EFFECTS OF SEASON, MATING AND PREGNANCY ON THE VOLUME AND PROTEIN CONTENT OF EWE OVIDUCT FLUID

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GAMETE transport, fertilization and the initiation of cleavage are important events which occur in the biochemical environment of the mammalian oviduct. Recently developed techniques for cannulating oviducts have made possible the study of their secretions (Clewe and Mastroianni, 1959; Black, Duby and Riesen, 1963; Perkins et al., 1965; Restall, 1966a). However, the relationship of oviduct fluid to the well-being of ova, spermatozoa and the preimplantation stages of the embryo remains obscure. Furthermore, our knowledge of factors affecting the secretion rate and composition of oviduct fluid is limited, and the effects of installing a cannula in the oviduct on fertilization and early embryo development have not been determined.

To date, most studies of oviduct fluid have been carried out with fluid obtained from oviducts ligated at the tubo-uterine junction. However, such data may not be representative of conditions existing in the oviduct after mating. It is becoming increasingly evident that the rate of fluid secretion by the oviduct is under hormonal control (Mastroianni et al., 1961; Perkins et al., 1965; Restall, 1966b). The ewe is seasonally polyestrous, and fertility is low at the beginning and end of the anestrous season. Therefore, it seems highly desirable to compare oviduct fluid from ewes cycling during the normal breeding season with fluid from ewes cycling during the "anestrous" season.

The objectives of this study were to compare the volume and protein content of oviduct fluid from ewes exhibiting estrus during different seasons of the year, to determine whether pregnancy could be established in ewes with cannulated oviducts, and to study the effects of mating and pregnancy on oviduct fluid.

Experimental Procedure

Trials I and II

Trials I and II were carried out during seasons of the year when the reproductive tract of the ewe is presumed to be under different hormonal regulation. Trial I, initiated February 2, was conducted during a period when the incidence of anestrous ewes is high in the Experiment Station Dorset flock. In contrast, Trial II was initiated July 1 after ewes had resumed regular estrous cycles.

Five and six Dorset ewes were utilized in Trials I and II, respectively. All ewes had satisfactory lambing histories. Daily heat checks with vasectomized rams established that all ewes were cycling at the beginning of each trial. The infundibular ends of both oviducts of each ewe were cannulated on day 9 or 10 of the estrous cycle according to the method of Perkins et al. (1965). In order to study the effects of mating on oviduct fluid, one oviduct in each ewe was ligated at the tubo-uterine junction so as to block the entrance of semen and assure that only oviduct secretions were collected. The remaining oviduct was not ligated.

Cannulae were passed through a single stab wound in the left flank and attached to collection chambers. Cannulae from the respective oviducts were identified, and collection chambers were enclosed within a polystyrene case. The exterior collection apparatus was held in place by a wide cloth bandage around the body of the ewe.

Fluid was collected at 24-hr. intervals, and volume was measured with a pipette. Each collection was stored in a tightly stoppered tube at $-5^\circ$ C. Protein was determined by the method of Gornall, Bardwill and David (1949) as modified by Caraway (1960). Whenever volume was adequate, protein analysis was carried out on each daily collection of fluid from both ligated and non-ligated oviducts. Duplicate determinations were made whenever possible.
Ewes were checked for estrus twice daily with vasectomized rams and were bred to a fertile ram at 12- and 24-hr. intervals after the onset of estrus. During the first 4 days after mating, fluids from the non-ligated oviducts were observed under a light microscope for the presence of sperm. Ewes were slaughtered at the end of each trial and the reproductive tracts were examined. Union of the cannula and oviduct was verified by injecting water into the free end of the cannula.

Estimates of fluid volume and protein concentration from Trials I and II contained several missing values and were not balanced. Therefore, the data were subjected to a least squares analysis of variance. The sources of variations analyzed were ewes, days of the estrous cycle, ligated vs. non-ligated oviducts, and season (Trial I vs. Trial II).

**Trial III**

Trial III was conducted to determine whether pregnancy could be established in ewes with a cannulated oviduct and to study the effects of pregnancy on the volume and protein content of oviduct fluid.

