INFLUENCE OF SEASON AND NUTRITION ON LUTEAL PLASMA PROGESTERONE IN RAMBOUILLET EWES

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PLASMA concentrations of progesterone in the ewe reach a plateau by about the 10th day of the estrous cycle, though there is considerable variation between days and animals (Stabenfeldt, Holt and Ewing, 1969; Thorburn, Bassett and Smith, 1969). Thorburn et al. (1969) observed variation in the luteal phase peak values of progesterone in the same ewes in successive cycles, which they speculated was due to variation in number of CL. Season and nutrition influence annual reproductive rhythms and also affect the suppressive action of exogenous progesterone on estrus and ovulation (Lamond, 1963, 1964). The purpose of the present study was to relate luteal phase plasma progesterone concentration to number of CL in ewes maintained for an 8-month period on three nutritional regimes.

Materials and Methods

Animals and Treatments. Grade Rambouillet ewes were obtained from Southwest Texas in 1969 and were allotted to three treatment groups in March, 1970, at 2 years of age. One group of 40 ewes was kept on coastal Bermuda grass pasture [P] and the other two groups, of 19 and 20 ewes, were kept in open pens. The pasture group was supplemented with hay as necessary to obtain a steady rate of growth so that their average live weight in November was at least 55 kilograms. One of the penned groups [H] received ad libitum a pelleted diet comprised of 10% dried coastal Bermudagrass, 10% oat gain, 5% soybean meal and 75% corn grain. Total digestible nutrients were estimated at 80%. The second group [L] was fed this diet every second day in amounts calculated to maintain body weights constant. All ewes had access to salt and mineral mixtures. As a safety precaution against trespass, night lights were switched off at dawn in the sheep pens and surrounding paddocks. All ewes in this experiment were exposed to these lights, which were 300 watt bulbs placed 15 ft. above ground.

The ewes averaged 45 kg live weight at the beginning of the experiment. They were checked for estrus twice daily (at 0800 hr. and 1600 hr.) with vasectomized rams. In four L, four H, and six P ewes blood was collected from the jugular vein at 0800 hr. on the 10th and 11th days after the end of the estrous cycle (estrus ranged from 12 to 60 hr.). The ovaries in these ewes were examined by laparotomy (Lamond and Urquhart, 1961) for number of CL after the ewes were bled on day 11. The ewes examined each time were those which happened to be in estrus nearest to June 8, July 16, September 1, and November 6. No ewe was examined twice. It was assumed that the data obtained from each sample of four to six ewes were representative of the treatment group at that time.

Commencing on October 1, estrous cycles in all ewes were suppressed with progesterone. The progesterone was dissolved in peanut oil (10 mg/ml) and was injected intramuscularly on alternate days for a total of eight injections. All groups received a dose of 2 ml for the first seven injections, and on the eighth and final injection, groups P and H received a 2-ml dose, whereas group L received a 1-ml dose. This variation in treatment served to bring the ewes into estrus at a similar time because underfed ewes are more readily suppressed with progesterone (Lamond, 1963). At the estrus following the synchronized one (first week in November), all ewes were mated to intact rams. Synchronization of the penultimate estrous cycle was carried out because 48 to 60 hr. after mating the ewes were subjected to laparotomy for recovery of ova, for a separate study.

Progesterone Determinations. Progesterone was measured using the competitive protein-binding assay. Plasma samples, 0.1 to 1.0 ml, were extracted once with 5 ml of petroleum ether. All plasma samples in a particular assay were extracted simultaneously. Gaddy (1971)

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showed that by testing batches of petroleum ether, it was possible to select a product which extracted progesterone separately from other steroids in ewe plasma. Procedural losses were estimated by the recovery of progesterone-1,2-3H added to each sample prior to solvent extraction.

