

Sperm transport and protein content of oviductal flushings in peripuberal ewe lambs
by Matthew A Lane

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Animal Science
Montana State University
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Abstract:
The objectives of this study were to determine: 1) if there is a difference in the ability of prepuberal estrogen-treated ewes (PPE), first (1E) or third (3E) estrus ewe lambs or mature (MAT) ewes to transport sperm through the anterior reproductive tract, and 2) if protein content of oviductal flushings differs among intact PPE, 1E or 3E ewe lambs and MAT ewes and ovariectomized estrogen-treated PPE, 1E or 3E ewe lambs and MAT ewes. Sperm transport was evaluated at either 2 or 22 h after artificial insemination in PPE, 1E, 3E and MAT ewes. Percentage of PPE ewes that transported sperm cells into the anterior reproductive tract was lower ($P < .10$) than that for postpuberal ewes (1E, 3E and MAT ewes) . Only 8.7% of all ewes transported sperm cells to the oviducts by 2 h after insemination. More postpuberal ewes transported sperm cells to the oviducts at 22 h after insemination than PPE ewes ($P < .05$). The distribution of sperm cells recovered from the uterus, isthmus and ampulla differed ($P < .05$) between 2 and 22 h but did not differ ($P > .10$) among postpuberal groups of ewes. Estrogen treatment did not affect protein per g or cell of oviductal tissue ($P > .10$) in any group. However prepuberal (PP) ewes had more protein per weight ($P > .10$) than did 1E and 3E ewes. Mature (MAT) ewes had more protein per oviductal cell ($P < .05$) than did 1E ewes but protein per cell did not differ from PP or 3E ewes ($P > .10$) . Prepuberal (PP), 3E and MAT ewes did not differ ($P > .10$) for protein per oviductal cell. In conclusion, sperm transport to the anterior reproductive tract does not change during the puberal transition in peripuberal ewe lambs. However7 oviductal protein content changes markedly during the puberal transition and this change may be a factor in influencing the ability of puberal ewe lambs to become pregnant.

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TABLE OF CONTENTS

LIST OF TABLES vii

LIST OF FIGURES viii

ABSTRACT x

INTRODUCTION 1

REVIEW OF LITERATURE 2

Puberty 2
Fertility at Puberty 3
Ovarian Function 7
Sperm Transport 11
Cervical and Hormonal Involvement . . . 11
Transport Through the Anterior Reproductive Tract 15
Oviductal Environment 23

STATEMENT OF PROBLEM 29

MATERIALS AND METHODS 31
Experiment 1 31
Statistical Analysis 36
Experiment 2 38
Statistical Analysis 41

RESULTS 43
Experiment 1 43
Experiment 2 48

DISCUSSION 55
Experiment 1 55
Experiment 2 59

LITERATURE CITED 64

LIST OF TABLES

Table		Page
1.	Experimental design and number of prepuberal estrogen-treated (PPE), first estrus (1E), third estrus (3E) and mature ewes (MAT) in each year for Experiment 1	32
2.	Experimental design and number of prepuberal (PP), first estrus (1E), third estrus (3E) and mature (MAT) ewes in each treatment in each year for Experiment 2	39
3.	Log_{10} number of sperm cells recovered from the uterus, isthmus and ampulla at time 2 and 22 h after insemination for prepuberal estrogen-treated (PPE), first estrus (1E), third estrus (3E) and mature (MAT) ewes	46
4.	Least square means of total Log_{10} number of sperm cells (TOTLOG) recovered from the anterior reproductive tracts of prepuberal estrogen-treated (PPE), first estrus (1E), third estrus (3E) and mature (MAT) ewes and the corresponding real number of sperm cells	47
5.	Least-squares means for total protein recovered from right and left oviducts (PTN), total protein per unit length of right and left oviducts (PTNLT) and total protein per unit weight of right and left oviducts (PTNWT) for prepuberal (PP), first (1E) and third estrus (3E) ewes in TRT 0 (intact) which were common to both years	48
6.	Least-squares means for total protein per oviductal cell (PTNCELL) for prepuberal (PP), first (1E) or third estrus (3E) and mature (MAT) ewes	50
7.	Least-squares means for total protein recovered from right and left oviducts (PTN), total protein per unit length of right and left oviducts (PTNLT), total protein per unit weight of right and left oviducts (PTNWT), total protein per unit area of right and left oviducts (PTNAR) and total protein per oviductal cell (PTNCELL) for mature (MAT) ewes in TRT 0, intact non-treated, TRT 1, hysterectomized estrogen-treated and TRT 2, hysterectomized corn oil treated	54

LIST OF FIGURES

Figure — Page

1. Percentages of ewes in prepuberal estrogen-treated (PPE), first estrus (1E), third estrus (3E) and mature (MAT) groups with sperm in their anterior reproductive tract at 2 and 22 h after insemination. Different letters above bars indicate differences ($P < .10$) 44

2. Percentages of prepuberal estrogen-treated (PPE), first estrus (1E), third estrus (3E) and mature (MAT) ewes with sperm cells in their oviducts at time 2 or 22 h after insemination. Different letters above bars indicate differences ($P < .05$) 45

3. Least-squares means Log_{10} number of sperm cells recovered from the uterus, isthmus and ampulla at time 2 or 22 h after insemination. Different letters above bars indicate differences ($P < .05$). PSE bar represents pooled standard error; df = 68 47

4. Least-squares means for protein per unit oviductal weight (PTNWT) recovered from prepuberal (PP), first estrus (1E), third estrus (3E) and mature (MAT) ewes. Different letters above bars indicate differences ($P < .10$). PSE bar represents pooled standard error; df = 46 49

5. Least-squares means for protein content of oviducts from intact (TRT 0) or hysterectomized estrogen-treated (TRT 1), prepuberal (PP), first estrus (1E), third estrus (3E) and mature (MAT) illustrating a RS by TRT interaction ($P < .05$). Different letters above bars indicate differences ($P < .05$). PSE bar represents pooled standard error; df = 46 51

6. Least-squares means for protein per unit length of oviducts (PTNLT) from intact (TRT 0) or ovariectomized estrogen-treated (TRT 1), prepuberal (PP), first estrus (1E), third estrus (3E) and mature (MAT) ewes illustrating a RS by TRT interaction ($P < .10$). Different letters above bars indicate differences ($P < .10$). PSE bar represents pooled standard error; df = 46 52

LIST OF FIGURES

Figure — Page

7. Least-squares means for protein per unit area (PTNAR) of oviducts from intact (TRT 0) or hysterectomized estrogen-treated (TRT 1), prepuberal (PP), first estrus (1E), third estrus (3E) and mature (MAT) ewes illustrating a RS by TRT interaction ($P < .05$). Different letters above bars indicate differences ($P < .05$). PSE bar represents pooled standard error; df = 21 53

ABSTRACT

The objectives of this study were to determine: 1) if there is a difference in the ability of prepuberal estrogen-treated ewes (PPE), first (1E) or third (3E) estrus ewe lambs or mature (MAT) ewes to transport sperm through the anterior reproductive tract, and 2) if protein content of oviductal flushings differs among intact PPE, 1E or 3E ewe lambs and MAT ewes and ovariectomized estrogen-treated PPE, 1E or 3E ewe lambs and MAT ewes. Sperm transport was evaluated at either 2 or 22 h after artificial insemination in PPE, 1E, 3E and MAT ewes. Percentage of PPE ewes that transported sperm cells into the anterior reproductive tract was lower ($P < .10$) than that for postpuberal ewes (1E, 3E and MAT ewes). Only 8.7% of all ewes transported sperm cells to the oviducts by 2 h after insemination. More postpuberal ewes transported sperm cells to the oviducts at 22 h after insemination than PPE ewes ($P < .05$). The distribution of sperm cells recovered from the uterus, isthmus and ampulla differed ($P < .05$) between 2 and 22 h but did not differ ($P > .10$) among postpuberal groups of ewes. Estrogen treatment did not affect protein per g or cell of oviductal tissue ($P > .10$) in any group. However prepuberal (PP) ewes had more protein per weight ($P > .10$) than did 1E and 3E ewes. Mature (MAT) ewes had more protein per oviductal cell ($P < .05$) than did 1E ewes but protein per cell did not differ from PP or 3E ewes ($P > .10$). Prepuberal (PP), 3E and MAT ewes did not differ ($P > .10$) for protein per oviductal cell. In conclusion, sperm transport to the anterior reproductive tract does not change during the puberal transition in peripuberal ewe lambs. However, oviductal protein content changes markedly during the puberal transition and this change may be a factor in influencing the ability of puberal ewe lambs to become pregnant.

INTRODUCTION

The ability of domestic female livestock to reproduce as soon as physiologically possible is an economically important event for livestock producers. This ability is affected by the attainment of puberty which is dependent upon physiological maturity, genetic potential, management of the replacement female and the interactions among these.

Current management practices dictate that replacement females should be bred as early in the breeding season in the first year of life as possible. This often results in breeding females at or shortly after puberty.

Pregnancy rates associated with breeding females at or soon after puberty are lower than those associated with breeding females at a more mature age. The factor or factors involved in this phenomenon of lower pregnancy rates at puberty are not known. The present study was undertaken to examine certain aspects of this phenomenon in young ewe lambs.

The following is a review of literature which includes various aspects of puberty, pregnancy rates at puberty and factors involved in fertilization which may be involved in lower pregnancy rates of the female ovine at puberty. Other species are included to provide a basis for discussion and where data for the ovine are not available.

REVIEW OF LITERATURE

Puberty

There are many definitions of puberty for female mammals in the literature, including; "the occurrence of changes whereby the two sexes become fully differentiated" (Marshall, 1922), "attainment of estrous activity and the ability to properly interact with males of the same species" (Crew, 1930), "the time at which reproduction first becomes possible" (Asdell, 1946), and "the initial production of viable ova and performing the appropriate role in sexual congress of the species" (Lewis, 1990). Implicit in the last definition is that the female must be able to ovulate, show estrus, and be able to participate in courtship behavior associated with that species in order that she might mate, conceive and carry a fetus to term.

The puberal transition from the prepuberal to the puberal reproductive state is thought to involve a change in the hypothalamo-hypophyseal-ovarian axis in response to estrogen. This change involves a desensitization of the negative feedback effect of estrogen on this axis which results in an increase of circulating levels of gonadotropins (Foster et al., 1986). It has been reported that the mechanism that drives female reproductive cyclicity which includes the ovary and the neuroendocrine system, develops very early after birth

and, under the appropriate circumstances, can respond in a similar manner as that observed in an adult female (Foster and Ryan, 1981). These authors reported that there is a neuroendocrine rhythm established very early in life which results in an hourly release of gonadotropin-releasing hormone (GnRH). The release of GnRH by the neurosecretory neurons should induce a release of luteinizing hormone (LH) from the anterior pituitary. The resultant LH pulses are not present at the expected heights following a pulse-release of GnRH. They proposed that the inhibitory feedback action of estrogen on the release of LH at this stage in development is extremely high. Consequently, LH rises only slightly after each release of GnRH and then quickly returns to basal levels. During the puberal transition the inhibitory action of estrogen decreases and hourly LH peaks become more pronounced. The overall increase in systemic LH levels is thought to cause one or more follicles to grow which results in an increase in estrogen production and release. The increase in estrogen is thought to trigger a preovulatory surge of LH causing the first ovulation and generally signals the beginning of the puberal transition for the female.

