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Gonadotrophic Potency of Ewe Pituitary Glands as Affected by Spaying, Season, and Breed.*

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The breeding season in ewes of most breeds is limited to the fall and early winter months.[1-4] During the remainder of the year, the ewe is anestrual although the ovaries often show a certain amount of activity, especially during the last few weeks before the breeding season begins.[1]

A lowered activity on the part of the pituitary gland has been postulated as a possible reason for anestrus in the ewe since injections of gonadotrophic hormones from pregnant mare serum or pituitary glands produce ovulation in a high percentage of anestrual ewes.[1,5] The great variability in response to injections of this kind,[6,7] however, raises a question as to whether other physiological factors may be involved.

The gonadotrophic hormone content of the pituitary gland of the male 13-lined ground squirrel increases greatly during the breeding season,[8] suggesting that at least in this species hormone content of the gland is correlated with secretory rate and the activity of the gonads. The gonadotrophic hormone content of the pituitary gland of the male cottontail rabbit increases during the breeding season, but that of the female does not.[9] The gonadotrophic hormone content of ewe pituitary glands remains about the same during the breeding season and during anestrus,[10] suggesting that a lack of pituitary gland activity may not be wholly responsible for anestrus in the ewe.

The time of the breeding season is evidently dependent upon environmental factors since it shifts when ewes are taken to the southern hemisphere.[5] The success which has been achieved in changing the breeding season by modifying the amount of light received is further evidence in this regard.[11] The physiological mechanism through which environmental factors operate to control sexual activity in the ewe has never been studied.

The hormonic secretions of active gonads normally lower the gonadotrophic hormone content of the pituitary gland and a pronounced rise occurs after gonadectomy.[12] Working upon the postulate that the pituitary gland of the anestrual ewe would indicate its potential activity by its ability to produce and store gonadotrophic hormones after spaying, the experiment reported here was performed in an effort to elucidate some of the factors responsible for the anestrual condition.

Materials and Methods. The experimental animals were 7-year-old ewes, half of each

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group being purebred Hampshires and half Lincoln X Rambouillet crossbreds. A group of 8 ewes was spayed in September, 1944, and maintained for a period of 90 days with a group of similar ewes serving as controls. At the end of this period, both groups were slaughtered and the pituitary glands removed for assay. All ewes in the spayed group had active corpora lutea in their ovaries at the time of the operation, and the control ewes had corpora lutea indicating recent ovulations at the time of slaughter. Thus, all ewes appeared to be in a reproductive condition normal for the breeding season.

A group of ewes from the same band was bred in the fall of 1944 and showed that they were reproductively normal by producing lambs during February and March of 1945. The lambs were weaned April 1, 1945 and on April 13 a group of 8 ewes was spayed. They too were maintained with a group of similar ewes which served as controls. All ewes were slaughtered after a period of 90 days and the pituitary glands removed for assay. At the time of spaying, all the ovaries removed were inactive with no follicle larger than 5 mm in diameter being observed. During the period from spaying until slaughter, an active painted teaser ram was kept with the control ewes and none showed an estrual period. Thus, all ewes were anestrual in a manner typical of the season. There was some ovarian activity since, at autopsy, one of the control ewes was found to have a fresh corpus luteum and 4 others had follicles as large as 8 mm in diameter. Three of 15 other ewes from the same band, slaughtered the same day, were found to have corpora lutea.

The pituitary glands were removed within 30 minutes after slaughter and immediately placed in an excess of acetone.† After being taken to the laboratory, usually a matter of from 4 to 5 hours, as much of the adhering connective tissue as possible was removed, the glands cut into small cubes, and the acetone changed. Three additional changes of acetone were made at intervals of 24 hours, after which drying was completed in desiccators containing calcium chloride.

After drying, the individual glands were ground with a mortar and pestle until fine enough to pass through an 80-mesh (to the inch) screen.

Assays of individual pituitary glands were carried out with rats and chicks. In the rat assays, the increase in ovarian weight of 21-day-old females was used as an endpoint. A total dosage of 20 mg of pituitary powder plus 4 mg of recrystallized hemin as an augmenter was suspended in 4½ cc of distilled water containing enough 1% NaOH to dissolve the hemin and injected subcutaneously in 9 equal injections over a period of 4½ days. Autopsies were performed 14 to 16 hours after the last injection. In the chick assays, the increase in testis weight of day-old White Leghorn cockerels was used as an endpoint. A total dosage of 10 mg of pituitary powder was suspended in 2½ cc of distilled water and injected subcutaneously in equal injections once per day over a 5-day period. Autopsies were performed 24 hours after the last injection.

The plan was to assay each gland on 2 rats and 5 chicks, but due to some death losses among the assay animals during the injection period, a few mistakes in sexing of the chicks, and a shortage of powder in 2 cases, data were actually available on a smaller number in some cases.

Results and Discussion. The results show that with both rats (Table I) and chicks (Table II) as assay animals the pituitary glands of spayed ewes of both breeds produced much greater responses than did the glands of the intact control ewes of the same breed. As indicated in Table III, these differences were highly significant. The differences between seasons were not significant in either breed in either spayed or control groups. There was no significant overall difference between seasons and the season-spaying interaction did not approach significance. Thus, the increase in gonadotrophic potency of the ewe pituitary glands after spaying was apparently of about the

† The cooperation of Armour and Co., Spokane, Washington, in making possible the collection of the glands in their plant is gratefully acknowledged.

