Sperm Survival and Transport in the Female Reproductive Tract

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ABSTRACT
Fertilization failure, mostly due to absence of sperm in the oviducts, is a major cause of reproductive inefficiency of farm animals. Sperm may be transported to the oviducts of cattle and sheep within a few minutes after mating or insemination, but these sperm probably fertilize few ova. Slower transport, with establishment of sperm populations in each segment of the reproductive tract, requires a few to several hours. In swine, sperm capable of fertilizing ova reach the oviducts in less than 1 h. Smooth muscle contractions of the reproductive tract, ciliary beats, fluid currents, and flagellar activity of sperm are primary mechanisms of sperm transport. Sperm become hyperactive in the oviducts in association with capacitation. Most sperm in an inseminate drain from the female reproductive tract within a few minutes or hours after insemination; remaining sperm are removed from the tract by slower drainage or phagocytosis.

Sperm survival and transport in estrous ewes is reduced drastically by pastures with high estrogen content and by regulating estrus with progestogen or prostaglandin F\(_2\alpha\). The cervix is the initial site of inhibition of sperm transport in ewes, and endocrine imbalances probably are the basis of inhibition. Sperm transport problems generally are associated with immobilization and death of sperm in the uterus and anterior segments of the cervix within 2 h after mating. After gilts are inseminated with frozen-thawed semen, relatively few sperm are retained in the reproductive tract, apparently accounting for lowered fertilization rates.

Sperm transport has been improved by adding to semen or administering to females such compounds as prostaglandin F\(_2\alpha\), oxytocin, estradiol, phenylephrine, or ergonovine. Estradiol, prostaglandin F\(_2\alpha\), phenylephrine, and ergonovine administered to rabbits at insemination each increased fertilization rates.

INTRODUCTION
Transport of sperm from site of deposition in the female to site of fertilization represents a critical phase of the reproductive process of farm animals. Sperm transport failures, which result in fertilization failure, account for a significant proportion of the loss of potential offspring in each major class of animal. Improving sperm transport and reducing fertilization failure could reduce intercalving intervals of cattle and increase lambing rate of sheep and average litter size of swine.

The term “sperm transport” properly means the movement of sperm by the female reproductive tract from the site of deposition of semen to the ampulla of the oviducts. However, the term often is used in a broader sense to define movement of sperm in the female regardless of whether the movement resulted from action of the female tract or from action of sperm. In fact, most studies on movement of live sperm through the female tract are not designed to separate the role of the female from that of the sperm. Therefore, “sperm transport” in this paper will encompass consequences of all physiological processes involved in movement of sperm in the female tract.

Sperm transport can be measured directly or indirectly in several ways. Fertility can be a crude measure of sperm transport if other factors that influence fertility are constant. The fertilization rate of ova and the number of...
accessory sperm embedded in or attached to the zona pellucida represent more quantitative measures of sperm transport, and the number of sperm in the various segments of the reproductive tract, preferably at more than one interval after insemination, is the most direct measure. Sperm numbers, usually calculated from microscopic counts of sperm in a portion of the washings or flushings of segments of the reproductive tract, are reliable only if the flushing procedures remove nearly all sperm from the tract. Sperm numbers in the reproductive tract, particularly in the oviducts, vary greatly among animals. This variability often necessitates assignment of large numbers of animals to experimental groups.

This review will be concerned primarily with sperm transport in cattle, sheep, and swine, particularly with aspects of sperm transport related to fertility. Sperm transport in laboratory animals will be cited mostly for principles and concepts not otherwise covered. However, several comprehensive and incisive reviews on sperm transport have been published in the past few years (5, 49, 50, 70, 77, 78).

SIGNIFICANCE OF SPERM TRANSPORT FAILURES IN FARM ANIMALS

Fertilization failure can account for significant reduction of reproductive efficiency of livestock. For dairy and beef cattle, fertilization failure has averaged nearly 15% (6, 15, 33, 89) and is second only to embryonic mortality as a cause of infertile services (6, 33). For sheep, the percentage of fertilization failure is usually 10% or more (8). For swine mated naturally or inseminated with fresh semen, fertilization failure has averaged about 5% in animals with fertilized ova, but both the percentage of gilts with no fertilized ova and the percentage of unfertilized ova in gilts with some fertilized ova increased considerably after insemination of gilts with frozen-thawed semen (78).

Fertilization failure can result from several factors including structural barriers to union of sperm and ova and, at least theoretically, to inability of sperm to penetrate ova or to unfertilizability of ova. However, there is reason to believe that failure of sperm transport in the female reproductive tract accounts for most fertilization failure. A few authors who investigated fertilization rates in cattle have stated that uncleaved ova contain no sperm in the zona pellucida (48, 93, 97). High rates of fertilization failure in sheep (87) and swine (80) have been associated with a lack of sperm in the oviducts near the time of ovulation. The association between the frequent absence of sperm in the oviducts around ovulation and reduced fertilization rates suggests strongly that the most common cause of fertilization failure in animals inseminated at the proper time is absence of sperm in the ampulla of the oviducts soon after ovulation.

Wiley et al. (103) questioned whether failure of sperm transport could account for fertilization failure of superovulated cows because sperm numbers in the oviducts did not differ significantly between superovulated and non-superovulated cows. However, their data showed that more ova were fertilized in superovulated cows in which sperm were found in the ampulla at 30 h after the beginning of estrus (14 of 23 ova fertilized, 61%) than in superovulated cows in which no sperm were found in the ampulla (3 of 12 ova fertilized, 25%).

DYNAMICS OF SPERM TRANSPORT

Sperm can be transported efficiently only in animals in estrus or in ovariectomized animals under the influence of estrogen (1, 5, 73). Noyes et al. (73), working with ovariectomized rabbits, noted that small amounts of estradiol increased the efficiency of sperm transport but that greater amounts of estradiol appeared to be detrimental.