Two standard solutions were used. One was a solution of progesterone in methanol (5 ng/ml), and appropriate dilutions were included in all assays in order to obtain a standard curve of authentic progesterone. The second was a pool of several hundred milliliters of luteal phase ewe plasma which was divided into 1-ml quantities for storage at −10°C until used. On the basis of 8 assays of this plasma, it was determined that the potency was 2.63 ng/ml (S.E.=±0.07 ng/ml). Aliquots of 0.2, 0.4 and 0.8 ml of the luteal phase plasma were run in duplicate with each assay. The potency of the luteal phase standard was determined from the standard curve of authentic progesterone and the value obtained was compared with the previously established potency for this plasma in order to obtain a correction coefficient for the assay. This method was used to eliminate procedural variation due to recovery of inaccurately mixed labelled progesterone and to compensate for possible variation in the accuracy of the extraction technique or the binding solution. It also enabled us to keep a close check on the quality of the petroleum ether used.

The binding solution was prepared by adding 1 μC corticosterone-1,2-3H and 0.5 ml of adrenalectomized dog plasma to 99.5 ml of glass distilled water and mixing gently. It remained stable for about 1 week when stored at 2°C. Gaddy (1971) had shown that plasma from an adrenalectomized dog increased the sensitivity of the assay so that the lower limit of sensitivity of the assay used in these experiments was less than 0.1 ng/ml. One milliliter of binding solution was added to each of the standard and sample tubes in each assay. The tubes were then shaken briefly on a vortex mixer and placed in a 45°C waterbath for 3 min. to facilitate dissolution of progesterone. They were again mixed briefly and then transferred to an ice bath for 10 minutes. Florisil (80 mesh) was used to separate the unbound radioactive corticosterone from the solution. In sequence at 60-sec. intervals, 80 mg of Florisil were added to each tube which was shaken for 15 sec. on a vortex mixer and returned to the ice bath. When mixing of all tubes was completed, a 500 μl aliquot of the supernatant from each tube was transferred to a glass scintillation counting vial at 60-sec. intervals. Exact timing was used to ensure that all samples were treated the same. Ten milliliters of dioxane-omnifluor scintillation fluid were added to each scintillation vial. The vials were counted in a Unilux II Liquid Scintillation Counter (Nuclear, Chicago) for 20 min. or 20,000 counts.

The time required for each of the standard samples to reach 20,000 counts was plotted against the log-dose of progesterone. The amount of progesterone in the unknown samples was determined by reading off the standard curve.

Results

Change in Live Weight. The average weekly gain from March to the end of November was 34 g for the L ewes, 155 g for the P ewes and 450 g for the H ewes.

Occurrence of Estrus. Estrous cycles were infrequent during March, April and May; however, more than 70% of the ewes were in estrus 20 to 26 days after shearing on May 17. Thereafter, the majority of ewes had estrous cycles throughout the period of observations.

The number of ewes which had a period of anestrus during July and August was 12 of 40 for the P group, 4 of 20 for the H group, and 5 of 19 for the L group. The length of estrous cycles in those ewes which continued to have estrous cycles throughout the period of June to September, inclusive, were submitted to regression analysis (table 1). There

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<th>TABLE 1. DURATION OF ESTRUS AND LENGTH OF ESTRUS CYCLES IN RAMBOUILLET EWES HAVING SIX SUCCESSIVE ESTRUS CYCLES DURING THE PERIOD JUNE TO SEPTEMBER</th>
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a Number of positive heat checks during estrus.
b Change in duration of estrus over six successive cycles.
c Change in length of cycles in successive cycles.
was no significant change in the length of the cycle for any of the groups over the period of the observations. However, the L group ewes averaged one day longer estrous cycles than P group ewes with the H group ewes intermediate (P<.001).

Over the same period of six successive estrous cycles, the duration of estrus was scored on the basis of the number of times (checked two times per day) that the ewes remained in estrus. Thus, if a ewe was in estrus for 2 full days, she had a score of four.

The durations of estrus were submitted to regression analysis (table 1), which showed a linear reduction in duration in all ewes over the period of observations. There were no significant differences between the three nutrition treatments either in position or slope of the regression lines. The average duration of estrus in June was approximately 44 hr. (3.7 score) and in November was 34 hr., approximately (2.8 score).

**Number of CL.** The numbers of CL observed on each occasion are shown in table 2. Only in November did any of the L ewes have two CL. The majority of H ewes had two CL during the period of observations. An analysis of variance indicated significant differences (P<.01) between the H group and the other two in respect to the average number of CL.

