Evidence for Microbial Transfer by Spermatozoa

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Ovulatory-phase cervical mucus columns demonstrate that microorganisms migrate in the cervical mucus with moving spermatozoa. Cultures obtained from the distal end of the mucus column after spermatozoal migration was complete yielded the same aerobic and anaerobic microbial isolates that were originally recovered from the seminal fluid. Exogenous aerobic bacteria added to the seminal fluid also appeared at the top of the mucus column. After removal of the spermatozoa, no bacteria were observed migrating through the mucus. It is concluded that spermatozoa may provide a vehicle for bacteria present in the seminal fluid prior to ejaculation and for those already present in the cervix or vagina. The significance of this finding is discussed, and one mechanism for the development of salpingo-oophoritis in the female is proposed. ***(Obstet Gynecol 59:556, 1982)***

There are many similarities between the bacterial flora of the male and female genital tracts. In previous studies from this laboratory, the authors have shown that the seminal fluid of asymptomatic infertile males is colonized with a wide variety of both aerobic and anaerobic bacteria.[1,2] The number of bacteria isolated from the seminal fluid of fertile males was significantly less.[2] This wide variety of bacteria has been recovered from the vagina and cervix of asymptomatic women [3–5] and has also been isolated from soft tissue infection sites of the female genital tract in which the disease was not transmitted sexually.[6,7]

Because bacteria in the male ejaculate is added to the already abundant flora of the vagina during intercourse, access of the bacteria to higher genital tract structures should be facilitated by the same factors that work for the normal vaginal flora. This study was designed to determine whether the bacteria isolated from semen or experimentally added bacteria can use spermatozoa, the motile component of the semen, to penetrate ovulatory cervical mucus.

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Materials and Methods

Ovulatory-phase cervical mucus was collected through a sterile Milex Pro-ception fertility cannula.* Under aseptic conditions, the mucus was drawn up into sterilized microhematocrit tubes, 75 mm in length and with an internal diameter of 1.1 mm. Semen specimens were obtained from patients visiting the MacLeod Laboratory for infertility consultation. The semen specimens were collected by masturbation into a sterile glass container after a 3-day period of continence. All patients were instructed to wash the glans penis with soap and water thoroughly before masturbation. Aliquots of the cervical mucus and semen were cultured for aerobic and anaerobic bacteria.

Linbro tissue culture multi-well plates† were used for the different seminal fluid suspensions. Seminal fluid with or without spermatozoa and seminal fluid with added microorganisms were placed into the wells. The spermatozoa and bacteria-free filtrate of the semen were obtained using a Gelman syringe filter holder fitted with filter discs having a pore size of 0.45 μ.

Mucus-filled capillary tubes were placed upright into the wells at the start of the experiment. To monitor sperm migration within the capillary tubes, a control mucus-filled tube was placed under the microscope with one end immersed in semen. The experiment was terminated—after approximately 1 hour—when the distal end of the capillary tube under the microscope showed adequate numbers of the spermatozoa present. Under sterile conditions, the upper third of each capillary tube was broken off, and the mucus columns were cut with sterile scissors. The upper third of the capillary tube, filled with mucus, was then cultured for aerobic and anaerobic bacteria (Figure 1).

Results

Table 1a summarizes the different microorganisms isolated from the semen specimens used in these

*Milex Products, Inc, Chicago, Illinois.

†Linbro Scientific, Inc, Hamden, Connecticut.

Table 1. Summary of Microorganisms Isolated from Semen Specimens*

a) Bacteria Recovered from 10 Semen Specimens Used in These Experiments*	b) Added Experimental Bacteria Migrating with Spermatozoa	c) Microorganisms Added to Sperm-Free Seminal Fluid—No Migration†
	Aerobic and facultative species	
Staphylococcus epidermidis	*Streptococcus faecalis*	*E. coli*
Streptococcus viridans	*Streptococcus viridans*	*P. vulgaris*
Diphtheroids	*Pseudomonas aeruginosa*	*Klebsiella* sp
β-hemolytic streptococci	*Proteus vulgaris*	
Escherichia coli	*Proteus mirabilis*	
Staphylococcus aureus	*E. coli*	
Neisseria sp	*Salmonella enteritidis*	
Klebsiella sp	*Enterobacter aerogenes*	
	Anaerobic species	
Peptostreptococcus	*Fusobacterium nucleatum*	
Peptococcus prevotii		
Propionibacterium		

* Present in seminal fluid and migrating with spermatozoa.
† In these experiments, none of the endogenous bacteria migrated.

