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INTERACTIONS BETWEEN SPERMATOZOA AND THE CRYPTS, CILIA, AND MUCUS OF THE CERVIX IN THE EWE

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Abstract

In ruminants, minor cervical folds, commonly called crypts, temporarily store spermatozoa for a short interval of time between insemination and fertilization. However, the mechanism by which spermatozoa are transported to these crypts and subsequently to the uterus is not known. To study this problem, cervical tissue, which was removed from ewes that were naturally inseminated by rams at estrus, was examined with the scanning electron microscope to determine the physical associations that occur between the spermatozoa and the structural features of the cervix. The study indicates that the spermatozoa generally are not oriented parallel to the longitudinal axis of the cervix, exhibit no consistent association with the cervical cilia, and do not lie in any well defined channels formed by the cervical secretions. Alternatively, the majority of spermatozoa occur as isolated aggregations that lie in or near the shallow folds or crypts of the cervix. The vast numbers of spermatozoa in these aggregations and the lack of any common orientation suggest that some form of external stimulus, such as cervical contractions, might be responsible for the initial mass movement and distribution of spermatozoa in the cervix of the ewe.

Keywords: Sperm, Spermatozoa, Cilia, Mucus, Cervix, Crypts, Ewe.

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Introduction

In 1932, Quinlan et al. (1932) proposed that the cervix of the ewe stored spermatozoa during an interval between insemination and fertilization. Since that time, several investigators concluded that minor folds or crypts in the cervix act as reservoirs for spermatozoa (Blandau, 1973; Hawk and Conley, 1975; Mattner, 1963, 1966). However, the mechanism(s) by which spermatozoa enter the crypts and are subsequently transported to the uterus remains unknown. Our lack of understanding of this phenomenon is not unique to the ewe; transport of spermatozoa through the cervix has not been completely defined in a single mammalian species (Blandau, 1973).

Attempts to define how the cervix affects transport and storage of spermatozoa have concentrated on the functional significance of several structural features; namely, the cilia of the epithelial cells, the flagella of the spermatozoa, and the mucus of the cervical mucosal cells. Numerous studies have suggested that movement of spermatozoa to and from the crypts is facilitated by their flagella. Other investigators have suggested that the ciliated epithelium of the cervix may also be involved in spermatozoal transport. A third suggestion is that the cilia may help to distribute and orient the cervical mucus, which forms channels that would guide the spermatozoa to and from the cervical crypts (Hafez, 1976). These conflicting views, regarding the role of the structural features of the cervix, may be partly due to the fact that their morphological and physical properties were often examined *in vitro*. For example, secretions were aspirated from the cervix and interacted with spermatozoa which traversed the mucus by their own mobility, thereby indicating that the flagella were primarily involved. However, the rate of movement for the spermatozoa was too slow to account for the speed at which they traversed the cervix (Smelser et al., 1974). Furthermore, aspirated mucus does not exhibit the same structural features as that *in situ* (Wergin, 1979).

In an attempt to gain a more complete understanding of the functional interactions between the cervix and spermatozoa *in situ*, ewes were naturally inseminated, the reproductive tract was then chemically fixed, and segments of the cervix were prepared for examination with the scanning electron microscope. The morphology of the cervix, and the physical interactions that occur between spermatozoa and the crypts, cilia and mucus were then observed.

Materials and Methods

Six parous ewes were naturally inseminated by rams at day 0 (first day of estrus). Two hours following insemination the ewes were sacrificed and the reproductive tracts were removed and immediately perfused through the uterine arteries with 3% glutaraldehyde in 0.05 M phosphate buffer at pH 6.8. Following perfusion, the cervices were dissected from the tracts and cut into 1–2 cm wide transverse segments. The anterior edge of each segment was stained with Wright's stain. Next, each segment was carefully opened to expose the luminal surface and trimmed into a 1 cm^3 specimen. The specimens were placed in fixative for an additional 24 h, dehydrated in an alcohol series and critical point dried from liquid carbon dioxide. The specimens, with their luminal surfaces exposed, were mounted on stubs that were etched to indicate the anterior and posterior ends of the tissue. Each specimen was coated with 20–30 nm of gold-palladium in a Hummer V sputtering device. The coated specimens were viewed in a Hitachi HHS-2R scanning electron microscope operating at 10 or 15 kV. Before photographing, each specimen was oriented to identify the longitudinal axis and anterior end of the cervical tissue.

Observations

Morphology of the Cervix

Folds and Crypts. The surface of the cervix is highly convoluted (Fig. 1). When the surface of the cervix is observed with the unaided eye numerous depressions, consisting of major folds, tend to lie parallel to the longitudinal axis of the cervix; however, microscopic examination reveals more numerous minor folds or crypts that occur randomly on the surface, but exhibit no consistent orientation with respect to the main cervical axis. The major folds, which may be several mm deep, are easily observed with the unaided eye when examining cross sections through the cervix. However, the minor folds or crypts, which may be only a few microns deep, become apparent only when the tissue is examined with the SEM (Fig. 2).

Ultrastructural Cell Patterns. At estrus, the surface of the cervix commonly exhibits three different structural patterns that consist either of the apices of the epithelial cells, the dilated secretory cells, or the mucus. Although the epithelial cells are also present at diestrous, the latter two patterns are only rarely encountered in the diestrous ewe (Wergin, 1979). The three patterns are not normally intermixed but rather each occurs independently in an area that may encompass several lobes of the cervix.