Fertility at Puberty

Pregnancy rates at puberal estrus has been reported to be lower than pregnancy rates at successive estrous cycles in

many mammalian species. Byerley et al. (1987) reported that pregnancy rates at first estrus (puberty or puberal estrus) were 21% lower than third estrus for beef heifers. Pregnancy rate was defined as a failure to return to estrus and a positive pregnancy diagnosis by rectal palpation. Archibong et al. (1987) reported that early embryonic mortality rates in first estrous gilts was markedly higher than third estrus early embryonic mortality rates. Early embryonic mortality rates were used for the pig because it is a litter bearing species.

In sheep, embryo survival rates associated with breeding at puberal estrus have been reported to be lower than survival rates associated with breeding at subsequent estrous cycles. Hare and Bryant (1985) found that embryonic survival increased by approximately 20% from first to second estrus in crossbred Suffolk ewe lambs with little improvement from second to third estrus. In this study, ewes were assigned to first (1E), second (2E) or third estrus (3E) and mated on the assigned estrus. These ewes were sacrificed at 25 days post-mating or when they returned to estrus. Presence of viable and non-viable embryos along with the number of corpora lutea or number of regressing corpora lutea were recorded. They reported an increase in embryonic survival from 34% at 1E (24 of 71) to 56% at 2E (38 of 68) and 3E (28 of 50).

Various factors have been examined to determine the limiting factor or factors involved in lower pregnancy rates

and decreased embryonic survival at first estrus in sheep. McMillan and McDonald (1985) conducted an experiment in which they examined the viability of zygotes from ewe lambs and mature ewes. This was accomplished by mating ewe lambs and mature ewes, removing zygotes at the 8 to 16 cell stage and transferring two zygotes, one from a mature ewe and one from a ewe lamb into the uterus of a ewe lamb. Fifty-two percent of zygotes from mature ewes developed to term compared to 25% for the zygotes from ewe lambs. Although there were no transfers into mature ewes for direct comparisons, the number of ewe lambs that did carry the transferred ova to term was 29 of 48 (60%) suggesting ability of the uterus to carry a fetus to term is not a major limiting factor in fertility for peripuberal ewes. This agrees with Quirke's (1979) findings for twinning rates between ewe lambs and mature ewes receiving two transferred adult ewe ova. Quirke reported a 41.1% twinning rate for ewe lambs compared to a 45.3% twinning rate for adult ewes. McMillan and McDonald (1985) reported that the ovulation rate of ewe lambs did not differ and of the 86 ewe lambs laparotomized in their study, 83 had ovulated at estrus. The occurrence of anovular estrus was 3.5% in ewe lambs compared to 0% for adult ewes indicating that failure to ovulate was not a major factor limiting reproductive performance in ewe lambs. This notion is supported by the work of Hare and Bryant (1985) who reported the number of corpora lutea (CL) on ovaries at slaughter or regressing CL at

return to estrus (ovulation rate) did not differ among first, second or third estrous ewe lambs and that all ewe lambs in their study had ovulated at estrus. Reports of Hare and Bryant (1985) and McMillan and McDonald (1985) are contrary to those of Edey et al. (1977) who reported that the occurrence of anovulation at puberal estrus ranged from 6.6% in Perendale ewe lambs to 33% in Merino ewe lambs.

Archibong et al. (1987) reported that the number of ovulations in puberal gilts was less than that of third estrus gilts but the occurrence of ovulation and fertilization rates of ovulated oocytes did not differ among these gilts. However, embryonic mortality did differ between first and third estrous gilts at both 15 and 30 days post-mating. Embryonic mortality for first estrus gilts was 22% at 15 days and 33.3% at 30 days post-mating while third estrus gilts had 4.9% and 10.6% embryonic mortality at each of these days, respectively. These findings suggest that the lower reproductive performance reported for porcine at their puberal estrus results almost entirely from early embryonic death and not anovulation or fertilization failure. However, these data do not entirely rule out improper or inadequate fertilization manifesting itself later as early embryonic mortality.

Supporting data for this notion comes from Hare and Bryant (1985). They reported a difference in ova wastage which was defined as a "CL not represented by viable embryos at necropsy" among three different groups of ewes: first

estrus (1E), second estrus (2E) and third estrus (3E). There was a decrease in ova wastage from 1E (56%) to 3E (27%). Of all the ova wasted, 78% was accounted for by pre-implantation losses. These findings suggest that a major factor in the decreased pregnancy rates of puberal sheep is not fertilization failure but early embryonic mortality, as was the case in gilts (Archibong et al., 1987).

Ovarian Function

A factor that could be involved in the reduced pregnancy rates of first estrous ewes is that of ovarian function. Onset of puberty and the number and type of estrous cycles in 30 5-month-old prepuberal crossbred ewe lambs was studied by Hare and Bryant (1982) as a means to evaluate the ovarian function in ewe lambs. They found that 28 of the 30 ewe lambs attained puberty during their first breeding season which lasted from November to January. The average number of estrous periods for all 30 ewe lambs including the two that did not cycle was 3.3. The number of normal length single cycles defined as a cycle lasting 14 to 19 days, was 65 out of 71 (92%). There were 4 apparent silent post-puberal ovulations that resulted in "multiple cycles" which are cycles lasting 27 to 37 days for a double cycle or 38 to 57 days for a triple cycle. For these to be considered multiple cycles and not a short period of anestrus, there had to be an indication of a rise in blood progesterone indicating a silent

ovulation had occurred. Of the 28 ewes that attained puberty, 10 were examined for prepuberal progesterone activity. In all 10 ewe lambs there was a rise in progesterone before the beginning of estrous activity, indicating that all 10 of these prepuberal ewe lambs had a silent ovulation before the initiation of estrous activity. This agrees with data reported by Berardinelli et al. (1980) who found that peripheral progesterone concentrations increase in prepuberal ewe lambs 1 to 4 days before puberty. From these results, Hare and Bryant (1982) concluded that inadequate ovarian function resulting in irregular cycles in ewe lambs was not different from data reported by Hafez (1952) for the occurrence of irregular cycles in adult ewes and consequently was not responsible for decreased pregnancy rates in the ewe lamb.

The previous studies measured ovarian function by occurrence and regularity of ovulations and progesterone release in ewe lambs. However, another ovarian steroid that may be important during the peripuberal period is estrogen. Changes in estrogen or estrogen to progesterone ratios may play a role in the puberal transition and the lowered pregnancy rates at puberty. Archibong et al. (1987) examined progesterone and estrogen concentrations in gilts bred at puberty or third estrus. They found that estrogen concentrations at Day 3 post-mating did not differ between gilts bred at their first or third estrus. However, estrogen

concentrations at 30 days post-mating did differ in that first estrous gilts had lower estrogen concentrations than did third estrous gilts. The lower estrogen concentrations found at Day 30 post-mating in first estrus gilts may have some impact on the ability of these first estrus gilts to maintain all the fetuses to term. Progesterone concentrations in first estrus gilts tended to be lower than for third estrus gilts but both had adequate circulating progesterone to maintain a gravid uterus at 3, 15 and 30 days post-mating.

In this same study, Archibong et al. (1987) examined the progesterone to estrogen concentration ratios for these animals. First estrous gilts reportedly had a lower ratio of progesterone to estrogen at Day 3 post-mating but this relationship changed on Day 15 and Day 30 post-mating. On these days, first estrus gilts had a higher ratio than that of third estrus gilts. The authors suggested that the low progesterone to estrogen ratio on Day 3 may alter the temporal synchrony between uterine secretory activity (environment) and the developing conceptus.

Another area that indirectly involves ovarian function and may impact pregnancy rates in female sheep is that of sexual behavior and its effect on the ewe's ability to go through the proper courtship rituals with the ram before mating. If there was a problem with the length or intensity of estrus, then the ability of ewe lambs to participate in proper courtship rituals may be adversely affected which could

decrease the chance of the ewe mating and conceiving. There have been contradictory reports concerning the duration of estrus in ewe lambs. Hafez (1952), Dýrmundsson (1978) and Edey et al. (1978) reported that the duration of estrus was shorter in ewe lambs than in mature ewes which might mean ewe lambs have less of an opportunity to mate than mature ewes. However, Land (1970) reported that the duration of estrus in Finn-cross ewes lambs was similar to that of mature ewes. Hafez (1951) reported that intensity of estrus was lower in ewe lambs than in mature ewes.

The hypothesis that inadequate expression of behavioral estrus causes a decrease in pregnancy rates because it would reduce the chance for a ewe lamb to mate was not supported by findings of McMillan and McDonald (1985) who reported that fertilization in ewe lambs is entirely adequate. Also, Archibong et al. (1987) and Hare and Bryant (1985) reported that the major cause of decreased pregnancy rates in ewe lambs was early embryonic mortality not fertilization failure. One consequence of an alteration in duration of estrus could be a shift in the temporal synchrony between the uterine environment and the developing conceptus. Shorter estrus lengths might affect the ability of ewe lambs to become pregnant by affecting the normal changes that occur in the uterine environment. An early change in the uterine environment might impede implantation and cause early embryonic mortality which has been shown to be the manner in

which reproductive performance is negatively affected in gilts (Archibong et al., 1987) and peripuberal ewes (Hare and Bryant, 1985).

Sperm Transport

Fertilization requires transportation of both gametes to the site of fertilization in the oviducts. Sperm cells must be capacitated, in a state of hyperactivity and non-acrosome reacted immediately before encountering an oocyte. The oocyte must be in its second meiotic arrested state (metaphase II) and union of the two gametes cannot be delayed. A delay in the union of the gametes causes aging of the oocyte which decreases its ability to become fertilized properly and increases the incidence of polyspermy. Because of these physio-temporal restraints, transportation of these gametes must be accomplished in such a way that the male and female gametes meet when they are most capable to fertilize or be fertilized.

Cervical and Hormonal Involvement. The first barrier encountered by sperm in animals which deposit their semen into the vagina is the cervix. Mattner and Braden (1969) reported that sperm must be motile for passage through the cervix. In their experiment, they inseminated ewes with fresh semen containing live sperm cells or semen that contained only dead sperm cells. Their findings showed that sperm must be alive

and have good progressive motility for sperm to reach the anterior half of the cervix within 30 min after insemination. Dead sperm inseminated into the first caudal cervical fold were found at 30 min only in the caudal one-half of the cervix and the caudal one-fourth at 4 h after insemination. In addition, ewes that had been inseminated with dead sperm cells were reported to have sperm recovered only in the cervical lumen whereas ewes inseminated with live sperm had sperm recovered from the cervical glands or between cervical villi. In the cow, sperm recovered from the uterus after insemination were more motile than sperm recovered from the vagina or cervix (See Hawk, 1987; Thibault, 1973). Results of these studies indicate that the cervix may play a role as a "filter" which primarily allows motile sperm to pass while it clears non-motile sperm and discharges them back into the vagina. Inert particles deposited into the vagina do not pass the cervix into the uterus of chickens, women and rabbits (See Thibault, 1973). However there is one report that indicated that inert radio-opaque particles pass through the cervix into the uterus when the particles are placed into the vagina of rabbits (Krehbiel and Carstens, 1939). However, it has since been suggested that this finding resulted from the dorsal recumbent restraint of the rabbits during experimentation.