In cattle and sheep, semen is deposited naturally in the anterior end of the vagina against the cervix. Sperm may be transported to the oviducts in two phases — an initial rapid phase in which small numbers of sperm are transported to the oviducts within a few minutes, followed by slower movement of sperm into and through the uterus and a gradual increase over several hours of sperm numbers in the oviducts. In swine, semen is deposited naturally or artificially in the cranial end of the cervix or in the uterus, and little evidence of two phases of sperm transport has been apparent.
Rapid Transport of Sperm to Oviducts

Several investigators recovered sperm from oviducts of farm animals within a few minutes after mating or artificial insemination. VanDemark and Moeller (102) found sperm in oviducts of cows a few minutes after mating or artificial insemination into the cervix. Mattner and Braden (65) and Mattner (61) found large numbers of sperm in oviducts of ewes killed 5 or 10 min after mating. First et al. (22) and Baker and Degen (2) and others recovered sperm from oviducts of sows 15 min or less after insemination.

Sperm need not be live to be transported rapidly to the oviducts. Mattner and Braden (65) recovered killed ram sperm from oviducts of ewes within a few minutes after artificial insemination, and First et al. (22) recovered killed sperm from oviducts of sows 30 min after artificial insemination into the uterus.

There seems to be an interval between the initial sperm reaching the oviducts and sperm entering the oviducts during later phases of transport. Mattner (61) counted more sperm in the oviducts than in the uterus of a group of ewes killed 10 min after mating. Schott and Phillips (92) found that more ewes had sperm in the anterior segment of the oviducts than in the anterior segment of the cervix or in the uterine horns at 15 to 40 min after mating. Similar results were obtained by Overstreet and Cooper (74) who found more sperm in the upper ampulla and fimbriae and on the ovaries of rabbits than in lower segments of the oviducts at 1, 15, and 90 min after mating. These results suggest that sperm reaching the oviducts within a few minutes, at least in sheep and rabbits, represent a small proportion of the inseminate that was transported rapidly through the tract while the main pool of sperm remained in the vagina and cervix.

Dobrowolski and Hafez (16) recovered large numbers of sperm from oviducts of heifers at 1 h after insemination into the anterior vagina. Although these sperm could have been remnants of an earlier rapid phase of transport, they were probably the beginning of the prolonged phase because the uterus and tubo-uterine junction were populated with sperm. First et al. (22) examined the reproductive tract of swine at 15 min and 2, 4, 8, and 24 h after insemination and found similar numbers of sperm in oviducts each time. Sperm in the oviducts at 15 min might have been a different population from sperm in the oviducts later.

Many investigators have found little or no evidence for a rapid phase of sperm transport in ewes. In numerous studies, few sperm were recovered from oviducts of ewes at 30 min or at 1 or 2 h after mating or artificial insemination, and no sperm were recovered from oviducts of a high proportion of the ewes (35, 36, 39, 52, 58, 82). In some or all of these studies, it is possible that sperm had been transported rapidly to the ampulla after mating or artificial insemination and had been moved into the peritoneal cavity by the time of necropsy. Perhaps more likely, a rapid phase of sperm transport in sheep often may be omitted or suppressed.

Hunter et al. (52) ligated and transected reproductive tracts of ewes near the tubo-uterine junction at 4 to 10 h after mating; no fertilized ova were recovered after transection at 4 or 6 h, but 30 and 100% of ova, respectively, were fertilized after transection at 8 and 10 h. If any sperm were transported rapidly to oviducts of these ewes, the sperm were apparently not in the ampulla at 4 or 6 h after mating or were not able to fertilize ova.

Overstreet and Cooper (74) found that most of the sperm in the ampulla and fimbria of rabbit oviducts at 1 and 15 min after insemination were nonmotile and had damaged membranes. The sperm soon were transported through the fimbria and into the peritoneal cavity. They concluded that the initial rapid phase of sperm transport in rabbits was not relevant to later fertilization of ova. It is not known whether sperm transported to the oviducts of at least some cattle and sheep within a few minutes after insemination are also nonviable.

In contrast to the damaged and dead sperm transported rapidly to oviducts of rabbits, sperm capable of fertilizing ova can be transported to oviducts of gilts as early as 30 min after mating. Hunter (51) ligated and transected oviducts of gilts above or below the tubo-uterine junction at 30, 45, and 60 min after mating. Nearly 50% of ova were fertilized after transection above the junction at 30 min; the percentage increased with transection below the junction at 30 min or delay in transection at either site until 60 min.
The inconsistent finding of a rapid phase of sperm transport in sheep simply may reflect variation of effectiveness of physiological mechanisms responsible for the rapid phase of transport. Stress on the ewe suppresses rapid transport of sperm to oviducts. Mattner (61) found that ewes conditioned to being handled had large numbers of sperm in the oviducts at 15 min after mating whereas unconditioned ewes had relatively few sperm in the oviducts; when conditioned ewes were disturbed deliberately, sperm were not in the oviducts at 15 min. Thibault and Wintenberger-Torres (100) found a similar effect of stress. In the work of Mattner (61), stress did not reduce the number of sperm in the cervix at any time after mating (15 min and 4, 24, or 48 h) and did not reduce the number of sperm in the oviducts at 24 or 48 h.

Sperm found in the oviducts within a few minutes after mating or artificial insemination by some investigators presumably were transported there by contractions of the reproductive tract, perhaps contractions that were stimulated by mating or insemination (56, 101). Whether stress affects sperm transport through uterine contractions is questionable, because Lehrer et al. (56) found that fright did not suppress uterine motility in ewes.

Rapid transport of sperm to the ampulla might be advantageous to fertility after insemination of sheep or swine with frozen-thawed semen. As will be discussed later, ram and boar sperm that have been frozen and thawed are retained in the female reproductive tract for a relatively short time.

