RELATIONSHIP OF ENDOCRINE STATE TO SPONTANEOUS MOTILITY OF SHEEP MYOMETRIUM IN VITRO

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It is generally considered that uterine motility is high during the follicular phase of the estrous cycle and relatively low during the luteal phase, although motility during the luteal phase varies somewhat among studies. In sheep, neither the variation in spontaneous uterine motility during the estrous cycle nor the effects of exogenous ovarian hormones on this motility have been studied extensively. Most reports on the subject deal primarily with uterine activity in response to pharmacological agents, with ovarian status given secondary importance (Alexander, 1945; Ambache and Hammond, 1949), although Polovceva (1942) reported that uterine motility, studied in vivo, was greatest during estrus and lowest on the fourth or fifth day post estrus.

The present study was conducted to determine the effect of the endocrine state and exogenous ovarian hormones on the spontaneous motility of sheep myometrium in vitro.

Materials and Methods

Myometrial motility was studied when experimental ewes were in the following endocrine states: estrus (E); luteal phase of the cycle (L); after ovariec-tomy (O); and after ovariec-tomy and treatment with estradiol (OE), progesterone (OP) or a combination of estradiol and progesterone (OEP). Estrous ewes were used during the first few hours of estrus; luteal-phase ewes were used 8 days post estrus, and ovariec-tomized ewes were used 4 to 5 wk. following bilateral ovariec-tomy. Hormones were injected subcutaneously at the rate of 500 mcg. estradiol, 10 mg. progesterone, or 500 mcg. estradiol and 10 mg. progesterone per day for 5 days, as split injections on each day. These ewes were killed the day following the last hormone injection.

At autopsy, the uterus was removed and placed immediately in Krebs-Ringer bicarbonate solution containing the following (mM./l.): NaCl, 118.46; KCl, 4.74; CaCl2, 2.54; KH2PO4, 1.18; MgSO4.7H2O, 1.18; NaHCO3, 24.87; and 100 mg. dextrose/100 ml. Within 15 min. after slaughter, 5 x 25 mm. longitudinal strips were excised from the antimesometrial wall of one uterine horn. The endometrium was removed and one end of the myometrial strip was tied in a fixed position to the oxygenator tube in a 50 ml. chamber in an isolated tissue-organ bath. The other end of the muscle strip was tied to an isotonic front end writing lever. The muscle was continually oxygenated by a mixture of 95% O2 and 5% CO2 bubbling through the Krebs-Ringer bicarbonate solution. Temperatures were maintained at 38.5° C.

Myometrial strips were allowed to equilibrate in the muscle chambers for 30 min. before recording began. Only that muscle activity recorded between 30 and 75 min. after the strip was first placed in the chamber was used for comparison purposes.

The qualitative effects of epinephrine, acetylcholine and oxytocin on the activity of the myometrium was also studied in vitro. The concentration of these substances per ml. of fluid was 0.2 mcg. of epinephrine or acetylcholine or 0.2 I.U. of oxytocin. After determining the effect of each drug on myometrial motility, tissue chambers were refilled with fresh Krebs-Ringer bicarbonate solution from two preheated reservoirs.

Spontaneous motility of the myometrium was measured by the amplitude of contraction (height in mm.), frequency of contraction (contractions per min.) and activity index (amplitude x frequency). Each vertical movement of the pen 1 mm. or greater represented a contraction.

Results

Tracings from one ewe from each group were selected to represent the mean values for that particular group (figure 1). Myometrial motility was greatest in E ewes, intermediate

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Figure 1. Representative tracings from sheep of each endocrine group. Tracings E, L and O are from estrous, luteal and ovariectomized ewes, respectively. Tracings OE, OP and OEP are from ovariectomized estradiol-treated ewes, ovariectomized progesterone-treated ewes and ovariectomized estradiol and progesterone-treated ewes, respectively. Time marked at 1 min. intervals. Scale: 1 mm. = 2.33 mm. on original tracings.

in O, OE and OEP ewes and lowest in L and OP ewes. Patterns of motility in OE ewes were similar to those of E ewes, but lower in magnitude. Contractions occurred less frequently in myometrium from O ewes than from E ewes, resulting in broader motility patterns. Motility was often totally absent in L and OP ewes. Contractions in OEP ewes were frequent and variable in amplitude.

Amplitude of Contractions. Amplitude variances among the six experimental groups were heterogeneous ($P<0.01$) according to Bartlett's test (LeClerg et al., 1962). The heterogeneity was due mostly to the high mean and correspondingly high variance for E ewes. After exclusion of E ewes, variances were no longer heterogeneous, so all amplitude means except that for E ewes were compared by Duncan's multiple range test (table 1). Mean amplitudes were similar for L, OP and OEP ewes, but lower than the mean for O ewes ($P<0.01$), suggesting that exogenous or endogenous progesterone decreased the amplitude of myometrial contractions. Estradiol, when administered to ovariectomized ewes (OE), did not increase the amplitude to the level of that in E ewes.