The effect of exogenous hormones on passage of sperm through the cervix in ewes was studied by Hawk and Conley (1975). They showed a clear relationship between low numbers

of sperm cells in the anterior cervix at 2 h after mating and reduced numbers of sperm cells in the oviducts at 22 h. Ewes treated with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), medroxyprogesterone acetate (MAP) and ewes with an intrauterine device (IUD) inserted into one horn had lower numbers of sperm in the anterior cervix than did control ewes which had not been treated with exogenous hormones or had an IUD. The overall number of sperm cells recovered from the cervix between the groups did not differ but the distribution of the sperm cells found within the cervix did. Ewes in the treated groups ($PGF_{2\alpha}$, MAP and IUD) had fewer number of sperm cells ($P < .05$) in the anterior cervix and the oviducts than did ewes in the control group. These findings were confirmed by Hawk et al. (1981) who reported that treating ewes with $PGF_{2\alpha}$ or MAP caused immobilization and death of sperm cells in the reproductive tract. This effect was most evident in the anterior one-third of the cervix and in the uterus. When exogenous estrogen was administered to control ewes, ewes treated with $PGF_{2\alpha}$ or MAP ewes, there was an increase in the number of sperm recovered from the reproductive tract (Hawk et al., 1978). The increase in number of spermatozoa recovered was significant only in the anterior cervix and was confined to those with regulated estrous cycles ($PGF_{2\alpha}$- and MAP-treated ewes).

In another experiment, Hawk et al. (1981b) reported that removing the ovary bearing the corpus luteum (CL) on either Day 3, Day 10 or Day 15 post-mating decreased the numbers of

sperm cells recovered from the reproductive tract of ewes at the ensuing estrus compared to control ewes in which the non-CL bearing ovary was removed at one of the three days. The decrease was much greater for ewes that were hemiovariectomized on Day 10 than for those hemiovariectomized on the either Day 3 or Day 15. They hypothesized that the decrease in the number of sperm cells recovered from the oviducts was a result of removing an ovary with either a fully functional CL, follicles or both as opposed to removing an ovary on Day 3 or Day 15 which would have neither a developing nor a regressing CL.

Sperm transport has also been studied in intact ewes, ovariectomized ewes and ovariectomized ewes treated with various hormones. Allison (1972) reported that ovariectomized ewes had fewer sperm cells recovered from all segments of the reproductive tract than did intact ewes following induction of artificial estrus and mating. Transport of sperm into the cervix was lower in ovariectomized ewes than in intact ewes, $\approx$ 6,000 vs 15,000 recovered from the caudal cervix. Very few sperm cells were recovered from the mid and cranial segments of the cervices of ovariectomized ewes. Inadequate transport of sperm into the anterior cervix in ovariectomized ewes presumably decreased the numbers of sperm cells recovered from the uterus and oviducts.

To evaluate the influence of ovarian steroids on sperm transport, ovariectomized ewes treated with varying dosages of

estrogen injections following different progesterone priming regimens were used (Allison, 1972). Number of sperm in the cervix was affected by the dosage of estrogen in an apparent dose-dependent manner. Proportions of ewes yielding large numbers of sperm (> 20,000) increased with increasing dosage of estrogen. Also, there was a dosage of estrogen by progesterone priming regimen interaction. This interaction indicated that high concentrations of progesterone were required for estrogen to fully express its effect.

The effect of exogenous hormones on transport of sperm through the anterior reproductive tract was reported by Quinlivan and Robinson (1969) and Croker et al. (1975). Quinlivan and Robinson (1969) reported no difference in either distribution or numbers of sperm recovered after 1 h between ewes treated or not treated with a progestagen. However, numbers of sperm recovered and their distribution varied greatly within treatment groups.

The function of the cervix in sperm transport in mammals that deposit semen into the vagina is that of a filter. Progressively motile sperm are allowed to enter the uterus while non-motile sperm are discharged back into the vagina. This ability is dependent upon both progesterone and estrogen which must be in the right order of appearance and at the correct levels for the cervix to fully function properly.

Transport Through the Anterior Reproductive Tract. The

ability of peripuberal ewe lambs to transport oocytes and fertilized ovum reportedly is "not abnormal" (McMillan and McDonald, 1985; Quirke and Hanrahan 1977). This conclusion was based on the fact that there were similar rates of ovum recovery, cell cleavage, and fertilization in mature ewes and ewe lambs in both studies.

Although ovum transport has been addressed in ewe lambs the ability of ewe lambs to transport sperm has not. Sperm transport has been examined in many species including sheep but exclusively in mature ewes. The mechanism of gamete transport to and through the oviduct is thought to be a diverse process involving smooth muscle contractions, ciliary activity and fluid currents in the oviducts (Blandau, 1973; Blandau and Gaddum-Rose, 1974; Hunter, 1989). Smooth muscle involvement in the uterus is thought to mainly be involved in mixing the sperm cells and uterine fluid within the lumen (Thibault and Wintenberger-Torres, 1967) and transport of the sperm cells to the tubouterine junction (Thibault 1973). Once sperm cells have been transported to the anterior portion of the uterine cornua, they are purportedly sequestered in either the caudal 1 to 2 cm of the isthmus for 17 to 18 h after arrival (Hunter 1989) or at the uterotubal junction within the uterus for approximately 18 h before being released to progress through the oviducts (Thibault and Wintenberger-Torres 1967). The mechanism of sequestering or storage is not known but some investigators report that penetration by sperm

cells into uterine glands may be involved. Thirty six percent of the spermatozoa reaching the upper portion of the uterus are reportedly found in uterine glands within 2 h of mating (Thibault and Wintenberger-Torres 1967). Others report a decrease in sperm motility in the caudal 1.5 - 2.0 cm of the isthmus and suggested this might be involved in the sequestering of sperm cells (Hunter 1989).

Regulation of the sequestering process appears to involve direct ovarian control. Specifically the pre- or peri-ovulatory follicle controls the activity of the sperm cells in the lumen of the isthmus by the release of gonadal hormones. These hormones act upon the oviductal epithelium to influence the sperm cells by affecting the secretions from the oviductal epithelium. These secretions are thought to affect the activity of the sperm cells in some as yet unexplained manner.

Recently, Smith and Yanagimachi (1991) have reported finding sperm cells attached to the lumenal epithelial surface of the isthmi of hamsters. This method of sequestering is also thought to aid in the capacitation of the sperm cells. The authors reported that only uncapacitated sperm cells would attach to the epithelial surface and that once these cells were capacitated they were released. They found almost no sperm in the ampulla suggesting the possibility that the findings of sperm cells in the ampulla in previous reports were artifacts.

Hunter and Nichol (1983) performed an experiment in which

the caudal isthmi of ewes were transected at 1.5 to 2.0 cm from the uterus at increasing time intervals from 10 to 26 hours after mating. One to three days after surgery, ova were recovered and the incidence of fertilized ova and number of accessory sperm were recorded. They recovered no fertilized ova (0 of 52) from oviducts transected between 10 to 21 h after mating. Only 2 of the 33 ova recovered from oviducts transected between 22 and 24 h were fertilized while 53.3% (16 of 30) ova recovered from oviducts transected at 25 to 26 h after mating were fertilized. These authors concluded that the caudal portion of the isthmus sequestered viable spermatozoa until just before ovulation. They suggested that the "trigger" allowing further progression by the sperm cells into the proximal isthmus is an ovarian follicular hormone or hormones released just before ovulation.

The method of transport of sperm cells through the isthmus in the pig is reported to rely on ad-ovarian muscular contractions (Blandau and Gaddum-Rose, 1974). These contractions act to pinch off a section of the isthmus and move the contents within that section ad-ovarianly much like transporting a bolus through the esophagus. The circular contractions responsible for this movement are thought to be so vigorous that they completely occlude the lumen. These peristaltic waves have also been reported in the rabbit (Blandau, 1973).

The role of cilia of epithelial cells in the isthmus is

not fully understood. Ciliated cells lining the lumen of oviducts in rabbits have been reported to beat both ad- and ab-ovarianly (Blandau, 1973). This phenomenon was discovered by removing the oviducts, splitting them longitudinally, placing them in a tissue culture medium and evaluating their ability to transport microspheres and Lycopodium granules. The spheres and granules, when flushed onto the ciliated mucosal surfaces at various subdivisions of the oviducts, were transported up and down the length of the tissues. This occurred only in the isthmic portion of the oviduct and was thought to be proof that there is a dual ciliary tract system which aids in transport of gametes both ab- and ad-ovarianly through direct contact with gametes and the fluid currents which resulted from the ciliary beating. These findings imply a very intricately controlled system within the oviduct which is involved in what initially appears to be a simple physiological event, i.e., gamete transport through the oviduct.

The speed at which sperm cells are transported through the reproductive tract of ruminants may be an important factor in optimizing fertilization and pregnancy rates. Data concerning the rapidity of sperm transport in these species are equivocal. Studies by Mattner (1963), Mattner and Braden (1963), Lightfoot and Restall (1971) and Quinlivan and Robinson (1969) in the ewe, and Dobrowolski and Hafez (1970) in the heifer support the notion that sperm are transported

rapidly through the anterior reproductive tract and are found in the oviduct within 30 to 60 min. On the other hand, data reported by Thibault and Wintenberger-Torres (1967), Hafez and Thibault (1974), Hunter (1985) and Hunter (1989) support the idea that sperm transport through the anterior reproductive tract and into the oviduct is a relatively slow process taking anywhere from at 2 to 8 h. The disparity in these results could be associated with the variable techniques used to collect this data. Many researchers use slaughter or euthanasia followed by collection of the reproductive tract as a means of collecting the tract, whereas others collect the reproductive tract surgically. The use of slaughter is thought to potentially obscure the data and any subsequent interpretations of such data (Hunter, 1989).

The mechanism of sperm transport involves contractions of the smooth muscle in the uterine walls. Use of slaughter as a technique used in the evaluation of sperm transport may confound the results and produce artifacts resulting from the massive smooth muscle contractions that can occur. Mattner and Braden (1963) reported finding sperm in the oviducts within approximately 8 min after natural service in the ewe. The method of collection of the reproductive tract and recovery of sperm was done by euthanizing the ewe five minutes after mating with an intracardiac injection of pentobarbitone and immediate removal of the reproductive tract. The recovery of sperm was completed by flushing the uterus, oviducts, and

cervix and searching the flushings for sperm. Five of seven mature Merino ewes had sperm in their oviducts after being euthanized. Mattner (1963) also reported that sperm were recovered from the oviducts within 15 min post-mating in mature Merino ewes that had been preconditioned to the confinement and handling involved in the experiment or those that had not been exposed to the laboratory environment. The procedure for the collection of the reproductive tract and recovery of sperm was similar to that described in the previous experiment of Mattner and Braden (1963).

Lightfoot and Restall (1971) reported sperm to be in the oviducts of mature ewes within 30 min of insemination with two fresh ejaculates into the external os cervix regardless of depth of insemination. The method of collection of the tract was euthanasia with an intracardiac injection and immediate removal of the tract. The oviducts, uterus and cervix were flushed and the flushings were examined for the presence of sperm.

Dobrowolski and Hafez (1970) reported finding sperm in the oviducts of heifers at 1 h after insemination into the external os cervix with 2.0×10^9 sperm cells. The method of collection in this experiment also involved collection of the reproductive tract and recovery of sperm after slaughter either by conventional means or by intracardiac injection.

Thibault and Wintenberger-Torres (1967) concluded sperm do not reach oviducts of sheep mated naturally by 2 h post-

mating. The method of collection of the reproductive tract and recovery of sperm involved slaughter and immediate collection of the reproductive tract but the method of slaughter is not known.

Hunter (1985; 1989) reported that at least 6 to 8 h are required for sperm to reach the oviducts of sheep after natural service. However, Hunter took a different approach to determining whether sperm had reached the oviducts. At predetermined times after mating and before ovulation, oviducts of ewes were ligated at the tubouterine junction and transected from the uterus. This prevented any further migration of sperm after the surgery. Ewes were allowed to recover and 1 to 2 d after mating ewes were slaughtered and oviducts collected. The oviducts were flushed to recover oocytes. These were evaluated to determine if fertilization had occurred and the number of sperm associated with the zona pellucida. This allowed for determination of viable spermatozoa present in the oviduct and an approximation of the total number sperm present from the number of accessory sperm associated with the zona pellucida of the oocytes.

