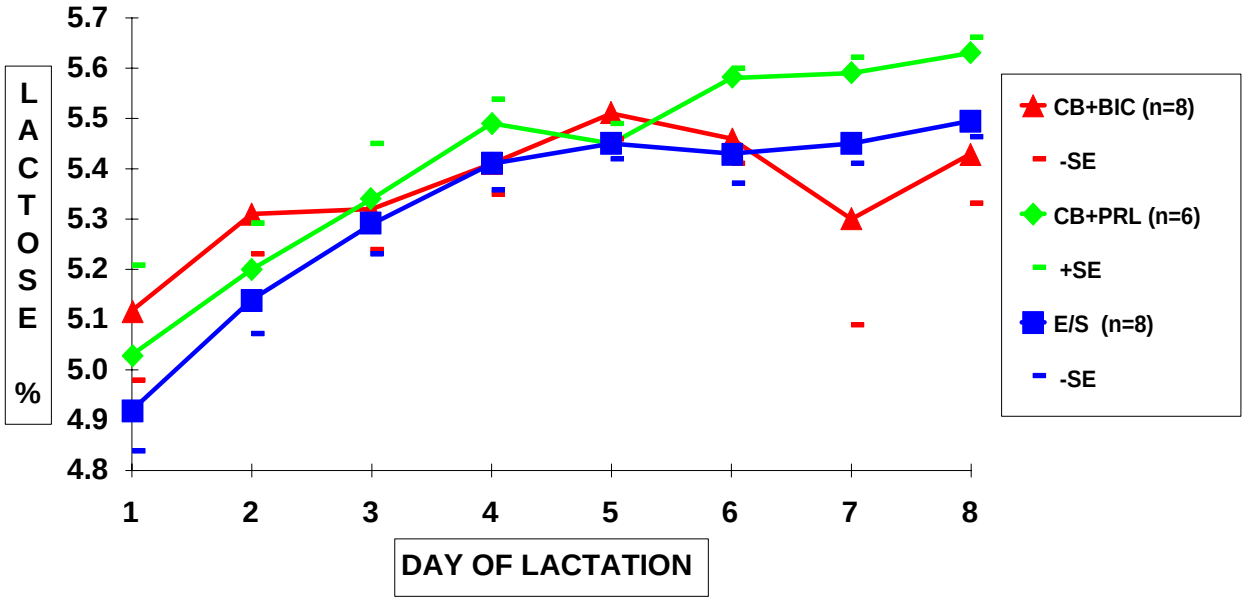


FIG 3.22 MEAN±SE MILK LACTOSE PERCENTAGE IN EWES TREATED PREPARTUM WITH CB154 (7d) PLUS BICARBONATE (2d)(CB+BIC), CB154 (7d) PLUS oPRL (2d)(CB+PRL), OR WITH ETHANOL/SALINE (7d)(E/S)



RANK EFFECTS

Plasma PRL concentrations did not differ significantly between single- and twin-bearing ewes (Figure 3.23) but milk yields were significantly ($P<0.05$) higher in twin-bearing ewes than in single-bearing ewes (Figure 3.24). Milk composition did not differ significantly between single- and twin-bearing ewes (data not shown).

FIG 3.23 MEAN±SE PLASMA PROLACTIN CONCENTRATIONS (ng/ml) IN SINGLE- AND TWIN-BEARING EWES

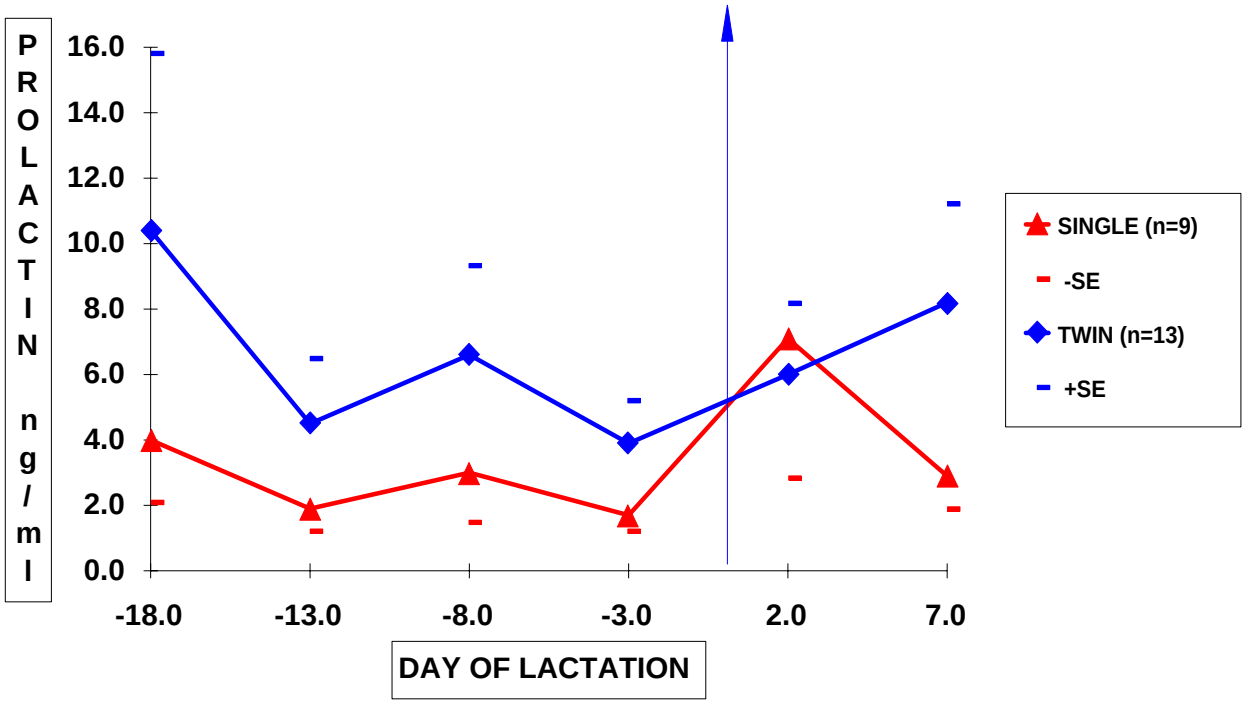
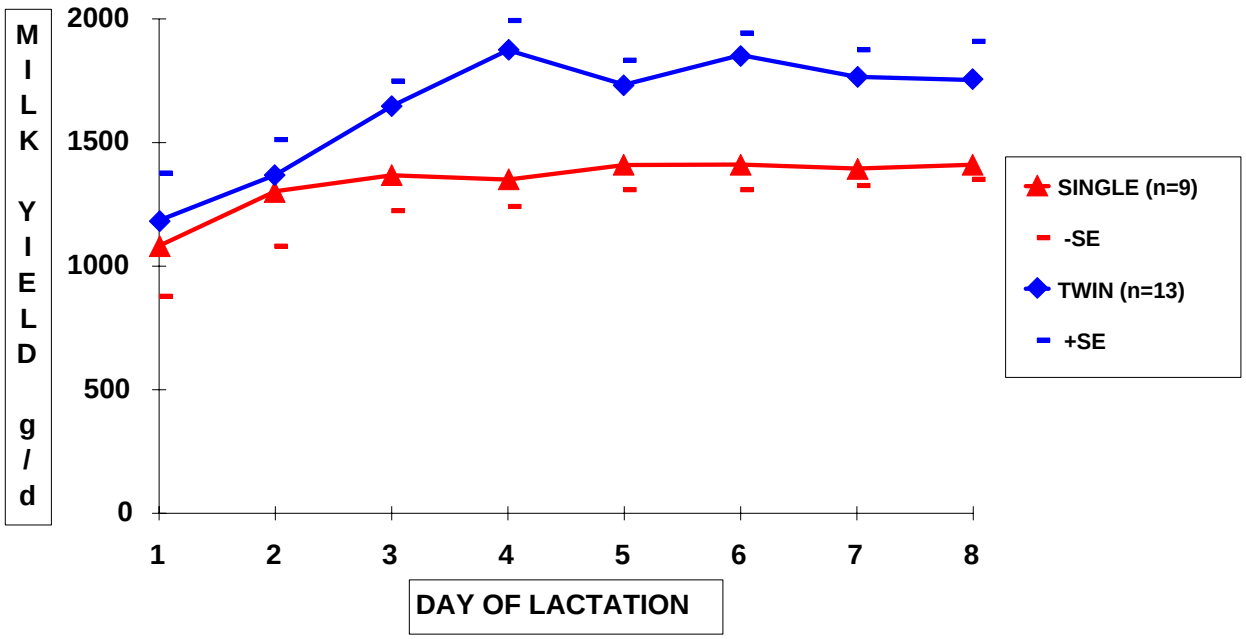


FIG 3.24 MEAN±SE MILK YIELDS (g/d) IN SINGLE- AND TWIN-BEARING EWES

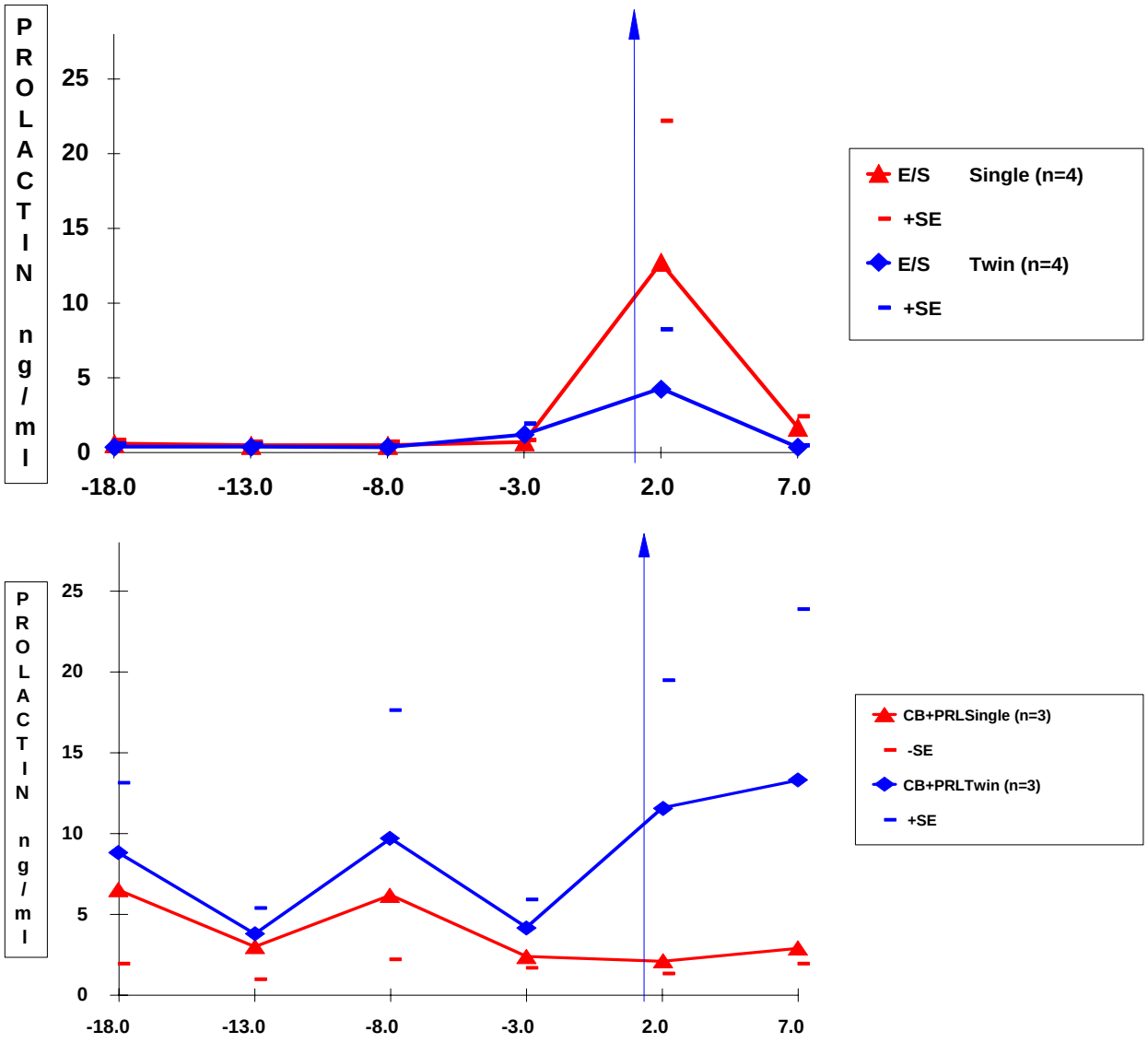


TREATMENT BY RANK INTERACTIONS

Plasma PRL data for single- and twin-bearing ewes in each group are presented in Figure 3.25. The group by rank interaction was significant ($P<0.001$), as was the group contrast CB+BIC versus E/S by rank interaction ($P<0.001$), but only when analysed over the postpartum period (d 2 and d 7). This indicated that plasma PRL levels were reduced to a greater extent in single-bearing ewes than in twin-bearing ewes by the CB154 treatment. The group contrast CB+PRL versus E/S by Rank interaction was significant ($P<0.001$) over the same period reflecting the fact that in CB+PRL twin-bearing ewes postpartum PRL levels were increased while single-bearing ewes had decreased plasma PRL concentrations relative to comparable E/S ewes. However, these results may be artefacts of the blood sampling interval which did not coincide with the mean date of parturition, as discussed above.

Single-bearing ewes were most affected by this length (mean 7 d) of CB154 treatment, producing only 64±12 g of milk on d 1 compared to 743±190 g in twin-bearing ewes. However there were too few single-bearing ewes (n=2) in the CB+BIC group to detect a significant effect (on milk yield) of the group by rank interaction, or of the group contrast CB+BIC versus E/S by rank interaction. Nevertheless, the milk yield data for single- and twin-bearing ewes in each group are presented (Figure 3.26) since, when considered along with the CB20 versus CB9xRank interaction reported in trial 1, they may contribute to the understanding of the latter effect.

FIG 3.25 MEAN±SE PROLACTIN CONCENTRATIONS (ng/ml) IN SINGLE- AND TWIN- BEARING EWES TREATED PREPARTUM WITH CB154 (7d) PLUS BICARBONATE (2d)(CB+BIC), CB154 (7d) PLUS oPRL (2d)(CB+PRL), OR WITH ETHANOL/SALINE (7d)(E/S)



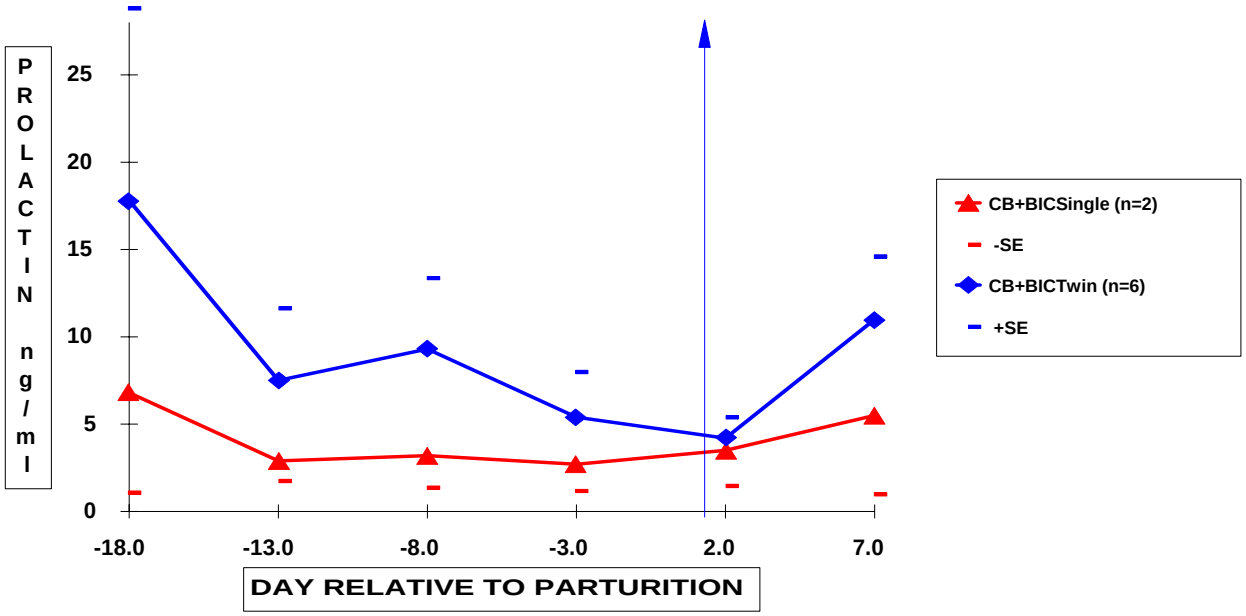
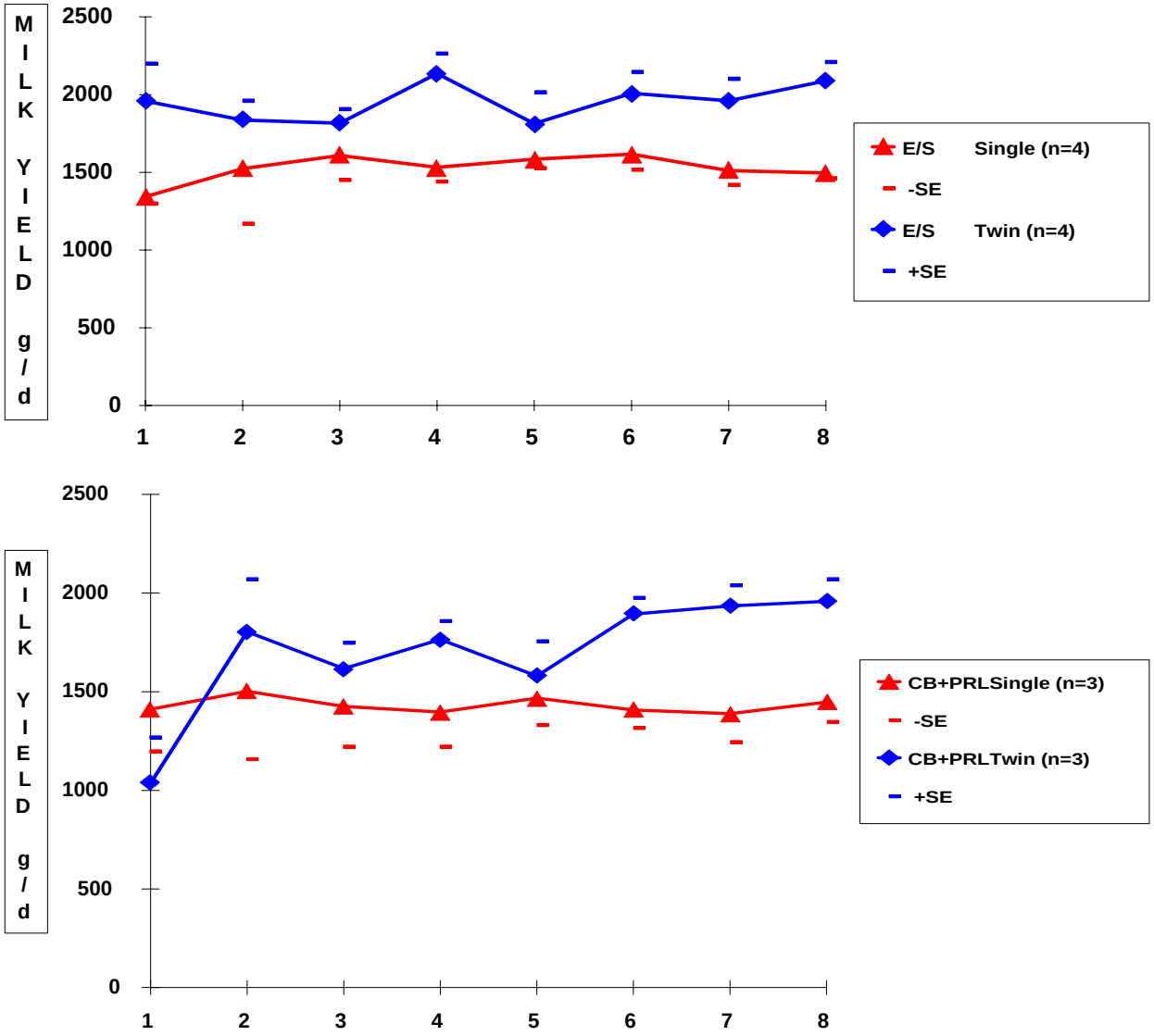
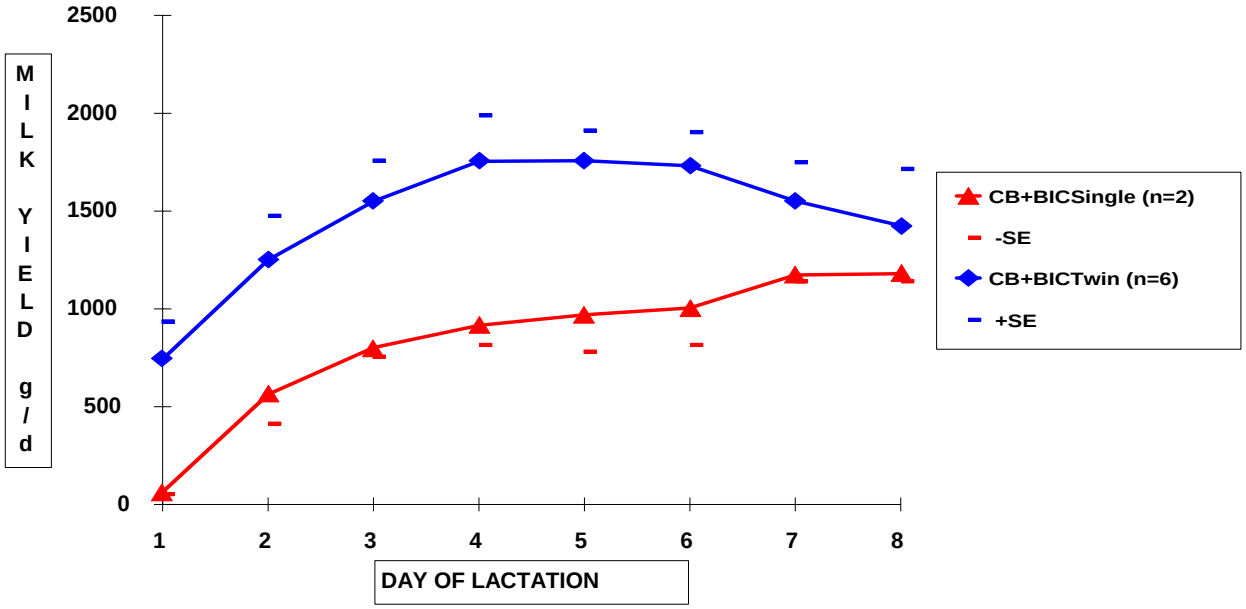

PROLACTIN ng/ml
25
20
15
10
5
0
-18.0
-13.0
-8.0
-3.0
2.0
7.0
DAY RELATIVE TO PARTURITION
CB+BICSingle (n=2)
-SE
CB+BICTwin (n=6)
+SE

FIG 3.26 MEAN±SE MILK YIELDS (g/d) IN SINGLE- AND TWIN-BEARING EWES TREATED PREPARTUM WITH CB154 (7d) PLUS BICARBONATE (2d)(CB+BIC), CB154 (7d) PLUS oPRL (2d)(CB+PRL), OR WITH ETHANOL/SALINE (7d)(E/S)




MILK YIELD g/d
2500
2000
1500
1000
500
0
1
2
3
4
5
6
7
8
DAY OF LACTATION
CB+BICSingle (n=2)
-SE
CB+BICTwin (n=6)
+SE

3.4 DISCUSSION AND CONCLUSIONS

The major objectives of trial 1 were to ascertain whether CB154 treatment inhibited PRL release in local ewes and whether this delayed lactogenesis. Secondary objectives were to determine the period of CB154 treatment necessary to delay lactogenesis and to identify any evidence for the existence of "type I" and "type II" ewes as described by Kann *et al.* (1978).