Five Dorset ewes were used in Trial III, which was initiated on August 24. On day 9 or 10 of the cycle, one oviduct in each ewe was cannulated at the infundibular end by the method of Perkins et al. (1965). The ovary adjacent to the cannulated oviduct was removed in order to insure that all subsequent ovulations would occur on the intact side of the tract. The remaining ovary always possessed at least one corpus luteum. Fluid samples were processed in the manner described for Trials I and II. Data on fluid volume and protein concentration were not subjected to statistical analysis, however.

Ewes were checked for estrus daily with a vasectomized ram and were bred to fertile rams at 12- and 24-hr. intervals after onset of estrus. Ewes failing to return to estrus were slaughtered at 24 to 30 days post coitum, and their reproductive tracts were examined. Embryos were removed and placed in physiological saline solution until estimates of the crown-rump length could be made. Ewes returning to estrus were rebred to fertile rams and were slaughtered on day 4 of the cycle. The reproductive tracts were removed and the non-cannulated oviducts were flushed with physiological saline. Ova recovered were observed under a light microscope.

**Results and Discussion**

**Trials I and II**

Data were obtained from both oviducts of four of the five ewes started in Trial I and from four of the six ewes started in Trial II. One ewe was eliminated from Trial I because the cannula became detached from the non-ligated oviduct shortly after it was installed. Infection in the oviducts of two ewes resulted in their elimination from Trial II. The reproductive tracts of the remaining ewes appeared normal at autopsy except for minor adhesions. One ewe in Trial I and two ewes in Trial II provided data from more than one estrous cycle. For the purpose of statistical analysis, each cycle was considered to represent a different ewe. Thus, ewes and estrous cycles were confounded to this extent.

**Fluid Volume.** The least squares means of fluid volume for individual ewes are presented in table 1. Differences among ewes were significant (P<.01) and volume from ligated oviducts was greater (P<.01) than that from non-ligated oviducts. Estimates of fluid volume from Trials I and II were combined, and secretion rates for ligated and non-ligated oviducts during the estrous cycle are summarized in figure 1. Differences attributable to days of the estrous cycle were significant (P<.01). A cyclic pattern of fluid secretion was evident in both ligated and non-ligated oviducts. The cyclic pattern and rate of fluid secretion by ewe oviducts ligated at the tubo-uterine junction are in agreement with data reported by Black et al. (1963), Perkins et al. (1965), Perkins and Goode (1966), Restall (1966b), Rowan and Goode (1967), Bellve and McDonald (1968) and Black et al. (1968). These reports and recent studies with exogenous hormones (Mastroianni et al., 1961; Restall, 1966b) clearly show that in intact females the cyclic variation in fluid secretion by the mammalian oviduct is the result of estrogen stimulation. A survey of reports dealing with the secretory activity of the oviduct suggests that reference to the luteal phase of the estrous cycle as the secretory phase is probably in error. The greatest secretory activity occurs at estrus rather than during the luteal phase.

A comparison of data from ligated and non-ligated oviducts provided information on the movement of fluid in the ewe oviduct. Restall (1966b) reported that the secretion rates of the right and left oviducts were approximately equal. In the present study,
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Fluid collection from the ligated oviduct represented total fluid production by the oviduct. Fluid collected from the non-ligated oviduct was considered to represent fluid flowing out the ovarian end of the oviduct. The difference in volume between ligated and non-ligated oviducts was considered to represent fluid flowing through the tubo-uterine junction into the uterus. From the data presented in figure 1, it is clear that fluid flows from the oviduct in two directions during the entire estrous cycle. Fluid flowing into the uterus was estimated to be 0.23 and 0.49 ml./24 hr. during Trials I and II, respectively. Maximum flow into the uterus occurred at 1 or 2 days after onset of estrus. These data show that the tubo-uterine junction of the ewe is not tightly closed. The remaining fluid, estimated at 0.23 ml./24 hr. for both trials, flows out the ovarian end of the oviduct into the peritoneal cavity. Bellve and McDonald (1968) also found that fluid moved from the oviduct in two directions. However, these authors reported that maximum flow into the uterus occurred on day 4 of the cycle, and that a larger proportion of fluid flowed into the peritoneal cavity.

