A seasonal difference in ovine peripheral plasma prolactin and progesterone concentrations in early pregnancy and in the relationship between the two hormones

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Summary. In the first 11 days of pregnancy, progesterone and prolactin levels were significantly correlated (P < 0.05), positively in December-mated animals (r = 0.889) and negatively in those mated in March (r = -0.865).

Behavioural oestrus and ovulation in the sheep can be induced during seasonal anoestrus by means of exogenous hormones, but fertility is lower than in ewes treated similarly during the natural breeding season (Allen & Lamming, 1960; Robinson, 1971). Whether or not the lower fertility is caused by seasonal differences in the endocrine status of the ewe in early pregnancy has not been resolved. Differences in peripheral plasma progesterone concentrations during early pregnancy in ewes mated in December and March have been reported (Rhind, Robinson, Fraser & Phillippo, 1977) but these were not attributable to ovulation rate, or to nutrition because diet and feeding regimens were the same for each time of year. Although there is evidence that prolactin is luteotrophic in the sheep (Denamur, Martinet & Short, 1973), studies of luteinized human granulosa cells in vitro indicate that while low concentrations of prolactin are required for progesterone production, high concentrations can have an inhibitory effect (McNatty, Sawers & McNeilly, 1974). We have therefore investigated seasonal differences in the concentration of plasma prolactin during early pregnancy in ewes mated at two times of year and in the relationship between plasma prolactin and progesterone concentrations.

Materials and Methods

The experimental animals were 12 Finnish Landrace × Dorset Horn ewes selected at random. They were housed under natural daylength conditions at 57°10'N 2°04'W and penned individually for the control of food intake. Oestrus in each ewe was synchronized by the insertion of an intravaginal pessary impregnated with 30 mg synthetic progestagen (SC 9880: G. D. Searle Ltd), plus 400 mg pure progesterone (Sigma Chemicals, St Louis, Missouri, U.S.A.). At pessary withdrawal after 12 days each ewe received an intramuscular injection of 400 i.u. PMSG (Folligon: Intervet Ltd). The ewes were mated by a fertile ram at approximately 48 and 60 h after pessary withdrawal on either 4 December (Group D, 6 ewes) or 19 March (Group M, 6 ewes). Blood samples were taken from each ewe by jugular venepuncture at 3- or 4-day intervals during the first 20 days of pregnancy and thereafter at weekly intervals until Day 62. The ewes were accustomed to frequent handling and care was taken at sampling to minimize stress. The blood samples were placed on ice until centrifugation.

The plasma progesterone concentrations were determined by a modification of the radioimmunoassay technique of Henricks, Dickey & Hill (1971). The antiserum (Y29/6) was raised in sheep to 6 β-hydroxyprogesterone-hemisuccinyl-BSA and diluted to 1:4000 in PBS 0.1% gelatin. Average recovery of [3H]progesterone for each of four concentrations within the range 0.5 to 9.5 ng/ml was 78 ± 0.7% (n = 12 for each concentration) and intra- and inter-assay coefficients of variation were 12% (n = 19) and 22% (n = 42) respectively. The sensitivity of the assay was 0.04 ng. Cross-reactions were 8–8% with 5β-pregnane-3,20-dione, 11.1% with deoxycorticosterone and ≤0.2% with other steroids.

The preparation of the antisera for the prolactin assay and of the assay procedures were as described by Chesworth (1977). The sensitivity of the assay was 0.03 ng and the intra- and inter
Days after mating

Text-fig. 1. Peripheral plasma prolactin (a) and progesterone (b) concentrations during the first 2 months of pregnancy in Finnish Landrace × Dorset Horn ewes mated in December (——) and March (---). Values are mean ± s.e.m. for 6 ewes.

assay coefficients of variation with this automated technique were 1·4% (n = 8) and 3·3% (n = 5) respectively.

The statistical significance of the seasonal differences in hormone concentrations were obtained by using the Mann–Whitney U-test.

Results

The mean prolactin and progesterone concentrations in the peripheral plasma for each of the 12 sampling times within each time of year of mating are given in Text-fig. 1. The overall mean plasma prolactin concentration for Group D was 23 ng/ml compared with 109 ng/ml for Group M. The corresponding values for plasma progesterone concentrations were 6·4 and 3·5 ng/ml respectively. Both these seasonal differences were significant (P < 0·001). Examination of the data given in Text-fig. 1 revealed that the largest differences in plasma progesterone concentrations between the two groups of ewes occurred between Days 4 and 11 (7·3 and 2·6 ng/ml), i.e. during the early life of the corpus luteum, and from Days 48 to 62 (8·3 and 4·1 ng/ml), i.e. when the placenta starts to contribute significantly to progesterone production in the ewe. To investigate the significance of these differences the data were divided into four periods, each consisting of three consecutive sampling times, and the mean plasma progesterone and prolactin concentrations were calculated for each ewe for each of...
the four periods. These mean values were used to calculate correlation coefficients between plasma prolactin and progesterone for the ewes within each mating group at each period.

There was a significant positive correlation \( r = 0.889, P < 0.05 \) between the two hormones in the 4–11 day period for the Group D ewes and a significant negative correlation \( r = -0.865, P < 0.05 \) during the same period for the Group M ewes. There was no evidence of correlation coefficients approaching significance in the remaining three periods. Furthermore, correlation coefficients for individual sampling times indicated that the significant relationships between plasma prolactin and progesterone were restricted to the first 10–11 days of pregnancy.

**Discussion**

Although a cause and effect relationship remains to be proved, the positive relationship between plasma prolactin and progesterone during early pregnancy in ewes mated in December, when prolactin concentrations were low, and the inverse relationship during the same period in ewes mated in March, when prolactin concentrations were approximately five times higher, is in agreement with the studies in vitro on human granulosa cells (McNatty et al., 1974) in which high prolactin concentrations were found to suppress progesterone production. The low plasma progesterone concentrations of the ewes mated in March and their associated decline with increasing plasma prolactin concentrations suggest that high plasma prolactin concentrations may have a luteolytic effect in the ewe in early pregnancy, an hypothesis consistent with the recent observations that high plasma prolactin concentrations are associated with low concentrations of plasma progesterone during early pregnancy in man (Seppala, Hirvonen & Ranta, 1976), and the observations of a luteolytic effect of prolactin in rats (Wuttke & Meites, 1971) and mice (Grandison & Meites, 1972). High prolactin levels in ewes bred out of season may therefore contribute to the reduced fertility at this time.

The gifts of progesterone antiserum from Dr C. Munro, Glasgow Veterinary School, Bearsden, Glasgow, and of ovine prolactin from the National Institutes of Health, Bethesda, Maryland, are gratefully acknowledged. S.R. was in receipt of an Agricultural Research Council scholarship during the course of this work.

**References**


Received 25 April 1977