The unencumbered epithelial cells form a pattern that covers more than half of the exposed surface of the cervix. Approximately 20% of these cells are ciliated and the remaining 80% have apices that are covered with short, stubby microvilli. The ciliated cell gives rise to a group of 50 to 150 cilia, 10 μm long (Fig. 3). Occasionally, the cilia arising from a single cell, as well as those from surrounding cells, tend to be aligned in a single direction. When this occurs, the common orientation exhibited by the cilia frequently lies perpendicular to a fold or crypt; they are not normally aligned in a direction that is parallel to the longitudinal axis of the cervix.

The second pattern of cells consists of dilated secretory cells (Fig. 4). Uniform populations of these cells are most frequently found along the folds or crypts. At estrus, they constitute about one-tenth of the surface that is exposed to the lumen of the cervix.

The remaining surface of the cervix is covered with mucus (Fig. 5). The mucus, which arises from the dilated secretory cells (Wergin, 1979), is frequently distributed over folds between adjacent lobes of the cervix. However, as the mucus flows toward the vagina, it frequently forms a layer that covers lobes of underlying epithelial cells whose apices terminate with cilia and microvilli (Fig. 6).

Distribution of Spermatozoa in the Cervix

Folds or Crypts. The vast majority of spermatozoa in the cervix of the inseminated ewe is found around the openings to the folds or crypts (Fig. 7). Neither the depth nor the volume of a fold can be determined when observing the luminal surface of the cervix with the SEM; however, the numerous spermatozoa that are frequently observed near the openings would suggest that thousands of these cells could easily occupy a single crypt. The spermatozoa that are observed near an opening, do not generally exhibit any uniform orientation with respect to the depth or direction of the underlying fold. The sperm, which are randomly oriented, form a mass that may be associated with

In Figures 1 through 18, the shafts of the arrows are parallel to the longitudinal axes of the cervices. The arrow heads point toward the anterior ends.

Fig. 1. Portion of the cervix illustrating the highly convoluted epithelial surface. Major folds separate adjacent lobes which also contain minor infoldings or crypts. Spermatozoa and/or mucus (arrows) frequently occur around the openings of the folds.

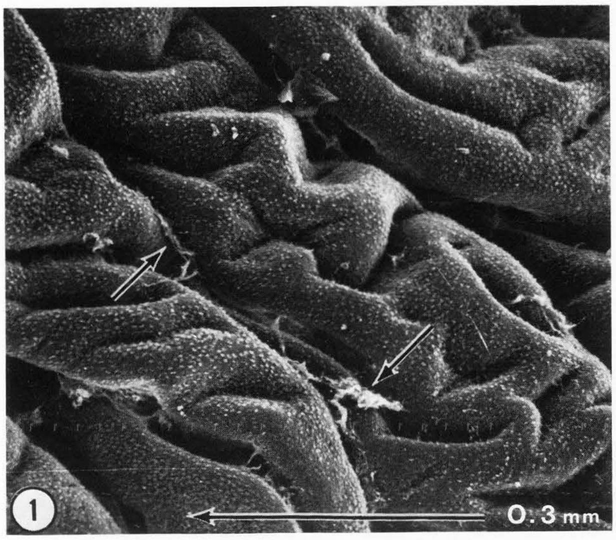
Fig. 2. Cross section through the wall of the cervix. When the surface of the cervix is examined, the number, depth, and volume of the folds can be ascertained. If this specimen were examined from above, one would observe a single fold only a few microns deep. However, in cross section, this single fold is actually an opening to several folds, several mm deep.

Fig. 3. Portion of the cervix illustrating the apices of the two types of epithelial cells that are normally present. Approximately 20% of the cells are ciliated; i.e., they terminate with 50–150 cilia which project into the lumen of the cervix. The remaining 80% of the cells have much shorter stubby microvilli which cover the apical membrane.

Fig. 4. Beginning two to three days before estrus, populations of dilated secretory cells develop in the cervix. These cells, which are most commonly found along the openings to the folds or crypts, give rise to a granular precursor of mucus.

Fig. 5. Region of the cervix that is partially covered with mucus. The mucus does not cover the entire surface of the cervix at estrus, but is preferentially distributed over the folds, and is less frequently found over the epithelial cells of the lobes.