The results of the present study indicate that the oviducts may be a major source of fluid found in the peritoneal cavity of the female. Oviduct fluid may also make significant contributions to the biochemical environment of the uterus. Perkins et al. (1965) reported that fluid secretion by the oviduct exceeded that of a uterine horn during the luteal phase of the cycle. Therefore, oviduct fluid may represent a large proportion of the fluid present in the uterus during the early stages of embryo development. The fact that oviduct fluid may make important physiological contributions during this period will have to be considered when attempting to define or characterize intrauterine environment.

The volumes of fluid collected from ligated oviducts in Trial I and II were compared to determine if season had an effect on secretion rate (figure 2). The volume of fluid produced by ewes in Trial II (normal breeding season) was greater (P<.01) than that produced by ewes in Trial I (anestrous season). The difference was most pronounced at 1 and 2 days after the onset of estrus. Two of four ewes in Trial I returned to estrus once during collection periods of 18 and 34 days. A third ewe exhibited estrus twice during a 43-day collection period. The absence of corpora lutea and follicular development on the ovaries at autopsy indicates that these ewes had entered anestrus during the trial. The remaining ewe exhibited estrus three times at approximately 17-day intervals. However, the rate of secretion decreased slightly with each successive estrous cycle. At autopsy after the...
third estrus, no corpora lutea or large follicles were found on the ovaries. In Trial II, ewes exhibited consecutive estrous cycles of approximately normal length, and corpora lutea were present on the ovaries at autopsy. The difference in secretion rate obtained in the two trials suggests that ovarian estrogen production is low in ewes exhibiting estrus during the anestrous season, or that the oviduct is refractory to estrogen stimulation.

The results of the present study should be valuable in the design and conduct of future experiments dealing with ewe oviduct fluid. The experimental conditions relating to season of the year and the breeding habits of the sheep involved should be carefully defined.

Moor and Rowson (1966) have suggested that a local luteolytic factor may be produced by the uterus. One conceivable route by which such a factor might reach the ovary is through reproductive tract fluids. However, the results of Trial II appear to rule out the Fallopian tube as the conductor of the factor if it does exist. In this experiment, normal estrous cycles were consistently observed in ewes in which the ovarian ends of both oviducts were cannulated, a procedure which effectively prevented direct contact of oviduct fluid with the ovary.

On each of the first 4 days after mating, fluid from non-ligated oviducts was examined under a microscope for the presence of spermatozoa. Sperm were not observed in any sample of fluid. Possible explanations for the absence of sperm include: (1) sperm were present in small numbers but were overlooked, (2) sperm were phagocytized, and (3) the presence of the cannula caused sperm breakage in the reproductive tract or interfered with sperm transport. It has been shown that only a relatively small percentage of ejaculated spermatozoa are found in the oviducts of mammals. Phagocytosis of sperm in the oviduct is suggested by the finding of leukocytes in oviduct fluid (Perkins and Goode, 1966). Conley and Hawk (1967) and Hawk (1967) reported that small plastic spirals placed in the sheep uterus resulted in breakage of a large proportion of sperm present. Spirals were also shown to inhibit sperm transport and fertilization (Hawk, 1967).

**Protein.** The least squares means of protein concentration for individual ewes are presented in table 1. Differences among ewes were significant (P < .01). Protein levels in this study were within the range of values reported by Perkins and Goode (1966) and Restall and Wales (1966).

Protein concentration varied markedly during the estrous cycle, but differences among days were not significant. However, since fluid volume is highest at estrus, the total amount of protein secreted by the oviduct is greatest when the reproductive tract is under the influence of estrogen. Differences between ligated and non-ligated oviducts were not significant. Therefore, mating did not affect protein concentration in fluid from non-ligated oviducts. Perkins and Goode (1966) and Restall and Wales (1966) also found that protein did not vary significantly during the estrous cycle.