These experiments evaluated the same aspect of fertilization, rapidity of sperm transport, but the results of the independent studies varied greatly from reports of sperm reaching the oviducts within minutes of mating to requiring several hours for them to be recovered from the oviducts. However, none of these studies examined sperm transport and

how it is affected by the puberal transition in sheep.

Oviductal Environment

The oviduct is not merely a passive conduit for gamete travel but plays an active role in transportation of gametes both ab- and ad-ovarianly, sometimes in both directions at the same time. Furthermore, the oviduct is thought to provide an environment which assists in or is essential to the establishment of pregnancy (Buhi et al., 1990). The lumenal environment of the oviduct facilitates sperm capacitation, fertilization and early embryonic development. Buhi et al. (1990) postulated that the control of this environment is manifested through secretions from the oviductal epithelium.

The composition of fluid recovered from the oviductal lumen has been reported to be a mixture of peritoneal, uterine and follicular fluid along with fluid that has its origins in the oviduct itself (Hamner, 1973). Oviductal fluid is a mixture from two different sources: a lymph and vascular transudate and fluid secreted from the secretory cells found in the oviductal epithelium. The transudative process in the oviduct is reported to be selective (Shapiro et al., 1971; Oliphant et al., 1978). Shapiro et al. (1971) and Oliphant et al. (1978) found that oviductal fluid and serum contained similar proteins but oviductal fluid contained proteins immunologically unique to oviductal secretory fluid.

Oviductal secretory fluid originates in the oviductal

epithelial secretory cells which fill with secretory granules just before and during estrus or as the result of exogenous estrogen. Secretory granules are discharged at either ovulation or after treatment with exogenous progesterone (Greenwald, 1958; Brower and Anderson, 1969; Fredriccson, 1969; Lambert et al., 1973).

The overall volume and composition of oviductal fluid has been studied in a variety of species. Black et al. (1962) found that the volume of the daily output of oviductal fluid was 1.4 ml/d in the ewe, 5.0 ml/d in the cow, and 1.2 ml/d in the rabbit. Stambaugh et al. (1969) characterized the oviductal fluid in the estrous rabbit as having an osmolarity of 302 to 310 mOsm and a dry matter content of oviductal fluid from estrogen-treated rabbits as 11.9%. Iritani et al. (1969) examined the gross chemical composition of oviductal fluid and reported that the major constituents were: sodium, chloride, potassium, magnesium, calcium, phosphate, bicarbonate, lactate, glucose, carbohydrates and proteins.

There have been many studies that have demonstrated a relationship between a factor (s) in the oviductal environment and successful fertilization (Lambert and Hamner, 1975; Greenwald, 1962; Kille and Hamner, 1973; Cline et al., 1977; Richardson et al., 1980; Van Winkle, 1985). The proteinaceous constituents of oviductal fluid have received the most attention in this regard. Oviductal fluids contain either sulfated mucopolysaccharides or mucoproteins (Shapiro et al.,

1974; Hanscom and Oliphant, 1976; Barr and Oliphant, 1981) or acidic sulfated glycoproteins (Brower and Anderson, 1969; Jansen and Bajpai, 1982; Fredriccson, 1969). Recently, these proteins have been characterized as oviductal specific proteins (OSP) which are glycoproteins (Sutton et al., 1984, ovine; Kapur and Johnson, 1988, murine; Robitaille et al., 1988, hamster; Gandolfi et al., 1989, ovine; Boice et al., 1990, bovine; Buhi et al., 1990, porcine).

The molecular weight of OSP varies among species. In sheep, there are reports of an OSP with a range of 90,000 to 92,000 Da (Sutton et al. 1984; Gandolfi et al., 1989). Molecular weights of these proteins for other species are: rabbits, 71,000 Da (Oliphant and Ross, 1982; Barr and Oliphant, 1981); pigs, 115,000 Da (Buhi et al. 1990); cattle, 97,000 Da (Boice et al., 1990a); and mice, 215,000 Da (Kapur and Johnson, 1985).

Stage of estrous has been reported to affect the secretory activity of the oviductal epithelium and consequently the composition of the oviductal lumenal fluid. Sutton et al. (1984) reported a fluctuation in the oviductal fluid flow rate in ewes. They reported that the oviductal fluid flow was cyclic and dependent upon the stage of the estrous cycle with the flow being higher (1.56 ± .35 ml/d) at or shortly after estrus compared with the flow at mid-cycle (.49 ± .29 ml/d). This report agrees with those given in a review by Leese (1988) in which it was stated that oviductal

secretory activity is dictated by ovarian steroids.

Sutton et al. (1986) studied the effect that the stage of the breeding season and exogenous estrogen had on peak oviductal fluid flow rate and on the estrus-associated glycoprotein (OSP) in ewes. The percentage of samples that contained this glycoprotein changed progressively depending upon the stage of the estrous cycle. They reported finding this glycoprotein only in fluid from ewes on Day -1 to Day 6 of the cycle (Day 0 = Day of estrus). On Day -1 only 5% of the samples contained the estrus-associated glycoprotein compared to 100% on Day 1 and 4% on Day 6. As the breeding season progressed, the mean peak flow determined from flow rates throughout the cycle dropped from 1.63 ± 0.5 ml/d early in the season to 1.38 ± 0.4 ml/d in mid-season and then to 0.85 ± 0.21 ml/d late in the season. The effect of exogenous estrogen on the presence of this protein and the fluid flow rate in anestrus, mid-cycle and ovariectomized ewes was also reported. An increase in flow rate and the appearance of a OSP 1 to 2 d after an injection of estrogen occurred in all groups of ewes. The duration of the response to exogenous estrogen was longer (8.4 ± 0.8 d) in the mid-cycle ewes compared with anestrus (7.9 ± 1.3 d) and ovariectomized ewes (6.8 ± 1.6 d) From these results the authors concluded that an increase in estrogen is the causative factor or the trigger for the appearance of the OSP in the oviduct of sheep.

Erickson-Lawrence et al. (1989) further demonstrated the

effect of steroid hormones on the secretion of OSP. They cultured rabbit oviductal tissue explants in vivo and tested the effects of estrogen and progesterone on the secretion of OSP by these explants. They reported that tissue treated with progesterone and estrogen secreted a greater amount of the OSP than did control tissue (non-treated). Also, they found a difference in the time to maximum response to these treatments between ampullary and isthmic explants; ampullary tissue showed the greatest response to estrogen and progesterone on Days 2 and 3 after injections, whereas isthmic tissue showed its greatest response on Day 4 post-treatment. Furthermore, maximum production of OSP by ampullary tissue was reported to be approximately 22 times greater than the maximum isthmic production. These results are supported by those of Hyde and Black (1986) who reported a difference in the secretory rate of ampullary and isthmic explants cultured in vitro with the radioactive labeled precursors [^{3}H]glucosamine and [^{35}S]sodium sulphate of rabbits. They found that ampullary tissue secreted a greater amount of labeled OSP than did isthmic tissue. The finding that both the ampulla and isthmus are involved in secreting OSP agrees with the report by Boice et al. (1990a) for OSP secretion in different segments of the cow's oviduct. However, these findings disagree with those of Jansen and Bajpai (1982) who reported that the secretion of a glycoprotein in the oviduct of the rabbit was confined to the isthmic area. The authors reportedly found that only the

epithelial tissue of the isthmic region and to some extent, the ampullary-isthmic junction contained secretory granules which stain positive for the acidic mucus glycoprotein.

There is still no sound biological explanation for the function of these glycoproteins. There have been reports that the glycoproteins become associated with the ovum as it passes through the oviduct in many different species including; murine (Kapur and Johnson, 1988), hamster (Robitaille et al. 1988), baboons (Boice et al., 1990b) and pigs (Hedrick et al., 1987). The importance of these proteinaceous investments are not known but they may play a role in the development of the embryo and the implantation process. If this is true, any deviation from the "normal" amount or type of proteins produced and secreted may have an impact on successful fertilization and subsequent pregnancy.

STATEMENT OF THE PROBLEM

Pregnancy rates at and just after puberty in many mammals are lower than pregnancy rates associated with estrous cycles later in life. The review of literature indicated that the factors involved in this phenomenon are not entirely clear or have not yet been identified. Two factors that may affect fertility during the puberal transition in sheep are sperm transport to the site of fertilization and the oviductal environment.

The functions of the oviduct include transport of gametes and provision of the proper environment for fertilization and early embryonic development. The ability of the female to transport both gametes to the site of fertilization is extremely important for fertilization to occur. Studies have demonstrated that the ability of peripuberal ewes to transport oocytes does not differ from that observed in mature ewes but no research has investigated their ability to transport sperm cells to the oviduct.

The environment of the oviduct appears to be critical for proper fertilization. Little research has focused on the changes that occur in the oviductal environment as ewe lambs mature.

The objectives of this study were to determine: 1) if there is a difference in the ability of prepuberal estrogen-treated ewes, first or third estrus ewe lambs or mature ewes in their ability to transport sperm through the anterior

reproductive tract; and 2) if there was a difference in the protein content of the flushings from the oviducts of intact and ovariectomized, estrogen-treated prepuberal, first or third estrus ewe lambs and mature ewes.

MATERIALS AND METHODS

Experiment 1

This experiment was conducted at the Fort Ellis Sheep Research Station, Bozeman, Montana from September through January of 1989-1990 (YR1) and 1990-1991 (YR2).

Spring-born ewe lambs were placed with epididectomized rams fitted with marking harnesses and observed for estrous activity twice daily both years. When a ewe lamb exhibited estrus, she was randomly assigned to be either inseminated immediately (1E) or at her third estrus (3E). In the first year, ewe lambs that had not yet shown signs of estrous activity were assigned to be injected intramuscularly with estradiol benzoate (2.2 ug/kg body weight) and inseminated 24 hours later (prepuberal estrogen-treated; PPE). In the second year, mature ewes (MAT) were inseminated immediately upon detection of estrus. Prepuberal ewes were included in YR1 only and mature ewes were included in YR2 only (See Table 1 for experimental design).

Inseminations were performed with fresh semen collected from two intact rams with the aid of an artificial vagina. Following collection the ejaculates were evaluated for concentration, progressive motility, gross morphology and percent live cells. Concentration was determined by diluting the fresh semen 1:500 with 3% saline. Dilutions were performed with a Toma pipette and these dilutions were then

Table 1. Experimental design and number of prepuberal estrogen-treated (PPE), first estrus (1E), third estrus (3E) and mature ewes (MAT) in each year for Experiment 1

Year	PPE	1E	3E	MAT
1	8	7	8	-
2	-	8	5	12
Total	8	15	13	12

evaluated for number of sperm cells using an Improved Neubauer Ultra-Plane hemocytometer (Hauser Scientific, Blue Bell, Pennsylvania). Percent progressively motile was subjectively determined by an experienced technician. This percentage was then considered to be the percentage of viable sperm in the ejaculate. Following evaluation, an equal volume of each ejaculate (≈400 ul) was pooled together and mixed thoroughly. The following steps were then taken to determine the volume of the pooled semen to use in order to inseminate the ewe with 7.50×10^8 progressively motile sperm cells:

Step 1: Concentration of Ram 1 (Cells/ul) X Percent Progressively Motile Cells for Ram 1 = Progressively Motile Cells/ul for Ram 1

Step 2: Volume of Semen for Ram 1 in Pooled Sample (ul) X Progressively Motile Cells/ul for Ram 1 = Number of Progressively Motile Cells in Pooled Sample for Ram 1

Step 3: Repeat Step 1 for Ram 2

Step 4: Repeat step 2 for Ram 2

Step 5: Sum the Number of Progressively Motile Cells in Pooled Sample for Ram 1 and 2 = Total Number Progressively Motile Sperm Cells in Pooled Sample

Step 6: (Total Number Progressively Motile Sperm Cells in Pooled Sample / 7.50×10^8) X 100 = Percent of the Pooled Sample to Use for Insemination.