### Prolonged Phase of Sperm Transport

It is doubtful that the initial rapid phase of sperm transport in some animals totally is separated in time from the prolonged phase. Initial stages of the prolonged phase of sperm transport probably begin within a few minutes or even seconds after insemination. Mattner (61) found large numbers of sperm in the cervix of the ewe at 15 min after mating, and it is likely that these were at least part of the sperm that penetrated the cervical folds and crypts to establish the basis for prolonged transport to the oviducts.

Tables of sperm numbers in cows and ewes over time after insemination can be found in the literature (16, 82). Data in Table 1 illustrate changes in ewes. Each ewe was mated to two rams within a few minutes, sperm were recovered at definite intervals of time after mating, and the cervix was divided into anterior, middle, and posterior thirds before recovery of sperm. Data in the table were pooled from several separate experiments. The total number of sperm in the cervix may have been maximal earlier than 2 h after mating, but sperm numbers in the cervix at 2 h were probably higher than at any later time. That is, by 2 h, more sperm probably were moving from the cervix to the vagina and uterus than were entering the cervix from the vagina. The number of sperm decreased in the posterior third of the cervix between 2 and 8 h and in the middle third between 8 and 22 to 24 h (Table 1). Declining sperm numbers in posterior and middle segments of the cervix presumably reflect some movement of sperm.

| Table 1. Number of sperm recovered from the reproductive tract of ewes at 2, 8, or 22 to 24 h after mating.\(^a\) |
|---|---|---|---|---|---|
| Hour and no. of ewes | Cervix | | | | |
| | Posterior third | Middle third | Anterior third | Uterus\(^b\) | Oviducts\(^b\) |
| | \((10^6)\) | \((10^6)\) | \((10^6)\) | \((10^3)\) | \((10^3)\) |
| 2 (78) | 28 | 14 | 1.4 | 29 (4) | 48 (19) |
| 8 (24) | 11 | 16 | 3.7 | 97 | 190 (9) |
| 22–24 (50) | 2 | 3 | 1.4 | 158 | 4450 (4) |

\(^a\)Adapted from (35, 36, 39, and unpublished data).

\(^b\)Figures in parenthesis indicate the number of ewes in the group in which no sperm were recovered from that segment of the tract.
anteriorly into the uterus and oviducts, but most sperm lost from the cervix probably moved into the vagina. Sperm numbers in the anterior third of the cervix may have increased from 2 to 8 h and declined from 8 to 22 to 24 h, but numbers remained reasonably constant until at least 22 to 24 h after mating, which was shortly after ovulation. Number of sperm in the uterus and oviducts gradually increased with sperm numbers in the oviducts increasing most markedly after 8 h. This increase accords with data of Hunter et al. (52), which showed that sheep ova consistently were fertilized after transection of the oviducts only when transection was delayed until 10 h postmating.

There must be nearly continual movement of sperm anteriorly from the cervix, because sperm numbers in the uterus and oviducts of cattle and sheep increase gradually between 1 and about 24 h after mating or insemination into the vagina (16, 82; Table 1). However, there is little information for any species about the rate at which sperm in the uterus or oviducts are lost by phagocytosis or by passage into the peritoneal cavity or back into the cervix. Unless the rate of sperm loss and replacement is extremely high, only a small proportion of sperm in the cervix and vagina is required to move anteriorly to increase and maintain sperm populations in the uterus and oviducts.

Information seems to be almost completely lacking about sperm transport in the reproductive tract of cows artificially inseminated with the commonly used inseminate of 10 to 20 million motile sperm deposited in the uterine body or uterine horns. Experimental data are needed on movement of sperm away from site of deposition, number of sperm retained in the tract after 1 or 2 h after insemination, and degree of population of the cervix by sperm deposited in the uterus. Information also is needed about whether high fertility requires a large population of sperm in the cervix or whether insemination into the uterus eliminates the cervix as a critical segment of the tract for sperm transport.

In swine, ejaculate of the boar fills the uterine lumen of the female. Edematous polypoid processes at the tubo-uterine junction prevent entrance of semen directly into the oviducts (50). Seminal plasma in the uterus, along with most of the sperm, disappears within 2 h, probably primarily by drainage to the exterior (49). After most of the ejaculate has disappeared, the greatest concentrations of sperm are in the tubo-uterine junctions and the isthmus of the oviducts (20, 50, 51, 86).

Sperm are transported into oviducts of pigs faster after mating around the time of ovulation than after mating earlier in estrus (51). Also, sperm move into the oviducts most rapidly around ovulation in several small animal species (4, 51). In contrast, sperm transport was less efficient in ewes mated late in estrus, around ovulation, than in ewes mated early in estrus (67).

Smooth muscle contractions and ciliary beats, along with flagellar activity of sperm, are probably the major factors in movement of sperm from site of deposition to the ampulla of the oviducts (4, 5, 7, 25). The precise role and relative importance of these different factors in movement of sperm through various segments of the reproductive tract are not established.

Sperm motility seems to be necessary for sperm to enter the cervical folds and crypts during the first few hours after insemination and establish the basis for the prolonged phase of sperm transport. Killed sperm deposited in the ewe enter the cervix only in relatively small numbers (58, 66), remain in the lumen and do not enter the cervical folds, and are soon lost, probably by drainage to the exterior (58).

Cervical mucus is an essential medium for movement of sperm into and through the cervix and for establishment of sperm populations in the cervical folds and crypts (64). Ovarian hormones control the structural, physical, and rheological properties of cervical mucus, with estrogen increasing the amount of mucus secreted and changing properties of mucus to facilitate sperm penetration.

Mullins and Saacke (72) described the bovine cervix as composed of a complex series of longitudinal crypts and folds that begin at the external cervical os, traverse the annular rings, and extend to the cervico-uterine junction. They postulated that sperm entering these crypts at the external cervical os can move along the base of the crypts and reach the uterus without entering the cervical canal. By moving through the cervix along this pathway, sperm would avoid exposure to mucus flowing through the cervical lumen and into...
the vagina. This postulation is consistent with high proportions of sperm in the cervix being located in the cervical crypts and folds (58, 62).