**TABLE 1. LEAST SQUARES MEANS AND STANDARD ERRORS OF FLUID VOLUME AND PROTEIN CONCENTRATION FOR INDIVIDUAL EWES**

<table>
<thead>
<tr>
<th>Item</th>
<th>Ewe number</th>
<th>Cycle number</th>
<th>Volume (ml./24 hr.)</th>
<th>Protein concentration (gm./100 ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ligated oviducts</td>
<td>Non-ligated oviducts</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ligated oviducts</td>
<td>Non-ligated oviducts</td>
</tr>
<tr>
<td>Trial I</td>
<td>1</td>
<td>1</td>
<td>0.47±0.05</td>
<td>0.36±0.05</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1</td>
<td>0.88±0.06</td>
<td>0.24±0.06</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1</td>
<td>0.22±0.05</td>
<td>0.14±0.05</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1</td>
<td>0.43±0.05</td>
<td>0.25±0.05</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2</td>
<td>0.29±0.05</td>
<td>0.13±0.05</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td></td>
<td>0.46±0.02</td>
<td>0.23±0.02</td>
</tr>
<tr>
<td>Trial II</td>
<td>5</td>
<td>1</td>
<td>0.50±0.15</td>
<td>0.29±0.13</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1</td>
<td>0.70±0.16</td>
<td>0.20±0.16</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2</td>
<td>0.77±0.13</td>
<td>0.11±0.13</td>
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<tr>
<td></td>
<td>7</td>
<td>1</td>
<td>0.85±0.13</td>
<td>0.28±0.13</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>2</td>
<td>1.29±0.13</td>
<td>0.24±0.13</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>1</td>
<td>0.19±0.13</td>
<td>0.27±0.13</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td></td>
<td>0.72±0.06</td>
<td>0.23±0.06</td>
</tr>
</tbody>
</table>
Protein in fluid from Trial I did not differ significantly from that obtained from Trial II. In contrast to fluid volume, season of the year did not affect protein concentration. These data, and the fact that protein did not vary significantly during the estrous cycle, show that physiological levels of ovarian hormones in intact ewes do not affect protein concentration in oviduct fluid. This does not imply, however, that individual components of the protein fraction may not change with stage of the estrous cycle or season. Perkins and Goode (1966) have reported that alkaline phosphatase activity varied significantly with stage of cycle.

**Trial III**

Three of five ewes in Trial III were pregnant at autopsy 24 to 30 days post coitum. Single embryos with crown-rump lengths of 8 mm. (day 24) and 16 mm. (day 30) were recovered from ewes 10 and 13, respectively. A single corpus luteum was present on the ovary of each ewe. Each embryo exhibited a heart beat, and development appeared to be normal. The ovary of ewe number 11 had two corpora lutea, and two embryos were present at autopsy on day 26. One embryo had a crown-rump length of 6 mm., did not exhibit a heart beat, and appeared to be degenerating. The other embryo was apparently normal, exhibited a heart beat and had a crown-rump length of 10 mm. Pregnancy in domestic animals in which oviducts were canulated has not previously been reported. However, Mastroianni and Wallach (1961), Hafez (1963) and Orsini and McLaren (1967) reported pregnancies in rabbits in which oviducts were ligated and/or canulated.

Trial III provides important information on questions raised in Trials I and II regarding the absence of spermatozoa in oviduct fluid. The fact that pregnancies were established in ewes in which one oviduct was canulated demonstrates that the presence of the cannula did not interfere with sperm transport in the other oviduct. It was also shown that a cannula in one oviduct did not alter conditions necessary for fertilization and the development of embryos in the reproductive tract. The techniques used in Trial III provide a suitable method for studying the secretory activity of the oviduct and the composition of oviduct fluid during early pregnancy.