Fig. 6. An area of the cervix that is covered with a thin layer of mucus. Occasional disruptions in the mucus reveal the cilia and microvilli from the underlying epithelial cells.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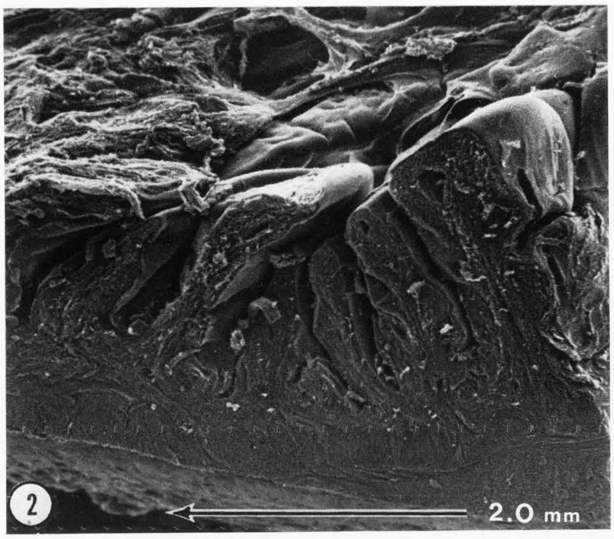
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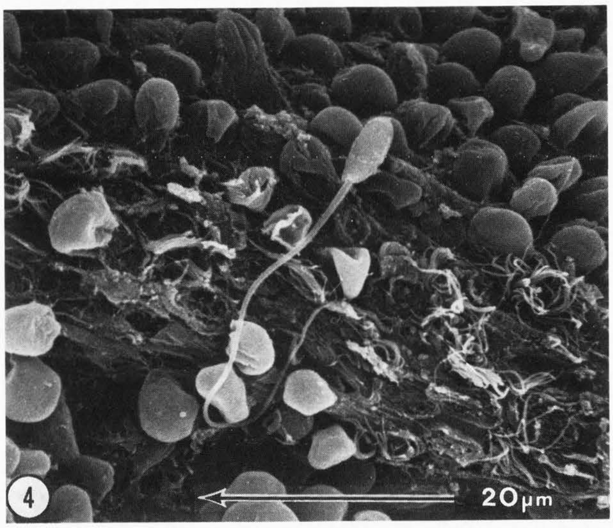
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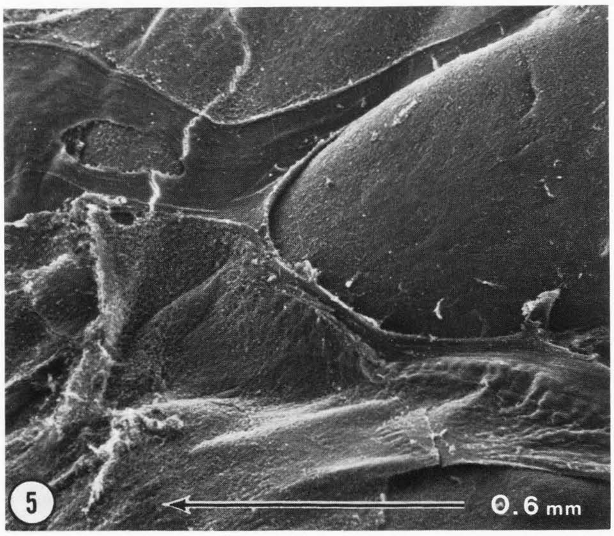
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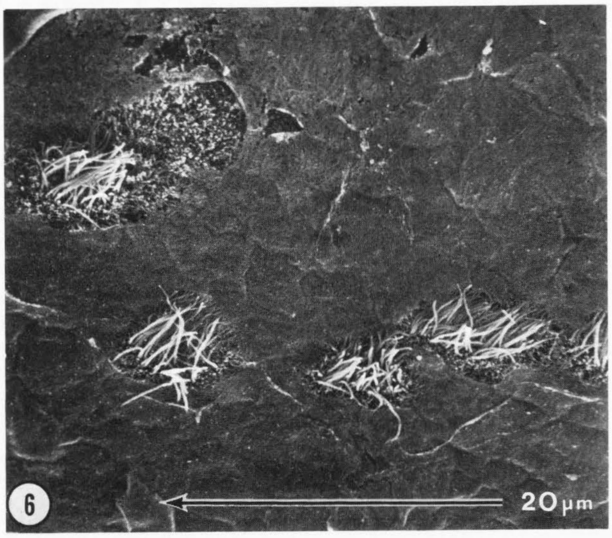
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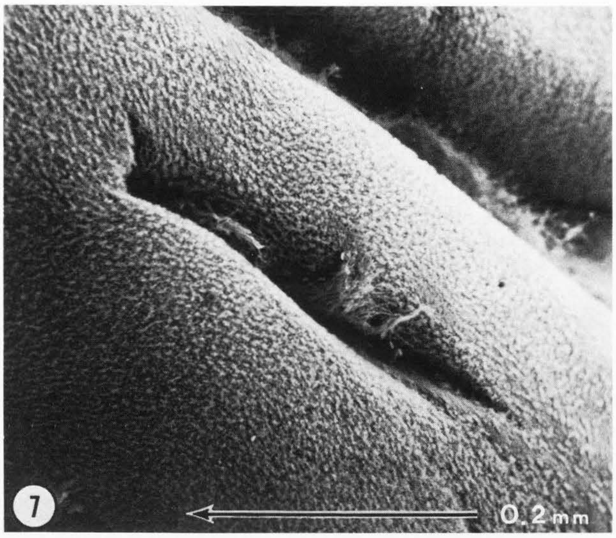
