Effects of season, lactation and plane of nutrition on prolactin concentrations in ovine plasma and the role of prolactin in the control of ewe fertility

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Summary. Plasma prolactin concentrations during the first 2 months after lambing, at oestrus, and during early pregnancy were investigated in 2 experiments in which Finn × Dorset Horn ewes were mated at an induced oestrus approximately 9 weeks after lambing.

Mean prolactin concentrations between lambing and mating were dependent on season, being >260 ng/ml plasma in lactating ewes mated in July and <150 ng/ml in those mated in October. Within 8 days of weaning of the lambs at 50 days post partum values declined to 122 and 30 ng/ml respectively. Plane of nutrition had little effect on prolactin levels. Higher prolactin values were recorded during oestrus in ewes mated in March or July, the normal period of anoestrus, than in December, the normal breeding season, mean values being approximately 200 ng/ml and 35 ng/ml respectively. The mean increases in the concentrations of prolactin during oestrus were smaller in lactating than non-lactating ewes.

It is suggested that these differences in prolactin levels may be responsible for the effects of season and lactation on ewe fertility.

Introduction

While there is evidence that prolactin is concerned with the establishment and maintenance of lactation in some species, including the sheep (Tucker, 1974; Kann, 1976), and may have luteotrophic and/or luteolytic effects (Denamur, Martinet & Short, 1973; Kann & Denamur, 1974), its precise functions in reproduction in sheep are uncertain. There is evidence that concentrations of prolactin in sheep plasma are elevated during the normal period of sexual inactivity (Pelletier, 1973; Ravault, 1976; Erb, Sitarz & Malven, 1977) and during lactation (Lamming, Moseley & McNeilly, 1974), particularly during and immediately after suckling (McNeilly, Moseley & Lamming, 1972). There is also some evidence that high plasma prolactin concentrations may depress oestrus and ovarian activity (Kann & Martinet, 1975). However, little is known about the pattern of change in prolactin concentrations during the interval between lambing and re-mating and how this is related, if at all, to reproductive activity. Furthermore, little is known about the role of the preovulatory surges of prolactin which occur before and during oestrus (Kann, 1971; Lamming et al., 1974) and how they differ with season and lactation status.

Rhind, Robinson, Chesworth & Phillippo (1980) have reported that the fertility of Finnish Landrace × Dorset Horn ewes in a frequent breeding programme was significantly affected by season of mating, lactation and the level of feed intake during lactation. The present paper reports the patterns of prolactin concentrations in the plasma of the same ewes during the first 2 months after lambing, oestrus and early pregnancy.

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Materials and Methods

The details of the 2 experiments are given by Rhind et al. (1980) and only those features relevant to the interpretation of the present data are described here.

*Ewes.* Each experiment involved two flocks each of 48 Finn × Dorset Horn ewes. They were maintained on a breeding interval of 7 months by using intravaginal pessaries impregnated with the progestagen SC 9880 (G. D. Searle Ltd) followed by an intramuscular injection of 400 i.u. PMSG (Folligon: Intervet Ltd) in 2 ml sterile saline at the time of pessary withdrawal. All matings were by Suffolk rams.

*Treatments.* In Exp. 1 lambs were removed from the ewes to end lactation at 35 and 15 days before mating or 7 and 35 days after mating. These correspond to durations of lactation of approximately 30, 50, 70 and 100 days and these treatments were designated W30, W50, W70 and W100. Matings took place on 4 December (Flock D) or 19 March (Flock M). During lactation the ewes were fed *ad libitum* and their mean daily metabolizable energy (ME) intakes were over 30 MJ. After weaning daily ME intakes were restricted to 14 MJ.