Treatment with CB154 in trial 1 was successful in reducing PRL to very low levels and in reducing early lactation milk yields. The lack of an effect of CB154 on progesterone concentrations, and the lack of a consistent effect on insulin concentrations, implies that differences in milk yields between the groups can probably be attributed to changes in circulating PRL levels. The fact that CB154 treatment has been found previously to have no effect on plasma concentrations of progesterone in ewes (Niswender 1974), or progesterone, placental lactogen, oestrone sulphate (Forsyth *et al.* 1985), GH, insulin or thyroxine (Hart 1976; Hart and Morant 1980) in goats, lends further support to this conclusion. However, reports that CB154 increased GH (Forsyth *et al.* 1985) and decreased insulin (Hart and Morant 1980; Johnsson *et al.* 1986) concentrations cannot be ignored, and the possibility remains that CB154 exerts its effects through some factor other than PRL. The situation with respect to insulin is confusing, since Hart has reported apparently conflicting results (Hart 1976; Hart and Morant 1980) relating to the same trial. The first report (Hart 1976) indicated no consistent effects of CB154 on insulin concentrations in goats, whereas the second report (Hart and Morant 1980) showed that long-term CB154 treatment (23 or 77 d, concomitant with oestradiol benzoate and progesterone treatment to induce lactation) reduced insulin concentrations during a subsequent 17-d milking period. This result is contrary to the finding in trial 1, in which CB154 treatment for 20 d (and perhaps also for 9 d) increased plasma insulin concentrations. No logical relationship is apparent between the higher prepartum insulin concentration in CB20 ewes and subsequent depressed milk yields.

As noted earlier (section 3.2), the statistical test for a delay in lactogenesis was the interaction CB20 versus E/S by time (i.e. testing the hypothesis that CB154 treatment depressed milk yields in early lactation but that CB154-treated ewes eventually attained the same milk yields as E/S ewes). In trial 1 this interaction was not significant, but there was a significant main effect of CB154 treatment. Moreover, visual examination of the data (Figure 3.4) suggests that milk yields of the 3 treatment groups were converging towards the end of the milking period. Thus it can be concluded that CB154 treatment was effective in delaying lactogenesis (i.e. in delaying the onset of normal milk secretion). Since the two CB154-treated groups did not differ in milk yield (averaged across ranks) it is apparent that the 9 and 20 d periods of CB154 treatment were equally effective in depressing milk yields.

CB154 treatment was, however, more effective in reducing milk yield in twin-bearing than in single-bearing ewes when used for the longer 20 d period (Figure 3.17). For example, the ratio of milk yield on day 1 of lactation for the E/S : CB9 : CB20 groups was 1 : 0.46 : 0.68 for single-bearing ewes whereas it was 1 : 0.46 : 0.11 for twin- bearing ewes. In fact, on the first day of lactation milk production was completely inhibited in two of the twin-bearing ewes, and almost completely inhibited in another two, but only completely inhibited in one single-bearing ewe. The 9 d CB154 treatment period reduced milk yields by about the same proportion in single- and twin-bearing ewes. In contrast, accumulation of precolostrum in the udder was not affected by long-term bromocriptine treatment in does carrying twin foetuses but in single-bearing does it was delayed about 4-6 weeks (Forsyth *et al*. 1985).

The mechanism by which long periods (20d) of CB154 treatment differentially affected milk yields in the single- and twin-bearing ewes is unclear. The interaction between effects of length of CB154 treatment (9 versus 20 d) and rank (single- versus twin-bearing) on plasma PRL levels was nonsignificant, indicating that different lengths of CB154 treatment had the same effects on plasma PRL concentrations in single- and twin-bearing ewes. Thus the differential effects on milk yield cannot be explained by corresponding effects on plasma PRL concentrations during the pre- or postpartum periods. This leaves two other possible explanations. Circulating PRL during the period 20 to 10 d prepartum may have an important effect on milk yield in twin- but not single-bearing ewes with the result that suppression of PRL levels during this period reduces milk yields in the former group but not in the latter group. Alternatively, the effect of the CB154 may be mediated via other hormones (but apparently not progesterone or insulin). For example, twin-bearing ewes have higher plasma concentrations of oPL than single-bearing ewes at this time (Gluckman *et al.* 1979; Oddy and Jenkin 1981) and CB154 treatment might have affected these levels. However, Martal and Lacroix (1978) and Forsyth *et al.* (1985) could find no effect of CB154 treatment on circulating PL concentrations in ewes and does respectively, although Martal and Lacroix (1978) reported that CB154 treatment increased placental oPL concentrations 4 to 6 times.

The lack of differences between the treatment groups in lactose percentage over the first 7 d of lactation indicates that lactose synthesis was inhibited by the treatments to the same extent as milk volume. Indeed, since lactose synthesis is the major factor determining milk yield (Linzell and Peaker 1971b) it is assumed that the CB154 treatment delayed lactogenesis by delaying the initiation of normal lactose synthesis. There is no apparent explanation for the difference in lactose percentage between the CB20 and CB9 groups over the weekly intervals.

Interpretation of other milk composition results was complicated by the differences in milk yields. The lack of differences in milk fat percentage suggests that milk fat synthesis was inhibited to the same degree as milk volume (i.e. lactose synthesis). The much higher protein content measured in the secretion collected during the first few days of lactation from the CB154-treated groups was

probably due initially to immunoglobulin secretion not being inhibited relative to milk volume. This is entirely possible since the immunoglobulins in colostrum are transferred from the blood rather than being synthesised in the glandular epithelium. The secretion collected from CB154-treated ewes had a thicker consistency than that from E/S ewes on the first day or two, and in ewes producing very low volumes it was often so thick as to make removal very difficult. However, since the milk composition analyses measured only total protein it is not possible to determine changes in glandular protein synthesis during the initial stage of lactation. Similar results have been reported in cows; prepartum CB154 treatment depressed the onset of lactation, reduced the concentration of lactose, increased protein concentration and altered the casein composition of colostrum (Karg and Schams 1974). Johke and Hodate (1983) reported that CB154 injections during the 2 weeks prepartum markedly decreased concentrations of α-lactalbumin and lactose in the colostrum but that ß-lactoglobulin and IgG concentration increased. Since large concentrations of immunoglobulins are normally present in milk only during the initial stage of lactation, when the pool of immunoglobulin which has accumulated during the prepartum period is washed out (Newstead 1976), the continued difference in protein percentage past the first few days in the present trial cannot readily be attributed to differences in immunoglobulin secretion. The immunoglobulin transfer from the blood is probably unaffected by CB154 while the synthesis of lactose, and hence milk volume, is delayed. This would result in an increase in the concentration of immunoglobulin in milk which, because of the increased viscosity and reduced milk volume, might take longer to be washed from the gland. This may only partly explain the increase in milk protein concentration since its continued elevation for 4 weeks indicates that protein synthesis was less affected by CB154 treatment than was milk volume (i.e. lactose synthesis). These results suggest that initiation of normal milk protein synthesis is not controlled by PRL alone.

Examination of the plasma PRL profiles of individual ewes for the presence of "type I" and "type II" ewes was necessarily limited to the E/S group, since CB154 treatment suppressed PRL concentrations in the other two groups. There was no suggestion, within this limited sample (n=9), of the existence of the two "types" of ewe. The progesterone profiles of all ewes were examined, since CB154 had no effect on mean progesterone concentrations, and these also provided no evidence of the existence of "type I" and "type II" ewes. However this trial was not specifically designed to identify such differences and no statistical test was considered appropriate.

Having determined that CB154 treatment for 9 d or more prepartum effectively reduced plasma PRL concentrations and delayed lactogenesis, it was necessary to establish that the responses to CB154 were effected by changes in circulating PRL concentrations. This was tested in trial 2, by comparing the effects of oPRL with those of bicarbonate in CB154-treated ewes, and with the effects of ethanol/saline treatment. Based on the results of trial 1, it was intended to administer

CB154 for 9 d prepartum, but ewes lambed on average 2 d earlier than expected, resulting in a mean prepartum CB154 treatment period of 7 days.

In trial 2, completed in autumn, plasma PRL concentrations were much lower than those in trial 1 despite the fact that ewes in both trials were exposed to the same photoperiod during the experimental period. Longer photoperiod increases PRL levels greatly in wethers (Trenkle 1978; Eisemann *et al.* 1984a and 1984b) and in both pregnant and lactating ewes (Munro *et al.* 1980; Perier *et al.* 1986). It is likely that the pattern of change in photoperiod prior to each trial had a carry-over effect on PRL levels. In fact, recent evidence indicates that circannual rhythms are generated by an endogenous process that is synchronised by exposure to long days during the previous spring and summer (Karsch and Wayne 1988; Malpaux *et al.* 1989; Wayne *et al.* 1990). It is also possible, although unlikely, that the different composition of the diet in the two trials (spring pasture versus autumn pasture) was responsible for the differences in circulating PRL levels. These possibilities will be addressed in Chapter 5.

Plasma PRL levels were lower in the E/S than in the CB154-treated ewes before treatment began (Figure 3.18) which can only have been due to a chance effect associated with the random assignment of ewes to treatment groups. This difference was essentially maintained from the start of CB154 treatment until parturition, suggesting that CB154 had no effect on plasma PRL concentrations. It is possible that this dose of CB154 was unable to decrease PRL concentrations further than the naturally low levels recorded in the autumn. Although blood sampling did not coincide with the mean date of parturition, the periparturient peak in plasma PRL concentrations was evident in three E/S ewes and it is therefore probable that all E/S ewes had such a peak whereas CB+BIC ewes did not. The oPRL administration would have restored plasma PRL levels in CB+PRL ewes to values similar to the normal peak, as indicated by the data presented in Figure 3.1.

The administration of oPRL (CB+PRL group) resulted in milk yields similar to those in the E/S ewes and significantly greater than those in the CB+BIC ewes, indicating that oPRL prevented the CB154-induced reduction of milk yields. Administration of PRL in CB154-treated cows also resulted in restoration of milk yields (Akers, Bauman, Capuco *et al.* 1981).

Milk yields in CB+BIC twin-bearing ewes were substantially reduced by CB154 treatment (e.g. to 38% of E/S group yields on d1), but the reduction was much greater in single-bearing ewes (e.g. to 5% of E/S group yields on d1). This difference must be interpreted with caution because there were only 2 ewes in the CB+BIC group and, perhaps as a consequence, the CB+BIC versus E/S by rank interaction was nonsignificant with respect to milk yield. However, plasma PRL concentrations were reduced by CB154 treatment to a greater extent in single- than in twin-bearing ewes during the period d 2 to d 7 postpartum (Figure 3.25). This may explain the low milk yields of single-bearing CB+BIC ewes during early lactation. However it does not explain the

inconsistency in effects of 9 d (trial 1) versus 7 d (trial 2) CB154 treatment on milk yields of single-bearing ewes. These results are likely to be confounded by the seasonal differences between trials 1 and 2. It was noted that milk yields were higher in the spring-lambing ewes than in the autumn-lambing ewes (2082 ±133 versus 1732 ± 92 g/d in the E/S ewes).

Treatment with CB154 had the same effect on milk composition in trial 2 as it did in trial 1. Milk fat and lactose content were unaffected while protein concentration was increased. Administration of oPRL prevented the CB154-induced increase in milk protein concentration, presumably by increasing milk yield (lactose synthesis) by a greater proportion than it did protein synthesis.

To summarise, these trials have shown that CB154 treatment for 7 d or more prepartum was effective in delaying or inhibiting complete initiation of lactogenesis. Since the injection of 0.5 mg/kg LWT oPRL resulted in plasma concentrations similar to those observed during the normal periparturient surge, and reversed the effects of CB154, it was concluded that PRL has an important role in enabling the rapid and complete initiation of lactogenesis. Varying responses to the different lengths of CB154 treatment, especially between single- and twin-bearing ewes, suggested that the timing of oPRL administration may affect subsequent lactation, but the success of the 2 daily oPRL injections indicated that this frequency and the doses used were adequate for subsequent trials. It was considered necessary to further investigate the relationship between the seasonal differences in PRL levels and milk yields, and to determine whether nutritional factors contributed to these differences. Furthermore, it was considered appropriate to investigate the effectiveness of alternative routes of oPRL administration, especially direct administration into the udder. This might confirm a direct action of oPRL on the mammary gland (without the need for an intermediate hormone) and could lead to an effective method of increasing milk yields by oPRL supplementation.

CHAPTER 4

THE EFFECT ON LACTOGENESIS OF INTRAMAMMARY ADMINISTRATION OF OVINE PROLACTIN AT PARTURITION IN CB154-TREATED EWES

4.1 INTRODUCTION

Although PRL is known from *in vitro* studies to act directly on the mammary gland tissue to stimulate the synthesis of all the major milk solids (Kelly *et al.* 1984), the possibility that, *in vivo*, it influences lactogenesis (or other processes) via one or more intermediate hormones has not been eliminated (Friesen *et al.* 1985; Rillema 1985; Vonderhaar *et al.* 1985; Vonderhaar 1987). Furthermore, no reports have established whether PRL is active when introduced directly into the mammary gland of ruminants, and no published work has reported an attempt to increase milk yields by peripartum oPRL supplementation of ewes (either with artificially reduced, or with normal, circulating PRL levels).

PRL has been previously administered to ruminants by continuous i.v. infusion (Hooley *et al.* 1978; Akers, Bauman, Capuco *et al.* 1981) and by a series of daily injections (Plaut *et al.* 1987). The former method is impractical for larger scale trials and the latter might be expected to provide an unnatural profile of plasma PRL concentrations. A preferable alternative would be a slow release implant to release the hormone at a steady, controlled rate, but this technology is presently not available for PRL. However if oPRL is effective when administered directly into the mammary gland of the ewe then problems associated with systemic administration can be avoided. Direct administration of oPRL into the mammary gland of pseudopregnant rabbits (via the teat duct) resulted in the secretion of milk and increased activity of lipoprotein lipase in the mammary gland (Falconer and Fiddler 1970) suggesting that oPRL acts directly on the mammary gland to initiate lactogenesis (at least in the rabbit).

If intramammary injection of oPRL were effective in increasing the milk yield of sheep, this route would have several major advantages. First, the amount of hormone required might be much lower than that for administration via other routes, since there would be no need to raise circulating concentrations of PRL. Second, the secretions within the gland might act as a reservoir for PRL, presenting it to the epithelial cells in high concentrations over an extended period (a natural slow release mechanism). Third, if the dose was correct, oPRL release into the circulation might be so slow that plasma levels would not rise significantly. Thus any response

obtained could be attributed directly to an action of PRL within the gland, not requiring the presence of a hypothetical intermediate hormone or systemic actions of PRL.

The objective of the trials reported here was therefore to establish whether the administration of oPRL via the intramammary route (at a dose which would not appreciably elevate circulating levels of PRL) was capable of eliminating the delay in the onset of lactogenesis caused by treatment with CB154.

4.2 MATERIALS AND METHODS

In order to determine a suitable intramammary dose of oPRL, which would not appreciably raise plasma concentrations of PRL, a preliminary trial was carried out to determine the effect on plasma PRL concentrations of intramammary administration of oPRL. It was necessary to carry out an empirical experiment since the rate constant for the release of oPRL from the mammary gland has not been determined in any animal, and the rate constant for the disappearance of PRL from the blood of the ewe has not been reported. Thus it was not possible to calculate the required dose. The pilot trial was carried out in May. Subsequently, two major trials were completed; the first in August-September and the second the following August, to examine the effect on lactogenesis of intramammary oPRL administration.

PILOT TRIAL

For the preliminary trial, two ewes were selected from a flock synchronised with progesterone-impregnated CIDRs, induced to ovulate using PMSG and mated over a 36-h period. Twin-pregnant (determined by ultrasound diagnosis) multiparous ewes, aged 4 or 5 years were selected. The ewes were housed indoors from 3-4 weeks prepartum until 14 d postpartum, and fed calculated requirements of pasture and sheepnuts. Photoperiod was set at 18L:6D. Both ewes were treated with daily subcutaneous injections of 2 mg CB154 (dissolved in 60% ethanol/40% saline) to inhibit PRL release, beginning 9 d before the expected mean date of parturition and continuing until the fifth day after they gave birth. Indwelling jugular cannulae were inserted 3 days after CB154 treatment began and, on the next day each ewe was treated with a single intramammary dose of 0.5 mg/kg LWT oPRL (10 mg/ml solution). This was the same dose used previously for s.c. treatment (Figure 3.1) and therefore allowed a comparison of circulating PRL levels following the two routes of administration. The hormone was injected via the teat duct of the right gland while the left gland was treated with an equivalent volume of excipient (0.03M bicarbonate in 0.15M NaCl, henceforth referred to as BIC). This route of PRL administration will henceforth be referred to as "i.mam." (intramammary via the intraductal route). Both ewes lambed 2 d earlier than expected, resulting in a prepartum CB154 treatment-period of 7 d, rather than the intended 9 d, and PRL administration at day -3 relative to parturition.

Blood samples were collected via the jugular cannulae at 15 min intervals for 30 min before oPRL administration (0900 h), then at hourly intervals for 24 h. Samples were taken every 4 h during the period 24-48 h after oPRL administration, and subsequently at 0900 h on each day for 12 d. All plasma samples were analysed for PRL in the same assay as the samples taken from ewes treated with a s.c. oPRL injection (see Chapter 3).

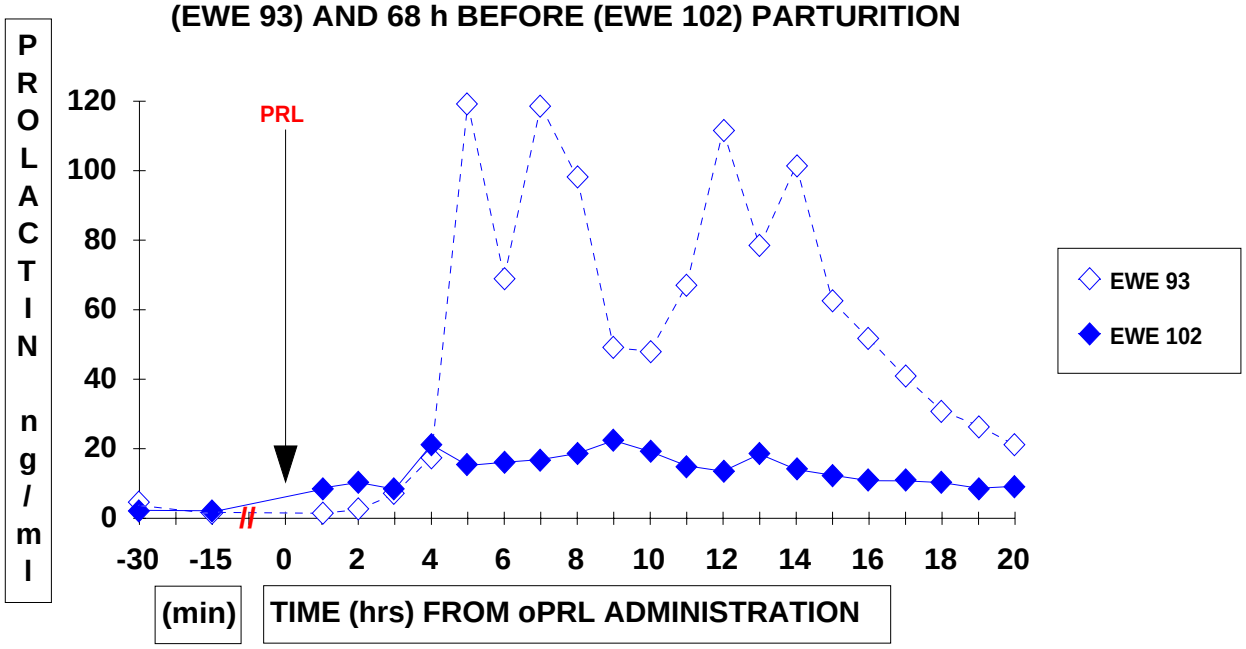
Ultrasound pregnancy diagnosis of the ewes in this trial proved to be inaccurate. Ewe 93 had only 1 lamb, and Ewe 102 had triplets, but one was stillborn. Ewes 102 and 93 lambed about 68 and 79 h after oPRL treatment respectively. During the 24-h period following oPRL treatment, examination revealed that the right (oPRL-treated) gland of each ewe was warmer than the left (control-treated) and, because of the possibility of intramammary infection, both ewes were given 6 ml of Streptopen (procaine penicillin 250,000 i.u./ml and dihydrostreptomycin sulphate 250,000 i.u./ml, Glaxo Animal Health (NZ) Ltd, Palmerston North, New Zealand) by s.c. injection, 10 h after PRL administration. Inflammation of the oPRL-treated glands may have affected the rate of release of oPRL into the circulation.