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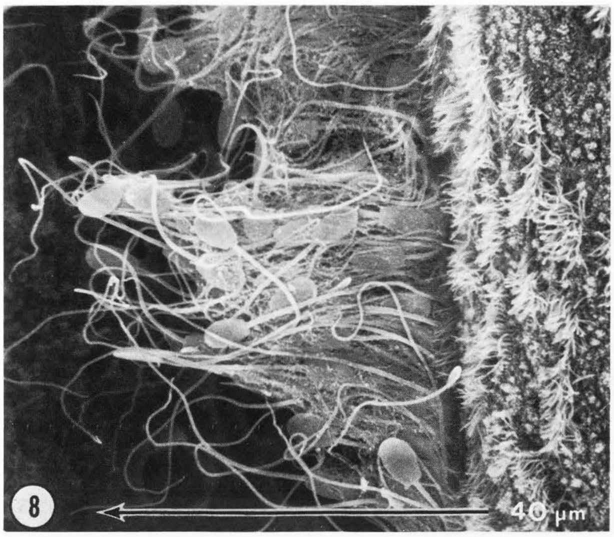
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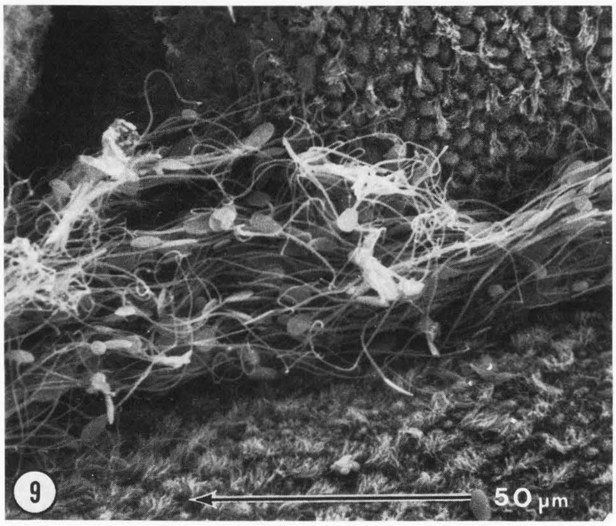
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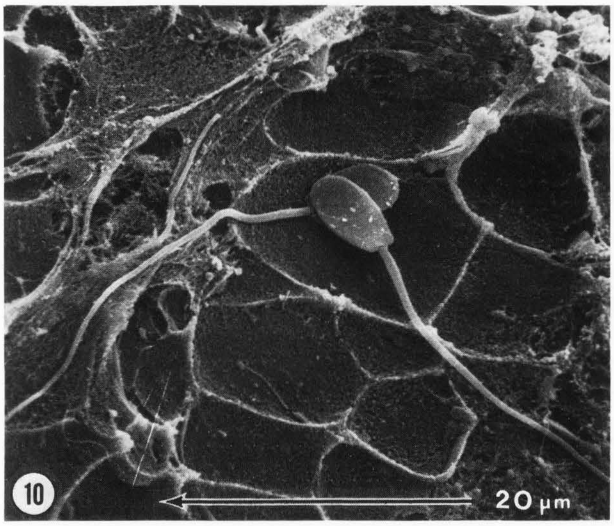
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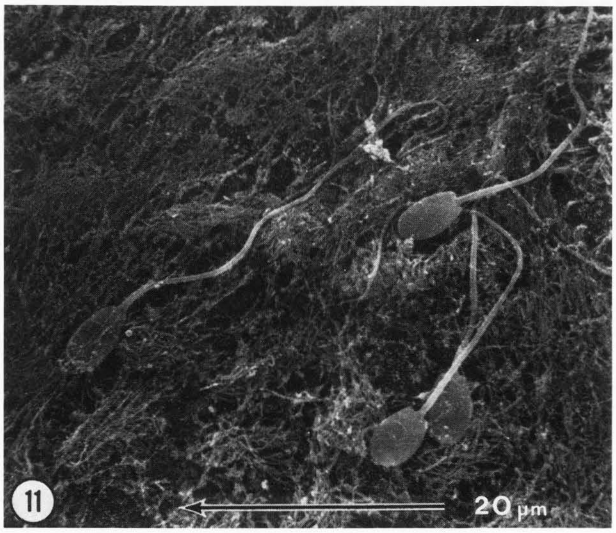
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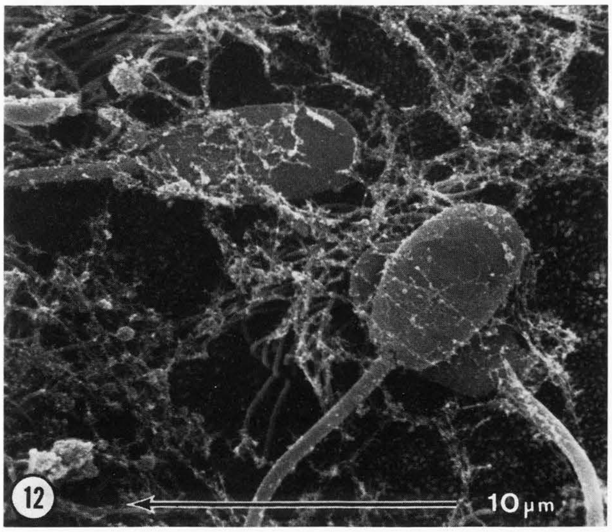
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Fig. 7. Lobe of the cervix that is interrupted by a minor fold or crypt. Spermatozoa, which have accumulated around the opening, appear to be a part of a larger mass that descends into the fold.