A volume of pooled semen containing 7.5×10^8 progressively motile sperm cells was loaded into an insemination pipette. Each ewe was inseminated non-surgically into the external os cervix after exposing the cervix with a vaginal speculum. Immediately after insemination, residual semen at the tip of the insemination pipette was observed for the presence of motile sperm cells. If sperm cells were observed to be non-motile when taken from the tip of the pipette then that ewe was eliminated from the study.

The first ewe inseminated in each group was randomly assigned to be hysterectomized at either 2 or 22 h after insemination. The next ewe in that group was then assigned to be hysterectomized at the converse time.

Hysterosalpingo-oophorectomy was performed on each ewe via a mid-ventral laparotomy under a surgical plane of anesthesia. A surgical plane of anesthesia was induced with an intravenous injection of sodium thiamylyl (10 ml; 4% in

0.9% sterile saline) and maintained with 2-bromo-2-chloro-1,1,1-trifluoroethane (Halothane; 2.0 to 2.5% and 400 to 600 cc O_2 in a closed system). To ensure that ewes in each experimental group were actually in that reproductive stage, ovaries of each ewe were examined for luteal and follicular structures immediately after exteriorizing the reproductive tract. Ewes assigned to the prepuberal estrogen-treated group could have no luteal structures or preovulatory follicles on their ovaries and were eliminated from the study if they had any of these. First estrus ewes could have no more than one corpus albicans (CA) and had to have a preovulatory follicle or a corpus haemorrhagicum (CH). Third estrus ewes had to have at least two and no more than three CA of different ages and either a preovulatory follicle or a CH. Mature ewes had to have either a preovulatory follicle or a CH on their ovaries.

Ligatures were placed just anterior to the internal os cervix, at the right and left tubouterine, isthmic-ampullar and ampullar-infundibular junctions. Ligations were placed to prevent further migration of sperm cells during the operation. The vascular supply to the reproductive tract was then ligated and the reproductive tract was excised. The left and right isthmi and ampullae were removed from the uterus. The uterus was carefully bisected from the external uterine bifurcation through the uterine body to yield the right and left uterine cornu and body. Each of these anatomical segment was flushed

with 0.9% saline and the flushings collected. The uterine cornu and body were flushed with approximately 50 ml per side, the ampulla and isthmus were flushed with approximately 5 ml per side. The right and left flushings of the isthmi and ampullae were pooled to yield one flushing for the isthmi and one flushing for the ampullae. All flushings were then centrifuged at 1,850 X g at 20° C for 20 min. Supernatants of each segment were decanted and each pellet was resuspended in a known volume of 0.1% Triton X-100 in 0.9% saline.

Both chambers of six Improved Neubauer Ultra-Plane (Hauser Scientific, Blue Bell, Pennsylvania) hemocytometers were loaded with the resuspended flushings for each segment and allowed to settle for 3 to 5 min. Hemocytometers were then evaluated under a light microscope at 400X for the presence and number of sperm cells. Only intact sperm cells were counted using the leukocyte counting method as described in Directions for the use of Levy and Levy-Hauser Corpuscle Counting Chamber (1974).

Number of sperm cells counted in each chamber of the six hemocytometers for each segment was recorded as cells per microliter counted. Total number of sperm cells recovered from each segment was then determined by multiplying the volume of resuspended flushings for a particular segment by the number of cells per microliter counted. One was added to all sperm counts so that data could be transformed to Log_{10}. This was necessary due to the extremely high variation in the

numbers of sperm cells recovered from the reproductive tracts of ewes and is an acceptable method of data analysis for sperm cell recovery (Quinlivan et al., 1969).

Statistical Analysis

Log_{10} transformed values were analyzed initially for a year effect using data from ewes in common groups (1E and 3E) for each year with General Linear Models (GLM) procedure of SAS (SAS, 1985). The model included year, group, year by group interaction, year by time interaction, group by time interaction and the year by group by time three-way interaction. Following the initial analysis, data for Log_{10} transformed sperm counts were then analyzed by analysis of variance for a completely randomized split-plot design using the General Linear Models procedure of SAS (SAS, 1985). The model included group, time after insemination and the group by time after insemination interaction in the main plot. The error term used to test these main plot effects was animal within time by group. Sub-plot effects were segments, the two-way interactions of segment by group and segment by time and the three-way interaction of group by time by segment. In order to make multiple comparisons of Log_{10} least squares means, Bonferroni's Inequality (Snedecor and Cochran, 1989) along with the PDIFF procedure of SAS (SAS, 1985) were used. The Log_{10} values for the total number of sperm cells recovered

from the entire anterior reproductive tract (TOTLOG) were analyzed with the General Linear Models procedure of SAS (SAS, 1985). The model included group, time after insemination and group by time after insemination. The Categorical Data Modeling procedure of SAS (SAS, 1985) was used to evaluate proportions of ewes among groups that had transported sperm cells to either the uterus, isthmus or ampulla at 2 or 22 hours. Fisher's "Exact Test" (Steel and Torrie, 1980) was used to make comparisons between proportions.

Experiment 2

Gross and histometric data used in this experiment were collected in a previous experiment (Lewis, 1990). Data collected by Lewis (1990) that were used in the present study included; total wet weight of the right and left oviducts (ODWT), total length of right and left oviducts (ODLT), total lumenal surface area of both the right and left oviducts (ODAR), and total number of epithelial cells from both the right and left oviducts (CELL). These data were from ewes in four different reproductive stages: prepuberal (PP), first estrus (1E), third estrus (3E) and mature (MAT) ewes. Ewes in each stage were subjected to one of two treatments: intact (TRT 0) or ovariectomized estrogen-treated (TRT 1). Mature ewes (MAT) were subjected to one other treatment; ovariectomized corn-oil treated (TRT 2). The experimental design and number of ewes in each group within each treatment is described in Table 2.

Following surgery, after detection of estrus, oviducts of each ewe were flushed by using a continuous infusion pump which flushed 0.9% saline through the lumen of the oviduct at a rate of 200 ul per minute. Each oviduct was flushed with a total of 2 ml so that approximately 4 ml was collected from each ewe. Flushings were centrifuged at 400 X g for 20 min to remove cellular debris. Each flushing was decanted into a sterile glass tube, frozen and stored at -20° C.

Table 2. Experimental design and number of prepuberal (PP), first estrus (1E), third estrus (3E) and mature (MAT) ewes in each treatment in each year for Experiment 2

	TRT 0[a]		TRT 1[b]		TRT 2[c]	
	YR 1	YR 2	YR 1	YR 2	YR 1	YR 2
PP	7	2	-	4	-	-
1E	9	3	-	4	-	-
3E	8	4	-	4	-	-
MAT	-	3	-	6	-	6

[a] TRT 0 = Intact non-estrogenized ewes.
[b] TRT 1 = Ovariectomized estrogen-treated ewes.
[c] TRT 2 = Ovariectomized corn oil treated ewes.

Volume of each sample was determined volumetrically with a Ranin Pipetman® (P 1000 and P 200). Protein concentration for each sample was determined by using the Pierce BCA® protein assay. The standard used in the assay was bovine serum albumin and standard concentrations ranged from 0 to 150 ul/ml. Samples were thawed at 4° C, vortexed and approximately 260 ul was removed from each sample. Two hundred fifty microliters of the sample was then diluted to 1000 ul with 750 ul of distilled water. From this, 100 ul aliquants of diluted sample were placed in two separate tubes and each diluted with 900 additional ul of distilled water. The standards were treated in the same manner with 100 ul of the standard being diluted with 900 ul of water in 2 duplicate aliquants. All tubes were then vortexed. Two ml of working

reagent was added to each tube and the tubes were incubated at 60° C in a water bath for 30 minutes as described in the Pierce BCA® Protein Assay manual. Samples were removed from the bath and allowed to reach room temperature. Absorbance of all standards and samples was determined at 562 nm using a Beckman Model 25 Spectrophotometer. Absorbance of the standards was regressed on the protein concentration of the standards to establish a standard curve. The protein concentration for each oviductal flushing was determined from the absorbance of the 2 samples for each oviduct. The mean protein concentration of the duplicates for each oviductal sample was multiplied by the total volume of that sample to get the protein content of that sample. Right and left oviductal protein content were added together for total protein content of both oviducts (PTN).

The PTN for each ewe was then divided by the ODWT and ODLT for that ewe to yield the total amount of protein per unit weight (PTNWT) and the total amount of protein per unit length (PTNLT) of both oviducts. For ewes in the second year PTN was also divided ODAR and CELL for each ewe to yield total protein per area (PTNAR) and the total protein per cell (PTNCELL) of both oviducts.

These variables were derived for the purpose of characterizing the relationship between histomorphic changes associated with oviducts and the amount of protein recovered from oviducts of ewe lambs during the puberal transition.

Statistical Analysis

Data that were common to both years, PTN, PTNLT and PTNWT for intact ewes (TRT 0) were initially analyzed with the GLM procedure of SAS (SAS, 1985). The model included year (YR), reproductive state (RS) and the YR by RS interaction. Multiple comparisons were made utilizing the PDIFF procedure of SAS (SAS, 1985) along with Bonferroni's Inequality (Snedecor and Cochran, 1989).

No year effect was found, therefore data for both years were then pooled and analyzed with the GLM procedure of SAS (SAS, 1985). Multiple comparisons were made utilizing the PDIFF procedure of SAS (SAS, 1985) and Bonferroni's Inequality (Snedecor and Cochran, 1989). All reproductive states were included, only treatment 0 and 1 were used. Treatment 2 was excluded because it was unique to RS 4. The dependent variables for this analysis were PTN, PTNLT, PTNWT, PTNAR and PTNCELL. The model included RS, TRT and the RS by TRT interaction.

To examine the effect ovariectomy with exogenous estrogen treatment (TRT 1) and ovariectomy with corn oil injection (TRT 2) had on the dependent variables PTN, PTNLT, PTNWT, PTNAR, and PTNCELL, MAT ewes only were analyzed using the GLM procedure of SAS (SAS, 1985). Multiple comparisons were made with the PDIFF procedure of SAS (SAS, 1985) along with Bonferroni's Inequality (Snedecor and Cochran, 1989). The

model included only TRT.

RESULTS

Experiment 1

There was no ($P > .10$) effect of year for sperm counts or percentage of ewes from which sperm cells were recovered in the various segments of 1E and 3E ewes (the only common groups in both years of the study); therefore, data from both years were pooled and analyzed together.

There was no time by group interaction ($P > .10$) for the percentage of ewes from which sperm were recovered from the anterior reproductive tract. The percentage of ewes from which sperm cells were recovered from the anterior reproductive tract at 2 h (83%) did not differ ($P > .10$) from the percentage of ewes from which sperm cells were recovered at 22 h (96%). Although there was no difference in the percentages of ewes from which sperm cells were recovered, there was a difference in the distribution of the sperm cells in the uterus, isthmus and ampulla at these two times. Ewes hysterectomized at 2 h had the majority of the sperm cells recovered from the uterus while the 22 h ewes had sperm cells recovered from the entire anterior reproductive tract. Percentages of ewes from which sperm cells were recovered from the uterus, isthmus and ampulla did not differ ($P > .10$) among postpuberal ewes but tended to be higher ($P < .10$) than that for PPE ewes (Figure 1).