TABLE I.
Ovarian Responses of Immature Rats to Injections of 20 mg of Pituitary Powder and 4 mg of Recrystallized Hemin.

Season in which donor ewes slaughtered	Type of donor ewes: Intact — No. ewes	No. rats	Avg ovarian wt. mg	Spayed — No. ewes	No. rats	Avg orarian wt. mg
Breeding season:						
Hampshire	4	8	15.0 ± 1.3*	4	7	34.1 ± 17.3
Crossbred	4	7	27.9 ± 8.2	4	8	134.5 ± 45.0
Anestrus:						
Hampshire	4	8	13.4 ± 1.8	4	8	36.0 ± 8.2
Crossbred	4	8	20.0 ± 5.7	4	8	83.7 ± 19.8

* Standard errors based on between ewe mean squares.

TABLE II.
Testicular Responses of Immature Chicks to Injections of 10 mg of Pituitary Powder.

Season in which donor ewes slaughtered	Type of donor ewes: Intact — No. ewes	No. chicks	Avg testis wt. mg	Spayed — No. ewes	No. chicks	Avg testis wt. mg
Breeding season:						
Hampshire	4	17	17.4 ± 2.4*	4	17	19.5 ± 2.8
Crossbred	4	16	19.1 ± 3.0	4	17	25.2 ± 4.6
Anestrus:						
Hampshire	4	12	14.0 ± 2.9	4	13	22.1 ± 2.8
Crossbred	4	13	18.0 ± 2.7	4	14	22.1 ± 1.6

* Standard errors based on between ewe mean squares.

TABLE III.
Significance of Various Factors Influencing the Gonadotrophic Potency of Ewe Pituitary Glands as Determined by Analysis of Variance.

	Type of assay: 21-day-old female rat	Day-old cockerel
Season	N.S.	N.S.
Breed	**	*
Spaying	**	**
Season × Breed	N.S.	N.S.
Season × Spaying	N.S.	N.S.
Breed × Spaying	N.S.	N.S.
Season × Breed × Spaying	N.S.	N.S.

Meanings of symbols are as follows:
N.S.—Not statistically significant.
*—Statistically significant $P < .05$.
**—Statistically highly significant $P < .01$.

same order during anestrus as during the breeding season.

The pituitary glands of the Lincoln × Rambouillet crossbred ewes were consistently more potent than those of Hampshire ewes, and as indicated in Table III, the differences were significant or highly significant as judged by the chick or rat assays, respectively.

The variances associated with the group means in the chick assay data were essentially homogeneous within the various groups and therefore well suited to the use of analysis of variance without transformation. The variances in the rat assay data were distinctly heterogeneous, and it was necessary to transform the ovarian weights to the logarithms of their logarithms before the mean squares seemed independent.‡

Qualitative as well as quantitative differences may exist between glands in their gonadotrophic hormone content. The degree of luteinization in the ovaries of the test rats was observed to be related to ovarian weight. Whether this represented a qualitative difference or merely a dosage effect is not known. Glands from spayed ewes in the 2 seasons were very comparable in their ability to produce luteinization.

‡ The interest and cooperation of Professor George W. Snedecor, Iowa State College, Ames, Iowa, in suggesting and making these transformations is gratefully acknowledged.

These data show that the pituitary gland of the anestrual ewe is comparable to that of the breeding-season ewe in having the ability to store gonadotrophic hormones in increased amounts following spaying. Thus the anestrual ewe has the ability to produce appreciable amounts of these hormones, and the suggestion is strong that this ability may be as great as in the breeding-season ewe. This latter point cannot be considered as proven, however, since the amount of hormone stored in the gland may reach an equilibrium in less than 90 days. The data provide no information on the rate of increase.

In view of the apparent ability of the anestrual ewe to produce gonadotrophic hormones, the current theory that anestrus is due to a lowered activity on the part of the pituitary gland may have to be modified. Quite possibly the immediate cause of the anestrual season is some changed physiological condition outside the pituitary gland which influences the ability of the ovary to respond to gonadotrophic hormones. Any attempt to discuss possible factors at this time would be mere speculation.

Summary. The gonadotrophic potency of pituitary glands from crossbred Lincoln × Rambouillet ewes was significantly higher than that of pituitaries from purebred Hampshire ewes. The gonadotrophic potency of glands from both breeds increased markedly during a 90-day period following spaying whether during the breeding season or during anestrus.

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Utilization of Glucose and Acetone Bodies by Gastrointestinal Tract in Fasting Normal and Diabetic Dogs.

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Although the method of arterio-venous differences has often been used to study the metabolism of muscle[1–5] and of brain[6,7] in both normal and diabetic animals and men, this procedure has apparently seldom been applied to the gastrointestinal tract. It is now generally recognized that the RQ of the brain is always close to unity, and that it continues to remove glucose from the blood and utilize this sugar as its chief, if not its only fuel, under a great variety of conditions including fasting and diabetes. In skeletal muscle, however, it has been found by all who have studied A-V differences in severe diabetes that either no glucose is removed or the amount removed is greatly reduced even after the administration of this sugar. Only Cavett and Seljeskog[4] report that as much glucose is removed in diabetic patients as in normal controls, but it seems improbable that they studied severe diabetes for only one of their 8 patients had a blood sugar level above 180 mg/ml. Others have also reported nearly normal values in mild diabetes,[1,3] but absent or reduced A-V differences in severely diabetic patients.

London[8] found that the portal blood of normal dogs contained 6 to 9 mg/100 ml

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