Ewe 102 suffered seriously from pregnancy toxaemia and scours, and was off her feed, beginning on the day of PRL administration. She was treated with Ketol (propylene glycol and glycerol, Veterinary Ethicals Ltd, Auckland, New Zealand) (120 ml orally, 8.5, 15.5 and 24 h after PRL administration) and with calcium borogluconate (25% solution, 85 ml s.c. and 15 ml i.v. at 15.5 h, and 12 ml i.v. at 48 h, after PRL administration). She recovered after parturition (which occurred at about 68 h after PRL administration).

The mean plasma PRL concentration in the two pretreatment blood samples was 2.4 ng/ml (range 1.8-4.7 ng/ml). Compared to the s.c. injection of oPRL previously described (see Figure 3.1), i.mam. administration resulted in a much slower and smaller rise in plasma PRL concentration (Figure 4.1). Ewe 98 reached peak concentrations of 120 ng/ml after 5 and 7 h. In Ewe 102 the rise was very small, reaching peak levels of 21 ng/ml and 22 ng/ml at 4 and 9 h post-treatment respectively. While these values are very low compared to those for the s.c. treated ewes reported in Chapter 3, they are higher than the mean values recorded for any of the three groups in trial 2, Chapter 3 (see Figure 3.18). This indicates that the i.mam. treatment of 0.5 mg/kg LWT oPRL increased plasma PRL concentrations above the basal levels characteristic of autumn-lambing ewes.

By 24 h post-treatment, plasma PRL concentrations in both ewes had declined substantially to values of 11 and 23 ng/ml. After a further 24 h, PRL levels in both ewes (data not shown) had reached basal levels similar to those seen in trial 2 (Chapter 3).

FIG 4.1 PLASMA PROLACTIN CONCENTRATIONS (ng/ml) IN 2 EWES TREATED FOR 7 d PREPARTUM AND 5 d POSTPARTUM WITH CB154, AND WITH 0.5 mg/kg LWT oPRL BY A SINGLE INTRAMAMMARY INJECTION VIA THE TEAT DUCT, 79 h BEFORE (EWE 93) AND 68 h BEFORE (EWE 102) PARTURITION



The mammary gland secretions of both ewes were examined 24 h post-treatment. Ewe 93 had a watery fluid in the BIC-treated gland while the secretion in the PRL-treated gland had the appearance of normal milk. Ewe 102 had a watery fluid in both glands. Although the secretion from the PRL-treated gland was cloudier than that from the contralateral gland, it did not have the appearance of normal milk.

The pilot trial therefore showed that the plasma concentration of PRL was elevated in both ewes following the intramammary injection of oPRL but that the size of the increase was much lower than that observed following the s.c. injection of the same dose per unit live weight (Figure 3.1). Nevertheless, it was apparent that the dose used in future trials needed to be considerably lower than 0.5 mg/kg in order to prevent an increase in plasma PRL concentrations that might be sufficient to initiate lactogenesis in the control gland.

The final i.mam. dose used was based on a consideration of expected oPRL concentrations in the gland. Assuming a gland volume of 500 ml (Anderson 1975) a dose of 10 mg would result in oPRL concentrations within the gland of about 20,000 ng/ml (assuming an even distribution throughout the gland). If the rate of transfer of the oPRL from the gland to the circulation were also dose-dependent, reducing the dose to 10 mg (c.f. 30 mg used in the pilot trial) would cause a peak circulating level of 40 ng/ml (based on Ewe 102) or 7 ng/ml (based on Ewe 93). These values are considerably lower than the recorded peripartum peak values and should not be

sufficient to systemically trigger lactogenesis. If this assumption were incorrect and the dose was sufficient to systemically trigger lactogenesis then oPRL-treated ewes would exhibit bilateral lactogenesis (compared to control ewes treated with BIC in both glands). Accordingly, a dose of 10 mg/gland was used in subsequent trials.

TRIAL 3

Following pregnancy diagnosis 29 ewes were selected from a flock synchronised with progesterone-impregnated CIDRs and mated over a 36-h period. The group included 24 primiparous (two year old) and 5 multiparous (3-7 years) ewes. Ewes were allocated to one of two groups at random, except that groups were balanced as much as possible for age, live weight and pregnancy rank. Twenty ewes were allocated to the oPRL treatment group (CB+PRL) and 9 to the control group (CB+BIC). They were grazed at pasture (mainly ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*)) throughout the trial.

In all ewes PRL release was inhibited using daily s.c. injections of 2 mg CB154 (dissolved in 60% ethanol/40% saline), beginning 18 d before the expected mean date of parturition and continuing until d 2 postpartum. The prepartum CB154 treatment period was increased from the 7 or 9 d periods used in trials 1 and 2 (Chapter 3) because it was evident that lactogenesis was little affected in the twin-bearing ewes by these shorter periods, compared to the delay produced by the 20-d period used in trial 1. The postpartum period of CB154 treatment was reduced from the 5 d used in trial 2 (Chapter 3) because the 5-d period was apparently no more effective in delaying lactogenesis than the 2-d period used in trial 1 (Chapter 3).

Each of the 20 ewes in the CB+PRL group received 10 mg of oPRL (dissolved in 1 ml of BIC) injected into one gland (10 ewes injected in the left gland and 10 in the right gland). The contralateral gland was treated with 1 ml BIC. The CB+BIC group (9 ewes) received BIC in both glands. In an attempt to minimise infection of the mammary gland, teats were cleaned first with a solution of Hibitane (Chlorhexidine gluconate 5% w/v. ICI PLC, Macclesfield, Cheshire, England) in water and then with 70% ethanol. Between uses, the needle used for intramammary injections was immersed in 70% ethanol. Because of the inability to accurately predict the lambing date of ewes, it was decided to administer the oPRL on the two consecutive days immediately prior to the expected mean date of parturition in order to ensure that intramammary oPRL concentrations were elevated at a time close to parturition. Injections were administered between 1100 and 1200 h on each day.

Following lambing, ewes were milked daily for 8 d and milk yields calculated as described previously (Chapter 2). Any ewes which developed mastitis were treated with 5 ml Streptopen administered by s.c. injection in the neck. Ewes were returned to pasture between morning and afternoon milkings, while lambs were held indoors and fed (as described in Chapter 2).

Beginning at the start of CB154 treatment, blood samples were collected by jugular venipuncture at 5-d intervals until the days of oPRL treatment, when samples were collected at 1030, 1800 and 2000 h on each of the 2 treatment days. Thereafter 2 further blood samples were taken at 5-d intervals.

TRIAL 4

Trial 4 was essentially a repeat of trial 3, carried out in spring one year later . Eleven ewes (5 twin-bearing and 6 single-bearing) which had been synchronised with progesterone-impregnated CIDRs and mated over a 3-d period were used in the trial. All were treated with 2 mg/d CB154 beginning 13 d before oPRL treatment and ending on d 2 postpartum. All ewes were treated on 2 consecutive days with oPRL i.mam. in one gland while the contralateral gland was treated with BIC (as in trial 3). Injections were administered between 1100 and 1200 h on each day. Due to the low number of ewes available (the consequence of a severe facial eczema outbreak) there was no separate control group. The BIC-treated gland within each ewe was therefore the control for comparison with the PRL-treated gland. On each day of oPRL treatment, all ewes received a s.c. injection of 5 ml Streptopen antibiotic. In addition, on the first day of milking each ewe was treated with 2.5 million i.u. of Leocillin (Penethamate Hydriodide, a slightly soluble hydriodide of an ester of benzylpenicillin which is actively taken up by the mammary gland (Edwards 1966)). Ewes which subsequently showed any sign of mastitis were given 10 ml Streptopen s.c. Ewes were milked each day for 10 d then subsequently on d 12, 14, 17, 21, 28, 35, 42, 49 and 56 postpartum.

Blood samples were collected by jugular venipuncture 6 d and 2 d before oPRL treatment and at 1100, 1800 and 2000 h on the days of oPRL treatment. Subsequently blood samples were collected at 2-d intervals until 21 d after the mean date of parturition.

Milk samples were analysed for milk fat, protein and lactose content, and plasma samples were analysed for PRL in both trials.

Data were analysed using the computer statistical package REG (Gilmour 1990). Multivariate (repeated measures) analysis of variance was used to analyse all time-series data. PRL data (log transformed) were separated into 3 periods, corresponding to pre-treatment, treatment, and post-treatment periods, for statistical analysis. Multivariate analyses were also carried out on all milk yield and composition data (the latter being arc sine transformed).

For analysis of PRL data in trial 3, ewes were classified into 2 treatment groups: those which received BIC (CB+BIC), and those which received oPRL in one gland and BIC in the other (CB+PRL). For analyses of milk yield and composition data, glands, rather than ewes, were classified into 3 treatment groups. Control group glands were those in ewes treated with BIC in both glands while BIC and PRL group glands were those in ewes treated with BIC and PRL in

contralateral glands respectively. In trial 4, since all ewes received the same treatment, there was no analysis of PRL data and glands were classified as either PRL-treated or BIC-treated. In both trials the test for a delay in the onset of lactogenesis was the interaction of group contrasts with time. In trial 4 there were insufficient d.f. for more than 8 repeated measures in each analysis.

4.3 RESULTS

TRIAL 3

Of the 29 ewes, 3 were removed from the trial, one due to a blocked teat canal, one due to a stillborn lamb and another due to lambing one oestrous cycle late. This left 26 ewes, 22 two-years old and 4 older ewes (aged 3, 4, 6 and 7 years), 18 of which were treated with oPRL and 8 with BIC. The PRL group comprised 10 single-bearing and 8 twin-bearing ewes while the BIC group contained 5 single-bearing and 3 twin-bearing ewes.

Ewes lambed over a period of 7 d and there was no significant difference between the CB+PRL and CB+BIC groups in mean lambing date. The mean length of the prepartum CB154 treatment period was 19 d and the mean day of parturition was 2 d after the second administration of oPRL or BIC. The day on which blood samples were collected relative to the day of parturition varied between ewes because the actual day of lambing could not be accurately predicted when the prepartum samples were taken. Thus reported sampling days in the figures are the mean ± (SE) 0.3 d of the actual sampling days.

TREATMENT EFFECTS

CB154 was effective in suppressing plasma PRL concentrations to very low levels during the period before PRL and BIC treatments were administered (Figure 4.2). In that period only 2 ewes (both in the CB+PRL group) exhibited values higher than 6 ng/ml while most ewes maintained plasma PRL concentrations below 1 ng /ml during this period.

Plasma PRL concentrations did not differ significantly between the CB+BIC and CB+PRL ewes when analysed over the period before PRL and BIC treatments were administered (d -19 to -4) or during the post-treatment period (which included d -2 (the last sample taken), d 3 and d 8) (Figure 4.2). Nor did circulating PRL levels differ significantly between groups on the 2 days when oPRL and BIC treatments were administered (Figure 4.3). The increase in PRL concentration in the CB+PRL group on the first day of treatment (d -3 prepartum, 6 h after the first oPRL injection) was due to a high concentration (339 ng/ml) recorded in one ewe (Ewe 81) which exhibited much higher plasma PRL levels than the others throughout the trial. The highest concentration found in the plasma of any other ewe at that time was 7.4 ng/ml, while 21 of the ewes had levels below the assay sensitivity (1 ng/ml). Thus, data for the CB+PRL ewes are presented both with (CB+PRL n=18), and without (CB+PRL n=17), data for Ewe 81.

FIG 4.2 MEAN±SE PLASMA PROLACTIN CONCENTRATION (ng/ml) IN EWES TREATED WITH CB154 FOR 19 d PRE- AND 2 d POST-PARTUM, AND WITH 10 mg oPRL VIA ONE TEAT DUCT AND 1 ml BICARBONATE VIA THE OTHER (CB+PRL), OR WITH 1 ml BICARBONATE VIA BOTH TEAT DUCTS (CB+BIC)

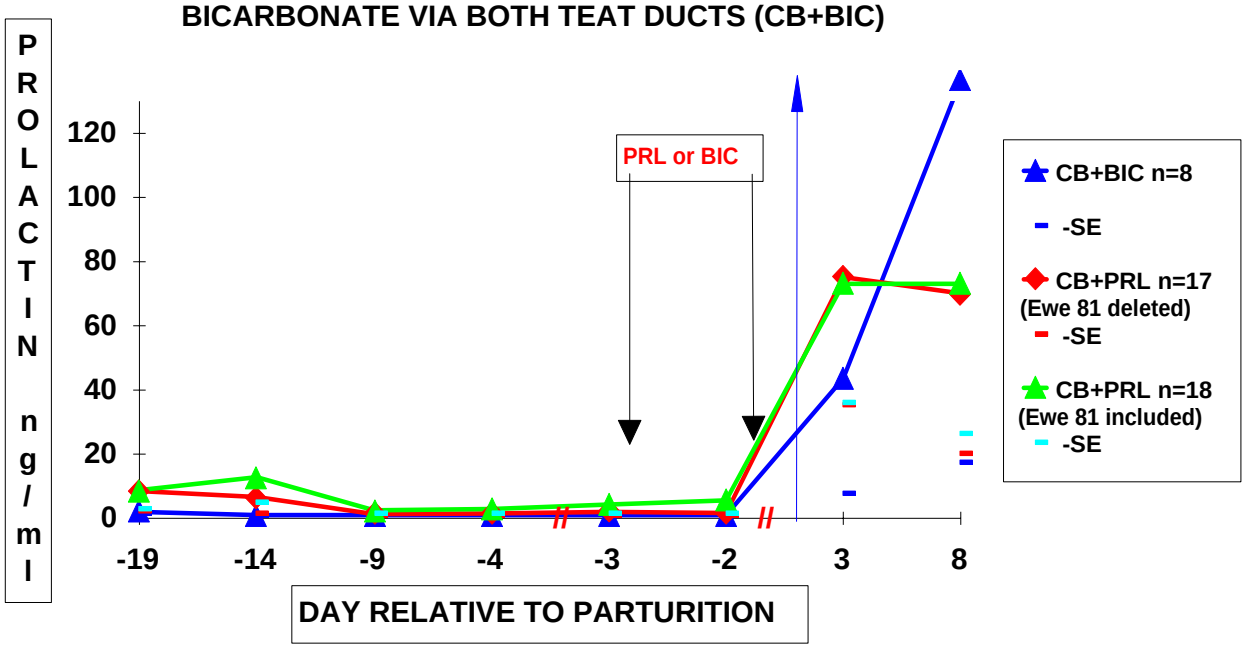


FIG 4.3 MEAN±SE PLASMA PROLACTIN CONCENTRATION (ng/ml) IN CB154-TREATED EWES, ON THE DAYS OF TREATMENT WITH oPRL VIA ONE TEAT DUCT AND BICARBONATE VIA THE OTHER (CB+PRL), OR WITH BICARBONATE VIA BOTH TEAT DUCTS (CB+BIC)

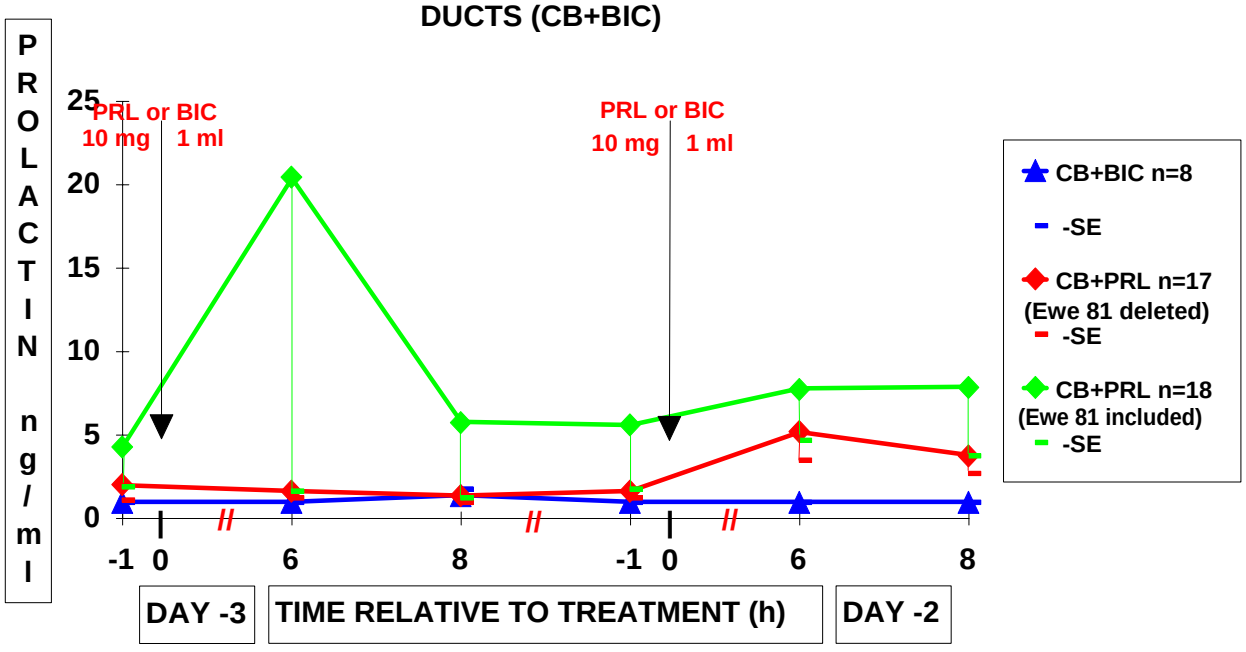
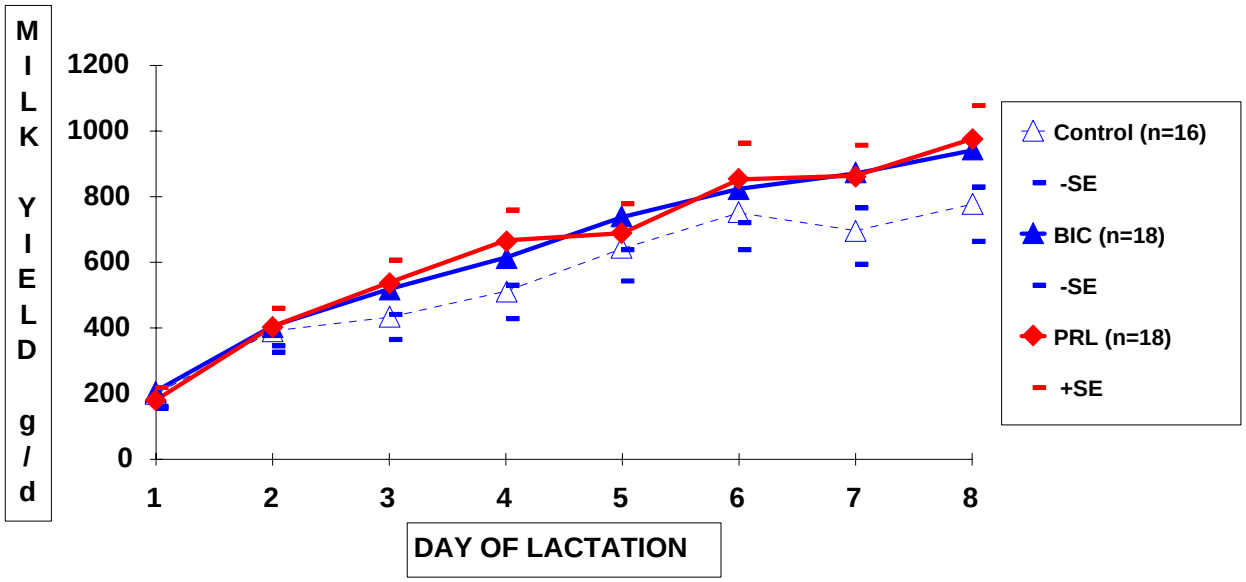


FIG 4.4 MEAN±SE MILK YIELDS OF MAMMARY GLANDS TREATED ON 2 d BEGINNING 3 d PREPARTUM, WITH 10 mg oPRL OR WITH 1 ml BICARBONATE ADMINISTERED VIA THE TEAT DUCT, IN EWES TREATED WITH CB154 FOR 19 d PRE- AND 2 d POSTPARTUM



Milk yields did not differ significantly between PRL- and BIC-treated glands (within the CB+PRL group) indicating that there was no effect of i.mam. oPRL administration on milk yields (Figure 4.4). Furthermore, there was no significant interaction of the group contrast PRL versus BIC with time, indicating that there was no delay in lactogenesis in the BIC-treated glands relative to the PRL-treated glands. There was no significant difference in milk yields between BIC-treated glands (in the CB+PRL group) and Control glands and no interaction of the group contrast BIC versus Control with time. Multivariate analyses with gland nested within ewe confirmed the lack of effect of oPRL on milk yields. There were no significant effects of oPRL treatment on milk fat, protein or lactose percentage (data not shown).

Clinical mastitis (blood and/or lumps detected on more than one occasion) was evident in 11 ewes, and suspected (detected only once) in a further 3 ewes. Mastitis was observed in 8 oPRL-treated glands and 5 BIC-treated glands (two ewes were infected in both glands). Antibiotic treatment (Streptopen) was administered a total of 21 times (to the 11 ewes) during the milking period.

TRIAL 4

All ewes lambed within a period of 7 d. Nine produced single lambs and two had twins. Due to the death of her lamb at birth, ewe 64 was milked twice daily for 21 d and again on d 28, 35 and 42 postpartum and her daily milk yields were estimated (by adjusting the weight of milk obtained for the time period between morning and afternoon milkings) in the same way as the milk yields of the other ewes were calculated. Ewe 130 was not milked after d 35 postpartum due to a skin infection on the udder.

The mean length of the prepartum CB154 treatment period was 15 d and the mean day of parturition was 2 d after the second administration of oPRL or BIC. The day on which blood samples were collected relative to the day of parturition varied between ewes because the actual day of lambing could not be accurately predicted when the prepartum samples were taken. Thus reported sampling days in the figures are the mean ± (SE) 0.5 d of the actual sampling days.

TREATMENT EFFECTS

Plasma PRL concentrations did not rise to detectable levels until 8 d postpartum (Figure 4.5). No increases in plasma PRL concentrations were detected in the samples taken 6 and 8 h after the intraductal oPRL administration.