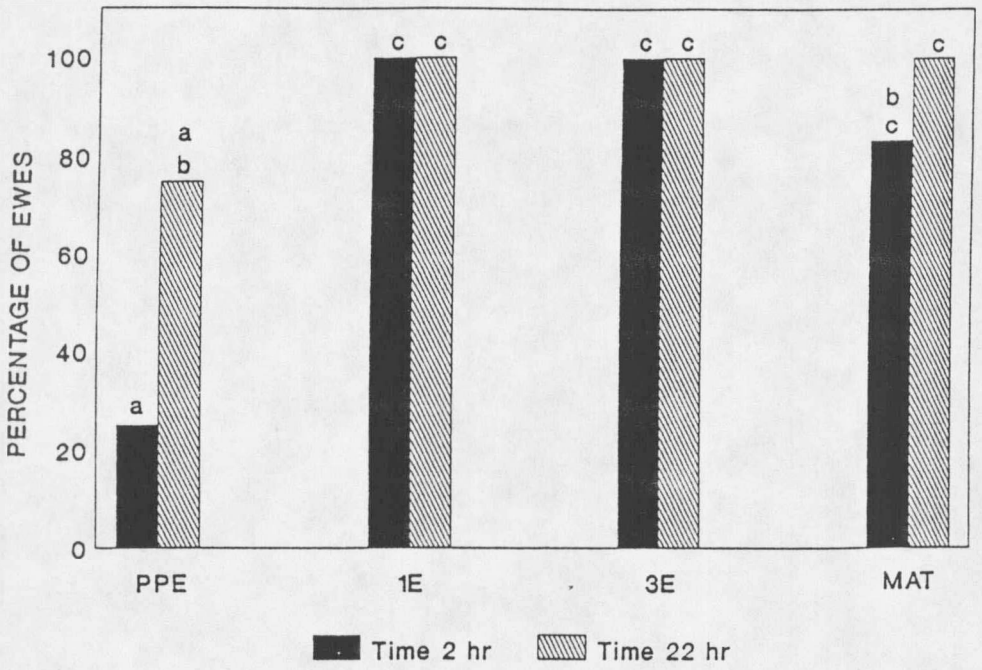


Figure 1. Percentages of ewes in prepuberal estrogen-treated (PPE), first estrus (1E), third estrus (3E) and mature (MAT) ewes with sperm in their anterior reproductive tract at 2 and 22 h after insemination. Different letters above bars indicate differences ($P < .10$).

At 2 h only 8.7% of all ewes (2 of 23) had transported sperm cells to the oviducts (Figure 2). The percentage of postpuberal ewes (90%) that had transported sperm cells to the oviducts by 22 h tended to be greater ($P < .10$) than the percentage of prepuberal ewes (25%). There was no difference ($P > .10$) among the percentages of 1E, 3E or MAT ewes that transported sperm cells to the oviducts (Figure 2) at either 2 or 22 h after insemination.

There was a time after insemination by group by segment interaction (Table 3; $P < .05$) for Log_{10} number of sperm cells recovered in the different segments of the reproductive tract

when all groups were included in the analysis. However, when only postpuberal ewes (1E, 3E and MAT) were included in the analysis the three way interaction was not significant ($P > .10$). In this analysis we found a time by segment interaction (Figure 3; $P < .05$) due to the extremely low numbers of sperm cells transported to the oviducts at 2 h (Figure 3).

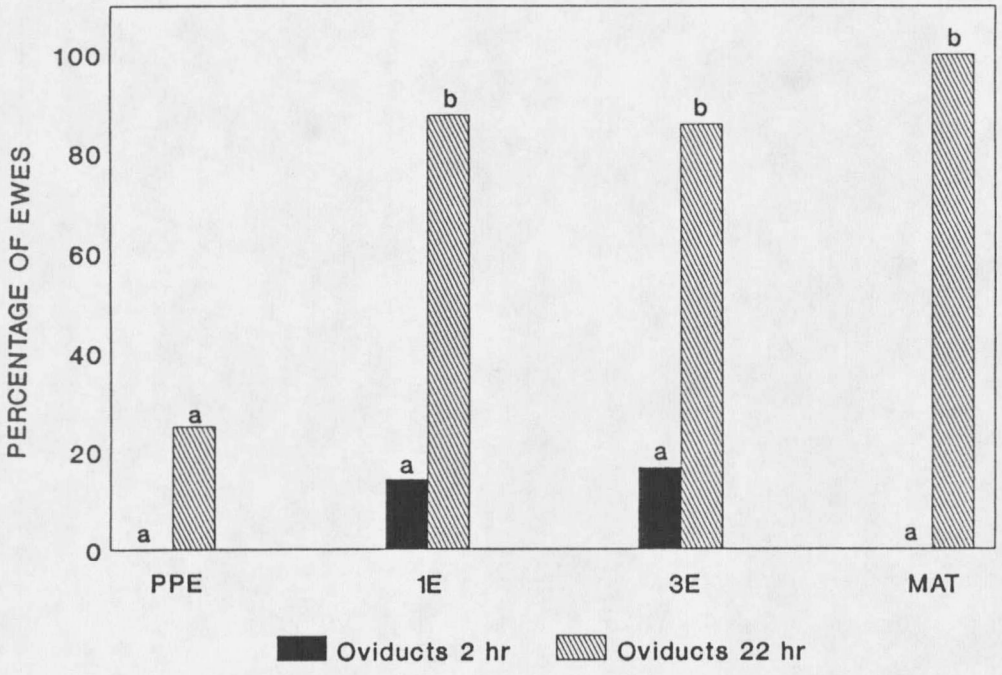


Figure 2. Percentages of prepuberal estrogen-treated (PPE), first estrus (1E), third estrus (3E) and mature (MAT) ewes with sperm cells in their oviducts at time 2 or 22 h after insemination. Different letters above bars indicate differences ($P < .05$).

Total number of sperm cells recovered from the entire anterior reproductive tract of ewes was analyzed as total Log_{10} number of sperm cells (TOTLOG). TOTLOG did not differ ($P > .10$) among postpuberal ewes, however, there was a difference ($P < .05$) between the prepuberal and postpuberal ewes (Table

4) with the postpuberal ewes having approximately a two-fold increase in the TOTLOG sperm cells recovered from the anterior reproductive tract.

Table 3. Log_{10} number of sperm cells recovered from the uterus, isthmus and ampulla at time 2 and 22 h after insemination[a] for prepuberal estrogen-treated (PPE), first estrus (1E), third estrus (3E) and mature (MAT) ewes

	Uterus		Isthmus		Ampulla	
	2	22	2	22	2	22
PPE	1.21[b]	2.40[b]	0.00	0.59[b]	0.00	0.00
1E	4.45[c]	4.44[c]	0.28	2.88[c]	0.00	0.94
3E	5.04[c]	5.00[c]	0.32	3.08[c]	0.00	1.04
MAT	3.39[c]	5.27[c]	0.00	3.27[c]	0.00	0.87

[a] Time by Group interaction ($P < .05$); Pooled Standard Error = 0.91; df = 80.

[b,c] Different superscripts within the same column indicate differences ($P < .10$). Columns with no super scripts indicate no differences ($P > .10$).

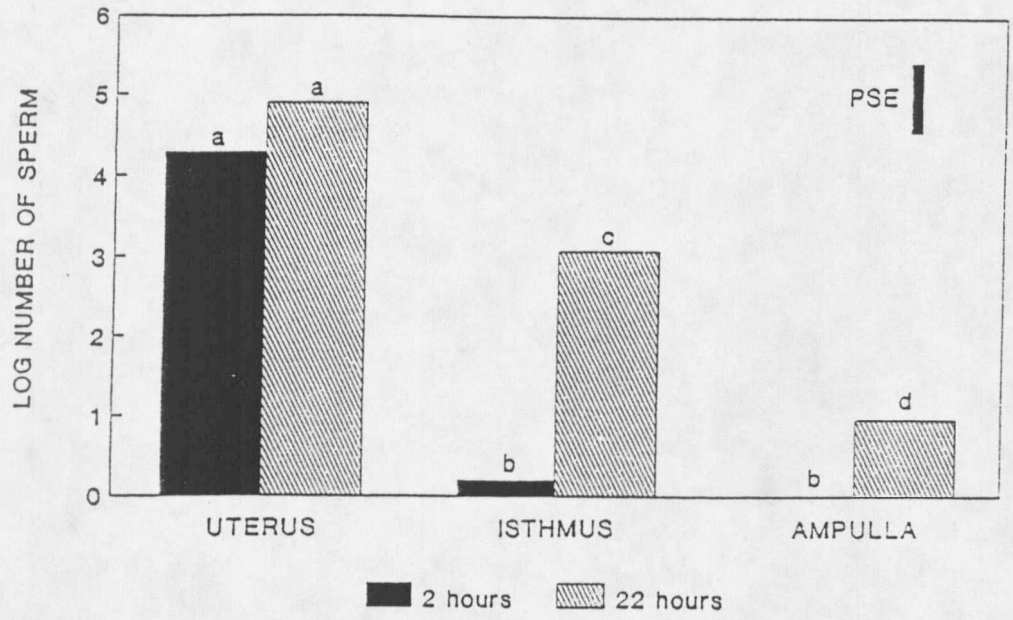


Figure 3. Least-squares means for Log_{10} number of sperm cells recovered from the uterus, isthmus and ampulla at time 2 or 22 h after insemination. Different letters above bars indicate differences ($P < .05$). PSE bar represents pooled standard error; df = 68.

Table 4. Least square means of total Log_{10} number of sperm cells (TOTLOG) recovered from the anterior reproductive tracts of prepuberal estrogen-treated (PPE), first estrus (1E), third estrus (3E) and mature (MAT) ewes and the corresponding real number of sperm cells[a]

Group	TOTLOG	Real #
PPE	2.05[b]	112
1E	4.46[c]	28,840
3E	5.04[c]	109,647
MAT	4.37[c]	23,442

[a] Pooled Standard Error for TOTLOG = 1.20; df = 40.
[b,c] Different superscripts indicate differences ($P < .05$).

Experiment 2

There was no YR effect or YR by RS interaction ($P > .10$) for PTN, PTNLT, or PTNWT for ewes common to both years (PP, 1E and 3E; TRT 0); therefore, data from both years were pooled and analyzed together. In this initial analysis prepuberal ewe lambs had more than twice the PTNLT and approximately three times as much PTNWT (Table 5; $P < .10$) as did either 1E or 3E ewes. Prepuberal ewes also had more than twice as much PTN recovered from their oviducts as did either 1E or 3E but because of the large variation in this variable they did not differ ($P = .13$).

Table 5. Least-squares means for total protein recovered from right and left oviducts (PTN), total protein per unit length of right and left oviducts (PTNLT) and total protein per unit weight of right and left oviducts (PTNWT) for prepuberal (PP), first (1E) and third estrus (3E) ewes in TRT 0 (intact) which were common to both years

	PTN	PTNLT	PTNWT
PP	1093[a]	39.8[a]	1.22[a]
1E	474[a]	16.5[b]	0.42[b]
3E	499[a]	16.8[b]	0.45[b]
SE[c]	554	18.4	0.42

[a,b] Different superscripts within the same column indicate differences ($P < .10$).
[c] df = 27

The analysis for all ewes from both years, excluding MAT ovariectomized estrogen-treated ewes because this treatment

was unique to MAT ewes, indicated that RS affected PTNWT and PTNCELL.

Prepuberal ewes tended ($P < .10$) to have more protein per unit oviductal weight than did either 1E or 3E ewes (Figure 4). However, protein per unit oviductal weight for PP ewes did not differ ($P > .10$) from PTNWT for MAT ewes. Protein per unit oviductal weight did not differ ($P > .10$) between 1E and 3E ewes.