Fig. 8. Spermatozoa that can be observed around an opening to a fold may be associated with a fine fibrillar component believed to be cervical mucus.

Fig. 9. Spermatozoa may occur in a randomly entangled meshwork of cells that does not exhibit any association with a fibrillar or granular matrix. They do not exhibit a consistent orientation toward the anterior end of the cervix or the depths of the fold.

Fig. 10. Spermatozoa found on remnants of the dilated secretory cells which have burst and released a small granular precursor of mucus.

Fig. 11. Newly formed fibers that constitute a loose network of mucus over the underlying epithelial cells.

Fig. 12. Spermatozoa associated with a loose fibrillar network. Spermatozoa do not exhibit accumulations of fibers along the apical regions of their heads nor do they form channels through the fibrous network. The loose random association between these two structures provides no evidence that transport of spermatozoa is either enhanced or impeded by the fibers.

a fibrillar component resembling cervical mucus (Fig. 8), and they also occur in an entangled meshwork that does not appear to be associated with mucus (Fig. 9).

Mucus. At estrus, different forms of mucus are found on the surface of the cervix. These forms result from structural transformations that begin with a granular component which is released from the dilated secretory cells. This component contributes to the formation of fibrils that initially are randomly dispersed, but gradually become similarly aligned in a thin film of mucus that covers the epithelial cells (Wergin, 1979). Spermatozoa are associated with all of these structural forms of mucus.

Spermatozoa can easily be distinguished from the newly emerging components of mucus, which include the granular material that is released from the dilated secretory cells (Fig. 10) and the loose network of fibers that covers the underlying epithelial cells (Fig. 11). The fibers occur evenly distributed across the spermatozoon head, which does not appear to accumulate the mucus along its apical region, or displace the fibers from the surface of the cervix (Fig. 12). The spermatozoa, which are associated with these two components of mucus, generally lie with their flattened heads parallel to the cervical surface. No regular orientation of the spermatozoa, with respect to the longitudinal axis or apical end of the cervix, has been noted.

When the fibers of mucus form a continuous sheet across the surface of the cervix, only spermatozoa that are either on the surface, or are slightly covered with mucus, can be observed (Fig. 13). These spermatozoa do not exhibit any polar orientation. Furthermore, no discrete channels or accumulations of fibers, which could enhance or impede spermatozoal transport, are apparent (Fig. 14).

Initially, the sheet of mucus, which forms on the surface of the cervix, consists of fibers that are randomly intermeshed. As this sheet flows across the surface of the cervix, the fibrils become aligned parallel to one another (Fig. 15). Although not all of the spermatozoa that may be present either within or below this sheet can be observed, those on the surface are oriented parallel to the longitudinal axis of the fibers (Figs. 15 and 16). However, this parallel orientation of the fibers and the spermatozoa is seldom consistent with the longitudinal axis of the cervix. Furthermore, the head-to-tail arrangement of the spermatozoa is generally not directed toward the apical end of the cervix.

Epithelial Surface. Occasionally, one to three spermatozoa are found in areas of the cervix not covered with mucus (Fig. 17). In these cases, the spermatozoal heads do not lie flat on the epithelial surface, but rather occur at more random angles among the cilia and microvilli. Approximately one-half of the spermatozoa have their heads in close contact with the cilia, whereas the remaining spermatozoa are free from these associations (Fig. 18). When cilia from a group of epithelial cells exhibit a common orientation, it does not parallel the apparent direction of spermatozoal movement.

Discussion

Reproductive tissue contains delicate structural features that are highly hydrated; consequently preparation of the specimens has the potential to produce artifacts. Two sources of structural alterations must be carefully considered: first, the physical damage that may occur during sampling, processing and mounting; and second, modifications that result from chemical fixation and dehydration. In this study the care taken to minimize physical damage resulted in preservation of delicate structures, such as the ciliated cells, spermatozoa and mucus microfilaments. The adverse effects of chemical processing of tissues, especially dehydration, are more difficult to ascertain. The potential alterations occurring in such structures as mucus, which consists of more than 90% water, have recently been reviewed by Daunter (1984). The observations and interpretations of mucus structure in this study are based on an awareness of these problems, and are consistent with the results presented in a previous more detailed study of cervical secretions (Wergin, 1979).

Tissue Orientation

The anterior and posterior ends of the cervix could be identified at all times; however, in general, neither the structural features of the surface nor the spermatozoa exhibited any consistent orientation with respect to the longitudinal axis of the cervix. Major folds, which tend to lie parallel to the longitudinal axis of the cervix, represent an exception; the minor folds and crypts appeared more randomly oriented. This orientation agrees with the light microscopic observations of Moghissi (1972) who observed that cervical crypts lie in oblique, transverse and longitudinal directions.

The majority of spermatozoa observed in the cervix were found near openings to the folds and crypts, where hundreds of these cells appeared randomly entangled. This observation is consistent with conclusions based on other types of studies in various species (Chretien et al., 1973, 1974; Mattner and Braden, 1969; Mattner, 1968; Moghissi, 1972, 1973, 1977; Overstreet and Katz, 1977) where following fertilization, the spermatozoa were found in crypts rather than in the lumen of the cervix.

Cilia

The ciliated epithelium occasionally exhibited a common orientation. When this occurred, the apparent direction of the ciliary beat was perpendicular to a minor fold. However, most of the cells did not show a common orientation, and only rarely exhibited any direct association with spermatozoa. This apparent lack of any consistent physical association that would implicate a role for the ciliated epithelium in the transport of spermatozoa is consistent with evidence from other types of studies. For example, *in vitro* studies (Hafez and Kanagawa, 1972) have shown that motile spermatozoa move against the effective stroke of the cilia. In addition, women who suffer from an abnormality, called Kartagener's syndrome, which is characterized by immotility of the body's cilia, are not necessarily fertile (Afzelius et al., 1978; Afzelius and Eliasson, 1983). These and similar observations (Hafez, 1973; Hafez and Kanagawa, 1972) suggest that although epithelial cilia do not directly assist in sperm transport, they may be involved in movement of secretions in the cervix (Hafez, 1973) and transport of the egg and zygote in other segments of the reproductive tract (Afzelius et al., 1978).