Milk yields were significantly ($P<0.05$) higher in the PRL-treated glands than in the BIC-treated glands during the first 8 d of lactation (Figure 4.6). PRL-treated glands produced on average 15% more milk than BIC-treated glands over this 8-day period. Milk yields recorded at weekly intervals (week 1-8) approached significance ($P<0.1$) (Figure 4.6). The interaction of group contrasts with time was not significant over either period reflecting the fact that the difference in milk yields between PRL- and BIC-treated glands did not decrease during the 8-week lactation.

Milk lactose percentage was higher in samples collected from PRL-treated glands on the first 5 d of lactation and the difference approached significance ($P<0.1$) when analysed over the first 8-d period (Figure 4.7). Milk fat and protein percentage did not differ significantly between PRL-and BIC-treated glands (data not shown).

There was little evidence of mastitis. Blood was detected in the milk of only 3 ewes (3 BIC-treated and 1 PRL-treated glands) and it disappeared rapidly following further antibiotic treatment.

FIG 4.5 MEAN±SE PLASMA PROLACTIN CONCENTRATION (ng/ml) IN EWES TREATED WITH CB154 FOR 15 d PRE- AND 2 d POST-PARTUM, AND WITH 10 mg oPRL VIA ONE TEAT DUCT AND 1 ml BICARBONATE VIA THE OTHER TEAT DUCT

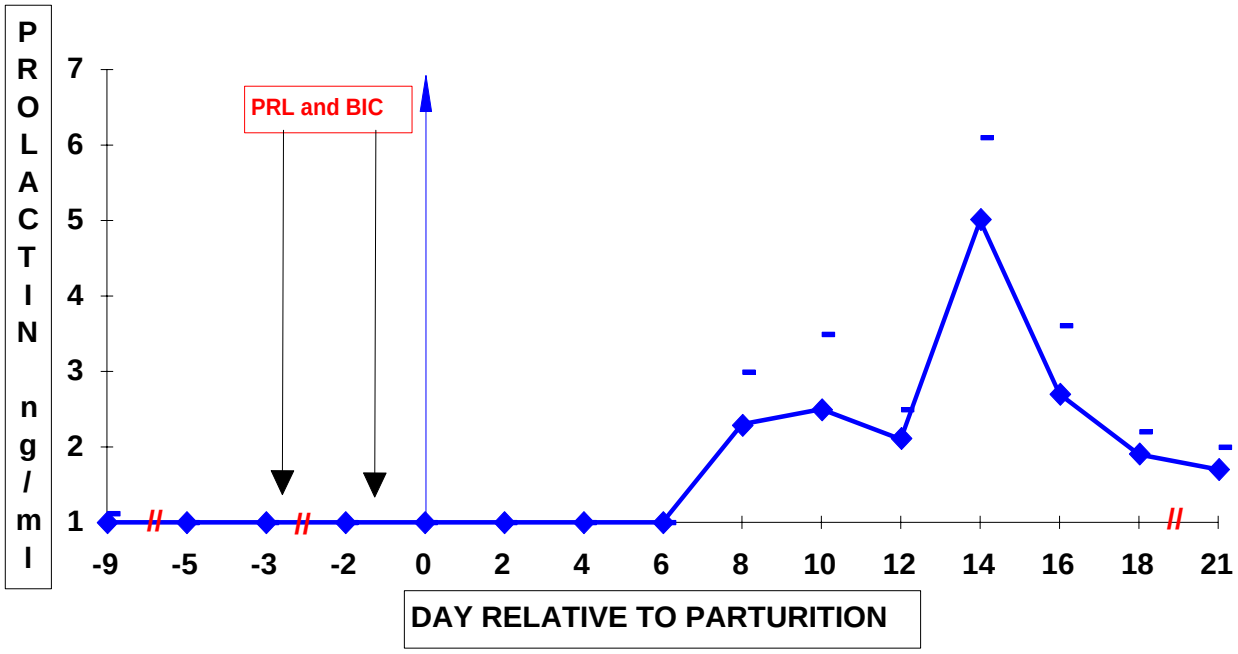


FIG 4.6 MEAN±SE MILK YIELDS OF MAMMARY GLANDS TREATED ON 2 d BEGINNING 3 d PREPARTUM, WITH 10 mg oPRL OR WITH 1 ml BICARBONATE ADMINISTERED VIA THE TEAT DUCT, IN EWES TREATED WITH CB154 FOR 15 d PRE- AND 2 d POSTPARTUM

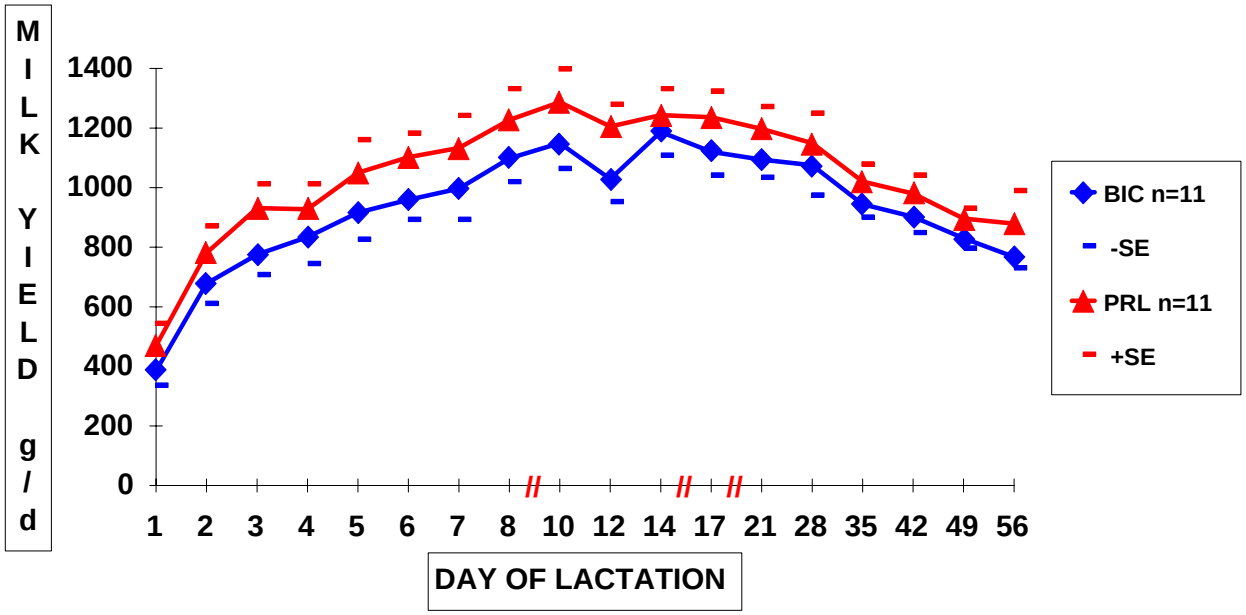
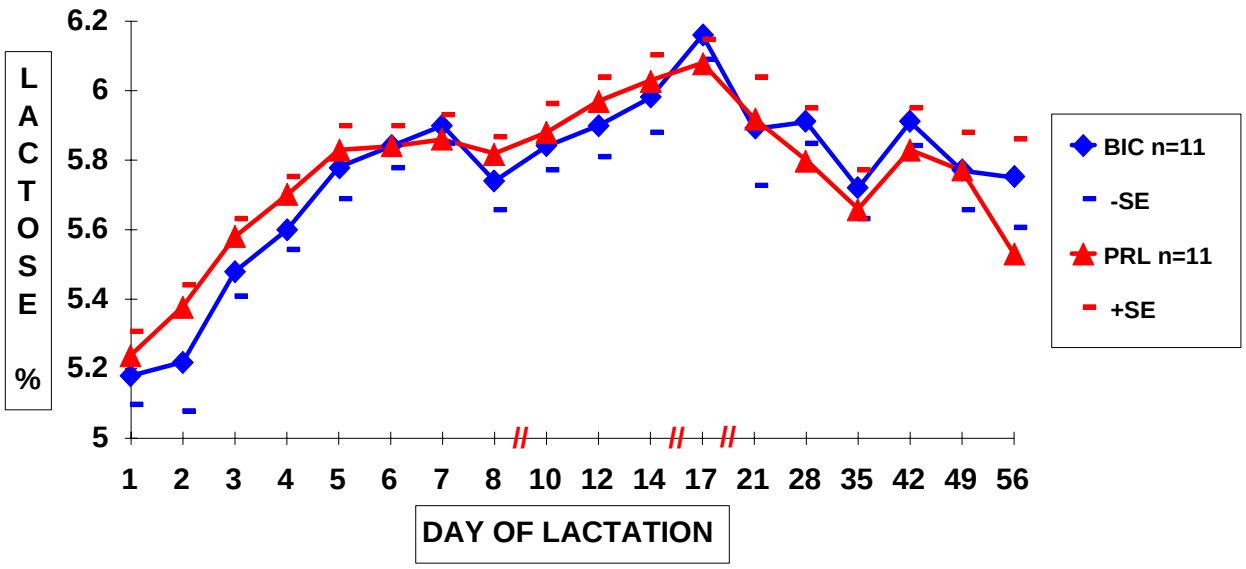


FIG 4.7 MEAN±SE LACTOSE PERCENTAGE IN MAMMARY GLANDS TREATED ON 2 d BEGINNING 3 d PREPARTUM, WITH 10 mg oPRL OR WITH 1 ml BICARBONATE ADMINISTERED VIA THE TEAT DUCT, IN EWES TREATED WITH CB154 FOR 15 d PRE- AND 2 d POSTPARTUM



4.4 DISCUSSION AND CONCLUSIONS

The first objective of trial 3 was to determine whether the empirically chosen 10 mg i.mam. dose raised circulating PRL concentrations, by comparing the plasma PRL levels of ewes treated with oPRL administered via the i.mam. route into one gland (CB+PRL), with those treated with BIC in both glands (CB+BIC). The second objective was to determine whether specific lactogenic responses to oPRL occurred in individual glands treated intraductally with the hormone.

It was evident that the dose of 10 mg/gland did not raise circulating PRL concentrations. Hence, any differences in milk yield between PRL- and BIC-treated glands could be attributed to the oPRL treatment. Furthermore, the milk yields of BIC-treated glands in CB+BIC ewes did not differ from those of BIC-treated glands in CB+PRL ewes, providing evidence that there were no effects of oPRL, transferred via the circulation from the treated gland, on the contralateral gland. Thus any effect of oPRL on lactogenesis in the oPRL-treated gland would not be masked by a carryover effect on the BIC-treated gland. However, milk yields did not differ between the oPRL- and BIC-treated glands in the CB+PRL ewes indicating that the i.mam. injection of oPRL had no effect on lactogenesis in this trial.

The reason for the failure of oPRL to affect lactogenesis is unknown, but it is probable that mastitis adversely affected milk yields in this trial and so it was decided to repeat the experiment,

taking greater precautions against mammary gland infection than in trial 3. In trial 4, however, prophylactic antibiotic treatment was apparently effective in minimising the incidence of mastitis.

As in trial 3, circulating PRL concentrations did not change following the i.mam. oPRL treatment in trial 4, so any difference between BIC- and PRL-treated glands could be attributed to the effect of treatment. The glands treated with oPRL produced significantly more milk than the BIC-treated glands, suggesting that oPRL stimulated lactogenesis when administered via the intraductal route (i.e. a local effect). The milk yield advantage in the oPRL-treated glands was maintained throughout the 8-week period, indicating that the effect did not merely represent the reversal of a CB154-induced delay in lactogenesis. Rather, it appeared that the oPRL had effected a permanent change in the ability of the gland to produce milk. This is consistent with the finding, in periparturient cows, that milk production during the subsequent lactation was correlated with the total PRL content of the mammary gland on the day of parturition (van Zyl *et al.* 1986).

Trial 4 thus demonstrated that oPRL can have lactogenic effects when administered directly into the mammary gland of CB154-treated ewes, apparently without the need for a systemic intermediate hormone such as synlactin (see Vonderhaar 1987). Furthermore, results indicate that the oPRL caused a permanent increase in milk yields (by an average 12%) in the treated gland (at least during the 8 week lactation period studied). It is therefore concluded that PRL is important to the complete initiation of lactogenesis in ewes, that it acts directly on the gland and that it may be necessary for establishing the maximum potential of the gland to secrete milk. Hartmann (1992) suggested that the endocrine system sets the upper limits to lactation while autocrine mechanisms down-regulate production. The mechanism by which oPRL might increase the potential milk secretion of the gland is unknown, but recent work by Molnaar *et al.* (1991) suggests a possibility. In mammary glands from pregnant and lactating ewes, a cRNA probe for the bovine α-lactalbumin gene revealed that α-lactalbumin gene expression was found in the secretory epithelium of some alveoli but not in others (Molnaar *et al.* 1991) suggesting that α-lactalbumin gene expression can be turned on and off in specific areas. Hence, it might be that PRL sets the upper limit during lactogenesis by determining the proportion of secretory cells which express the α-lactalbumin gene.

Further studies are required to determine if supplementary oPRL administered to normal (not CB154-treated) ewes can increase milk yields. Since low circulating PRL levels in autumn-lambing ewes are associated with their low milk yields (compared to those of spring-lambing ewes) (see Chapters 3 and 5) it would be logical to test the effect of supplementary oPRL, administered via the teat duct, in autumn-lambing ewes. This will be reported in Chapter 6. It would also seem logical to use a cRNA probe to test the effect of oPRL on a-lactalbumin gene expression.

CHAPTER 5

MILK PRODUCTION AND PLASMA PROLACTIN LEVELS IN SPRING- AND AUTUMN-LAMBING EWES

5.1 INTRODUCTION

In Chapter 3 (trials 1 and 2) it was noted that spring-lambing ewes appeared to produce higher milk yields than autumn-lambing ewes and that these higher yields were associated with higher plasma PRL concentrations. Although those trials were not designed to measure differences between spring- and autumn-lambing ewes, the experimental conditions (other than those associated with season) were very similar during the two trials.

Compared with the autumn-lambing control ewes (those not treated with CB154 or oPRL prepartum), spring-lambing control ewes had higher mean daily PRL levels (168±28 versus 20±9 ng/ml), higher mean milk yields (2082±133 versus 1732±92 g/d), lower milk fat (8.45±0.53 versus 10.66±0.28%) and protein (6.03±0.05 versus 6.54±0.15 %) concentrations, but similar mean lactose concentrations (5.23±0.08 versus 5.30±0.03 %). Although there was no difference between spring- and autumn-lambing ewes in postpartum live weight (60.4±1.2 versus 62.1±3.9 kg), spring-born lambs were heavier at birth (4.47±0.23 versus 3.81±0.26 kg), grew more rapidly over the first week of life (227±18 versus 184±12 g/d) and were thus 1.0 kg heavier at 7 d of age, than autumn-born lambs.

The cause of these differences between spring- and autumn-lambing ewes was not clear, but could reflect differences in photoperiod during the period before the trial, or differences in feed intake or composition of the diet.

This chapter describes a trial carried out to determine whether autumn-lambing ewes have lower milk yields than spring-lambing ewes when offered the same diet in both seasons and, if so, whether this is associated with differences in plasma PRL levels.

5.2 MATERIALS AND METHODS

Trial 5 involved studies carried out in the spring and the autumn and was specifically designed to identify possible seasonal differences in milk production and plasma PRL concentrations caused by differences other than dietary composition. Ewes studied in each season were sourced from the same flock and allocated randomly to either the autumn or spring trial.

For trial 5, groups of at least 50 ewes were synchronised with progesterone-impregnated CIDRs and mated to lamb in April or in August. Following ultrasound pregnancy diagnosis, 14 ewes from each group, selected on the basis of mating date and number of foetuses, were housed indoors in individual pens under constant photoperiod (18L:6D) from 3 weeks prepartum. The photoperiod was selected to enable all necessary activities, especially monitoring of lambing, to be carried out in light conditions, and because the 18L:6D photoperiod was used in trials 1 and 2. This trial differed from trials 1 and 2 in that ewes were fed chaffed meadow hay (50% of ME requirement) and concentrate (sheep nuts) (50% of ME requirement) from the same batches in both seasons. Daily feed allowances were based upon individual energy requirements calculated using equations derived from data for pregnant and lactating ewes (Rattray 1986) and adjusted for the reduced maintenance requirements of ewes housed indoors (Coop 1961), but not for changes in individual milk yields (see Chapter 2). Feed refusals were measured and the ME deficit (difference between ME requirement and ME intake) calculated for each ewe. Beginning 18 d before the expected mean date of parturition, jugular blood samples were collected once daily by venipuncture at 5-d intervals until 7 d postpartum (6 samples per ewe in total) and analysed for PRL. Following lambing, milk yields were measured on the first 8 d of lactation. Milk samples were analysed for fat, protein and lactose content. Lambs were weighed at birth and at weekly intervals until weaning at 8 weeks of age. Ewes which failed to adjust to the diet, delivered dead lambs, delivered a different number of lambs from that which was predicted or did not lamb within a week of the predicted time, were removed from the trial. This left 10 April-lambing (7 single-, 3 twin-bearing) and 10 August-lambing (7 single-, 3 twin-bearing) ewes in the trial.

Data were analysed using the computer statistical package REG (Gilmour 1990) . Multivariate (repeated measures) analysis of variance was used to analyse all time-series data. PRL data were log transformed and milk composition data (percentages) were arc sine transformed for repeated measures analyses. Regressions of mean milk yield (over the first 8 d of lactation) and mean PRL concentrations (over all 6 blood samples) on ewe live weight were calculated using untransformed means. Homogeneity of the within-season regression lines was tested according to Searle (1971). The same model was applied to moduli of residuals to test for heterogeneity of variances in the two seasons.

5.3 RESULTS

The spring-lambing ewes lambed over a 12-d period and the autumn-lambing ewes lambed over a 6-d period. The day on which blood samples were collected relative to the day of parturition varied between ewes because the actual day of lambing could not be accurately predicted when the prepartum samples were taken. Thus reported sampling days in the figures are the mean ± (SE) 0.4 d of the actual sampling days. The postpartum live weight of ewes was significantly

(P<0.001) higher in spring-lambing (62.2±1.7 kg) than in autumn-lambing (52.9±1.1 kg) ewes due to summer pasture shortages affecting liveweight gain in the latter group. Mean air temperatures in the months of August and April were 9.8 and 14.4 ^{O}C respectively.

Spring-lambing ewes had significantly (P<0.01) higher plasma PRL concentrations than autumn-lambing ewes over the experimental period (Figure 5.1) and had significantly (P<0.05) higher milk yields than autumn-lambing ewes over the first 8 d of lactation (Figure 5.2). Mean milk yields in the spring- and autumn-lambing ewes were 2041±114 and 1563±109 g/d respectively. However, neither the plasma PRL or the milk yield differences were significant when adjusted for the seasonal difference in ewe live weight. The regressions of mean PRL concentration on postpartum live weight were not statistically significant within seasons (when seasons were analysed individually) and the test of homogeneity of regression slopes for spring- and autumn-lambing ewes was non-significant (P>0.10) indicating that the regression lines did not have different slopes (Figure 5.3). Error variance was homogenous in both seasons. However, when data for both seasons were combined the regression of mean PRL concentration on postpartum live weight was statistically significant (P<0.01).

The regression of mean milk yield on postpartum live weight was significant (P<0.05) for the spring-lambing ewes but not for the autumn-lambing ewes (P>0.10) and the test of homogeneity of regression slopes for spring- and autumn-lambing ewes was non-significant (P>0.10) indicating that the regression lines did not differ in slope (Figure 5.4). Error variance was homogenous in both seasons. When data for both seasons were combined, the regression of mean milk yield on postpartum live weight was statistically significant (P<0.01).

There were no significant differences in milk fat, protein or lactose percentages between spring- and autumn-lambing ewes over the 8-d period (data not shown). Milk fat, protein and lactose yields were significantly (P<0.01) higher in spring-lambing ewes than in autumn-lambing ewes (data not shown), but these differences were not significant when adjusted for the corresponding liveweight differences.

Over the trial period, the spring-lambing ewes had a significantly (P<0.01) higher mean energy deficit (106±19 MJME over 22d) than autumn-lambing ewes (73±9 MJME over 22d) reflecting the fact that the spring-lambing ewes consumed less feed than the autumn-lambing ewes (data not shown). Mean energy deficit, however, had no significant effect on milk yields or PRL concentrations (either fitted before or after season in the model).