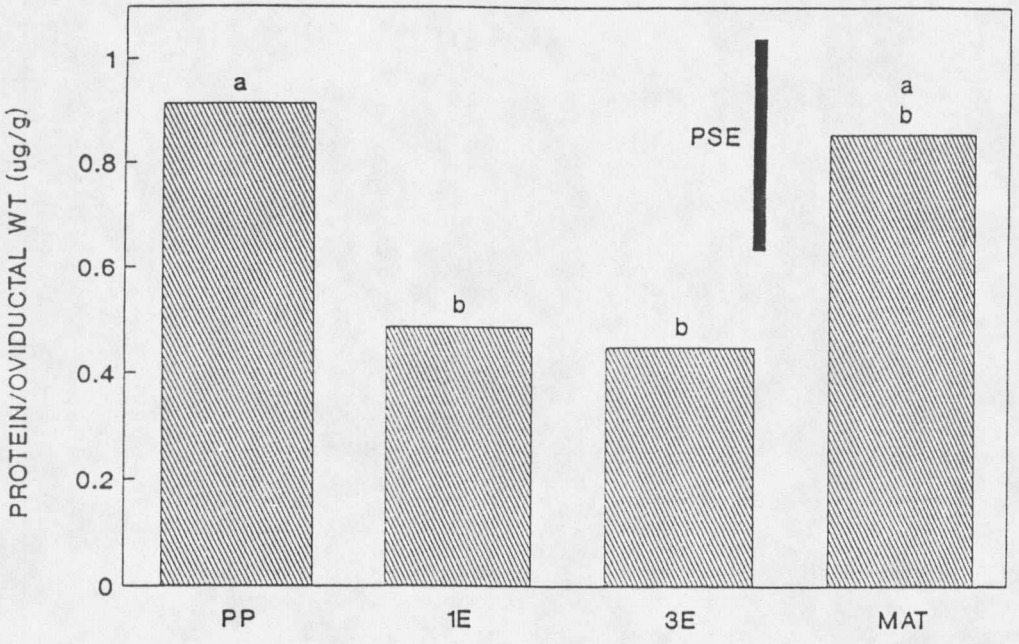


Figure 4. Least-squares means for protein per unit oviductal weight (PTNWT) recovered from prepuberal (PP), first estrus (1E), third estrus (3E) and mature (MAT) ewes. Different letters above bars indicate differences ($P < .10$). PSE bar represents pooled standard error; df = 46.

Mature ewes had more ($P < .05$) PTNCELL than did 1E ewes (Table 6), but MAT ewes did not differ ($P > .10$) in their PTNCELL from PP or 3E ewes.

Table 6. Least-squares means for total protein per oviductal cell (TOTCELL) for prepuberal (PP), first (1E) or third estrus (3E) and mature ewes (MAT)

	PTNCELL ($\times 10^{-7}$ ug/cell)
PP	5.9550[a,b]
1E	2.6217[a]
3E	3.5788[a,b]
MAT	7.7367[b]

[a,b] Different superscripts within the same column indicate differences ($P < .05$).

There was a RS by TRT interaction ($P < .05$) for PTN from the oviducts of all ewes in both TRT 0 and 1 (Figure 5). Ovariectomized mature ewes receiving estrogen had more ($P < .05$) protein in their oviducts than did any other group or treatment. This large response relative to the response to ovariectomy and estrogen-treatment for the other groups of ewes appears to be the main cause of this interaction.

A RS by TRT interaction ($P < .05$; Figure 6) was also found for PTNLT. Again this interaction appears to result from the response of ovariectomized mature ewes to estrogen-treatment. Mature ovariectomized estrogen-treated ewes had a much larger ($P < .05$) increase in the PTNLT relative to the intact ewes than ewes in other groups.

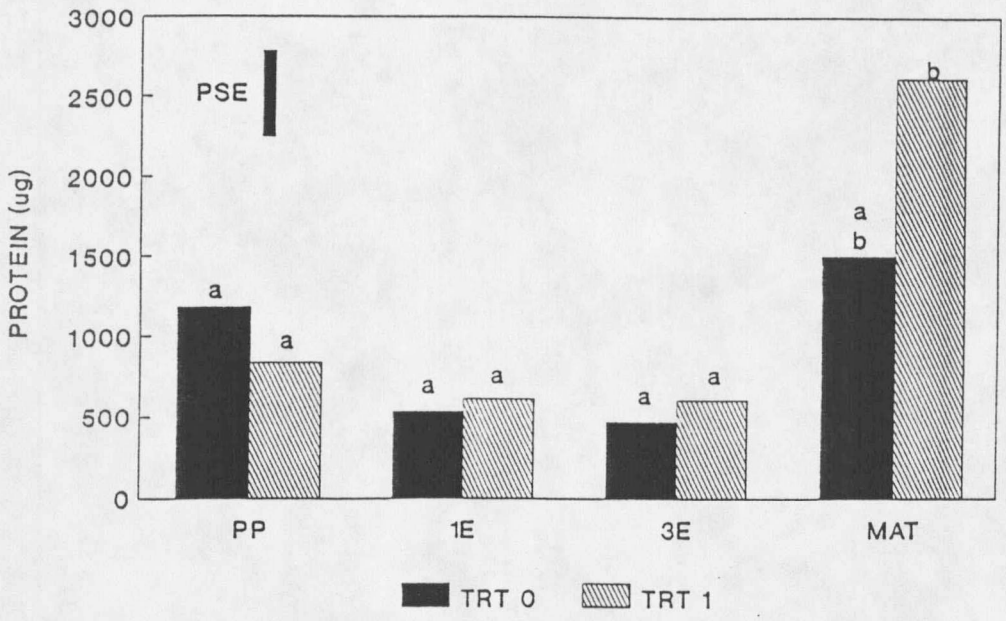


Figure 5. Least-squares means for protein content of oviducts from intact (TRT 0) or hysterectomized estrogen-treated (TRT 1), prepuberal (PP), first estrus (1E), third estrus (3E) and mature (MAT) ewes illustrating a RS by TRT interaction ($P < .05$). Different letters above bars indicate differences ($P < .05$). PSE bar represents pooled standard error; df = 46.

Protein per unit oviductal surface area was affected by a RS by TRT interaction ($P < .05$; Figure 7). The trend appears the same as for PTN and PTNLT in that the main contributor to this interaction was the response of mature ovariectomized ewes to estrogen. These ewes responded much greater relative to the mature intact ewes than ewes of any other treatment. Protein per unit area of oviductal lumen for MAT ewes in TRT 1 did not differ ($P > .10$) from PTNAR of PP ewes in both TRT 0 and TRT 1.

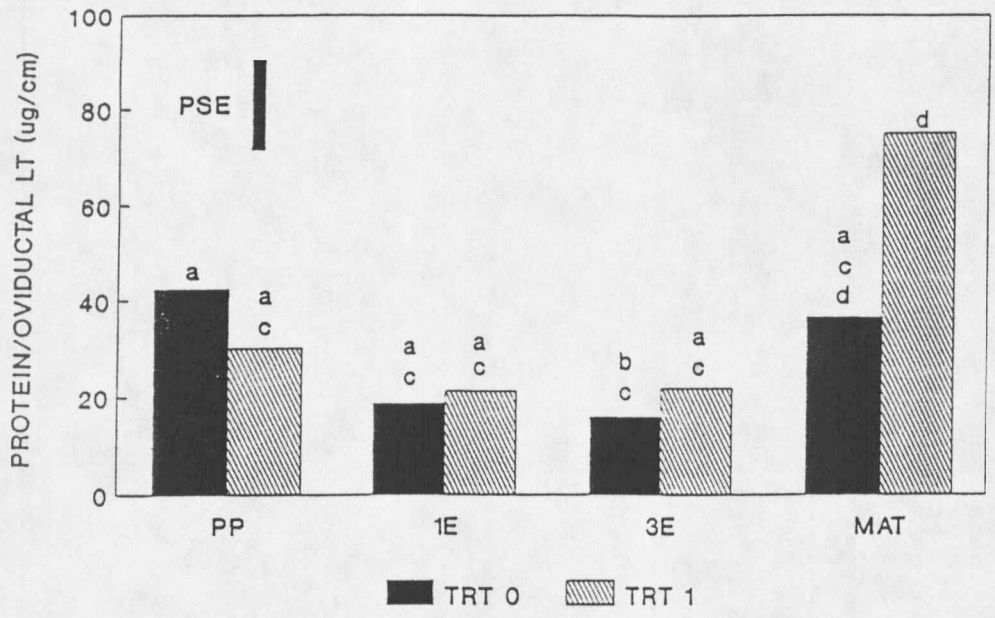


Figure 6. Least-squares means for protein per unit length of oviduct (PTNLT) from intact (TRT 0) or ovariectomized estrogen-treated (TRT 1), prepuberal (PP), first estrus (1E), third estrus (3E) and mature ewes (MAT) illustrating a RS by TRT interaction ($P < .10$). Different letters above bars indicate differences ($P < .10$). PSE bar represents pooled standard error; df = 46.

In an analysis that included all three treatments which were common only to MAT ewes, TRT was found to have an affect on PTN and PTNLT ($P < .05$) and, to a smaller extent on PTNAR ($P < .10$) but did not affect ($P > .10$) PTNWT or PTNCELL (Table 7).

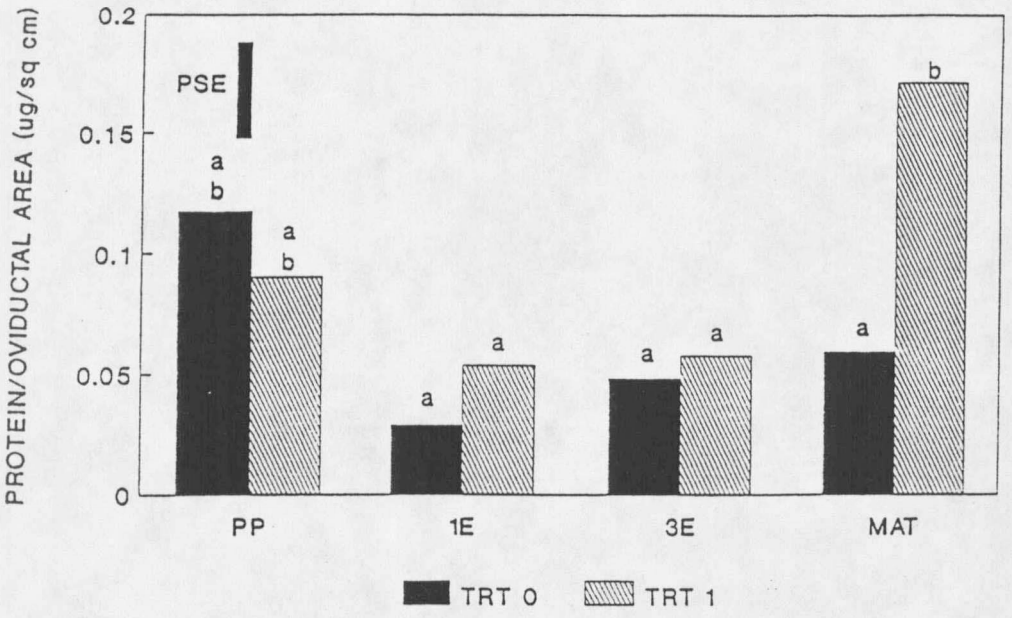


Figure 7. Least-squares means for protein per unit area of oviduct (PTNAR) from intact (TRT 0) or hysterectomized estrogen-treated (TRT 1), prepuberal (PP), first estrus (1E), third estrus (3E) and mature (MAT) ewes illustrating a RS by TRT interaction ($P < .05$). Different letters above bars indicate differences ($P < .05$). PSE bar represents pooled standard error; df = 21.