In Exp. 2 the ewes were mated on 1 July (Flock J) or 15 October (Flock O). During the first 50 days of lactation all ewes were fed *ad libitum*, and their daily ME intakes were about 25 MJ. Lambs were removed from some of the ewes to end lactation after 50 days (W50) and all ewes were allocated to a high (H) or low (L) level of feeding from Day 50 until Day 100 *post partum*. All ewes were mated 9 weeks *post partum* and those that were still lactating had their lambs removed 7 days later (W70). The treatments were designated W50H, W50L, W70H and W70L.

*Blood sample collection.* Samples were collected from the jugular vein into 10 ml evacuated glass tubes using a 20-gauge needle. Each tube contained 100 i.u. heparin (Boots Ltd, Nottingham). Samples were immediately placed on ice and then centrifuged at 4°C within 1 h of collection. All ewes were accustomed to frequent handling and venepuncture and care was taken to minimize stress.

In both experiments, blood samples were collected from all ewes at 3-h intervals from the time that the first ones were observed in oestrus until 27 h after onset of oestrus for each ewe.

The relationship between prolactin and progesterone during the first 10 days of pregnancy was also investigated. Blood samples were collected on three occasions at 3- or 4-day intervals during this time from randomly selected ewes from Flocks D, M, and O.

In Exp. 2, samples were also collected at 10-day intervals between parturition and re-mating.

*Hormone determinations.* Plasma prolactin concentrations were determined by radio-immunoassay using polymerized second antisera. Details of the preparation of the antisera and of the assay procedures are given by Chesworth (1977). The sensitivity of the assay was 0.03 ng and the intra- and inter-assay coefficients of variation with the automated technique that was used were 1.4% (*n* = 8) and 3.3% (*n* = 5) respectively. Cross-reactions with related hormones were negligible. A modification of the method of Henricks, Dickey & Hill (1971) was used for progesterone determinations. The antiserum (Y29/6) was raised in sheep to 6β-hydroxyprogesterone-hemisuccinyl–BSA and diluted to 1:4000 in phosphate-buffered saline–0.1% gelatin. Cross-reactions were 8.8% with 5-pregnane-3,20-dione, 11.1% with deoxycorticosterone and ≤0.02% with other steroids. The mean recovery rate of [*3H]*progesterone was 78 ± 0.7% (*n* = 12 for each of 4 concentrations between 0.5 and 9.5 ng/ml). The sensitivity of the assay was 0.04 ng/tube and intra- and inter-assay coefficients of variation were 12% (*n* = 19) and 22% (*n* = 42) respectively.

*Statistical methods.* The main effects of season and treatment were tested for significance using a 2-way classification analysis of variance. To obtain homogeneity in the variance between treatment groups, the values analysed were the natural logarithms of the prolactin values plus one. Furthermore, the data for each season were analysed separately to examine the treatment effects on concentrations of plasma prolactin independently of the highly significant differences between seasons. Before converting logarithmic values to absolute values, a standard adjust-
ment was made to allow for bias arising from averaging values on a logarithmic scale and transforming back. The adjustment consisted of adding 0.5 (r.s.d.)² to the logarithmic value before transformation (Kendall & Stuart, 1961).

**Results**

Although the design of Exps 1 and 2 differed, the results complement each other and allow the development of an overall hypothesis on the role of prolactin in the breeding ewe. For this reason the data for both experiments are presented concurrently.

**Plasma prolactin during the first 2 months post partum**

Since all the ewes were treated similarly during the first 50 days post partum in Exp. 2 plasma prolactin concentrations for each sampling time were pooled for each flock. The mean values, together with those recorded subsequently for each treatment, are given in Text-fig. 1. There was a large seasonal difference in prolactin concentrations (P < 0.01), mean values during the first 50 days post partum, when all the ewes were lactating, being >300 ng/ml in Flock J ewes and <150 ng/ml in Flock O ewes. While mean plasma prolactin concentrations decreased (P < 0.001) after weaning, the size of the decrease was not significantly affected by plane of nutrition.