FIG 5.1 MEAN±SE PLASMA PROLACTIN CONCENTRATION (ng/ml) IN 10 SPRING- AND 10 AUTUMN-LAMBING EWES FED THE SAME DIET (MEADOW HAY AND CONCENTRATES) TO INDIVIDUAL ME REQUIREMENTS, WHILE HOUSED INDOORS UNDER CONSTANT PHOTOPERIOD (18L:6D)

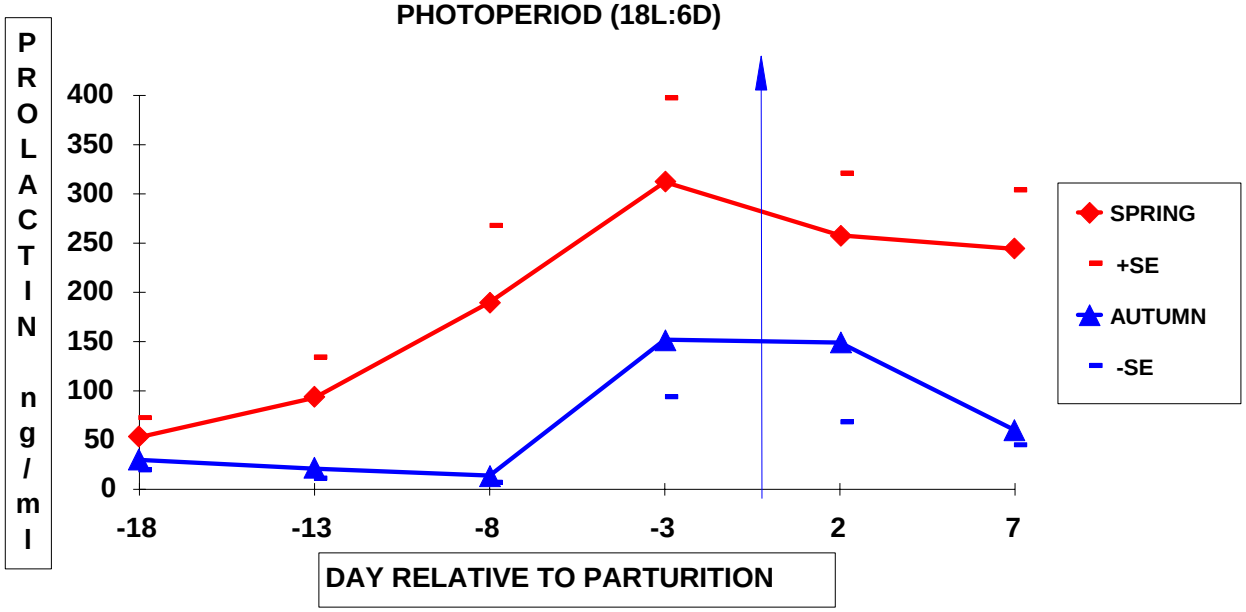


FIG 5.2 MEAN±SE MILK YIELD (g/d) OF 10 SPRING- AND 10 AUTUMN-LAMBING EWES FED THE SAME DIET (MEADOW HAY AND CONCENTRATES) TO INDIVIDUAL ME REQUIREMENTS, WHILE HOUSED INDOORS UNDER CONSTANT PHOTOPERIOD (18L:6D)

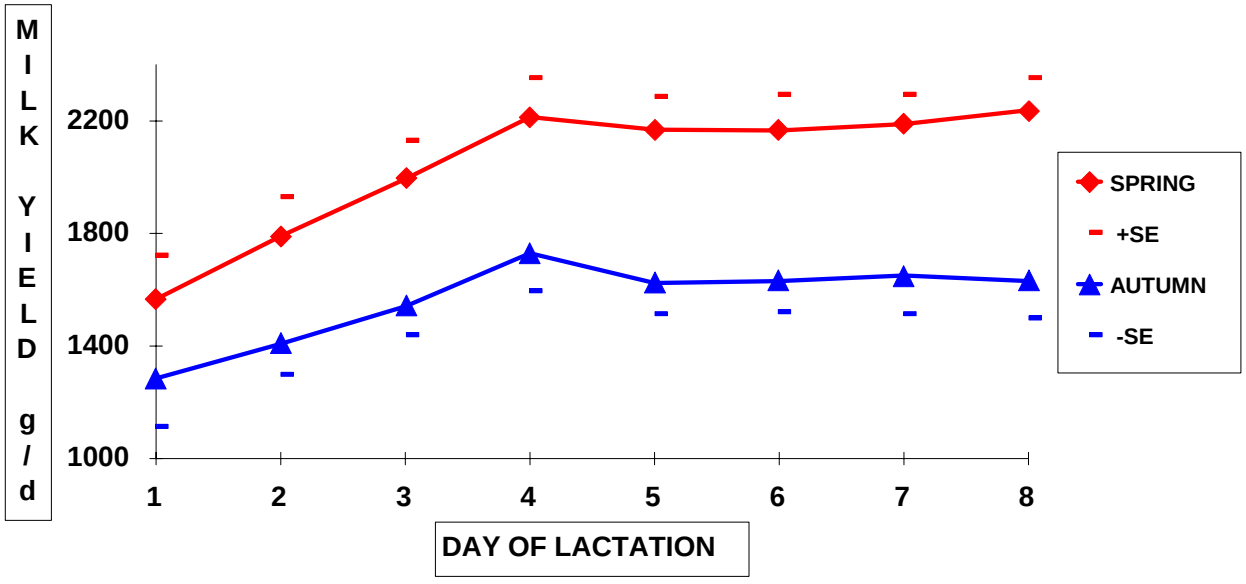


FIG 5.3 WITHIN-SEASON REGRESSION LINES OF MEAN PLASMA PROLACTIN CONCENTRATION (ng/ml) ON LIVEWEIGHT (kg) OF 10 SPRING- AND 10 AUTUMN-LAMBING EWES FED THE SAME DIET WHILE HOUSED INDOORS UNDER CONSTANT PHOTOPERIOD (16L:8D)

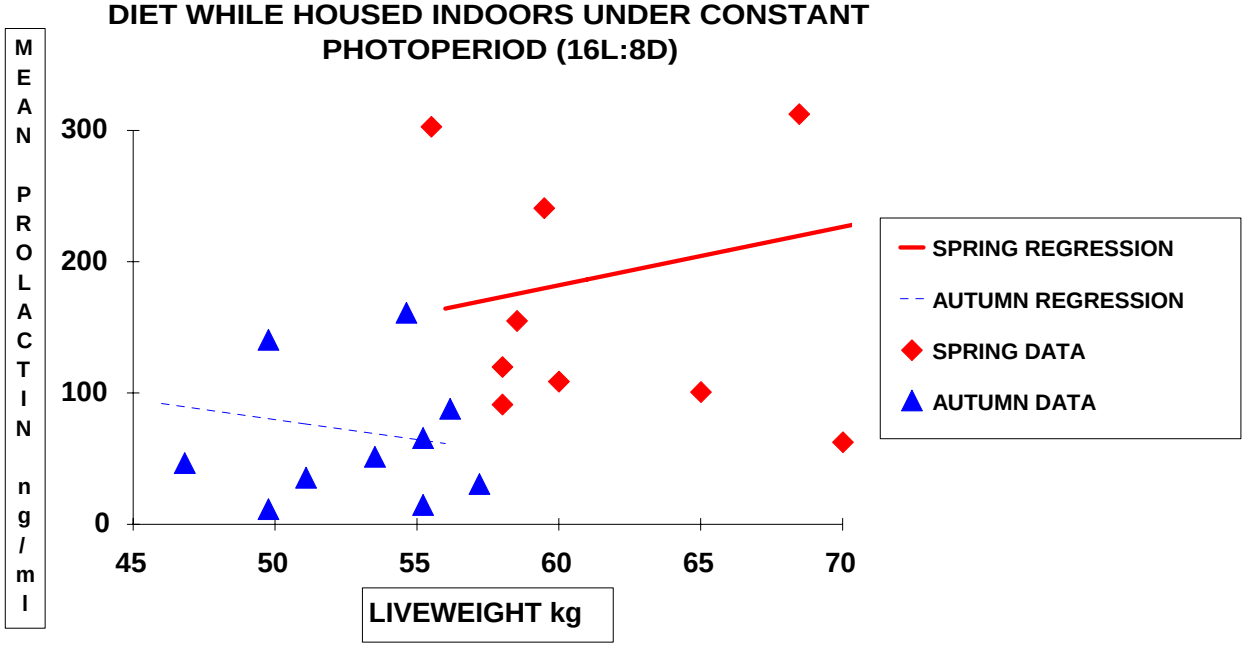


FIG 5.4 WITHIN-SEASON REGRESSION LINES OF MEAN MILK YIELD (g/d) ON LIVEWEIGHT (kg) OF 10 SPRING- AND 10 AUTUMN-LAMBING EWES FED THE SAME DIET WHILE HOUSED INDOORS UNDER CONSTANT PHOTOPERIOD (16L:8D)

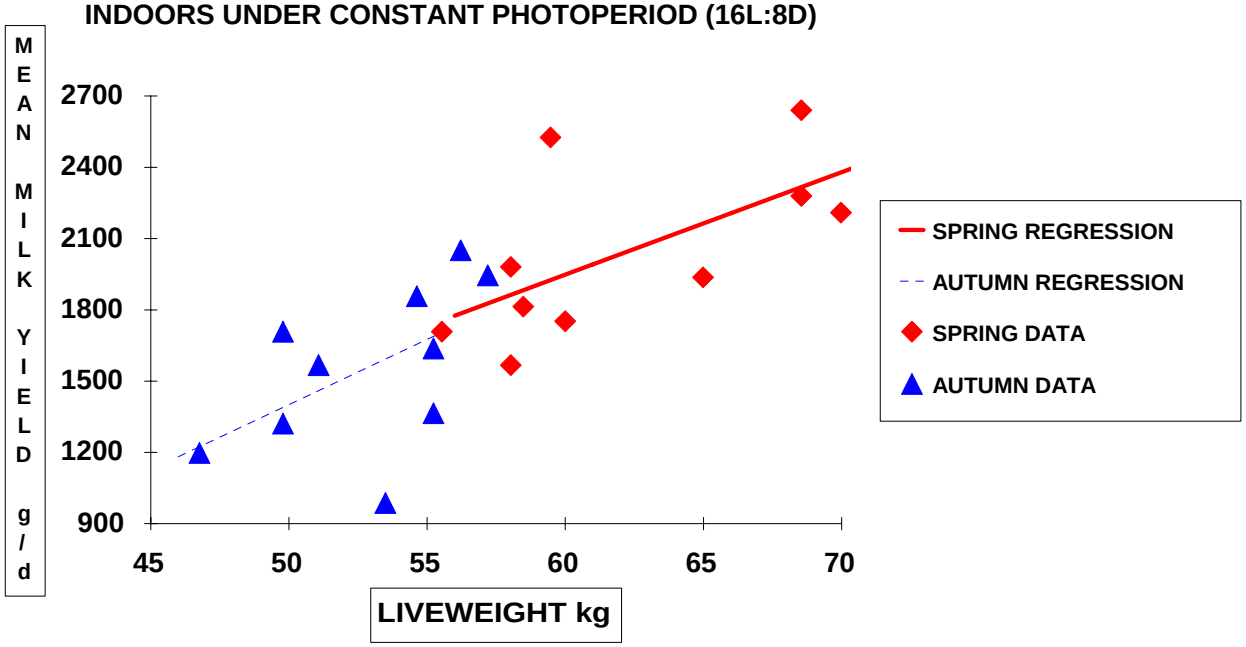
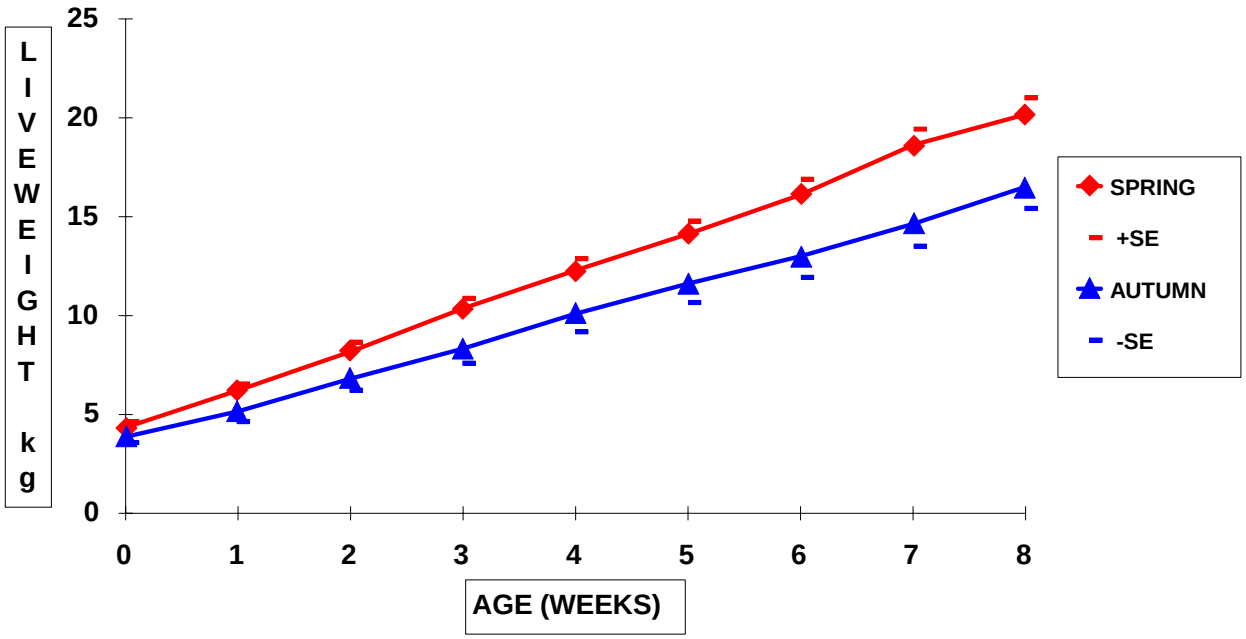


FIG 5.5 MEAN±SE LIVEWEIGHT (kg) OF LAMBS BORN IN SPRING (n=13) OR IN AUTUMN (n=12)



Lamb live weights are shown in Figure 5.5. Birthweight of lambs (adjusted for birthrank (single versus twin) and sex of lamb) was not significantly different between spring (4.34±0.25 kg) and autumn (3.87±0.33 kg), but lamb growth rates (adjusted for birthweight, birthrank and sex of lamb) over the 8 weeks from birth to weaning were significantly ($P<0.001$) higher in spring (282±12 g/d) than in autumn (225±15 g/d), resulting in spring-born lambs being 3.7 kg heavier ($P<0.001$) at 8 weeks of age than autumn-born lambs.

5.4 DISCUSSION AND CONCLUSIONS

Plasma PRL levels in dry ewes are widely considered to be determined primarily by photoperiod, reaching minimal concentrations in autumn, remaining low throughout winter and spring then rising to peak levels in summer (Munro *et al.* 1980). In pregnant ewes this pattern is interrupted by a peak at parturition. Level of nutrition is not expected to affect PRL concentrations since plasma levels of PRL were not affected by nutritional treatments in twin-pregnant ewes (Mellor *et al.* 1987) and marked underfeeding or *ad libitum* feeding did not affect PRL levels in ovariectomised ewe lambs (Foster *et al.* 1989). Other non-nutritional dietary factors may, however, affect circulating PRL levels. For example, high endophyte levels in pasture depress circulating PRL concentrations in grazing sheep (Fletcher and Barrell 1984).

In trials 1 and 2 (Chapter 3) there were considerable differences between spring- and autumn-lambing ewes in plasma PRL concentration, milk yield, milk composition, lamb birthweight and

lamb growth rate. In the trial reported here, in which the same diet was fed in both seasons, similar differences in plasma PRL concentration, milk yield and lamb growth rate were evident (all were lower in autumn than in spring). Thus, despite the fact that both the autumn- and spring-lambing ewes were housed under conditions of equal and constant photoperiod during the trial, their plasma PRL levels apparently continued to reflect the normal seasonal differences in photoperiod. Longer photoperiod has been shown to increase PRL levels greatly in wethers (Trenkle 1978; Eisemann *et al.* 1984a and 1984b), rams (Barrell and Lapwood 1979) and in both pregnant and lactating ewes (Munro *et al.* 1980; Perier *et al.* 1986). Also, recent French reports (Bocquier *et al.* 1986; Perier *et al.* 1986) indicate that longer photoperiod increases milk yields of ewes. It is likely, however, that the pattern of change in photoperiod prior to the trial reported here had a carry-over effect on PRL levels. Recent evidence indicates that circannual rhythms are generated by an endogenous process that is synchronised by exposure to long days during the previous spring and summer (Karsch and Wayne 1988; Malpaux *et al.* 1989; Wayne *et al.* 1990). Previously, it was concluded that photoperiod was the major factor governing the milking-induced PRL release in goats, but the seasonal changes in photoperiod and temperature were confounded (Hart 1975). Since low ambient temperature has recently been shown to reduce plasma PRL concentrations in goats independently of photoperiod (Forsyth 1992), it is also possible that the temperature difference between the seasons affected circulating PRL levels in the ewes during trial 5. However, it is difficult to envisage how the temperature difference could have caused the differences in circulating PRL levels since the recorded mean air temperature was higher during April than during August. If temperature had a major effect during this trial it would have been expected to reduce the apparent difference in plasma PRL levels between spring- and autumn-lambing ewes. The effect of temperature on plasma PRL concentration will be discussed further in Chapter 7.

The lower live weights of the autumn-lambing ewes complicate interpretation of the results from this trial. After adjusting for the difference in live weights between the two seasons, the PRL and milk yield differences were no longer statistically significant. It is questionable, however, whether there is sufficient biological justification for adjusting the PRL and milk yield data to a common ewe live weight. There is no evidence in the literature that live weight or plane of nutrition influence plasma PRL concentrations (whereas there are several reports of photoperiod-related differences and a possible temperature effect). Thus it is unlikely that the statistical adjustment of PRL levels to account for liveweight differences is biologically meaningful. This conclusion is supported by the finding that the within-season regressions of plasma PRL concentration on ewe live weight were not significantly different from zero and had slopes of opposite sign. Therefore, it seems likely that seasonal differences in PRL concentration are not related to corresponding differences in live weight in a cause-and-effect manner.

Intuitively one would expect that statistical adjustment of milk yield for live weight is biologically justified (i.e. that milk yield is causally related to live weight) but published reports are

contradictory. Bonsma (1939) recorded a significant positive correlation between the milk production and live weight of ewes. In his review, Wallace (1948) presented data on 14 Suffolk ewes which indicated a positive correlation (although no statistical analysis was reported) between the mean live weight and total lactation milk yield. He cited only the results of Bonsma (1939) and of several studies in dairy cows in support of the assumed relationship between live weight and milk yield. Barnicoat *et al.* (1949) found that the correlation between milk yield and live weight of 70 Romney ewes grazed at pasture was not significant. Thomson and Thomson (1953) reported that regressions of total milk yield on postpartum live weight of 56 housed ewes were not significant and, in five studies of the effects of nutrition on the onset of lactation, McCance and Alexander (1959) found that the rate of milk secretion was correlated with prepartum live weight only in a group of ewes which were losing 1.6 kg/week over the last 6 weeks of gestation, as a result of low feed intakes. Indeed, even nutrition during the latter part of gestation has a variable effect on subsequent lactation, poor prepartum nutrition either resulting in decreased milk yields (Barnicoat *et al.* 1949; Barnicoat 1952; Thomson and Thomson 1953; Treacher 1970) or having no effect (Peart 1967 and 1970; Geenty and Sykes 1986). Severe prepartum undernutrition delays the onset of lactation (McCance and Alexander 1959; Treacher 1970). Prepartum nutrition is, however, not as important as nutrition during lactation (Barnicoat *et al.* 1949; Geenty and Sykes 1986), and restricting intake during the first week delays the onset of lactation and peak milk yield (Jagusch *et al.* 1972). Thus, it has been suggested (Treacher 1970) that body weight or condition at parturition are not in themselves important factors affecting potential milk yield unless the level of nutrition during lactation is low. In trial 5 there was no evidence that any of the ewes were underfed. To the contrary, the reported energy deficits indicate the extent to which the ewes failed to consume the feed provided and suggest that they were fed to appetite. The energy deficits had no statistically significant effects on milk yield. Furthermore, the autumn-lambing ewes consumed more feed than the spring-lambing ewes, the opposite result to that expected if the lower autumn milk yield was a result of underfeeding. The results of trial 5 are in agreement with the finding that, with the same food intake during lactation, exposure of ewes to long photoperiod increased milk yields and plasma PRL concentrations relative to those exposed to short photoperiod (Bocquier *et al.* 1986; Perier *et al.* 1986).

Because the ewes were fed concentrates and hay from the same batches during both seasons, the feed offered to the autumn-lambing ewes was necessarily older than that given to the spring-lambing ewes. If the nutritive value of the diet had declined during the period between the seasons, this could have contributed to the lower autumn milk yields. The feedstuffs were stored in a dark, dry, cool room during the intervening period and, although no laboratory analyses were performed, there was no visible deterioration of the feedstuffs. Since is believed that a major decline in the quality of the diet would be necessary to cause the observed seasonal differences, it is considered very unlikely that the ageing of the diet was a significant factor.

In summary, while poor peripartum nutrition may reduce milk yields there is no evidence that ewes in this trial where underfed and, furthermore, live weight or body condition are unlikely to affect milk yields unless the level of nutrition is low. It is thus concluded that statistical adjustment of the milk yield data to a common ewe live weight is not justified in these circumstances.

Assuming, based on the above arguments, that liveweight differences between the seasons were not causally related to the corresponding differences in plasma PRL concentration and milk yield, and that the milk yield differences were not a function of differences in energy status, one is left with an apparent relationship between seasonal differences in plasma PRL concentration and milk yield. It is not, of course, possible to determine from the present trial whether this relationship was causal in nature. The key point is, however, that the differences observed in trial 5 paralleled those observed in the comparison of trials 1 and 2, but did so in the absence of the previously confounding differences in diet. This would suggest that the differences observed in the informal comparison of trials 1 and 2 were not a reflection of the different diets used. Similarly, the seasonal differences in milk composition, which were evident in trials 1 and 2, may have been prevented in trial 5 by autumn-lambing ewes eating more than spring-lambing ewes, or by the provision of the same diet in both seasons. However, the confounding effect of the differences in postpartum live weight between the spring- and autumn-lambing ewes prevents clear conclusions being reached regarding effects on milk composition.

Birthweights were higher in spring-born lambs than in autumn-born lambs in trials 1 and 2. This observation is consistent with the findings of Reid *et al.* (1988) and Morris *et al.* (1993). In trial 5, birthweights did not differ significantly between seasons but growth rates were higher in spring than in autumn. Lower growth rates have been reported in autumn- than in spring-born lambs (Reid *et al.* 1988; Cruickshank and Smith 1989; Morris 1992) and may be influenced by factors, other than the milk yield, such as lamb prenatal and neonatal PRL levels, which also respond to prenatal photoperiod (Bassett *et al.* 1988; Ebling *et al.* 1989). Furthermore, lambs of heavier birthweight demand and consume more milk (Bonsma 1939; Wallace 1948; McCance and Alexander 1959; Peart 1967) thus the higher spring birthweights and lamb growth rates may have contributed to the higher milk yields in the spring-lambing ewes compared to those in the autumn-lambing ewes through increased demand by the spring-born lambs, although machine milking would have minimised any such effect. It remains unclear, however, why there was no effect of season on lamb birthweight despite such an effect having been observed in other trials (Reid *et al.* 1988; Morris *et al.* 1993) and in the informal comparison of trial 1 versus trial 2.

In summary, the lower milk yields and PRL levels in autumn- than in spring-lambing ewes in trial 5 suggest that the reduced autumn milk yields and lamb growth rates are associated with lower circulating maternal PRL concentrations rather than with dietary differences. However, the confounding effects of differing dietary composition in trials 1 and 2, and differing live weight and ME intake in trial 5, prevent clear conclusions being drawn. Further work is required to establish

causal relationships among plasma PRL levels, milk yields, birthweights and lamb growth rates. Supplementation of autumn-lambing ewes with PRL may provide definitive information on these relationships, and is addressed in chapter 6.