Mature ewes of TRT 1 had more ($P < .05$) oviductal PTN than did ewes in TRT 0 or 2. The same pattern was found for PTNLT. in that TRT 1 differed from TRT 2 ($P < .05$). Protein per unit area from the oviducts was greatest in ewes of TRT 1 but differed from that of ewes in TRT 0 only ($P < .10$).

Table 7. Least-squares means for total protein of oviducts (PTN), total protein per unit length of oviducts (PTNLT), total protein per unit weight of oviducts (PTNWT), total protein per unit area of oviducts (PTNAR) and total protein per oviductal cell (PTNCELL) for mature ewes in TRT 0 (intact non-treated) and TRT 1 (ovariectomized estrogen-treated) and TRT 2 (ovariectomized corn oil-treated)

TRT	PTN (ug)	PTNLT (ug/cm)	PTNWT (ug/g)	PTNAR (ug/cm^2)	PTNCELL (ug/cell)
0	1503.48[a,b]	36.48[a,b]	0.633	0.059[a]	4.5×10^{-7}
1	2620.28[b]	74.97[b]	1.077	0.171[b]	11.0×10^{-7}
2	1307.85[a]	35.88[a]	0.698	0.096[a,b]	6.5×10^{-7}
SE[c]	702.49	25.51	0.539	0.066	7.9×10^{-7}

[a,b] Different superscripts within the same column indicate differences ($P < .10$), columns with no superscript indicate no difference ($P > .10$).

[c] df = 12.

DISCUSSION

Experiment 1

Proportion of ewes from which sperm cells were recovered from the anterior reproductive tract at 2 or 22 h after artificial insemination did not differ. This indicates that there was no sequestering of sperm cells by the cervix for later release. If there had been sequestering of sperm cells for later release by the cervix at some time after 2 h and before 22 h after insemination then a greater proportion of ewes would have transported sperm cells to the anterior reproductive tract at 22 h than at 2 h. Although the proportion of ewes from which sperm cells were recovered did not differ between the two times that ewes were examined, there was a greater number of sperm cells recovered from ewes hysterectomized at 22 h than at 2 h. This result differs from previous reports of Hawk and Conley (1975) for mature ewes in which there was no significant increase in numbers of sperm cells recovered from the uterus at 2 and 22 h after insemination. However, results of the present experiment agree with those of Dobrowolski and Hafez (1973) who found a steady increase in numbers of sperm cells recovered from the uterus between 1 and 24 h after insemination in the beef heifer.

Sperm cells were recovered from the anterior reproductive

tract of more postpuberal ewes than from PPE ewes indicating that PPE ewes do not pass sperm cells as readily through the cervix as postpuberal ewes. The finding that there was no difference among 1E, 3E, and MAT ewes in the proportion of ewes that transported sperm cells through the cervix and into the anterior reproductive tract demonstrated that there is no apparent improvement in the passage of sperm cells through the cervix and anterior reproductive tract with subsequent estrous cycles.

The low number of ewes with sperm cells in their oviducts at 2 h (2 of 23) differs from data reported by Quinlivan et al. (1969) and Lightfoot and Restall (1971) but agrees with reports of Hunter (1985) and Thibault (1973) in which it was reported that more than 2 h were required for sperm cells to reach the oviducts. However, by 22 h, 90% of postpuberal ewes had transported sperm cells to their oviducts but only 25% of PPE ewes had transported sperm cells to their oviducts. These findings indicate that PPE ewes do not readily pass sperm cells through the tubouterine junction. There was no difference among the proportion of postpuberal ewes that transported sperm cells to the oviducts which suggests that there is no maturational increase from 1E to 3E to MAT ewes in their ability to pass sperm cells through the tubouterine junction.

The group by time after insemination by segment interaction for Log_{10} number of sperm cells recovered was

primarily a result of the inability of PPE ewes to transport sperm cells to the oviducts by 22 h. All other groups were able to transport sperm cells to the oviducts by 22 h. The time by segment interaction resulted from a change in the pattern of appearance of sperm cells from 2 to 22 h with very few sperm cells being recovered from the isthmus and ampulla at 2 h. At 22 h there appeared to be a linear decrease in the Log_{10} number of sperm cells recovered from the uterus, isthmus and ampulla; however, this decrease was quadratic in appearance at 2 h. These patterns suggest rapid transport of sperm cells through the cervix and a delay in progression through the tubouterine junction into the oviducts. This result agrees with Thibault (1973) who reported fast sperm transport to the tubouterine junction with subsequent storage of sperm cells in the uterine cornua at the tubouterine junction for later release into the oviducts at approximately 18 h after insemination. Further support of our data were given by Hunter and Nichol (1983) and Hunter (1989) who reported sequestering of sperm cells in the tubouterine area for 20 to 22 h, but they found that sperm cells were stored in the caudal 1.5 to 2.0 cm of the isthmus and not in the uterine cornua.

The total Log_{10} number of sperm cells recovered (TOTLOG) in the anterior reproductive tract did differ among postpuberal groups of ewes in this study. Similarities in TOTLOG among 1E, 3E and MAT ewes were consistent with the

similarity in proportions of ewes from which sperm cells were recovered for these three groups. Postpuberal ewes did not differ in their ability to transport large numbers of sperm cells through the anterior reproductive tract. Although PPE ewes transported sperm into the uterus, the number of sperm cells recovered was much lower than that recovered in postpuberal ewes. These results suggest one of three things; 1) the estrogen treatment of the prepuberal ewes was not sufficient to simulate the basic endocrinological changes at estrus that occur in postpuberal ewes, 2) the prepuberal ewe experiences some physiological change such as a change in steroid sensitivity at puberty that enhances their ability to transport sperm cells into and through the anterior reproductive tract or 3) a combination of these possibilities.

Experiment 2

Treating ovariectomized prepuberal, 1E, 3E or mature ewes with estrogen did not affect PTNWT or PTNCELL in any of these ewes which indicates that the amount of protein production relative to the weight of or the number of cells in the oviducts of sheep is not dependent upon the presence of the ovary or treatment with estrogen but predetermined by physiological occurrences that the oviducts have experienced in the past, i.e., reproductive state of the animal. The difference in PTNWT and PTNCELL between the oviducts of prepuberal ewe lambs and first or third estrus ewe lamb suggests that a change occurs in the protein production and/or transudative process in oviducts of sheep at puberty. This change affects the production per epithelial cell and weight which can be viewed as a decrease in the "efficiency" of the oviduct as a secretory and/or a transudative organ. These variables also differed between the first and third estrus ewe lamb oviducts and mature ewe oviducts. It would appear that PTNWT and PTNCELL, at some time between third estrus and maturity, return to levels very similar to those seen in prepuberal ewe lambs. Whether this change is reciprocal to the change seen at puberty is not known.

The three variables PTN, PTNLT and PTNAR all responded differently within different groups when subjected to different treatments. The pattern of response for all three

variables was identical for ewes ovariectomized and estrogen-treated ewes. When prepuberal ewes (PP) were ovariectomized estrogen-treated, there was a decrease, relative to intact ewes in for PTN, PTNLT and PTNAR, whereas ovariectomy and estrogen-treatment resulted in an increase in PTN, PTNLT and PTNAR in 1E, 3E and MAT ewes. This contributed to the interactions observed in these variables but the response of mature ewes to ovariectomy and estrogen treatment (TRT 1) appears to be the main cause of the interaction. Mature ewes treated with estrogen following ovariectomy responded to a much greater extent than that observed in ovariectomized estrogen-treated ewes in either the first or third estrous groups. This supports the notion that a change occurs in the oviduct of ewes with respect to its response to estrogen at some time between third estrus and some later estrous period that results in different secretory and/or transudative activity.

The different responses seen for these variables between peripuberal ewes and mature ewes resulted from a greater cellular response to the ovariectomy and estrogen treatment in mature ewes. A possible explanation for the different response to ovariectomy and estrogen-treatment in mature ewes includes a more "efficient" secretory or transudative process or a shorter lag time for response after estrogen treatment resulting in greater protein release into the oviductal lumen. An example of being more "efficient" in

the secretory process could be that the oviductal cells of mature ewes are more capable of gathering either the amino acids or substrates for the sugar moieties of the glycoproteins to be secreted. This could result in these cells having a larger store of the proteins to be released. The mature ewe oviducts could also be more proficient in transporting macromolecules ad-lumenally for transudation. The resultant cascade of events following administration of estrogen including the intracellular second messenger network could be more efficient in mature ewes by having more biochemical messengers constructed and ready for release. However, the response could be a latent result of the ovary itself. If the mature ovary, in addition to the positive effect of the estrogen, exerted some inhibitory effect on the oviduct which is greater than that from peripuberal ewe lamb ovaries, then the removal of the ovary in a mature ewe could result in a greater response to similar dosages of estrogen compared to the response of peripuberal ewe lambs.

The similarities among prepuberal and first or third estrus ewes for PTN, PTNLT and PTNAR is not clear. Differences would be expected between these different groups of ewes as was noted for the variables PTNWT and PTNCELL. The lack of difference among these groups for these variables could mean that the change from 1E and 3E to MAT is not reciprocal to the change from PP to 1E and 3E but more likely the similarity among these ewes could be the result of the

high variation in these variables.

The effect of estrogen itself was only evaluated in MAT ewes, the only group with all three treatments. This can be done by comparing ovariectomized estrogen-treated ewes (TRT 1) with ovariectomized corn-oil-treated ewes (TRT 2). The only difference between these two groups was the administration of exogenous estrogen. The pattern exhibited by these ewes when comparing the three treatments is consistent throughout all five variables that were investigated (PTN, PTNWT, PTNLT, PTNAR and PTNCELL). Ovariectomy and estrogen treatment increased the levels of PTN, PTNWT, PTNLT, PTNAR and PTNCELL relative to levels for intact ewes. When the treatment included only ovariectomy with corn-oil injection the levels for all variables were similar to those for intact ewes. This pattern is seen in all variables but significant only for PTN, PTNLT and PTNAR. This is consistent with the findings for intact and ovariectomized estrogen-treated ewes in all reproductive states in which ovariectomy and estrogen-treatment was found to not affect PTNWT and PTNCELL but did affect PTN, PTNLT and PTNAR.

These responses do not completely agree with earlier reports on the effect of estrogen on the oviductal secretory function. Greenwald (1958), Brower and Anderson (1969), Fredriccson (1969) and Lambert et al. (1973) reported that estrogen caused the epithelial secretory cells to fill and progesterone was required for the expulsion of the content of

these granules. If this were entirely true, the estrogen treatment should not directly affect the protein recovered from the oviductal lumen. Oliphant et al. (1978) reported hormonal status did not affect the concentration of transudative proteins found in the oviductal lumen. Because of this, it can be assumed that the changes in protein content in these ewes results from the secretory protein(s).

The return of all variables to levels seen in intact ewes when ewes are ovariectomized and injected with corn-oil supports the theory that the presence of the ovary is not the deciding factor in determining the protein production but is predetermined by the reproductive state of the oviducts.

In conclusion, we found that it takes more than 2 h for sperm to reach the oviducts of sheep after artificial insemination into the anterior os cervix and that peripuberal and mature ewes did not differ in their ability to transport sperm cells into and through the anterior reproductive tract. There are however, differences in the protein content of oviductal flushings of peripuberal ewes and mature ewes. From these data one could hypothesize that lowered pregnancy rates of peripuberal ewes relative to mature ewes may not be a result of inadequate sperm cell transport. However, pregnancy rates may differ between peripuberal and mature ewes as a result of differences in oviductal environments.

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