![Text-fig. 1. Mean concentrations of prolactin in the plasma of ewes between lambing and mating in July (O) or October (●). The arrow indicates time of weaning for ewes in Groups W50H and W50L and the time when nutritional treatments were started (W50 and W70 = weaned after 50 and 70 days of lactation respectively; L = low plane, H = high plane). The mean coefficient of variation was 44%.](image)

**Plasma prolactin concentrations during oestrus**

Not all of the ewes of Flock D were sampled at 12 and 18 h after the onset of oestrus because of technical difficulties, and mean prolactin values at these times were therefore based on fewer ewes than at other times for this flock.
Mean prolactin concentrations in the plasma of ewes of Flocks D and M were not significantly different for ewes which subsequently lambed to the induced oestrus and those that did not. The data for the ewes of the two categories were therefore pooled. Mean prolactin concentrations for Flock M ewes were significantly higher ($P < 0.01$) than those for Flock D ewes throughout the period of sampling (see Text-fig. 2). Similarly, in Exp. 2 the concentrations were higher in Flock J than Flock O (see Text-fig. 3). Within each flock there were no significant differences between ewes on different planes of nutrition. For this reason only the pooled data for each flock are illustrated in Text-fig. 3. Mean prolactin concentrations were significantly higher ($P < 0.001$) for 12 h or more after onset of oestrus than before oestrus in ewes of Flock D and in Group W30 of Flock M. The trend was either absent or greatly reduced in the other groups.

![Text-fig. 2](image)

**Text-fig. 2.** Mean concentrations of prolactin in the plasma of ewes which had their lambs weaned at 35 (——) or 15 (———) days before mating, or 7 or 35 (———) days after mating in December (●) or March (○). The mean coefficients of variation were 86% and 54% for the respective flocks. Significant differences between non-lactating and lactating ewes in March are indicated: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; N.S., not significant.

Role of the preovulatory surge of prolactin

To investigate the possibility that the preovulatory prolactin surge may influence luteal function, the relationship between mean prolactin values for the first 21 h of oestrus and mean progesterone values for the first 20 days of pregnancy of ewes which conceived to the induced oestrus was investigated. As there were no significant differences between treatments in the progesterone profiles at each time of year, the investigation was based on the pooled values for all treatments. No significant relationship was found at any of the four times of year.
Text-fig. 3. Mean concentrations of prolactin in the plasma of lactating (▲, ●) and non-lactating (▲, ○) ewes during induced oestrus in July (▲, △) or October (●, ○). The mean coefficients of variation were 48% and 41% for the respective flocks. The significance of the differences between non-lactating and lactating ewes is indicated for July (above) and October (below): *P < 0.05; **P < 0.01; ***P < 0.001; N.S., not significant.

Text-fig. 4. Mean plasma concentrations of progesterone during the first 10 days of pregnancy plotted against the corresponding mean prolactin values in ewes mated in December (●), March (□) or October (●).
Prolactin and progesterone levels during early pregnancy

As shown in Text-fig. 4, the high prolactin values in March were associated with low progesterone values. In contrast, the highest levels of progesterone were recorded when prolactin concentrations were lowest, i.e. in December.

The fertility of lactating ewes at the induced oestrus was lower than that of non-lactating ewes (Table 1). The difference was greatest when prolactin concentrations were high and when the difference in mean concentrations between the ewes of the two categories was greatest, i.e. in March and July.

<table>
<thead>
<tr>
<th>Month of mating</th>
<th>Non-lactating ewes</th>
<th>Lactating ewes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of ewes conceiving (%)</td>
<td>Prolactin conc. (ng/ml)</td>
</tr>
<tr>
<td>December</td>
<td>20 (90)</td>
<td>33.1</td>
</tr>
<tr>
<td>March</td>
<td>21 (88)</td>
<td>167.7</td>
</tr>
<tr>
<td>July</td>
<td>10 (56)</td>
<td>135.4</td>
</tr>
<tr>
<td>October</td>
<td>13 (72)</td>
<td>68.9</td>
</tr>
</tbody>
</table>

Discussion

The most notable feature of the results was the very large seasonal changes in the mean prolactin concentrations, particularly in lactating ewes. With no significant effect of plane of nutrition on prolactin, these seasonal changes in the concentration of the hormone cannot be attributed to seasonal changes in nutritional state. Seasonal changes in prolactin have been reported previously in rams (Pelletier, 1973; Ravault, 1976) and in lactating ewes (Erb et al., 1977) but they were smaller than those recorded in the present study. The causes of the discrepancies between the results are not clear but differences in breed and latitude may be responsible.