CHAPTER 6

THE EFFECT OF SUPPLEMENTARY oPRL ADMINISTERED TO AUTUMN-LAMBING EWES

6.1 INTRODUCTION

In Chapter 4 (trial 4) it was shown that oPRL has lactogenic effects when administered directly into the mammary gland of CB154-treated ewes. Because the resulting increase in milk yield was still apparent 8 weeks after oPRL treatment ended, it was considered possible that PRL sets the upper limit for mammary gland synthetic functions during lactogenesis. In trial 5 (Chapter 5) it was found that reduced milk yields and lamb growth rates were associated with lower circulating PRL concentrations in autumn-lambing ewes versus spring-lambing ewes. However, these seasonal differences were confounded with corresponding differences in live weigh. Nevertheless, the results are in agreement with recent French reports (Bocquier *et al.* 1986; Perier *et al.* 1986) indicating that longer photoperiod increases milk yields of ewes. If the peripartum plasma PRL concentration is responsible for determining the potential milk production of the gland, then administration of supplementary oPRL into the glands of autumn-lambing ewes (with naturally low plasma PRL levels) would be expected to result in increased milk yields. This would also provide a means of uncoupling the seasonal effects on PRL levels and live weight which made interpretation of trial 5 results difficult. Although PRL has been previously administered during established lactation to CB154-treated ewes by continuous i.v. infusion (Hooley *et al.* 1978) and to cows (not treated with CB154) by a series of daily injections (Plaut *et al.* 1987), no published work has reported an attempt to increase milk yields by peripartum oPRL supplementation (in animals with either artificially reduced, or normal, circulating PRL levels).

This chapter describes two trials carried out to determine whether supplementary oPRL, administered to autumn-lambing ewes during the peripartum period by subcutaneous or intraductal injection, affected subsequent milk yield and composition.

6.2 MATERIALS AND METHODS

Two concurrent trials were carried out in autumn-lambing ewes. Trial 6 involved intramammary injection of oPRL into one gland and excipient into the contralateral gland of each ewe. Trial 7 compared ewes treated with subcutaneous injections of oPRL versus untreated ewes.

For both trials a flock of ewes, which were synchronised with progesterone-impregnated CIDRs, and induced to ovulate using PMSG, were mated during November to lamb in April. Following ultrasound pregnancy diagnosis, multiparous ewes (aged from 4-7 years), selected on the basis of mating date and number of foetuses, were allocated to the 2 trials at random, except that both trials, and the 2 groups within trial 7, were balanced as much as possible for age, live weight and pregnancy rank. Ewes were grazed at pasture (mainly ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*)) throughout both trials.

TRIAL 6

Eleven ewes were initially allocated to trial 6 for the intramammary oPRL treatment. Ewes which delivered dead lambs, did not lamb within a week of the predicted time or whose lamb(s) died during the early postnatal period, were removed from the trial. This left 8 ewes (7 single-bearing, 1 twin-bearing) in the trial. Each of the 8 ewes received 10 mg of oPRL (dissolved in 1 ml of 0.15M sodium chloride and 0.03M sodium bicarbonate) injected into one gland (4 ewes injected in the left gland and 4 in the right gland). The contralateral gland was treated with 1 ml of the excipient (0.15M saline, 0.03M bicarbonate, henceforth referred to as BIC) as a control to the oPRL treatment. In an attempt to minimise infection of the mammary gland, teats were first cleaned with a solution of Hibitane (Chlorhexidine gluconate 5% w/v. ICI PLC, Macclesfield, Cheshire, England) in water and then with 70% ethanol. Between uses the needle used for intramammary injections was immersed in 70% ethanol.

TRIAL 7

Twenty ewes were initially allocated to trial 7 (ten to the subcutaneous oPRL treatment (PRLsc) group and 10 to the control group). Ewes which delivered dead lambs, did not lamb within a week of the predicted time or whose lamb(s) died during the early postnatal period, were removed from the trial. This left 9 ewes (6 single-bearing, 3 twin-bearing) in the PRLsc group, and 8 ewes (6 single, 2 twin) in the control group. The PRLsc group received subcutaneous injections (in the shoulder region) of 30 mg oPRL dissolved in 3 ml of BIC. Control group ewes received no oPRL or BIC injections.

All of the ewes from both trials were run together as one flock and, apart from the treatment differences described above, were subjected to the same management and sampling regimen. Because of the inability to accurately predict the lambing date of ewes, the oPRL was administered on two consecutive days immediately prior to the expected mean date of parturition in an attempt to elevate intramammary or plasma PRL concentrations at a time close to parturition. Injections were administered at about 1700 h. On each day of oPRL treatment all ewes received a s.c. injection of 5 ml Streptopen antibiotic. In addition, on the first or second day of milking each ewe was treated with 2.5 million i.u. of Leocillin (Penethamate Hydriodide, a

slightly soluble hydriodide of an ester of benzylpenicillin which is actively taken up by the mammary gland (Edwards 1966)). Ewes which subsequently showed any sign of mastitis were given 5 ml Streptopen s.c.

Following lambing, ewes were milked on days 1-6, 8, 10 and 12 of lactation, milk samples collected and milk yields calculated as described previously (Chapter 2). Ewes were returned to pasture between morning and afternoon milkings, while lambs were held indoors and bottle-fed (as described in Chapter 2).

On each of the 2 treatment days, blood samples were collected by jugular venipuncture from all ewes before oPRL treatment. Thereafter 2 further blood samples were taken at 5-d intervals. Milk samples were analysed for milk fat, protein and lactose content, and plasma samples were analysed for PRL.

Data were analysed using the computer statistical package REG (Gilmour 1990) . Multivariate (repeated measures) analysis of variance was used to analyse all time-series data. PRL data were log transformed and all milk composition data were arc sine transformed.

For analyses of trial 6 milk yield and composition data, glands, rather than ewes, were classified into groups according to whether they were treated with oPRL (PRL-group glands) or BIC (BIC-group glands). Milk yield and composition data from PRL- and BIC-treated glands were analysed with glands nested within ewes. In trial 7, ewes were grouped according to treatment for data analyses. PRLsc-group ewes were those ewes treated with s.c. oPRL injections and the Control group were untreated ewes. The test for a delay in the onset of lactogenesis was the interaction of group contrasts with time.

6.3 RESULTS

All ewes lambed within a 4-d period, 16 of them lambing on one or other of the 2 days when oPRL treatments were administered. The day on which blood samples were collected relative to the day of parturition varied between ewes because the actual day of lambing could not be accurately predicted when the prepartum samples were taken. Thus reported sampling days in the figures and tables are the mean ± (SE) 0.2 d of the actual sampling days. The mean daily air temperature during April was 14.4 ^{o}C.

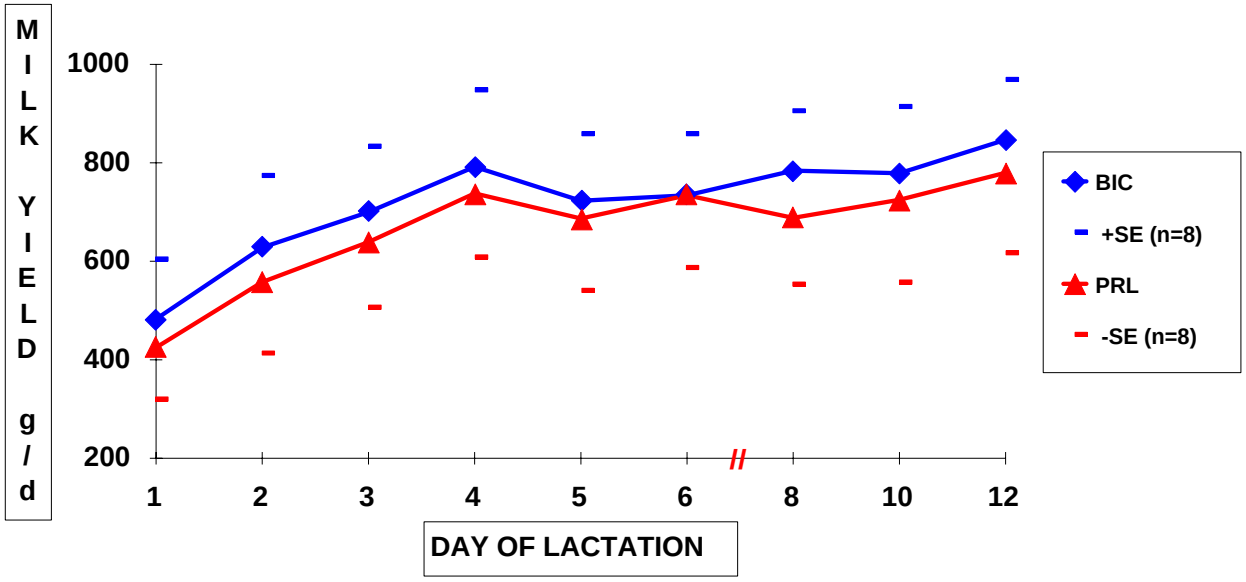
TRIAL 6

Mean plasma prolactin concentrations peaked at 376±127 ng/ml on day 1 of lactation (Table 6.1). Milk yields did not differ significantly between the PRL- and BIC-treated glands (Figure 6.1). Milk fat, protein and lactose percentages did not differ between the PRL- and BIC-treated glands (data not shown).

TABLE 6.1 MEAN±SE PLASMA PROLACTIN CONCENTRATIONS (ng/ml) IN AUTUMN-LAMBING EWES (TRIAL 6) TREATED VIA THE TEAT DUCT ON 2 DAYS PERIPARTUM WITH 10 mg oPRL IN ONE GLAND AND WITH 1 ml BICARBONATE IN THE CONTRALATERAL GLAND

DAY OF LACTATION			
0	1	5	10
324±110	376±127	182±71	72±32

FIG 6.1 MEAN±SE MILK YIELDS (g/d) OF INDIVIDUAL GLANDS OF AUTUMN-LAMBING EWES (TRIAL 6) TREATED VIA THE TEAT DUCT ON 2 DAYS PERIPARTUM WITH 10 mg oPRL IN ONE GLAND (PRL) AND WITH 1 ml BICARBONATE (BIC) IN THE CONTRALATERAL GLAND



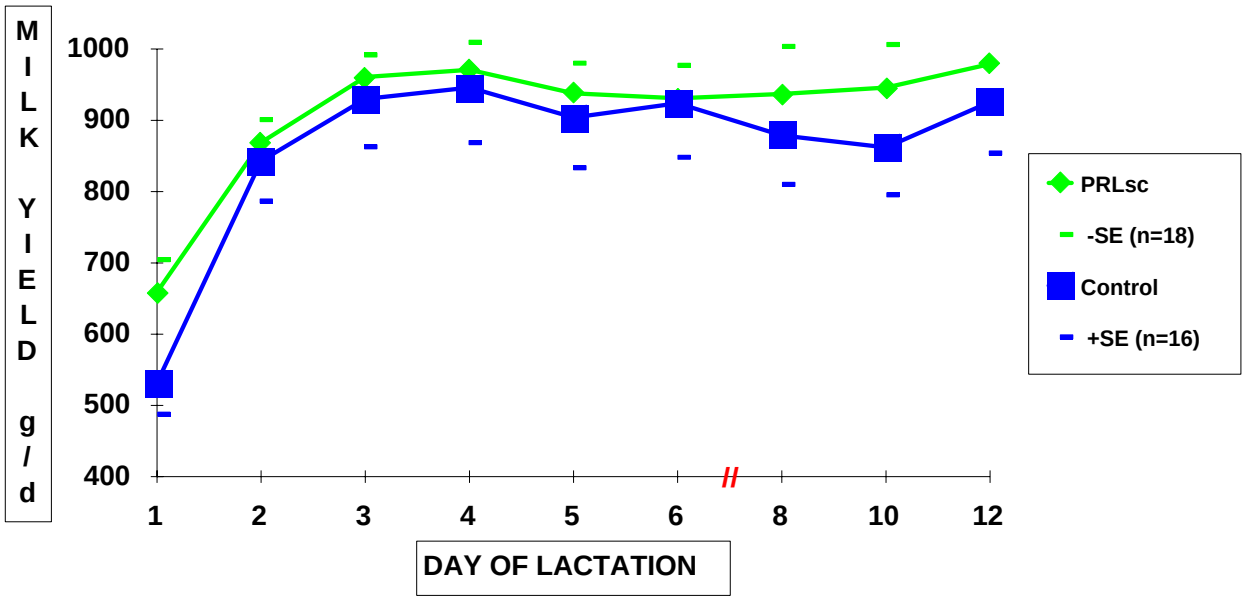
TRIAL 7

Plasma PRL concentrations did not differ significantly between groups (Table 6.2). Milk yields in PRLsc-group ewes did not differ from those in Control-group ewes (Figure 6.2). Milk fat, protein and lactose percentages did not differ between the 2 treatment groups (PRLsc or Control) (data not shown).

TABLE 6.2 MEAN±SE PLASMA PROLACTIN CONCENTRATIONS (ng/ml) IN AUTUMN-LAMBING EWES (TRIAL 7) TREATED ON 2 DAYS PERIPARTUM WITH 30 mg oPRL VIA SUBCUTANEOUS INJECTION (PRLsc) AND IN UNTREATED (Control) EWES

	DAY OF LACTATION			
	0	**1**	**5**	**10**
PRLsc	238±80	204±53	70±28	43±15
Control	437±165	261±87	137±49	73±34

FIG 6.2 MEAN±SE MILK YIELDS* (g/d) OF AUTUMN-LAMBING EWES (TRIAL 7) TREATED ON 2 DAYS PERIPARTUM WITH 30mg oPRL BY SUBCUTANEOUS INJECTION (PRLsc) AND OF UNTREATED (Control) EWES



* Milk yields are for individual glands to permit comparison with Figure 6.1

6.4 DISCUSSION AND CONCLUSIONS

The objective of these trials was to measure responses to supplementary oPRL in intact autumn-lambing ewes which, based on previous results (Chapter 5), were expected to have low plasma PRL concentrations and milk yields relative to spring-lambing ewes. Two routes of oPRL supplementation were used in order to test the effectiveness of elevating local or peripheral levels of PRL. Thus, in trial 6, the comparison was between intramammary treatment with oPRL in one gland and with excipient in the contralateral gland of each ewe while, in trial 7, responses in ewes supplemented systemically with oPRL were compared with those in untreated (control) ewes.

Plasma PRL concentrations were considerably higher in trials 6 and 7 (Tables 6.1 and 6.2) than those recorded in previous autumn trials (trials 2 and 5; see Figures 3.18 and 5.1). However, this may be because the sampling times in trials 6 and 7 corresponded much more closely with the mean date of parturition than they did in the previous autumn trials. Thus, the high levels found in trials 6 and 7 on the mean day of parturition and the following day are not directly comparable with values found in the previous trials three days prior to, or two days after, the mean date of parturition. Furthermore, the plasma prolactin levels in this trial may not be directly comparable with those in other trials since they were determined in different assays. However, pooled samples present in all assays differed little in mean PRL concentration, indicating that the difference between assays probably cannot explain the relatively large apparent differences between trials 6 and 7 and the previous autumn trials (trials 2 and 5). Results of trials 6 and 7 also indicate that the peripartum PRL concentrations in these autumn-lambing ewes were similar in magnitude to those recorded in control (E/S group) ewes lambing in spring (trial 1; see Figure 3.2). This is despite the finding in trial 5 that PRL levels in samples collected from autumn-lambing ewes between 2 weeks prepartum and 1 week postpartum (but not coinciding with the mean date of parturition) were significantly lower than those in spring-lambing ewes. This raises the question of whether circulating PRL concentrations are, in fact, consistently lower in autumn- than in spring-lambing ewes. Since circulating PRL concentrations in goats have recently been shown to respond to seasonal changes in ambient temperature independently of photoperiod (Forsyth 1992), it is possible that warm temperatures during trials 6 and 7 prevented the expected decrease in plasma PRL levels. However, this is considered unlikely since trials 5, 6 and 7 were carried out concurrently and, the mean daily temperature during the previous autumn trial (trial 2, also carried out in the month of April) was only 1.4 ^{o}C lower. Further studies involving frequent peripartum blood sampling under conditions of controlled temperature will be required to establish conclusively the effects of temperature in sheep.

Administration of supplementary oPRL directly into the gland did not increase the milk yields, or change the fat, protein or lactose composition of milk, compared to glands treated with BIC. Neither did subcutaneous injection of oPRL increase milk yields, or alter the fat, protein and lactose percentages of milk compared to Control group ewes. These results suggest that the circulating level of PRL, and the intramammary concentration of PRL, in autumn-lambing ewes are not limiting lactogenesis. However, the circulating levels of PRL were apparently much higher than those recorded in previous autumn-lambing trials in which the ewes were housed indoors, under artificial lighting, and fed different diets compared to the ewes grazed on pasture in the present trials. Thus, it is not possible to reach firm conclusions regarding possible effects of supplementary oPRL in ewes with naturally low plasma PRL concentrations.

CHAPTER 7

DISCUSSION AND CONCLUSIONS

The objective of this experimental programme was to examine the role of PRL in the control of ovine lactogenesis. At the outset of these studies it was apparent that published reports differed in their conclusions as to the importance of PRL for lactogenesis in the sheep. However, on balance it appeared that PRL is essential for complete, normal lactogenesis as evidenced by the effects of CB154 (although, as will be noted later, abolition of the PRL surge with CB154 may have potentially confounding side effects). There was ample evidence indicating that prepartum treatment with CB154 has delaying or inhibitory effects on lactation, and some evidence indicated that alteration of the peripartum PRL surge has long-acting or carry-over effects on milk yields during the subsequent lactation. This included at least one report (Maule Walker 1984b) suggesting that subsequent milk yields may be substantially improved by CB154-treatment. However there were no published reports in which oPRL had been administered to CB154-treated ewes around the time of parturition. This "positive control" treatment was required to conclusively establish that the effects of CB154 on lactogenesis are mediated through PRL and not through effects on some other factor. Furthermore, no published work had reported an attempt to increase milk yields by peripartum oPRL supplementation, and there were no reports establishing whether PRL is active when introduced directly into the mammary gland of ruminants. It was known that PRL can act directly on the mammary gland (of rabbits, whose lactation is very sensitive to PRL), but the possibility that it influences lactogenesis via an intermediate hormone had not been eliminated.

Therefore, the first major objective of the programme was to establish whether PRL is essential for complete initiation of lactogenesis by comparing milk yields in ewes whose PRL secretion was inhibited by CB154 treatment with milk yields of ewes treated with CB154 plus concurrent administration of oPRL (the positive control). Subsequently, the major objectives were to determine whether milk yield could be increased by short term peripartum administration of oPRL to ewes with normal PRL secretion at that time and to establish whether oPRL acts directly on the mammary gland by administering oPRL directly into the gland (via the teat duct) at doses which precluded a marked rise in circulating oPRL concentrations.

These studies required an experimental approach involving substantial modification of the animal's normal environment and physiology. It is therefore appropriate to examine aspects of the experimental protocols which may have influenced the measured parameters since these aspects ultimately place constraints upon the validity of the results and their interpretation.

In order to study the in vivo effects of PRL it is necessary to alter the circulating levels of the hormone by administration of exogenous PRL, by inhibiting or enhancing endogenous secretion, or by a combination of these methods.

Since PRL could not be synthesised until recently, most studies have used hormone obtained from pituitary extracts and early preparations were often contaminated by other pituitary hormones. Although separation and purification techniques have now virtually eliminated the problem of impurities, reliance upon pituitary extracts from slaughtered animals as the source of PRL naturally limits the amount of the hormone available for experimental purposes. Thus, researchers are limited in the number and size of animals that can be used in their studies. Ovine PRL is the most widely studied PRL molecule and the size of the sheep allows economical use of the available stocks of the hormone in a homologous system. Particularly important in the choice of oPRL for this research programme was the availability of oPRL derived from the pituitary glands of New Zealand sheep (Raiti 1990), thus avoiding the need for strict quarantine and subsequent slaughter of the experimental animals. In the future it is expected that recombinantly derived PRL of various species will be available in sufficient quantities and purity that researchers will not be bound by the above constraints.

PRL has been administered to ruminants by continuous i.v. infusion (Hooley *et al.* 1978; Akers, Bauman, Capuco *et al.* 1981) and by a series of daily injections (Plaut *et al.* 1987). The former method is impractical for larger scale trials and the latter might be expected to provide an unnatural profile of plasma PRL concentrations. A preferable alternative would be an implant to release the hormone at a steady, controlled rate, but this technology is presently not available for PRL. Direct administration of oPRL into the teat duct of pseudopregnant rabbits resulted in lactogenesis (Falconer and Fiddler 1970) suggesting that oPRL acts directly on the rabbit mammary gland. If oPRL is effective when administered directly into the mammary gland of the ewe then problems associated with systemic administration could be avoided. This route would have several major advantages. First, the amount of hormone required might be much lower than that for administration via other routes since there would be no need to raise circulating concentrations of PRL. Second, the secretions within the gland might act as a reservoir for PRL, presenting it to the secretory epithelial cells in high concentrations over an extended period (a natural slow release mechanism). Third, if the dose was correct, oPRL release into the circulation might be so slow that plasma levels would not rise significantly. Thus any response obtained could be attributed directly to an action of PRL within the gland, not requiring systemic actions of PRL or the presence of a hypothetical intermediate hormone.

Enhancement of plasma PRL concentrations in sheep has also been achieved using a variety of psychopharmacologic PRL releasers (Bass *et al.* 1974; Peel *et al.* 1977) and hormones, including thyrotropin-releasing hormone (Lamming *et al.* 1974), oestradiol (Fell *et al.* 1972), PGF (Field *et al.* 1977; Fulkerson *et al.* 1977) and corticotrophin-releasing hormone (Naylor *et al.*

1990). None of these, however, is specific for PRL so that it is impossible to attribute the responses obtained solely to the actions of PRL. When the identity of the prolactin-releasing hormone is known, and if it is specific to PRL, enhancement of endogenous PRL secretion by administration of its releasing hormone will be a valuable tool for the study of PRL-mediated effects.

Classical techniques in early endocrinology involved the ablation of the gland(s) secreting a particular hormone to remove the endogenous source and the subsequent reversal of the effects of ablation by the exogenous administration of the hormone in order to identify the specific actions of that hormone. Such methods do not provide particularly conclusive results in the case of pituitary hormones since hypophysectomy also eliminates the many other pituitary hormones. The identification of specific inhibitors for some hormones has provided an easier and more direct means of studying the physiological roles of those hormones. Simultaneously with the discovery of human PRL, bromocriptine was developed in the late 1960's specifically to inhibit PRL secretion (Flückiger *et al.* 1982). Thus, the PRL inhibitor CB154 (2α–bromoergocriptine mesylate) has been the method of choice for the study of the *in vivo* effects of PRL in many investigations, including the present programme. It is therefore appropriate to consider the effectiveness of CB154 in specifically inhibiting PRL secretion and to identify any side-effects which may influence lactogenesis.