**Plasma Progesterone Concentration.** The mean plasma progesterone concentrations for the groups of ewes sampled on each occasion are shown in table 2. The data were submitted to analysis of variance from which it was concluded that blood levels of progesterone were significantly (P<.01) higher in November than in July in all groups of ewes, and the other months were intermediate. Ewes subsequently shown to be pregnant had similar progesterone levels in November as non-pregnant ewes; hence, the higher average concentrations were unrelated to conception 10 days previously. The progesterone concentrations in the L group were significantly higher throughout the series of observations than the remaining two groups, the difference being greatest in November (P<.01).

**Relationship Between Number of CL and Plasma Progesterone Concentrations.** The correlation coefficient between number of CL and the level of progesterone in the peripheral plasma was 0.01. Thus, blood levels of progesterone were of no value in determining if ewes had one or two CL.

**Discussion**

The seasonal change in duration of estrus was independent of level of feeding. The longer duration (by about 10 hr.) in June compared with September may have been related to the age of the ewes, their previous management, their live weights, or the presence of night lights. Since the mechanisms controlling seasonality of reproduction in ewes are not fully understood, it is not possible to indicate why our environmental conditions caused seasonality in duration of estrus. Nevertheless, the results indicate a wide degree of variation in duration of estrus which might reasonably be studied in relation to hormone dynamics, particularly progesterone-estrogen relations prior to the onset of estrus.

Limited information in the literature supports the observation that ewes on maintenance rations have longer estrous cycles than those given growing rations (Allen and Lamming, 1961; Tassell, 1967). However, the results are not surprising because Lamond (1963) showed that the interval from the end of a series of injections of progesterone to onset of estrus was longest in underfed ewes. The studies reported here showed that underfed ewes had highest plasma progesterone concentrations. Presumably, therefore, the longer estrous cycles were due to a slower rate of maturation of follicles after regression of the CL. It is of interest that Cuming et al. (1971) reported elevated plasma progesterone concentrations in underfed ewes in early pregnancy.
We can offer no explanation for the higher average progesterone concentrations in November, other than to speculate that pituitary-ovarian function would be expected to reach an optimum (in terms of reproductive fitness) in the breeding season. The thin ewes might have higher blood concentrations of progesterone because the extra-vascular pool would be limited in such animals by low body fat content.

The seasonal change in plasma progesterone levels might be expected to have resulted in seasonal changes in length of estrous cycles. However, this apparent paradox can be resolved on the basis of the well-established seasonality in responsiveness to progesterone (Lamond, 1964). Thus, within nutrition treatments, the length of estrous cycles remained constant because the seasonal change in responsiveness to progesterone was balanced by the seasonal change in plasma concentrations.

The effect of nutrition on ovulation rate is well documented (Foote et al., 1959; Allen and Lamming, 1961; Tassell, 1967; Edey, 1968). It is apparent, however, that plasma progesterone concentrations were not higher in ewes with two CL. Contrary to the view expressed by Thorburn et al. (1969) who did not actually observe ovulation rate, the use of blood progesterone as an index of ovulation rate is not feasible. This conclusion does not hold for the cow because the majority with 2 or 3 CL have markedly elevated progesterone levels (Lamond and Gaddy, 1972).

**Summary**

Rambouillet ewes were allotted to three nutritional regimes (pasture—P; a balanced diet ad libitum—H; a maintenance ration—L) in March, and various aspects of reproductive function were observed through November. Average live weight gains per week were: P=155 g; H=450 g; L=34 g. The incidence of anestrus during the summer was not affected by the treatments. The duration of estrous did not differ between groups but all ewes had more prolonged estrus in June than September. The L ewes consistently had longer estrus cycles than the others. The H ewes had highest ovulation rates. Plasma progesterone on days 10 and 11 of the estrous cycle were consistently highest in the L ewes; also, the average concentration for all ewes was higher in fall than summer. Ewes with 1 or 2 CL had similar progesterone levels.

The results demonstrate seasonal variation in incidence and duration of estrus and plasma progesterone concentrations. Effects related to feed supplies were observed in length of estrous cycles, ovulation rate and plasma progesterone.

**Literature Cited**