Contractions of smooth muscle are probably primarily responsible for moving sperm through the uterus (5). Uterine contractions of cows and ewes are weak and localized during the luteal phase of the estrous cycle (32, 90). Contractions during estrus, under estrogenic influences, are frequent, strong, and usually propagated lengthwise along the uterine horn (13, 32, 90, 91). During the first few hours of estrus in ewes, the majority of uterine contractions originate in the uterine body or further posterior and move anteriorly (13, 32). By 26 to 48 h after the beginning of estrus, an increasing proportion of contractions originate near the tubo-uterine junction and move posteriorly (13, 32). Administration of estradiol prevents the change in site of origin and direction of contractions (32). Rexroad (83, 84), by the use of inhibitors of estrogenic action in the ewe, dissociated the mechanism that controls the frequency of contractions from that which controls the origin and direction of contractions. Estrogenic induction of an increased frequency of contractions is rapid and does not require activation of the genome, whereas estrogenic induction of a posterior site of origin of uterine contractions is slower and requires RNA and protein synthesis.

Sperm motility, ciliary beat and fluid currents, and muscular contractions all may be important in the transport of sperm through the tubo-uterine junction and the isthmus of the oviducts (4, 7, 25). In the rabbit, movement of sperm from the isthmus to the ampulla of the oviducts is associated with an increase of sperm motility (75), and Cummins (14) and others have recovered hyperactive sperm from oviducts of sheep and several other species around the time of ovulation and fertilization. Katz and Yanagimachi (54) calculated that "activated" sperm could traverse the hamster oviduct in a few minutes. Activation of sperm is associated with capacitation and may increase the chances of sperm contacting ova within the oviducts (14).

Sperm Reservoirs

Each segment of the reproductive tract must act, at least temporarily, as the reservoir from which sperm pass to the next anterior segment of the tract. Certain segments of the tract generally are believed to provide reasonably safe havens for sperm. Quinlan et al. (81) identified the cervix as the site of the sperm reservoir in the ewe because large numbers of motile sperm were maintained there. Mattner (61, 62, 63) suggested, on the basis of sperm numbers and motility in the reproductive tract, that the cervix serves as the sperm reservoir in cattle, sheep, and goats.

Data in Table 1 indicate that the anterior third of the cervix of the ewe contains a reasonably constant sperm population until after ovulation. The establishment of a sperm population of normal size in the anterior third of the cervix of the ewe soon after mating or insemination is essential for subsequent movement of normal numbers of sperm to the oviducts (12, 35). Low sperm numbers in the anterior segment of the cervix at 2 h after insemination reduces the number of sperm in the oviducts around ovulation and reduces fertility (12, 35, 88). Apparently, the anterior segment of the cervix is the site of an essential sperm reservoir in the ewe.

Several investigators obtained data that suggest that the tubo-uterine junction and the isthmus of the oviduct act as sperm reservoirs in the cow, pig, and some other species (20, 50, 52, 77, 86, 99). The suggestions are based upon prolonged maintenance of high sperm numbers in these areas.

In the rabbit, physiological mechanisms may restrict sperm to the isthmus of the oviducts for several hours and regulate the time of movement of sperm to the ampulla to a period near ovulation (29, 75, 76). Hunter et al. (52) proposed that similar mechanisms in the ewe may release sperm from the isthmus to the ampulla near the time of ovulation.

Fate of Sperm Deposited in the Female

Only a small percentage of sperm deposited in the vagina of the cow or ewe at natural mating enter the cervix, and only a small percentage of sperm that enter the cervix reach the uterus and oviducts. Most sperm are quickly lost to the exterior by expulsion or drainage. After inseminating heifers with 2 billion sperm at the external os of the cervix, Dobrowolski and Hafez (16) recovered an average of only
13.4% from the entire reproductive tract 1 h later. The percentage dropped to 3.8% at 8 h and to .9% at 24 h. After inseminating ewes with 500 million sperm in the external cervical os, Quinlivan and Robinson (82) recovered only about 3% of the sperm from the entire tract 1 h later. By 12 and 24 h, the proportion recovered averaged about .25%. In a study with ewes in this laboratory (34), a ligature was placed under the mucosa at the vulvovaginal junction at artificial insemination to prevent drainage to the exterior; 62% of the sperm in the inseminate was recovered from the entire reproductive tract 24 h later. In contrast, only .5% of the sperm in the inseminate was recovered from the entire tract of unligated control ewes. First et al. (22) deposited semen in the uterus of sows and 15 min later recovered from the uterus less than half of the sperm in the inseminate. At 30 min after artificially inseminating rabbits, Morton and Glover (71) could recover only about 20% of the sperm in the inseminate from the entire reproductive tract and concluded that most of the sperm had been lost by drainage to the exterior.

The proportion of sperm lost to the exterior after artificial insemination of the cow with 10 to 20 million motile sperm deposited in the uterus is not known. A high proportion of the sperm may pass through the cervix and vagina to the exterior.

Phagocytosis by leukocytes (5) probably accounts for the fate of most of the sperm in the female tract that are not lost to the exterior by drainage. Unknown numbers of sperm, undoubtedly a small proportion of the inseminate, pass through the oviducts and into the body cavity. Sperm may be incorporated into cells lining the reproductive tract in some species (5).

Sperm remain in the reproductive tract of the ewe, cow, and gilt for at least 48 to 72 h after insemination (50, 61, 82, 99). Most of these sperm are probably in folds and crevices in the tract and partially protected from phagocytes (50).

**INHIBITION OF SPERM SURVIVAL AND TRANSPORT IN THE FEMALE**

Sperm transport and survival in the female reproductive tract sometimes are reduced drastically. Most of the information has been acquired from sheep, an animal in which mechanisms of sperm transport may be more susceptible to disruption than in cattle or swine.