It is well established that CB154 inhibits pituitary PRL secretion and, in most studies, it has been assumed that pituitary PRL is the only significant source of endogenous PRL. However, PRL of nonpituitary origin is found in high concentrations in the amniotic fluid of primate species (Flückiger *et al.* 1982; Riddick 1985). When maternal circulating PRL concentrations are suppressed during pregnancy by CB154 treatment or ablation or radiation of the pituitary gland, amniotic fluid PRL levels remain high and in the normal range. This PRL, apparently identical to pituitary PRL, is produced in the human by the normal endometrium, gestational decidual endometrium, myometrium and rectus fascia (connective tissue). Furthermore, the known modulators of pituitary PRL synthesis (TRH, dopamine, CB154) have no effect on PRL production by decidua *in vitro* (Riddick 1985). This raises the question of whether there are other nonpituitary sources of PRL which are unresponsive to CB154. Nonpituitary PRL sources have been identified in certain clinical conditions (pharyngeal hypophyses, lung and kidney carcinomas, jejunal mucosa of patients with celiac disease) and in neurons in the hypothalamus and preoptic area of the rat (Flückiger *et al.* 1982). Intestinal mucosa, skin and liver of normal humans produced no PRL *in vitro* during tissue culture (Riddick 1985). Nonpituitary sources of PRL have not, apparently, been reported in the sheep. Indeed, since CB154 treatment invariably reduces plasma PRL concentrations to very low levels (as confirmed in this research programme) it may be concluded that, even if such nonpituitary sources do exist, they probably do not secrete sufficient PRL to significantly affect peripheral PRL-responsive mechanisms. The possibility should not be ignored, however, that nonpituitary PRL sources may exist to exert

autocrine or paracrine control over local mechanisms. Although it is generally accepted that PRL is not synthesised in the mammary gland to any great extent (Vonderhaar 1987), treatment of cows with ergot drugs to deplete pituitary and serum PRL levels did not reduce PRL levels in the milk (Malven 1983). Also, prepartum treatment of ewes with sulpiride[9] failed to raise serum or milk concentrations of PRL (van der Walt *et al.* 1990). According to Forsyth (1992) transfer of PRL from the blood to milk occurs through a receptor-mediated saturable mechanism and the efficiency of gland uptake is increased as blood PRL levels decline. Although CB154-treatment of goats markedly reduced whey PRL levels it also greatly reduced the ratio of blood PRL: whey PRL (Forsyth 1992). Thus, it is evident that milk concentrations of PRL are relatively insensitive to factors outside the mammary gland. While it is known that PRL is transferred from plasma into milk (Malven 1983; Forsyth 1992), the possibility that the gland itself produces significant amounts of PRL has apparently not been eliminated. The existence of such a local PRL source in the mammary gland of sheep might explain why CB154 treatment has a relatively small effect on lactation in the ewe compared to the effects in some other species (see section 1.9.2). Although research is required to examine this possibility, *in vivo* studies may be impractical due to the need to completely eliminate all extra-glandular PRL sources, and the mechanism (if it exists) may not operate in isolated mammary tissue *in vitro.*

Few side-effects of CB154 treatment have been reported. CB154 treatment has no effect on plasma concentrations of progesterone (Niswender 1974; Buys *et al.* 1990) , GH, TSH, T_3, T_4 or rT_3 in cycling ewes, progesterone or oestradiol concentrations in periparturient ewes (Burd, Ascherman *et al.* 1978) or progesterone, placental lactogen, oestrone sulphate (Forsyth *et al.* 1985), GH, insulin or thyroxine (Hart 1976; Hart and Morant 1980) in goats. There was no effect of CB154 treatment on circulating PL concentrations in ewes (Martal and Lacroix 1978; Schams *et al.* 1984) and does (Forsyth *et al.* 1985), although Martal and Lacroix (1978) reported that CB154 treatment increased placental oPL concentrations 4 to 6 times. There were no significant differences in serum concentrations of Na, K, Cl or bicarbonate between groups of non-breast-feeding women treated for 14 d postpartum with either CB154 or with a placebo (Kulski *et al.* 1978). Bromocriptine had no effect on the peripartum changes in mammary blood flow, on the time of onset of labour or delivery in ewes (Burd, Ascherman *et al.* 1978), on the liveweight gain or food conversion efficiency of growing lambs (Johnsson *et al.* 1986), or on the proportion of epithelial tissue or size of secretory epithelial cells in the mammary gland of does (Lee and Forsyth 1987). However, reports that CB154 increased GH (Forsyth *et al.* 1985) and decreased insulin (Hart and Morant 1980; Johnsson *et al.* 1986) concentrations cannot be ignored, and the possibility remains that CB154 exerts its effects through some factor other than PRL.

The situation with respect to insulin is particularly confusing, since Hart has reported apparently conflicting results (Hart 1976; Hart and Morant 1980) relating to the same trial (see Chapter 3 for

[9]Sulpiride is a psycopharmacologic PRL releaser.

further discussion of this conflict). Additional indirect evidence suggesting that CB154 may alter plasma insulin levels was provided by Vernon and Flint (1983) who reported that bromocriptine administration to lactating rats resulted in an increase in both the number of insulin receptors and the rate of fatty acid synthesis in adipocytes. Furthermore, CB154 caused a slight reduction in plasma insulin levels in six normal men (Zampa *et al.* 1981). Finally, CB154 treatment for 20 d in trial 1 (Chapter 3) of this research programme resulted in higher prepartum insulin concentrations than in ewes treated with ethanol/saline or those treated for only 9 d with CB154. When all of these results are considered, no consistent relationship is apparent between CB154 treatment and subsequent circulating insulin concentrations. Nevertheless, there is sufficient evidence to suggest that considerable care should be taken when attributing the experimental effects of CB154 solely to an effect mediated through reduction of plasma PRL concentrations. Studies specifically designed to identify possible effects of CB154 on plasma insulin concentrations in ewes are required.

A fundamental constraint affecting the ability to study lactogenesis in sheep is the seasonal nature of reproduction in that species. In order to expedite the present research programme it was necessary to carry out more than one trial in each calendar year. To evaluate the results of each trial sufficiently before proceeding with the next, it was also necessary to stagger the trials throughout the year and, on one occasion (trial 2), to mate ewes outside of their normal breeding season to lamb in the autumn. There being no published reports indicating differences in milk yield between spring- and autumn-lambing ewes, and one report indicating no difference between milk yields of January- and April-lambing ewes in the northern hemisphere (Bass *et al.* 1984), it was not possible to predict the seasonal milk yield differences which resulted (see Chapter 3). The seasonal differences themselves became the object of later studies (trial 5, Chapter 5) and the basis for the final trials (trials 6 and 7) being carried out in autumn.

Seasonal differences in circulating PRL levels were known to exist prior to the start of these studies. In particular, longer photoperiod had been shown to increase PRL levels greatly in goats (during milking)(Hart 1975), in wethers (Trenkle 1978; Eisemann *et al.* 1984a and 1984b) and in both pregnant and lactating ewes (Munro *et al.* 1980; Perier *et al.* 1986). It was for this reason that the ewes were housed under conditions of equal and constant photoperiod immediately prior to, and during, the initial trials. Recent evidence indicating that circannual rhythms are synchronised by exposure to long days during the previous spring and summer (Karsch and Wayne 1988; Malpaux *et al.* 1989; Wayne *et al.* 1990) was not available when the programme commenced. Although long-term changes in circulating PRL are determined by previous seasonal photoperiod changes, both basal and periparturient peak plasma PRL concentrations in pregnant dairy heifers were increased by exposure to longer photoperiod from d 128 of gestation (Newbold *et al.* 1991), indicating that plasma PRL levels do respond to photoperiod changes in the medium term. Similarly in ewes, exposure to long photoperiod from d 90 of gestation resulted in increased plasma PRL levels (Perier *et al.* 1986). However, the 3 week prepartum

acclimatisation period used during this research programme was probably not long enough to facilitate significant changes in plasma PRL concentrations so that circulating PRL levels continued to reflect the normal changes associated with the natural photoperiod.

Until recently it was widely believed that photoperiod was the major factor causing the seasonal changes in circulating PRL levels (Cowie *et al.* 1980) but, in most studies, temperature effects have not been adequately controlled. Based on studies with New Zealand Romney ewes grazed outdoors for 11 months, Munro *et al.* (1980) concluded that PRL concentrations were primarily determined by photoperiod. No positive correlations were found between plasma PRL levels in weekly samples and mean daily air temperature in pinealectomised ewes. Interestingly, they reported a significant negative correlation between plasma PRL concentration and air temperature in 2 of the 4 animals. It is likely that there were too few animals, inadequate blood-sampling frequency and too many sources of uncontrolled variation in that trial to permit a positive conclusion regarding the effect of seasonal temperature variation on plasma PRL levels in ewes. In housed ewes, subjected to controlled photoperiod, Brown and Forbes (1980) found a positive effect of long photoperiod on plasma PRL concentration in intact wethers which was not evident in pinealectomised wethers. In addition a surge in PRL levels was observed at dusk in both intact and pinealectomised animals, indicating that the diurnal variation was not controlled by photoperiod. Although temperature was not controlled in that study, the groups of sheep were held in rooms of similar temperature. The same relationship between long photoperiod and plasma PRL levels was found in wethers by Eisemann *et al.* (1984a and 1984b) but the diurnal variation exhibited was the opposite of that reported by Brown and Forbes (1980). Plasma PRL levels peaked in early afternoon and reached a nadir between 0100 and 0800 h independently of photoperiod. Again, temperature was not controlled but the authors noted that the plasma PRL concentrations followed exactly the temperature fluctuation in the building (Eisemann *et al.* 1984a). Hart (1975) concluded that photoperiod was the major factor governing the milking-induced PRL release in goats but failed to control temperature. In trials during which ambient temperature was maintained at 15^{o}C, plasma PRL levels exhibited a nocturnal rise in sham-operated rams, which was abolished by pinealectomy (Barrell and Lapwood 1978). Similarly, both unoperated and sham-operated rams displayed a direct relationship between photoperiod (normal, even or reversed) and plasma PRL levels which was diminished (although PRL levels were generally higher) in pinealectomised rams (Barrell and Lapwood 1979). Thus it is clear that photoperiod is an important factor in the control of PRL secretion, but these studies above have not established the relative importance of temperature in controlling seasonal or short-term changes in plasma prolactin concentrations.

Although circulating PRL concentration has been shown to increase with ambient temperature in three-month heifers (Wettemann and Tucker 1974), non-pregnant dairy cows induced to lactate (Kensinger *et al.* 1979), lactating goats (Sano *et al.* 1985) and growing pigs (Dauncey and Buttle 1990), the possibility that temperature has a major seasonal effect on plasma PRL levels was

apparently not considered seriously until recently. This situation is largely explained by the fact that, in most trials, photoperiod, temperature and physiological state have been confounded. Since circulating PRL concentrations in goats have recently been shown to respond to seasonal changes in ambient temperature independently of photoperiod (Forsyth 1992), it is possible that temperature is a major factor in the control of plasma PRL concentrations in sheep. This also raises the question of whether circulating PRL concentrations are, in fact, consistently lower in autumn- than in spring-lambing ewes. One might further speculate that ambient temperature, wind-chill factor and length of fleece during the immediate peripartum period may significantly alter circulating PRL levels and influence the rate of onset of lactogenesis in ewes, whatever the season. Definitive trials are required to answer these questions.

Since it is well established that nutrition has a major effect on milk yields, it is necessary to control the feed intake of individual ewes in most studies of lactogenesis. This fact, combined with the need to control photoperiod, was the main reason for housing the sheep indoors in individual pens in several trials in this research programme. Housing also facilitates the frequent blood sampling, drug administration, milking of ewes and the separation of the ewes from lambs, as well as the close monitoring of health and parturition required during many trials. On the other hand, in trials which involve the comparison of milk yields and composition from udder halves, the nutritional and photoperiod effects are not important factors since both udder halves are subjected to the same environment.

Housing and cannulation causes stress, as indicated by the plasma cortisol responses to adrenaline or ACTH injections which are higher in newly housed sheep, but which diminish over a period of two weeks (McNatty and Thurley 1973). Since plasma PRL concentrations are elevated by stress (Lamming *et al.* 1974; Trenkle 1978), it is necessary to allow housed ewes an adjustment period of about three weeks prior to parturition in studies involving PRL.

Housing pregnant and lactating ewes calls for a good supply of feed of reasonable quality. Ideally, for reasons of convenience, this would be provided by a concentrate diet, but the process of acclimatising pasture-fed sheep to such a diet invariably requires a considerable period of time for the ewes to accept the new feed and for their rumen fermentation to adjust. Furthermore, a proportion of animals fail to adjust quickly enough to the dietary change. In pregnant ewes, which are prone to pregnancy toxaemia, this can result in death of the ewes and or foetus(es). In the initial trials of this programme it was decided to use a diet of fresh cut pasture and hay so that the ewes would require less adaptation. However, despite this precaution some ewes did not adapt and were removed from the trial. Others showed signs of pregnancy toxaemia but recovered and remained in the trials. While there were no obvious effects on the parameters measured, it is possible that results of the trials were affected to some extent by the nutrition of the housed ewes. Since ewes grazed at pasture apparently produce higher milk yields than those fed concentrate diets indoors (Jagusch *et al.* 1972), it is likely that the absolute levels of milk yield

recorded in housed ewes during the present programme were not representative of grazing ewes. Nevertheless, since animals were allocated to treatment groups at random, and within trials, all were offered the same diet, it is most likely that the between-group comparisons, and the conclusions derived from them, are valid.

Handling and venipuncture are well known to raise plasma PRL concentrations (Trenkle 1978). On this understanding it would seen unwise to use venipuncture to obtain blood samples in trials designed to evaluate PRL and its effects. However, the only other viable alternative, sampling from indwelling venous cannulae, does not offer a solution to the problem. Lamming *et al.* (1974) reported that the stress of blood sampling via indwelling jugular catheters, or even injecting 1 ml of saline into the catheter, elevated plasma PRL concentrations, even though the sheep were accustomed to handling and to the sampling procedure for a period of weeks, and seemed unafraid. These observations indicate the care needed when conducting such trials and interpreting short term changes in plasma PRL concentrations. Given the additional stress of the cannulation procedure, the limited frequency of sample collection during most trials in this programme and the problems of maintaining patency of the cannulae over long periods, it was not considered practical to use cannulae for blood sampling. Remote sampling was therefore not possible. Instead samples were collected by venipuncture. This was done as quickly as possible (usually within 30 seconds) in order to reduce any effects on plasma PRL levels due to stress. While it is acknowledged that this method of sampling is bound to be stressful for the sheep, it was considered to be the only practical alternative. Routine blood samples were collected prior to milking or administration of drugs in an effort to minimise the influence of stress-related responses on the parameters measured. Since the same blood sampling methods were used throughout the research programme it is assumed that the stress effects were constant across treatment groups.

Administration of drugs is subject to the same problems as blood-sampling. The injection of drugs by the subcutaneous, intramuscular or intravenous routes, combined with the associated handling of the animals, is likely to elicit release of stress-related hormones which may in turn alter the measured parameters. In particular, the excipient used to dissolve and administer CB154 in the current research programme apparently caused a brief period of discomfort in ewes to which it was administered. Since this was a matter of daily routine some ewes seemed to adapt to the procedure while others became more agitated at the approach of humans. The long-acting version of CB154 (del Pozo *et al.* 1986) was not available and oral administration of CB154 has not been reported in ruminants so there was no alternative to daily injections. Since intramuscular injections seemed to cause more stress in the ewes than subcutaneous injections, the latter route was employed for administration of CB154, oPRL, excipient and Streptopen. Leocillin was administered intramuscularly (as per the manufacturers instructions) due to the relatively large volume involved. On the other hand, the intravenous route was chosen for the administration of oxytocin because it caused less stress than the alternative routes and because

it provided a more rapid milk let-down response with a minimal dose. It was considered important to minimise the dose of oxytocin used because of the possible lactogenic and galactopoietic effects of that hormone (see section 1.7.2). The intraductal route of administration, used for oPRL and excipient, probably caused less discomfort to the ewes than other methods of injection but, because of the need to tip the ewe on her rump and hold her for a considerable period, this was probably as stressful as the other methods employed. The intraductal route also introduced a possible source of infection and it was therefore necessary to treat all ewes with prophylactic antibiotics. While it has been suggested (Wilde 1991) that the antibiotics (Leocillin in particular) may have increased PRL transport from the plasma into the mammary gland via the paracellular route, all ewes, and indeed all glands, were subjected to the same treatment.

Several methods have been used to estimate milk yields in experimental animals (Cowie and Tindal 1971). Studies of lactogenesis usually require estimates of potential milk yield and, since new-born lambs are not capable of removing all of the available milk, methods reliant on the removal of milk by the young (the weigh-nurse-weigh and litter growth methods) are not satisfactory. On the other hand, permanent removal of the lambs (to be either sacrificed or hand-reared) so that total milk yields can be determined entirely by milking, involves a considerable economic cost. Thus, in many instances, a compromise method is used in which the lambs are separated from the ewes for a period of several hours each day, during which time the milk production is measured by milking. The actual milk yields are then adjusted to obtain an estimate of the daily milk yield or the rate of secretion is calculated.

The presence of lambs may affect the parameters measured in an experiment in various ways. The release of PRL at suckling may be reinforced by visual, olfactory and auditory stimuli (Cowie 1969). Milk production of ewes with lambs is likely to be altered by the ability of the lambs to remove milk. Since lambs of heavier birthweight present a higher demand for milk (Bonsma 1939; Wallace 1948; McCance and Alexander 1959; Peart 1967) estimates of milk yield obtained from ewes with lambs may be influenced by lamb birthweights. The size of the litter is also well known to affect milk yields. Hence treatment groups should be balanced with respect to the litter size and birthweights of lambs. Other uncontrolled factors such as the vigour of the lambs or their skill at suckling may add to variation in milk yields.

Different milkers and methods of milking can also be expected to contribute to variation in estimates of milk yield, both by presenting a differing demand for milk (or stimulus to milk production) and by their varying abilities to remove all of the milk from the gland. The importance of differences in hand-milking technique or skills can be reduced by machine milking and/or the use of oxytocin. Nevertheless, since machine milking frequently fails to remove all of the milk, hand-stripping is still necessary and the milker's skill can still affect the amount of milk removed. Furthermore, since milking elicits the release of oxytocin (Cowie and Tindal 1971) and PRL (Fell *et al.* 1972), the duration of machine milking and hand-stripping should be as uniform as possible

to avoid differential effects on these hormones. Milking skills and techniques designed to minimise variation in milk yields were previously discussed in Chapter 2.

This research programme commenced with a trial designed to determine whether CB154 treatment inhibited PRL release in local ewes and whether this delayed lactogenesis. Secondary objectives were to determine the period of CB154 treatment necessary to delay lactogenesis and to identify any evidence for the existence of "type I" and "type II" ewes as described by Kann *et al.* (1978).

Treatment with CB154 in trial 1 was successful in reducing PRL to very low levels and in reducing early lactation milk yields. It was thus concluded that CB154 treatment was effective in delaying lactogenesis. The 9- and 20-d periods of CB154 treatment were equally effective in depressing milk yields (averaged across ranks), but CB154 treatment was more effective in reducing milk yield in twin-bearing than in single-bearing ewes when used for the longer 20-d period. The differential effects on milk yield cannot be explained by corresponding effects on plasma PRL concentrations during the pre- or postpartum periods. This leaves two other possible explanations. Circulating PRL during the period 20 to 10 d prepartum may have an important effect on milk yield in twin- but not single-bearing ewes or, alternatively, the effect of the CB154 may be mediated via other hormones (but apparently not progesterone or insulin since they were not consistently affected by CB154). It is possible that oPL may be involved in this effect since twin-bearing ewes have higher plasma concentrations of oPL than single-bearing ewes at this time (Gluckman *et al.* 1979; Oddy and Jenkin 1981) and CB154 treatment has been reported to increase placental oPL concentrations considerably (Martal and Lacroix 1978). From this information, however, no supportable hypothesis can be offered as to the mechanism by which oPL could bring about the CB154-mediated depression of milk yields in the twin-bearing ewes relative to the single-bearing ewes.

In trial 1, CB154 treatment for 20 d, but not 9 d, increased plasma insulin concentrations. No logical relationship is apparent between the higher prepartum insulin concentration in ewes treated with CB154 for 20 d and subsequent depressed milk yields. Furthermore, this result is contrary to the findings of Hart and Morant (1980) and Johnsson *et al.* (1986) who reported that CB154 treatment decreased plasma insulin levels. However, it is apparent that possible effects of CB154 on insulin should be considered in future studies.

The lack of an effect of CB154 on progesterone concentrations is in agreement with previous reports (see above). Examination of the plasma PRL and progesterone profiles of individual ewes provided no evidence of the existence of "type I" and "type II" ewes. Because there has been no further published evidence supporting the differences reported by Kann *et al.* (1978) it is probable that the suggested different "types" do not exist.

The results of this trial indicate that further studies are required to clarify the interaction between effects of length of CB154 treatment (9 versus 20 d) and pregnancy rank (single- versus twin-bearing) on milk yield. Future investigations should attempt to replicate the current findings and examine possible effects on oPL while obtaining more detailed data on PRL and insulin levels. Administration of oPRL to single- and twin-bearing ewes for various periods prepartum may also be informative in this respect.

The main objective of the second trial was to establish that the milk yield responses to CB154 observed previously were effected by changes in circulating PRL concentrations. This was addressed by comparing the effects of oPRL with those of bicarbonate in CB154-treated ewes, and with the effects of ethanol/saline treatment. The administration of oPRL resulted in milk yields similar to those in the control (E/S) ewes and significantly greater than those in the bicarbonate-treated ewes, indicating that oPRL prevented the CB154-induced reduction of milk yields.

Interpretation of the results was complicated by the apparently chance occurrence of lower plasma PRL levels in the E/S than in the CB154-treated ewes and the fact that the timing of routine blood-sampling did not coincide with the expected peak PRL concentration in most of the E/S ewes. Thus it was impossible to demonstrate conclusively that CB154 had suppressed plasma PRL levels relative to those in the E/S ewes. Nevertheless, there are no published reports of the failure of CB154 to suppress PRL secretion or of the failure of the periparturient surge in PRL levels to occur. Thus it is assumed that these events did occur, in the appropriate groups of ewes, in trial 2.