Cervical Mucus

Cervical secretions, commonly referred to as mucus, have also been implicated in spermatozoal transport, but their role is controversial (Lee et al., 1977a, 1977b; Moghissi, 1971). Investigators have indicated that the structural, physical and rheological properties of cervical mucus are important to the establishment, retention, and protection of a cervical spermatozoal reservoir (Chretien and David, 1978; Mattner, 1973; Daunter and Counsilman, 1980). In the ewe, Adams (1973) concluded that the structure of cervical mucus determines the orientation of spermatozoal migration, and thus the efficiency of spermatozoal transport. Similarly, Hafez (1976) suggested that mucus arranged in micelles may help direct spermatozoa to the crypts. This process could be further enhanced by oscillations along the micelles (Hafez, 1976; Kenemans and Hafez, 1984). Another explanation suggests that aqueous cavities in the mucus convey sperm at minimum energy expense through the mucus (Odeblad, 1962). Finally, investigators have proposed that the function of the mucus may be to filter selectively the morphologically abnormal spermatozoa (Perry et al., 1977) and/or to provide antibacterial properties needed at estrus (Vickery and Bennett, 1968; Wolf et al., 1978).

Flagellar Action

In vitro experiments show that spermatozoa can traverse cervical mucus by their own motility (Blandau, 1973; Mattner and Braden, 1969); however, the rates calculated are too slow to account for the speed at which they traverse the cervix (Smelser et al., 1974). In some species, the flagella do have a direct role. For example, men afflicted with Kartagener's syndrome, which is characterized by immotile cilia, are sterile. This phenomenon is attributed to spermatozoa that lack motile flagella (Afzelius et al., 1978). In poultry, fertility rates were decreased following cryopreservation, which is also associated with structural damage to spermatozoa (Bakst and Sexton, 1979). The observations made during this study would indicate that the waved tails that characterize the isolated spermatozoa, found in the cervix, could be effective during movement along the surface. However, the numerous entangled spermatozoa that are found near openings to the cervical crypts would suggest some other form of mass movement.

Fig. 13. Spermatozoa on the sheet of mucus that covers the cervix. No uniform orientation of the sperm is apparent.

Fig. 14. Spermatozoa can be observed on the surface of mucus. When this occurs, the flattened head of a spermatozoon generally lies parallel to the sheet of mucus.

Fig. 15. Portion of the cervix where most of the spermatozoa lie on the mucus along a single axis which is diagonal to that of the cervix. When such alignments are encountered, examination of the head to tail arrangement reveals that the individual spermatozoa are oriented in both directions along the axis.

Fig. 16. When spermatozoa tend to be aligned along a single axis on the mucus, as observed in Fig. 15, close examination reveals that the fibers, which compose the layer of mucus, are also aligned along a single axis that is parallel to that of the spermatozoa.

Figs. 17 and 18. Occasionally, spermatozoa are not associated with mucus, but lie on the apical surfaces of the epithelial cells (Fig. 17). When this occurs, the spermatozoal heads may come in close physical contact with cilia from the epithelial cells or can occur free from these associations on the microvillar surface of the intervening cells (Fig. 18). No consistent orientation or associations have been noted.

Conclusions

The absence of any structural associations that would account for the movement of numerous spermatozoa to and from the cervical crypts would suggest that some other mechanism(s) might be involved. In 1974, Blandau and Gaddum-Rosse (1974) proposed that smooth muscle activity could be an important factor in effective transport of spermatozoa. This observation is supported by the studies of Hawk (1975), who observed contractions in exteriorized uteri of the ewe that began 5 hours after the onset of estrus; these contractions originated near the cervix and moved anteriorly. *In vitro* testing showed that PG $F_{2\alpha}$ and oxytocin could also induce similar contractility of cervical strips from the ewe (Edqvist et al., 1975). More recent studies with implanted electrodes in living ewes indicate that mechanical and electrical activities in the cervix occur independently from those in the uterus, and can be altered by exogenous hormone treatments (Garcia-Villar et al., 1982).

These observations in the ewe, as well as studies in other ruminants, have led investigators to suggest that rapid movement of large numbers of spermatozoa to the cervical crypts may largely result from cervical contractions. The absence of any obvious structural features that would account for the movement of spermatozoa, would tend to support that concept, and would suggest that ciliated cells and cervical mucus do not have a direct role in spermatozoal transport.