Frequency of Contractions. Variances of frequencies were homogeneous. Myometrium from E ewes or those injected with estradiol (OE and OEP) contracted more frequently than did the myometrium from L or OP ewes (table 1).

Activity Index. The activity index variances were heterogeneous ($P<0.01$), due again to high amplitude and variation of contractions in E ewes. Therefore the mean for E ewes was again excluded from the multiple range test (table 1). Exogenous estradiol (OE and OEP groups) did not raise the activity index above
TABLE 1. RANKED MEANS ON THE EFFECT OF ENDOCRINE STATE ON SPONTANEOUS MOTILITY OF MYOMETRIAL STRIPS IN VITRO *

<table>
<thead>
<tr>
<th>Factor</th>
<th>Endocrine state b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitude, e height, mm.</td>
<td>L 0.57 1.00 2.40 3.78 5.10 16.65</td>
</tr>
<tr>
<td>Frequency, d contraction per min.</td>
<td>L 0.65 2.22 3.27 4.43 5.18 6.20</td>
</tr>
<tr>
<td>Activity index, e amplitude x frequency</td>
<td>L 1.15 2.71 14.83 16.17 17.94 60.62</td>
</tr>
</tbody>
</table>

a Means not underscored by the same line are significantly different at the 1% level for amplitude and activity index and at 5% level for frequency.
b Number of ewes in each group: four estrous ewes and six in each other endocrine state.
c Estrous group mean not included in multiple range test because its inclusion caused heterogeneity of variances; estrous group standard error: 7.39; standard errors range for other groups: 0.23 to 0.91.
d Estrous group standard error: 16.00; standard error range among endocrine groups: 0.33 to 0.83; estrous group mean not included in multiple range test because its inclusion caused heterogeneity of variances.

e Estrous group standard error: 16.00; standard error range for other groups: 0.81 to 3.33; estrous group mean not included in multiple range test because its inclusion caused heterogeneity of variances.

that of O ewes, but exogenous progesterone alone lowered the activity index in O ewes to a level similar to that of L ewes.

Qualitative Effects of Epinephrine, Acetylcholine and Oxytocin on Muscle Activity. Acetylcholine or oxytocin added to the bath caused a sustained contraction by the myometrium from ewes of all endocrine states. This was followed by partial relaxation in ewes of all endocrine states. In all except L and OP ewes, the frequency of subsequent contractions was increased, while the amplitude was lowered.

The response of myometrial strips to epinephrine varied with the endocrine state of the ewe. Myometrium from O ewes relaxed and contractions stopped. Myometrium from OP and L ewes had a small initial contraction, followed by a sustained contraction of high amplitude, which was usually followed by relaxation. In all ewes under the influence of estrogen (E, OE, and OEP ewes) epinephrine caused an immediate contraction of high amplitude and short duration, followed by partial relaxation. Subsequent contractions were of high frequency and low amplitude.

Discussion

Earlier studies have suggested that spontaneous motility of the sheep uterus is greatest during estrus, diminishing with the development of a corpus luteum (Polovceva, 1942; Ambache and Hammond, 1949). In addition to confirming these published observations, the present results indicate that the uterus is moderately active without ovarian hormonal influences. Based on the spontaneous activity of the myometrium from ovariectomized sheep, there is little doubt that ovarian hormones of estrus stimulate uterine motility and those of the luteal phase inhibit motility.

Progesterone injected into ovariectomized ewes diminished total uterine activity. Estradiol in the dosage used increased the frequency, but not the amplitude of contractions, thus only partially reproducing the effects of endogenous ovarian hormones in intact E ewes.

There appears to be some species variation in spontaneous activity of the myometrium during the luteal phase of the reproductive cycle. In most species studied, uterine motility is diminished during the luteal phase (Reynolds, 1949). Uterine motility in the cow, studied by the use of balloons inserted into the uterine lumen, did not vary significantly during the estrous cycle (Hays and VanDemark, 1953), but cow myometrium, studied in vitro, did show lowered motility during the luteal phase (Cupp and Asdell, 1944). In sheep, the study by Polovceva (1942) using in vivo methods and the present study both indicate diminished uterine motility during the luteal phase.

Summary

Spontaneous motility of myometrial strips incubated in vitro was studied during the estrous cycle and following ovariectomy. The amplitude of contraction, frequency of contraction and an activity index (amplitude x frequency) were used to assess uterine motility. Among estrous, luteal phase and ovariectomized ewes, amplitude, frequency and activity index were each greatest in estrous ewes and lowest in luteal-phase ewes. Estradiol injected into ovariectomized ewes increased the frequency but not the amplitude of contraction, while progesterone injected into ovariectomized ewes decreased both frequency and amplitude of contraction. Acetylcholine or oxytocin caused sustained contraction of myometrium of ewes from all endocrine groups. Epinephrine had effects similar to acetylcholine or oxytocin in estrous and luteal phase ewes, but stopped activity in ovariectomized ewes.
Literature Cited