In trial 2, completed in autumn, plasma PRL concentrations were much lower than those in trial 1 despite the fact that ewes in both trials were exposed to the same photoperiod during the experimental period. As noted earlier, recent evidence indicating that circannual rhythms are synchronised by exposure to long days during the previous spring and summer was not available when the programme commenced and the possibility that the parturient peak in plasma PRL concentrations may have been reduced in autumn-lambing ewes compared to spring-lambing ewes was not anticipated. Nevertheless, it is considered unlikely that the results of trial 2 were compromised by the fact that it was carried out in autumn. Although the mean blood-sampling time did not exactly correspond with the time of parturition, there was evidence that the expected peak in plasma PRL concentrations did occur in the E/S ewes but was absent in the CB154-treated ewes. Thus, the conclusion that administration of oPRL prevented the CB154-induced reduction of milk yields is believed to be valid.

Contrary to the findings in trial 1, the reduction of milk yields by CB154 treatment was much greater in single-bearing ewes than in twin-bearing ewes in trial 2. Furthermore, plasma PRL concentrations were reduced by CB154 treatment to a greater extent in single- than in twin-

bearing ewes during the period 2 to 7 d postpartum and may explain the low milk yields of single-bearing CB154-treated ewes during early lactation. However it does not explain the inconsistency in effects of 9-d (trial 1) versus 7-d (trial 2) CB154 treatment on milk yields of single-bearing ewes. These results are likely to be confounded by the seasonal differences between trials 1 and 2. Not only were plasma PRL concentrations higher, but milk yields were also higher, in the spring-lambing ewes than in the autumn-lambing ewes. However, any such comparisons between trial 1 and 2 can only be informal since the trials were not designed specifically to allow such comparisons. Formal comparisons between spring- and autumn-lambing ewes were subsequently carried out in trial 5.

Trial 2 demonstrated that the administration of oPRL to CB154-treated ewes prevented the CB154-induced reduction of milk yields. This is the first time that this has been demonstrated in sheep and confirms the earlier result of Akers, Bauman, Capuco *et al.* (1981) obtained in cows. This result is important because it establishes that PRL is essential for complete, normal lactogenesis in ewes and, further, that the effect of CB154 on lactogenesis in ewes is caused by the suppression of plasma PRL concentrations and not by effects on some other hormone(s).

Having established that PRL is essential to the normal process of lactogenesis in the ewe, the next step was to determine whether it acted directly on the mammary gland or whether lactogenesis required high circulating PRL concentrations, perhaps because the effects of PRL were mediated through some other intermediate hormone. It was decided to inject oPRL directly into the mammary gland via the teat duct. Although intramammary administration of PRL has not been previously reported in ruminants, lactogenesis has been stimulated by direct administration of oPRL into the mammary gland of pseudopregnant rabbits via the teat duct (Falconer and Fiddler 1970) suggesting that oPRL acts directly on the rabbit mammary gland. The intraductal route of administration has several advantages over alternative methods of raising the mammary arterial concentration of PRL (such as s.c. or i.m. injection or jugular vein, mammary or pudendal artery infusion). In particular, the intraductal method is relatively non-invasive in that it does not involve the degree of pain or stress associated with injections or surgical procedures. Furthermore, it calls for a considerably lower dose than might be required to raise circulating hormone concentrations sufficiently to affect mammary gland function and provides a natural slow release mechanism which should maintain high local oPRL concentrations in the vicinity of the secretory epithelium. Finally, it ensures that oPRL is delivered directly to the secretory cells and enables any local mammary responses to be attributed to the direct action of the hormone on the gland (as long as significant amounts do not enter the peripheral circulation).

The first objective of trial 3 was to determine whether the 10 mg intramammary dose, chosen following an empirical pilot trial, raised circulating PRL concentrations. This involved comparing the plasma PRL levels of ewes treated with oPRL administered via the intramammary route into one gland, with those of ewes treated with BIC in both glands . The second objective was to

determine whether specific lactogenic responses to oPRL occurred in individual glands treated intraductally with the hormone.

The dose of 10 mg/gland did not raise circulating PRL concentrations. Furthermore, the milk yields of BIC-treated glands in CB+BIC ewes did not differ from those of BIC-treated glands in CB+PRL ewes, demonstrating that there were no effects of oPRL, transferred via the circulation from the treated gland, on the contralateral gland. Hence any differences in milk yield between PRL- and BIC-treated glands could be attributed to the oPRL treatment. However, milk yields did not differ between the oPRL- and BIC-treated glands indicating that the intramammary injection of oPRL had no effect on lactogenesis in this trial. In retrospect, given the results of trial 4, this was most probably due to the high incidence of mastitis observed amongst the ewes.

In trial 4, which was similar in design to trial 3, prophylactic antibiotic treatment was apparently effective in minimising the incidence of mastitis. The glands treated with oPRL produced significantly more milk than the BIC-treated glands and the increased milk yield was maintained throughout the 8-week period, indicating that the oPRL had effected a permanent change in the ability of the gland to produce milk. This is consistent with the finding that milk production of cows was correlated with the total PRL content of the mammary gland on the day of parturition (van Zyl *et al.* 1986).

Trial 4 thus demonstrated that oPRL can have lactogenic effects when administered directly into the mammary gland of CB154-treated ewes, apparently without the need for a putative intermediate hormone. This result has not been previously reported in the literature (at least with respect to ruminants). Furthermore, results indicate that the oPRL caused a permanent increase in milk yields. It is therefore concluded that PRL is important to the complete initiation of lactogenesis in ewes, that it acts directly on the gland and that it may be necessary for establishing the maximum potential of the gland to secrete milk. The mechanism by which oPRL might increase the potential milk secretion of the gland is unknown. Recent studies relevant to this problem will be discussed later in this chapter.

At the conclusion of trial 4 it was evident that further studies were required to determine if supplementary oPRL administered to normal (i.e. not CB154-treated) ewes could increase milk yields. Since low circulating PRL levels in autumn-lambing ewes were associated with low milk yields (compared to those of spring-lambing ewes) (see Chapters 3 and 5) it seemed logical to test the effect of supplementary oPRL, administered via the teat duct, in autumn-lambing ewes. This was subsequently examined in trials 6 and 7.

The possibility that dietary differences were responsible for the seasonal differences in plasma PRL concentrations, milk yields, milk composition, lamb birthweight and lamb growth rate, observed in the informal comparison of trials 1 and 2, was examined in trial 5. A severe outbreak of facial eczema, and the associated pasture management practices adopted to minimise the

numbers of animals affected by the disease, resulted in the available autumn-lambing ewes being considerably lighter on average than the spring-lambing ewes. Despite this, the seasonal differences (except for milk composition and lamb birthweight) were again evident in trial 5 but they were confounded with corresponding differences in ewe live weight and it was not possible to determine whether dietary differences contributed significantly to the differences in lactational parameters. Furthermore, it is possible that the pattern of change in photoperiod prior to the trial had a carry-over effect on PRL levels as discussed earlier. In retrospect, the seasonal PRL and milk yield differences were probably also confounded with seasonal temperature differences. Although photoperiod has a major role in seasonal changes, the possibility that temperature has an important effect on seasonal plasma PRL levels has only recently been suggested (Forsyth 1992) (see discussion earlier in this chapter).

The failure of this trial to answer the original questions, and the subsequent consideration of the reasons for that failure, have highlighted the lack of definitive information on the effects of photoperiod, ambient temperature and diet on productive processes in the sheep. It is clear that carefully designed trials will be necessary to elucidate the relative importance of these factors. Such investigations would involve housing spring- and autumn-lambing ewes, under conditions of constant temperature while feeding them the same diet, from mating until lambs are weaned.

In Chapter 5, reported effects of live weight and nutrition on milk yield were discussed. Although reports differ as to the importance of these factors in determining milk yields, several studies showed that poor prepartum nutrition reduced subsequent milk production. Examination of the endocrine basis for the effect of poor nutrition during late pregnancy on subsequent milk yields may improve understanding of the mechanisms controlling lactogenesis. However, such studies would risk problems with pregnancy toxaemia as discussed earlier. Furthermore, they would be extremely difficult to interpret since the energy status of pregnant and lactating animals is confounded with physiological state.

The objective of the final trials was to measure responses to supplementary oPRL in normal (i.e. not treated with CB154) autumn-lambing ewes which, based on previous results (Chapter 5), were expected to have low plasma PRL concentrations and milk yields relative to spring-lambing ewes. Two routes of oPRL supplementation were used in order to test the effectiveness of elevating peripheral or local levels of PRL. Thus, in trial 6, the comparison was between intramammary treatment with oPRL in one gland and with excipient in the contralateral gland of each ewe while, in trial 7, responses in ewes supplemented systemically with oPRL were compared with those in untreated (control) ewes.

Administration of supplementary oPRL directly into the gland did not increase the milk yields, or change the composition of milk, compared to glands treated with BIC. Neither did subcutaneous injection of oPRL increase milk yields, or alter the milk composition, compared to Control group

ewes. These results suggest that the circulating level of PRL, and the intramammary concentration of PRL, in autumn-lambing ewes are not limiting lactogenesis.

However, plasma PRL concentrations were considerably higher in trials 6 and 7 than those recorded in the previous autumn trials (trials 2 and 5).This may have been because the sampling times in the various trials did not correspond well with each other and so were not directly comparable. Results of trial 6 and 7 also indicate that the peripartum PRL concentrations in these autumn-lambing ewes were similar in magnitude to those recorded in control (E/S group) ewes lambing in spring (trial 1). This raises the question of whether circulating PRL concentrations are, in fact, consistently lower in autumn- than in spring-lambing ewes. Since circulating PRL concentrations in goats have recently been shown to respond to seasonal changes in ambient temperature independently of photoperiod (Forsyth 1992), it is possible that warm temperatures during trials 6 and 7 caused higher than normal plasma PRL levels in the autumn-lambing ewes. However, meteorological records indicate that mean daily temperatures differed little between the three autumn trials carried out during this research programme. While ambient temperature may alter circulating PRL levels in sheep, the effect of temperature is expected to be smaller than the photoperiod effect since spring-lambing ewes usually have higher plasma PRL levels than autumn-lambing ewes, despite the fact that mean daily August temperatures are about 5 ^{o}C lower than those in April (data from the New Zealand Meteorological Service, Palmerston North). Further studies involving blood sampling under conditions of controlled temperature, photoperiod and nutrition will be required to establish conclusively the relative importance of seasonal factors in determining the seasonal changes in plasma PRL concentration in sheep.

Because the plasma PRL concentrations in the ewes were so high in trials 6 and 7, it was not possible to reach firm conclusions regarding possible effects of supplementary oPRL in ewes with naturally low plasma PRL concentrations. Nevertheless, the results do indicate that raising the intramammary concentration of PRL around the time of parturition, in ewes with circulating PRL levels characteristic of normal spring-lambing ewes, does not enhance lactogenesis. This is the first report of PRL supplementation, carried out at the time of lactogenesis, in animals which have normal plasma levels of PRL, although there are two reports of PRL supplementation during established lactation. PRL has been previously injected into lactating cows (Plaut *et al.* 1987) and infused into goats (after milking)(Jacquemet and Prigge 1991), without affecting milk yields.

To determine the effect of PRL supplementation in ewes with naturally low plasma levels of PRL it will be necessary to ensure that plasma PRL levels are, in fact, low before the administration of PRL. Recently it was demonstrated that both basal and periparturient peak plasma PRL concentrations in pregnant dairy heifers were increased by exposure to longer photoperiod from d 128 of pregnancy (Newbold *et al.* 1991). Hence, future trials will be best attempted in ewes subjected to short photoperiod and constant, low ambient temperature throughout the majority of the gestation period.

Finally, it is appropriate to reconsider the meaning of the term "lactogenesis", to summarise the role of PRL in that process (as it is currently understood) and to speculate briefly upon aspects that may lead to better understanding in the future.

In reviewing the literature, it was found necessary to carefully consider the meaning of the term "lactogenesis" (see Chapter 1). The more general definition of lactogenesis, as the initiation of milk secretion (Cowie *et al.* 1980) was, for the purposes of this study, too broad. The distinction between lactogenesis type I, the gradual appearance of precolostrum in the gland, and lactogenesis type II, the onset of copious milk secretion (Fleet *et al.* 1975) is more applicable and hence the term "lactogenesis" has been used herein synonymously with "lactogenesis type II". As was discussed in Chapter 1, this definition of lactogenesis involves a quantitative aspect as well as a temporal component. For semantic reasons it is tempting to further establish the limits of these temporal and quantitative aspects of lactogenesis, but the setting of arbitrary limitations can prejudice the understanding of the true processes involved.

If the final stages of alveolar epithelial growth and differentiation are considered to be a component of lactogenesis (rather than of mammogenesis) it may be that the delaying effects of CB154 treatment on lactogenesis can be largely attributed to a delay in the completion of differentiation of the epithelial cells (even if PRL has no other actions on lactogenesis). If, on the other hand, the final stages of alveolar epithelial growth and differentiation are considered to be a component of mammogenesis, then the effects of prepartum CB154 treatment can be entirely attributed to an effect on mammogenesis. However, it is likely that arbitrary definitions of mammogenesis and lactogenesis are not biologically sustainable and that, since the development of the epithelium is a continuous process, PRL is probably required over a considerably longer period prepartum than would be expected for the classical definition of lactogenesis (stage II). Put more simply, mammogenesis and lactogenesis overlap in time. From this point of view, the results of Hooley *et al.* (1978)(see Chapter 1, table 1.2), which conflict with most other reports, are understandable. They concluded, from the sum of their results, that PRL was important for mammogenesis and galactopoiesis, but not for lactogenesis. Since the development of the epithelium requires a longer period of PRL stimulation than the induction process allows, it is not surprising that inhibition of PRL secretion during the trigger phase failed to affect the onset of milk production. Thus, the disagreement between their conclusions and those of others in the literature is not really about the action of PRL, but about the definition of the process of lactogenesis. This emphasises how important it is for researchers to define carefully the terms they are using and the processes they are studying.

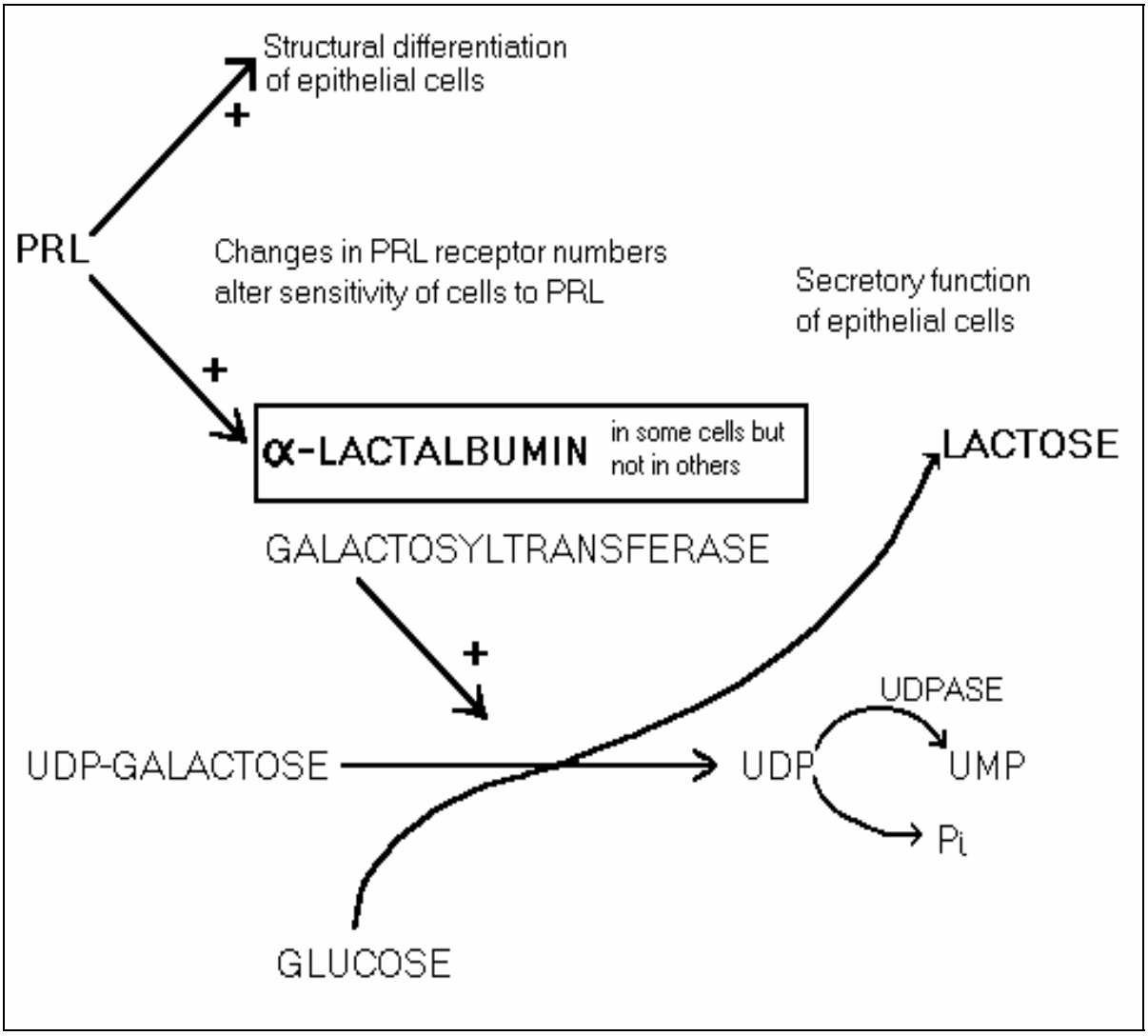
During recent years the appearance of relevant publications in the international literature indicates that researchers continue to seek to further define the role of PRL in the control of aspects of lactational performance. Selected salient reports indicate some areas of research which may lead to a better understanding of the role of this hormone.

Hartmann (1992) suggested that the endocrine system sets the upper limits to lactation while autocrine mechanisms down-regulate production. The mechanism by which oPRL might increase the potential milk secretion of the gland is unknown, but the following reports suggest a possibility. It has been suggested (Kuhn and White 1977) that the rate limiting step in lactose synthesis is the utilisation of UDP-galactose by Golgi vesicles in lactose synthesis:

UDP-galactose + glucose ----------> UDP + lactose

This reaction is catalysed by the enzyme galactosyltranferase in the presence of α-lactalbumin, which are the A and B protein subunits of lactose synthetase (Linzell and Peaker 1971b). The fall in circulating progesterone concentrations at the end of pregnancy permits the stimulation of α-lactalbumin synthesis by PRL, completing the lactose synthetase unit and so the catalysis of the final step in lactose synthesis (Cowie and Tindal 1971). PRL receptor numbers in goat mammary glands increase in response to thrice-daily milking and decrease following incomplete milking relative to the contralateral glands which are milked twice daily (McKinnon *et al.* 1988). This unilateral response shows that potential sensitivity to PRL can be modulated independently in the two glands. Recently, in mammary glands from pregnant and lactating ewes, a cRNA probe for the bovine α-lactalbumin gene revealed that α-lactalbumin gene expression was found in the secretory epithelium of some alveoli but not in others (Molnaar *et al.* 1991) suggesting that expression of this gene can be turned on and off in specific areas of the gland. Hence, it may be that PRL sets the upper limit to lactation during lactogenesis by determining the proportion of secretory cells which express the α-lactalbumin gene. It would also seem logical to use a cRNA probe to test the effect of oPRL on α-lactalbumin gene expression. Possible relationships between these various findings are illustrated in Figure 7.1.

FIG 7.1 POSTULATED MECHANISM BY WHICH PROLACTIN MAY SET THE UPPER LIMITS FOR LACTATION THROUGH THE PROPORTION OF CELLS EXPRESSING THE α-LACTALBUMIN GENE



During the course of this research programme, potentially promising areas for future research were identified. Several proposals have been outlined previously but it is appropriate to suggest others at this point.

Since unilateral intramammary oPRL administration has demonstrated the importance of PRL for lactogenesis in CB154-treated ewes, it would be interesting to attempt to induce lactation in one gland of non pregnant ewes by progesterone withdrawal combined with unilateral oPRL treatment. Comparison of milk yields between glands should confirm the importance of oPRL in the initiation of lactogenesis. Furthermore, comparison of oPRL treated glands in late-pregnant versus non-pregnant ewes induced to lactate (if the above procedure were effective) may quantify the importance of oPRL in relation to other lactogenic hormones. This relies on the assumption that other lactogenic hormones would not be active in non-pregnant ewes and would not assume their roles upon the administration of oPRL to pregnant ewes.

A further study worthy of investigation is the possibility of raising circulating PRL levels by the administration of antibodies specific to PRL receptors or by immunising ewes against their own PRL receptors (see Shiu and Friesen 1980). This should raise plasma PRL concentrations without the need for oPRL administration and could be used to examine the long-term effects on lactation of elevated PRL levels.

Finally, since human GH binds specifically to oPRL receptors better than does oPRL, and also binds well to oPL receptors (Emane *et al.* 1986) it may be more effective than oPRL for initiating lactogenesis in ewes. If this is the case it may prove to be a valuable tool for studying the mechanisms controlling lactogenesis. Furthermore, it may have practical commercial application when genetic manipulation of sheep becomes economically viable.

In conclusion, this research programme has clarified the role of PRL in ovine lactogenesis. It has dispelled any doubt that PRL is important for the normal, rapid and complete onset of copious milk secretion in ewes. The first reported administration of PRL to peripartum CB154-treated ruminants has established that the effect of CB154 on lactogenesis is mediated through suppression of PRL secretion and not by effects on some other hormone. The first reported intramammary administration of PRL to CB154-treated ruminants has shown that PRL acts directly on the mammary gland without the need for a putative intermediate hormone, that changes in local oPRL levels (but not in systemic levels) are a prerequisite for lactogenesis, and that intramammary PRL concentrations during lactogenesis may have long-lasting effects on lactation. Finally, the first reported administration of supplementary PRL to periparturient ewes with normal plasma PRL concentrations has indicated that mammary PRL levels during lactogenesis are not limiting milk production in normal ewes. Thus the administration of exogenous oPRL during the periparturient period does not appear to be a viable means of increasing milk yield in the ewe.

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