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

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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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), Dyrmundsson (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