Regulation or synchronization of estrus in cattle by synthetic progestogens often lowers fertility at the regulated estrus (104). Part of the infertility has been ascribed to failure of fertilization (48), presumably caused by problems of sperm transport. However, Wishart (104), using semen from a bull with known high fertility, found no evidence of increased fertilization failure in heifers in progesterone-regulated estrus. Fertility of cattle in prostaglandin F2α-regulated estrus generally has been as high as that of control cattle when time of insemination was based upon detected estrus (28, 69). In swine, there seems to be no evidence that regulation of estrus with methallibure, synthetic progestogen, or prostaglandin F2α reduces fertility at the regulated estrus (26, 27, 79).

Regulation of estrus in ewes with either progestogen or prostaglandin F2α often results in low fertility, particularly after insemination of ewes with diluted semen (9, 87). Lowered fertilization rates seem to account for all or most of the lowered fertility (9, 21, 87). Robinson and coworkers (87) reported that failure of sperm transport was the cause of low fertilization rates in ewes inseminated at progestogen-synchronized estrus. Problems of failure of sperm transport were similar in ewes in prostaglandin F2α-regulated estrus (31, 35, 39).

In addition to regulation of estrus with progestogen or prostaglandin F2α, four other treatments disrupt sperm transport mechanisms in the ewe. Lightfoot et al. (57) and Lightfoot and Wroth (59), working on the problem of low fertility in ewes grazing on subterranean clover with a high estrogen content, found lower fertilization rates and fewer sperm in cervix, uterus, and oviducts of ewes grazing clover pastures than in those of ewes grazing oat pastures. Likewise, administration of 25 µg of estradiol per day for 14 days before estrus reduced sperm numbers in middle and anterior thirds of the cervix at 2 h after insemination and in oviducts at 24 h (12). Insertion of a plastic intrauterine device (IUD) into the lumen of the uterus before estrus reduces drastically both number of sperm
transported to oviducts and fertilization rate
(30). The IUD interferes with sperm transport
through mechanisms other than physical
blockage of the uterus, because sperm fail to
reach either oviduct of ewes with an IUD in one
uterine horn. Removing the corpus luteum-
bearing ovary in mid-cycle (day 10), causing an
abrupt decline in plasma progesterone con-
centrations, drastically disrupted sperm trans-
port mechanisms at the ensuing estrus 2 days
later (45). Removing the corpus luteum-bearing
ovary at day 3 or 15 had less effect on sperm trans-
port.

The cervix seems to be the initial site of
inhibition of sperm transport in ewes. The
reduction of sperm numbers can be seen as
early as 2 h after mating or insemination when
the number of sperm in the anterior third of
the cervix of ewes with inhibited sperm trans-
port averages only a fraction of the number in
control ewes (11, 12, 35, 39, 88). Effects of
treatments on sperm transport in the ewe
are in Table 2. At 2 h after mating, sperm
numbers were reduced in the anterior segment
of the cervix in ewes of all four treatment
groups and in the middle segment of the cervix
in ewes of some groups. No significant reduction
in sperm numbers was seen in the posterior
third of the cervix. Low numbers of sperm in
the anterior cervix at 2 h were followed at 22
to 24 h, shortly after ovulation, by low numbers
in the uterus and oviducts (Table 2).

The cause of inhibited sperm transport in
the ewe is not definitely known, although
possible causes have been considered. Cervical
mucus has been investigated, and the use of
synthetic progestogen to regulate estrus has
both decreased (95) and increased (85) mucus
production. Exogenous estradiol given for 12
days before estrus or an IUD had no apparent
effect on mucus secretion (85).

Uterine contractions in estrous ewes with
inhibited sperm transport have differed from
contractions in control ewes. Generally, the
number of contractions originating in the cervix
or body of the uterus and moving toward the
oviducts has been reduced, and the number
originating near the tubo-uterine junction and
moving toward the cervix has been increased
(10, 31, 47). Despite the association of changes

TABLE 2. Effect of regulation of estrus with progestogen or prostaglandin F2α, presence of an intrauterine
device (IUD), or removal of the corpus luteum-bearing ovary on the number of sperm in the cervix of the ewe
at 2 h after mating and the number in the uterus and oviducts at 22 to 24 h.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Treatment \textsuperscript{b}</th>
<th>No. of ewes</th>
<th>Posterior third (10^6)</th>
<th>Middle third (10^6)</th>
<th>Anterior third (10^6)</th>
<th>No. of sperm recovered from cervix</th>
<th>22–24 h after mating</th>
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<tr>
<td>MAP</td>
<td>46</td>
<td>28</td>
<td>16</td>
<td>1.9</td>
<td>54</td>
<td>154</td>
</tr>
<tr>
<td>PGF2α</td>
<td>32</td>
<td>17</td>
<td>7</td>
<td>.3</td>
<td>20</td>
<td>24</td>
</tr>
<tr>
<td>IUD</td>
<td>10</td>
<td>33</td>
<td>12</td>
<td>.2</td>
<td>30</td>
<td>22</td>
</tr>
<tr>
<td>CLX</td>
<td>15</td>
<td>12</td>
<td>2</td>
<td>.04</td>
<td>...</td>
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</tr>
</tbody>
</table>

\textsuperscript{a}Adapted from (31, 35, 39, 45 and unpublished data). Within individual experiments, each treatment re-
suced sperm numbers significantly in the anterior third of the cervix at 2 h and in the uterus and oviducts at
22 to 24 h. PGF2α and CLX reduced sperm numbers significantly in the middle third of the cervix at 2 h.

\textsuperscript{b}MAP = intravaginal sponge, containing 60 mg of 6-methyl-17-acetoxyprogesterone, from approximately
days 10 to 22 of an estrous cycle; PGF2α = 10 or 15 mg of prostaglandin F2α injected intramuscularly on day
10 of an estrous cycle; IUD = plastic spiral IUD in the lumen of one uterine horn; CLX = corpus luteum-bearing
ovary removed on day 10 of an estrous cycle. Ewes were in estrus and were mated 2 or 3 days after withdrawal
of MAP and 2 days after PGF2α treatment or removal of the corpus luteum-bearing ovary. IUD-bearing ewes
were mated during estrus 2 weeks or more after surgical insertion of the IUD.