The two non-pregnant ewes returned to estrus 21 days after the previous estrous period. These sheep were rebred at 12 and 24 hr. after the onset of estrus and were slaughtered 3 days later. Two ova were flushed from the oviduct of ewe number 9, but neither was cleaved and spermatozoa were not observed in the zona pellucida. The canulated oviduct of this ewe was severely infected. In ewe number 12, the non-canulated oviduct was blocked by an adhesion to the surface of the ovary. Consequently, no ova were recovered from this ewe. Thus, in each of the non-pregnant sheep, probable causes for reproductive failure were clearly present.

Data on fluid volume and protein concentration from Trial III are summarized in table 2. The pattern of fluid secretion at estrus was similar to that shown in figures 1 and 2. Volume reached a maximum at 1 or 2 days after onset of estrus and then declined rapidly. After the initial decline, the rate of secretion in pregnant ewes remained at a level characteristic of the luteal phase of the cycle.

Fluid volume and protein concentration varied markedly among pregnant ewes. However, within ewes protein level was relatively stable during the trial and apparently was not affected by pregnancy. Because of infection and adhesions, data from the non-pregnant ewes are of doubtful value.

The results of this study and other recent reports (Perkins and Goode, 1966; Restall and Wales, 1966; Rowan and Goode, 1967) show that secretion rate and the concentrations of several constituents of oviduct fluid vary markedly among apparently normal ewes. Pregnancy data obtained in Trial III demonstrated conclusively that fertilization...
and early embryo development can occur under highly variable conditions. Therefore, if oviduct fluid is important in reproduction, all available data suggests that its effects are exerted through: (1) a specific metabolite required for a particular stage of reproduction, or (2) some substance having a stimulatory or inhibitory effect at a critical phase of reproduction.

Summary

Data on the volume and protein concentration of ewe oviduct fluid were obtained from two trials conducted during different seasons of the year. Cannulae were installed in the ovarian ends of both oviducts of each ewe. One oviduct was ligated at the tubo-uterine junction. The remaining oviduct was not ligated and, at mating, the passageway of semen into the oviduct was unobstructed. Fluid volume varied markedly among ewes in each trial (P<.01). Least squares means for individual ewes ranged from 0.19 to 1.29 ml./24 hr. in ligated oviducts and from 0.11 to 0.36 ml./24 hr. in non-ligated oviducts. A cyclic pattern of fluid secretion was evident in both ligated and non-ligated oviducts, and differences among days of the estrous cycle were significant (P<.01).

The volume of fluid secreted was higher (P<.01) in ewes exhibiting estrus during the normal breeding season (Trial II, 0.72 ml./24 hr.) than in ewes exhibiting estrus during the anestrous season (Trial I, 0.46 ml./24 hr.).

A comparison of the daily volume of fluid collected from ligated and non-ligated oviducts of the same ewes showed that some fluid (estimated at 0.23 and 0.49 ml./24 hr. in Trials I and II, respectively) flows through the tubo-uterine junction throughout the cycle. Maximum flow into the uterus occurred about the time of estrus. The remaining fluid flows out the ovarian end of the oviduct into the peritoneal cavity.

Least squares means of protein concentration for individual ewes ranged from 0.94 to 2.86 gm./100 ml. of fluid (P<.01). Protein did not vary significantly between seasons of the year (1.77 and 1.87 gm./100 ml. for Trials I and II, respectively), among days of the estrous cycle, or between ligated and non-ligated oviducts. Mating did not affect protein concentration in fluid from non-ligated oviducts.

In a third trial, three of five ewes with cannulated oviducts became pregnant and contained living embryos at autopsy 24 to 30 days post coitum. In pregnant females, fluid volume declined from the characteristic peak obtained at estrus and then remained relatively stable at levels typical of the luteal phase of the cycle. Estimates of fluid volume in pregnant females ranged from 0.26 to 0.66 ml./24 hr., and protein concentration ranged from 0.79 to 4.31 gm./100 ml. of fluid. Protein concentration did not appear to be affected by pregnancy. These data show that the presence of a cannula in the oviduct does not prevent sperm transport, fertilization and early embryo development in the opposite oviduct.

Literature Cited


Perkins, J. L. and L. Goode. 1966. Effects of stage of the estrous cycle and exogeneous hormone