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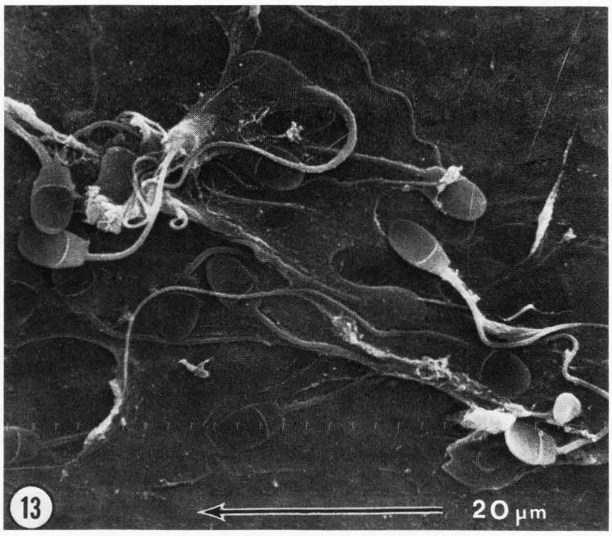
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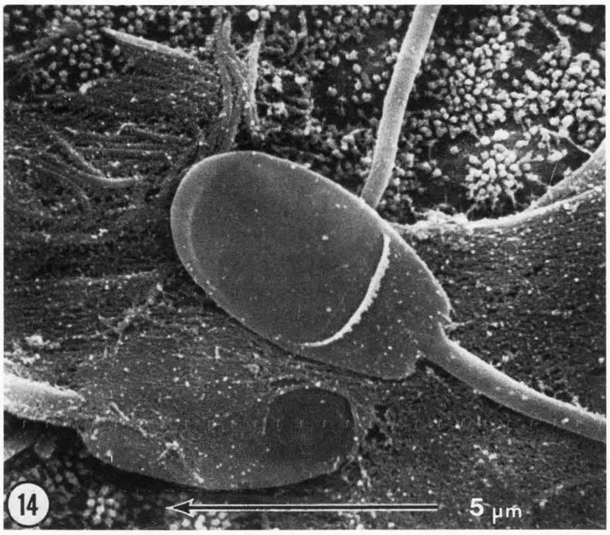
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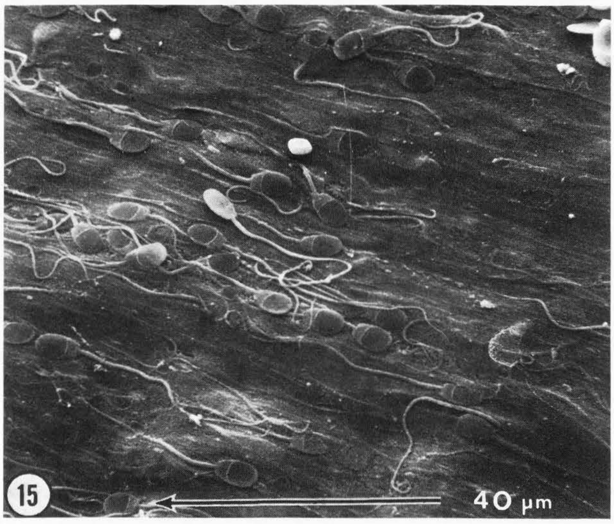
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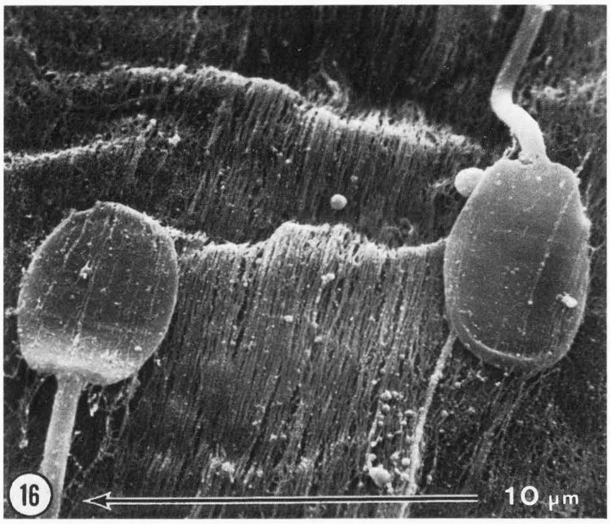
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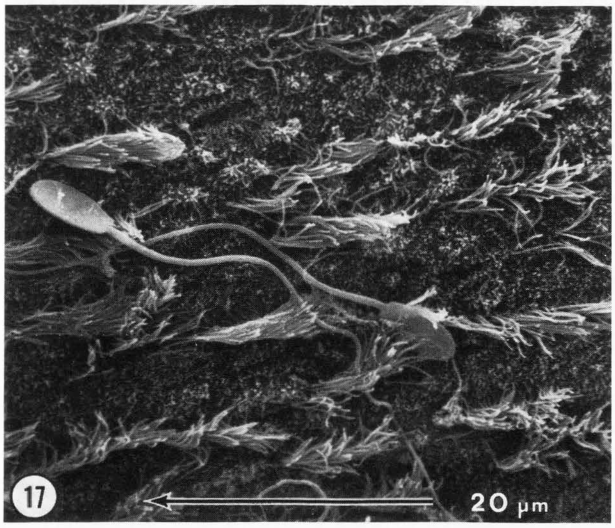
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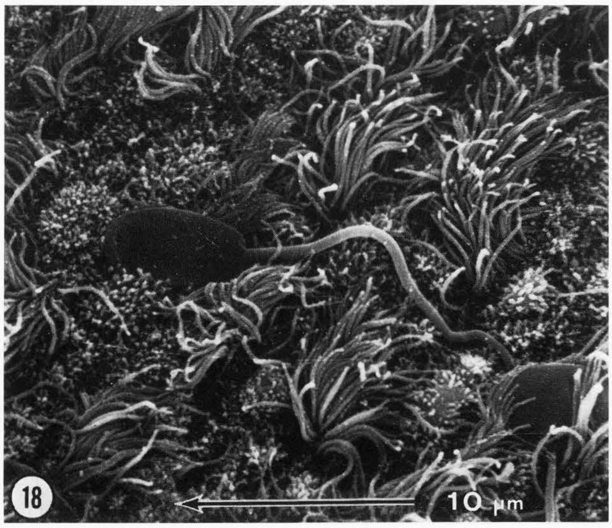
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Discussion with Reviewers

K.G. Gould: The author concludes that ciliated cells and cervical mucus have little direct role in sperm transport. Has he obtained any information on the surface structure and mucus structure in the depth of the crypts? It seems possible that ciliary action or mucus orientation could play a greater role when expressed in the strict confines of the crypts themselves. Such information might be obtained using tangential sections of the cervix.