\textsuperscript{c}Numbers in parentheses indicate number of ewes in the group from which no sperm were recovered.
of uterine contractions and problems of sperm transport, it is not certain that the direction of contractions is related to efficiency of sperm transport.

Most sperm recovered from the uterus and anterior cervix of ewes with inhibited sperm transport were immotile and dead or damaged. Ewes were in estrus after prostaglandin F2α-induced regression of corpora lutea (44), after removal of progestogen-impregnated intravaginal sponges (44), or after removal of the corpus luteum-bearing ovary (45). Other ewes had an IUD in the uterine lumen (46). Sperm were recovered from the uterine body and from anterior, middle, and posterior thirds of the cervix at 2 h after mating and evaluated for motility, response to live-dead staining, and acrosomal morphology. Each of the four treatments greatly reduced percentages of motile and live sperm recovered from the uterine body and anterior cervix (Table 3). Percentages were reduced moderately in the middle third of the cervix but were not reduced in the posterior third. These treatments also increased the proportion of sperm with damaged membranes recovered from the anterior cervix (44, 45, 46). The detrimental effects on sperm in the anterior cervix were largely independent of the number of sperm in that segment of the cervix (44).

The immobilization and death of sperm in the cervix and uterus could have resulted either from an unidentified spermicidal factor or condition or from the absence of protective agents. The lower viability of sperm recovered from the uterine body and anterior third of the cervix than from the middle and posterior thirds of the cervix suggests that any spermicidal factor might have originated primarily in the uterus and diffused into the cervix. Immobiliza-

<table>
<thead>
<tr>
<th>Treatment and condition of sperm</th>
<th>No. of ewes</th>
<th>Posterior third</th>
<th>Middle third</th>
<th>Anterior third</th>
<th>Uterine body</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motile</td>
<td></td>
<td>41</td>
<td>46</td>
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<td>28</td>
</tr>
<tr>
<td>Live</td>
<td></td>
<td>56</td>
<td>61</td>
<td>61</td>
<td>54</td>
</tr>
<tr>
<td>MAP</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motile</td>
<td></td>
<td>35</td>
<td>26</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Live</td>
<td></td>
<td>42</td>
<td>38</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>PGF2α</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motile</td>
<td></td>
<td>35</td>
<td>26</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Live</td>
<td></td>
<td>48</td>
<td>45</td>
<td>19</td>
<td>7</td>
</tr>
<tr>
<td>IUD</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motile</td>
<td></td>
<td>30</td>
<td>34</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>Live</td>
<td></td>
<td>56</td>
<td>45</td>
<td>23</td>
<td>2</td>
</tr>
<tr>
<td>CLX</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motile</td>
<td></td>
<td>36</td>
<td>19</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Live</td>
<td></td>
<td>44</td>
<td>28</td>
<td>14</td>
<td>3</td>
</tr>
</tbody>
</table>

*Adapted from (44, 45, 46). Within individual experiments, each treatment reduced the percentages of motile and live sperm significantly in the uterine body and anterior and middle thirds of the cervix compared to the percentages for the control ewes.

**MAP** = intravaginal sponge, containing 60 mg of 6-methyl-17-acetoxyprogesterone, from approximately days 10 to 22 of an estrous cycle; PGF2α = 10 or 15 mg of prostaglandin F2α injected intramuscularly on day 10 of an estrous cycle; IUD = plastic spiral IUD in the lumen of one uterine horn; CLX = corpus luteum-bearing ovary removed on day 10 of an estrous cycle. Ewes were in estrus and were mated 2 or 3 days after withdrawal of MAP and 2 days after PGF2α treatment or removal of the corpus luteum-bearing ovary. IUD-bearing ewes were mated during estrus 2 weeks or more after surgical insertion of the IUD. Percentages in this table were obtained from ewes necropsied 2 h after mating.
tion and death of sperm in the cervix may have been largely responsible for the inability of normal numbers of sperm to populate the anterior cervix of the treated ewes. Results of these experiments indicated a close relationship between decreased viability of sperm soon after insemination and inhibition of sperm transport to oviducts.

Disruption of sperm transport in the ewe is probably a result of an upset of the finely tuned endocrine control of sperm transport mechanisms. The amounts and balances of estrogen and progesterone influencing the ewe determine the efficiency of sperm transport (1, 11). Progestogen treatment that extended the estrous cycle increased estrogen concentrations of blood before estrus (18). Progestogen treatment that did not extend the cycle but inhibited sperm transport at the ensuing estrus (39) certainly changed the balance of steroids affecting the reproductive tract. Treating luteal-phase ewes with prostaglandin $F_2\alpha$ or removing their corpus luteum-bearing ovary changes the natural patterns of hormone secretion before estrus. The presence of an IUD causes several changes in the sheep uterus that resemble responses to estrogen (30). Thus, each of the four treatments known to increase the death of sperm in the ewe alters normal amounts, sequences, balances, or effects of ovarian hormones acting on the reproductive tract. These changes of endocrine influences may be the basis of both increased sperm death and decreased efficiency of sperm transport.

Ewes grazing clover high in estrogen content (57) and ewes receiving injections of estradiol for 2 wk before estrus (12) also must be unbalanced endocrinologically to some degree. Defects in sperm also may reduce the efficiency of sperm transport in the female. Retention of sperm in the reproductive tract of ewes and gilts is reduced by insemination of frozen-thawed ram or boar semen. Twenty-four hours after inseminating ewes with 100 million live sperm, either fresh or frozen-thawed, Mattner et al. (68) recovered an average of 12,000 sperm from oviducts of ewes inseminated with fresh semen but none from oviducts of ewes inseminated with frozen-thawed semen. Fertilization rates in ewes were low after intrauterine insemination with 3 million frozen-thawed sperm but normal after insemination with 30 to 150 million frozen-thawed sperm. Low fertilization rates with the lower numbers of sperm apparently were caused by retention of too few sperm in the reproductive tract to assure fertilization of ova.