While the higher mean prolactin values recorded in lactating than non-lactating ewes were probably due to the prolactin surges which occur in ewes during and immediately after suckling (McNeilly et al., 1972), the suckling stimulus may also have resulted in a higher basal secretion rate (Hart, 1972).

The reduced fertility of ewes mated in March and July, in particular those that were lactating at the time of mating, was associated with high concentrations of prolactin. At these times of year, prolactin concentrations were much higher in the lactating than in the non-lactating ewes. In contrast, the higher fertility of lactating and non-lactating ewes mated in October and December was associated with low concentrations of prolactin and with only minor effects of physiological state on the hormone. Furthermore, the rapid decline in plasma prolactin concentrations following weaning in June, after 50 days of lactation, was associated with an improved conception rate at mating 15 days later. All of these observations are consistent with the hypothesis that high plasma prolactin concentrations are detrimental to fertility.

The absence of any relationship between prolactin concentrations and fertility within each flock may be a function of the considerable variation in the data. This may partly be the result of stress-induced surges of prolactin in some animals at sampling (Lamming et al., 1974), but differences in the sensitivity of target organs of individuals and short-term changes in the patterns...
of prolactin release due to variation between individuals in milk production and suckling frequency may also be involved.

It has been suggested that high prolactin concentrations may depress fertility by interfering with LH release (Kann, Martinet & Schirar, 1976, 1977). There was little evidence to support this in the present study for neither season of breeding nor physiological state influenced the pattern of LH release (Rhind et al., 1980). Walton, McNeilly, McNeilly & Cunningham (1977) have suggested that prolactin may have an effect on follicular growth, but studies on ewes (Rhind, Chesworth & Robinson, 1978), women (Seppala, Hirvonen & Ranta, 1976) and human granulosa cells cultured in vitro (McNatty, Sawers & McNeilly, 1974) indicate that high prolactin levels are often associated with reductions in progesterone production and luteal function. In the present study, prolactin concentrations were low in December and progesterone levels in early pregnancy were high while in October and March when prolactin levels were much higher, the corresponding progesterone concentrations during early pregnancy were depressed. It is possible that when plasma prolactin concentrations are more than about 50 ng/ml, progesterone secretion is suppressed.

Kann & Denamur (1974) suggested that the preovulatory prolactin surge was involved in the establishment and function of corpora lutea (i.e. high levels may be necessary at this time). Haresign, Foster, Haynes, Crighton & Lamming (1975) considered that abnormalities in luteotropic factors at about the time of ovulation may have been responsible for the reduced luteal activity recorded in ewes treated with LH-RH during anoestrus. In the present study the functional activity of the corpora lutea, as estimated by peripheral progesterone levels, appeared normal following prolactin surges of widely different magnitude, but this does not preclude the possibility that corpora lutea formed after small prolactin surges may be subsequently more susceptible to luteolytic factors. The high prolactin concentrations during pregnancy which were characteristic of ewes mated during the anoestrous period may have had such a luteolytic effect, as has been demonstrated in rats (Wuttke & Meites, 1971) and mice (Grandison & Meites, 1972). Support for the suggestion that prolactin levels may affect luteal function and fertility in this way in sheep is provided by the higher incidence of luteal regression, before and after the normal time (14–21 days after mating), in lactating than in non-lactating ewes (Rhind et al., 1980).

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