Author: Although the term "crypt" is widely used in the literature pertaining to the cervix of ruminants, this study failed to reveal any unique or discrete structures that would distinguish a crypt from a small or minor fold in the cervical lumen. Furthermore, one might imagine that these crypts or minor folds are not static structures, but rather that at any one time they may result from muscular contractions or cervical undulations. As such, the epithelial surface of the crypts or folds displays the same morphological characteristics as the remaining cervical epithelium. The quantity and orientation of mucus, as well as that of the cilia, appear the same in the crypts as on any other surface area of the cervix.

S.V. Nicosia: Some micrographs show entangled spermatozoa near the openings of endocervical crypts. How can we be sure that these are viable spermatozoa capable of forward motility and not spermatozoa destined to be filtered out?

Author: One cannot distinguish whether the entangled spermatozoa are either viable and capable of forward motility or are destined to be "filtered out". However, an interesting aspect is how were these entanglements formed? Lack of uniform orientation among spermatozoa and the sparsity of mucus near the entanglements would indicate that neither unidirectional motility of spermatozoa nor aqueous channels formed by mucus were major contributors. Alternatively, a form of massive mechanical action, such as cervical contractions, might account for the formation of the entangled spermatozoa.

B. Afzelius: A method to determine in scanning micrographs the direction of the effective stroke of the cilia is to observe the direction of their curved distal tip; the curvature is in the direction of the effective stroke. It is generally believed that the cervical cilia beat in the posterior direction and the curvature of the distal ciliary tips in those figures where it can be determined (figures 3 and 18) is in agreement with this belief. Is it generally true that the ciliary tips bend posteriorly when one scrutinizes a larger number of electron micrographs?

Author: Generally the curvature of the cervical cilia do not appear to be synchronized in a single direction. In figure 18, they appear more random than uniform. However, when areas are observed that appear to exhibit cilia which are synchronized or uniform such as in figures 3 and 17, the apparent direction of beat is most often oriented towards a minor fold or crypt rather than with the posterior direction of the cervix.

B. Afzelius: Judging by the same criterion as above the spermatozoa in figures 17 and 18 can be seen to swim in a direction that is opposite to the effective ciliary stroke direction. This is also something that is consistent with generally held opinions (e.g., data by Hafez and Kanagawa, 1972), but is inconsistent with the claim by the author of an 'apparent lack of any consistent physical association that would implicate a role of the ciliary epithelium in the transport of the sperm'. In other words, the figures by the author could be used as a support for the generally held dogma rather than as evidence against it. The number of spermatozoa in the figures is too small for a (relatively) safe conclusion of this feature. What is the percentage of spermatozoa that are swimming against the direction of the ciliary effective stroke?

Author: Figure 17 illustrates two spermatozoa that appear to be swimming in opposite directions, whereas the effective beat of the cilia illustrated in figure 18 does not appear uniform. From these and other numerous inconsistencies between the apparent effective ciliary beat and the direction of sperm movement, one must conclude that the role of the ciliary epithelium in transport of spermatozoa should be questioned. Furthermore, other micrographs, which presumably show spermatozoa swimming in mucus, reveal that the spermatozoa are randomly oriented (e.g., Figs. 13 and 14) or diametrically opposed (e.g., Figs. 15 and 16). Again, the underlying ciliated epithelial cells associated with these spermatozoa would not appear to be effective in promoting any single directional orientation.

S.V. Nicosia: You suggest that cervical contractility plays an important role in sperm transport. However, the cervical wall of most mammals is composed mainly of dense collagenous and elastic fibers. Only a variable but generally not significant number of smooth muscle cells are dispersed among such fibers. Is it possible that mechanical transport of spermatozoa may be aided by vaginal contractions or by uterine suction?

Author: The suggestion that cervical contractility plays a possible role in transport of spermatozoa is based on the distribution of spermatozoa observed during this study and the contractility of cervical strips observed *in vitro* (Edqvist et al., 1975). As you suggest, vaginal contractions could also aid in this process or uterine contractions might conceivably result in suction; however, I know of no experimental evidence that relates either of these mechanisms to the distribution of spermatozoa on the cervix of the ewe.

B. Afzelius: All spermatozoa in figures 13 and 15 show a clear demarcation line between the acrosomal zone and the postacrosomal zone. Such a line is not seen or only barely visible in the other figures. Any explanation or comments?

Author: The demarcation line, which is more frequently pronounced when the spermatozoa are associated with mucus, is generally found along the postacrosomal margin at the base of the equatorial segment. This "line" appears to be a separation or break in the plasma membrane (see Fig. 14) and could be associated with the initial stage of capacitation.

S.V. Nicosia: Is the amount of mucus visible on the endocervical mucosa grossly similar in unmated estrous ewes? We have observed that semen triggers mucus hypersecretion at least in the rabbit (Scanning Electron Microscopy 1984/II:1321–1328).

Author: No attempt was made to quantitate or to compare the amount of mucus present on the endocervical mucosa in the inseminated and uninseminated ewes. However, observations made during this study of the inseminated ewes did not leave any impression that the amount or distribution differed from that previously described (Wergin, 1979) in the uninseminated ewe.