Pursel et al. (80) inseminated gilts with frozen-thawed or fresh boar semen. At 4 and 14 h after insemination, fewer sperm were recovered from the oviducts, tubo-uterine junctions, and uteri of gilts inseminated with frozen-thawed semen than of gilts inseminated with fresh semen (Table 4). The detrimental effect of freezing-thawing semen on the number of sperm in the oviducts was particularly striking at 14 h. Poor sperm retention was associated with damage to sperm membranes (Table 4); high percentages of sperm recovered from oviducts had missing apical ridges. At 24 h after insemination, the fertilization rate was much lower in gilts inseminated with frozen-thawed semen.

Pursel et al. (80) could not ascribe the rapid loss of frozen-thawed sperm from the reproductive tract of gilts to phagocytosis. Perhaps

### Table 4. Effect of freezing and thawing boar semen on the fertilization rate and number and condition of sperm in the oviducts of gilts at 4, 14, and 24 h after insemination.

<table>
<thead>
<tr>
<th>Semen</th>
<th>No. of sperm in oviducts</th>
<th>% Sperm with NAR in oviducts</th>
<th>% Fertilized ova recovered at 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 h</td>
<td>14 h</td>
<td>4 h</td>
</tr>
<tr>
<td>Fresh</td>
<td>1030</td>
<td>1980</td>
<td>74</td>
</tr>
<tr>
<td>Frozen</td>
<td>230</td>
<td>40</td>
<td>33</td>
</tr>
</tbody>
</table>

a Adapted from (80). Observations on 6 to 8 gilts per mean. Each mean for frozen semen was significantly lower than the mean for fresh semen.

b NAR = Normal apical ridge.
sperm that have been frozen and thawed are less able than sperm in fresh semen to adhere to the epithelium of the female reproductive tract or to enter folds and crevices in the tract and, thus, protect themselves from expulsion or drainage to the exterior.

**IMPROVEMENT OF SPERM RETENTION AND TRANSPORT**

Numerous investigators have attempted to improve sperm transport to oviducts. Successes reported in the literature may be selective because unsuccessful attempts are less likely to be reported.

Several compounds, when added to semen used for insemination or injected into females near insemination, have increased the number of sperm recovered later from oviducts. These compounds include prostaglandin E₁ or prostaglandin F₂α added to rabbit semen or injected into does (41, 60, 96), prostaglandin E₁ and F₂α combined and added to ram semen or injected into ewes (19), and estradiol-17β injected into rabbits and sheep (37, 38, 40). The addition of prostaglandin F₂α and prostaglandin E₂ to ram semen improved fertility of artificially inseminated ewes (17). Addition of carbacholine to boar semen used for inseminating gilts increased the number of sperm in the oviducts 16 h after insemination (3) and increased the conception rate (94).

Several compounds have been inconsistent in their effects on fertility, fertilization rate, or number of sperm in the reproductive tract. The addition of amylase or glucuronidase to bull semen has improved fertility significantly in some but not in all experiments (24). Administration of estradiol to ewes near mating increased number of sperm in the oviducts or anterior cervix in some experiments (36, 37), but exogenous estradiol given near insemination had no beneficial effect on fertility (23, 53, 55). Injection of oxytocin into ewes near insemination had no significant effect on number of sperm in uterus and oviducts at 30 min or 2 h after insemination (58) or on number of sperm in the zona pellucida (100), whereas injection of oxytocin into gilts at insemination improved the rate of fertilization (98). Addition of oxytocin to boar semen used for insemination improved conception rate in one study (94) but had no beneficial effect on number of sperm in oviducts or fertilization rate in another study (3).

In this laboratory, several compounds have been tested for their ability to increase number of sperm in oviducts of the rabbit at 2 to 3 h after mating or artificial insemination and to improve the fertilization rate (42, 43). These compounds (Table 5) also were tested for effects on uterine motility in conscious does by a physiograph and strain gauge force transducers attached to the uterus to record uterine contractions.

Data on sperm numbers in Table 5 were obtained from does in which each compound was administered by intramuscular injection near artificial insemination with about 100 million sperm. Estradiol, prostaglandin F₂α, phenylephrine (an α-adrenoceptor agonist), and ergonovine (an ergot derivative) increased significantly the number of sperm recovered from oviducts at 2 to 3 h after insemination. These compounds varied both in segments of the reproductive tract in which they increased sperm numbers significantly and in their effect on uterine contractions. Both estradiol and prostaglandin F₂α increased the number of sperm recovered from each segment of the reproductive tract (40, 41). The increased retention of sperm presumably was caused by reduced drainage of semen to the exterior and usually resulted in recovery from the treated does of at least 50% of the sperm in the inseminate compared to about 16% from the control does. It is not known whether prostaglandin F₂α- and estradiol-induced retention of sperm in the vagina and cervix provided the sole basis for increased numbers of sperm in the uterus and oviducts or whether these compounds actively enhanced sperm transport.

Phenylephrine consistently increased sperm numbers in oviducts, uterus, and cervix, and ergonovine increased sperm numbers in oviducts and uterus (42). It is not known whether differences among compounds in the site of their effects on sperm numbers may indicate partially different sites or mechanisms of action.

Contractions of smooth muscle in the female reproductive tract almost certainly play an important role in sperm transport to the oviducts (5). Whether intensified contractions could account for all of the induced increases
### TABLE 5. Effect of several compounds on sperm numbers and uterine contractions in rabbit does.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of uterine contractions between 10 and 30 min after treatment</th>
<th>No. of sperm recovered at 2 to 3 h after AI</th>
<th>Segments of reproductive tract with significant increase in sperm numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Uterus</td>
<td>Oviducts</td>
</tr>
<tr>
<td>Saline</td>
<td>36</td>
<td>92</td>
<td>4</td>
</tr>
<tr>
<td>Estradiol-17β (3 μg)</td>
<td>... c</td>
<td>214</td>
<td>18</td>
</tr>
<tr>
<td>Prostaglandin F₂α (.75 mg)</td>
<td>51</td>
<td>766</td>
<td>65</td>
</tr>
<tr>
<td>Phenylephrine (5 mg)</td>
<td>94</td>
<td>860</td>
<td>43</td>
</tr>
<tr>
<td>Ergonovine (6 mg)</td>
<td>42</td>
<td>558</td>
<td>23</td>
</tr>
</tbody>
</table>

aData adapted from (38, 40, 41, 42, 43). Within individual experiments, each treatment significantly increased the number of sperm recovered from the uterus and oviducts over the number recovered from saline-treated does. Prostaglandin F₂α and phenylephrine significantly increased the number of uterine contractions.

Estradiol-17β (3 μg) was injected intramuscularly 1 hr before artificial insemination. Prostaglandin F₂α, phenylephrine HCl and ergonovine maleate were injected intramuscularly immediately after insemination. Number of does per treatment: 22 to 74 for sperm numbers and 7 to 15 for uterine contractions.

CAI = Artificial insemination with approximately 100 million sperm in .2 ml of fresh semen.

d = Oviducts, u = uterus, c = cervix, v = vagina.

cNumber of contractions in 20-min periods for estradiol-treated estrous does: 34 before treatment; 25 about 2 h after treatment; 32 about 4 h after treatment.

The similarity between estradiol and prostaglandin F₂α in increasing the number of sperm throughout the reproductive tract suggests that sperm retention in the vagina and cervix may have been the common factor responsible for increasing the number of sperm reaching the oviducts. What physiological mechanism may be involved in the greatly increased sperm retention caused by estradiol and prostaglandin F₂α is not known. Retention might involve an increase of adhesion of sperm to the epithelium of the reproductive tract or an increase of the ability of sperm to enter folds and crevices of the tract. Mediating mechanisms might include an effect of the compounds on secretions of the tract, on adhesive properties of sperm membranes, or on metabolic activity of the sperm themselves.

Effects of prostaglandin F₂α and estradiol on sperm numbers in Table 5 were not measured in the same experiment, but the means suggest that prostaglandin F₂α had a greater effect on sperm numbers than did estradiol. The difference, if real, could have been caused by the contractile response to prostaglandin F₂α and the lack of contractile response to estradiol.

Increases of sperm numbers in uterus and oviducts induced by ergonovine may depend upon a change of a contractile characteristic that was not apparent on contraction tracings in these experiments, alteration of sperm numbers in the oviducts in Table 5 seems to be questionable. Uterine contractions could have mediated the increases in sperm numbers induced by prostaglandin F₂α and phenylephrine (Table 5), but the effect of exogenous estradiol on sperm numbers was not associated with an apparent increase in the number or strength of uterine contractions. Likewise, ergonovine given intramuscularly in an amount that consistently increased sperm numbers in the uterus and oviducts elicited no apparent contractile response during the 90-min recording period after treatment (42). Conversely, methoxamine, an alpha-adrenoceptor agonist with properties similar to those of phenylephrine, caused a significant increase of the number of uterine contractions but little if any increase of sperm numbers in any segment of the reproductive tract (42).
TABLE 6. Ovum fertilization in rabbits after insemination with low numbers of sperm and treatment with prostaglandin F2α, phenylephrine, or ergonovine.a

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total no.</th>
<th>No. of does with cleaved ova</th>
<th>No. of ova cleaved</th>
<th>Accessory sperm/cleaved ovum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Does</td>
<td>Ova</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>17</td>
<td>136</td>
<td>3</td>
<td>26 (19%)</td>
</tr>
<tr>
<td>Prostaglandin F2α</td>
<td>9</td>
<td>79</td>
<td>8</td>
<td>60 (76%)</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>17</td>
<td>173</td>
<td>14</td>
<td>111 (64%)</td>
</tr>
<tr>
<td>Ergonovine</td>
<td>8</td>
<td>75</td>
<td>6</td>
<td>47 (63%)</td>
</tr>
</tbody>
</table>

aData adapted from (42, 43). Average inseminate per doe: 89,000 sperm. Each treatment significantly increased the proportions of does with cleaved ova and the proportions of ova cleaved over those for saline-treated does.

bProstaglandin F2α (.75 mg), phenylephrine HCl (5 mg), or ergonovine maleate (.6 mg) was injected intramuscularly immediately after insemination.

contractions at a site other than the uterus, or noncontractile responses of some kind.

Compounds that increase sperm numbers in all or some segments of the reproductive tract of the rabbit appear generally to be optimally effective over a narrow dose range. Such was the case with estradiol (40, 73), prostaglandin F2α (43), phenylephrine, and ergonovine (42). Doses higher than the optimal one either caused no additional beneficial effect or had little beneficial effect at all.

Compounds that consistently improved sperm transport in rabbits were tested for beneficial effects on ovum fertilization. The rabbit does were inseminated with low numbers of sperm to obtain a low fertilization rate in controls so that any beneficial effects of treatment could be detected. The experimental reduction of fertilization rates may be analogous to circumstances in animals with high fertilization failure such as repeat-breeder cows and ewes in regulated estrus.

Estradiol-17β (40), prostaglandin F2α, phenylephrine, and ergonovine (Table 6) increased the proportion of does with cleaved ova, the proportion of total ova that were cleaved, and the number of accessory sperm. Results demonstrated that these compounds could increase fertilization rate, apparently by increasing the number of sperm in oviducts around ovulation.

The degree to which fertility might be improved in farm animals by the use of these compounds or others is not known. However, these results, as well as those cited earlier, have indicated that physiological mechanisms in the reproductive tract of the female can be manipulated, at least under some circumstances, to improve sperm transport and fertilization rates.

REFERENCES