experiments. All these bacteria were shown to migrate through the mucus column in the presence of motile spermatozoa. No bacterial migration could be detected in tubes where seminal fluid with bacteria but without spermatozoa was used. Experimental bacteria added to semen specimens in concentrations of 10^7 to 10^8/ml needed 1 to 3 hours' incubation at 37C to show migration in association with spermatozoa through the mucus columns (Table 1b). When the same bacteria were isolated from both seminal fluid and cervical mucus used in some of the experiments, the species were disregarded in the migration experiment and are not listed in Table 1. Spermatozoal migration was observed through menstrual plasma but no spermatozoa penetration and no bacterial migration could be demonstrated when luteal phase or pregnancy cervical mucus were used. Similarly, mucus collected from 5 women taking oral contraceptives did show only minimal penetration by spermatozoa.

Discussion

Cervical mucus has always been considered an effective mechanical and immunologic barrier between the bacterial flora of the vagina and the upper genital tract. This was demonstrated in vitro in this study by the failure of bacteria to migrate in the columns of cervical mucus when spermatozoa were absent. There was confirmation in vivo of this cervical mucus barrier with the rare isolation of bacteria from upper genital tract structures in nonpregnant women undergoing hysterectomy.[8] The association of gram positive and gram negative round and rod-like structures with spermatozoa has long been observed in this laboratory in specimens from asymptomatic males (Figure 2). The current study shows conclusively that aerobic and anaerobic bacteria obtained from semen specimens could easily penetrate this barrier when active spermatozoa were present. This migration of bacteria also

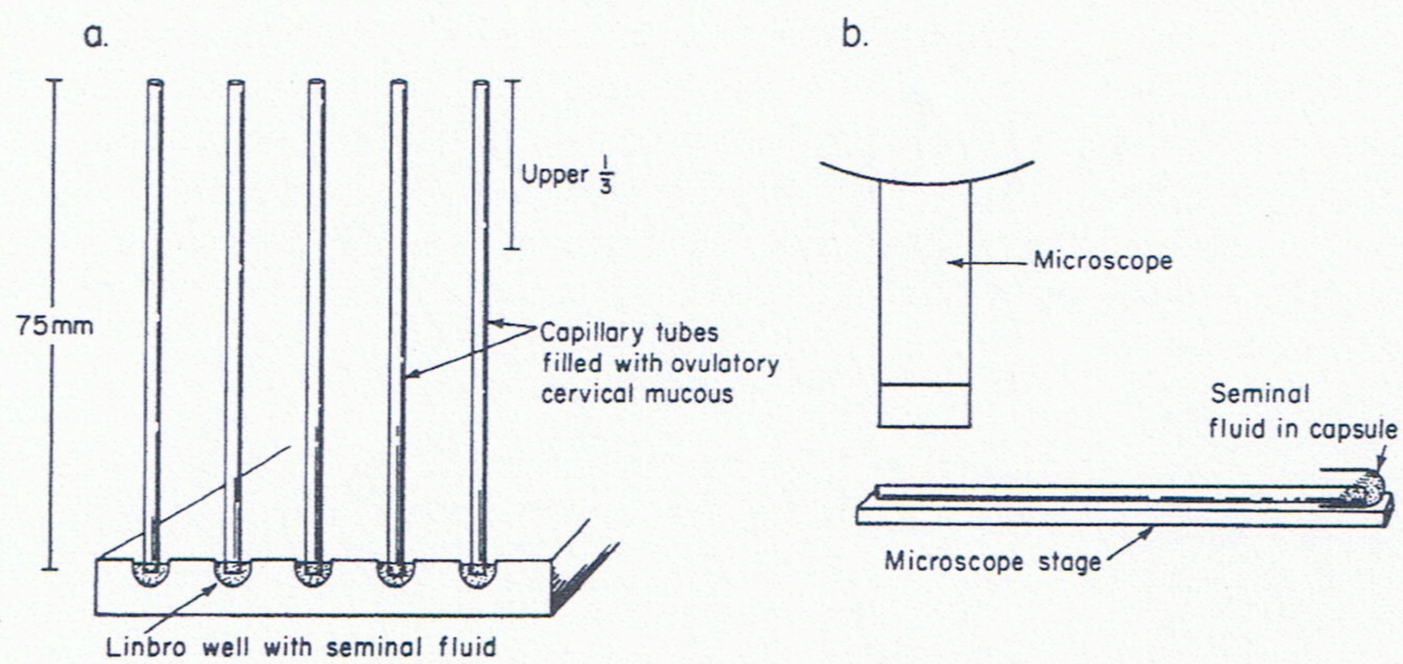

Figure 1. a) Mucus-filled capillary tubes are placed upright in wells with seminal fluid. b) Control tube arranged under microscope.

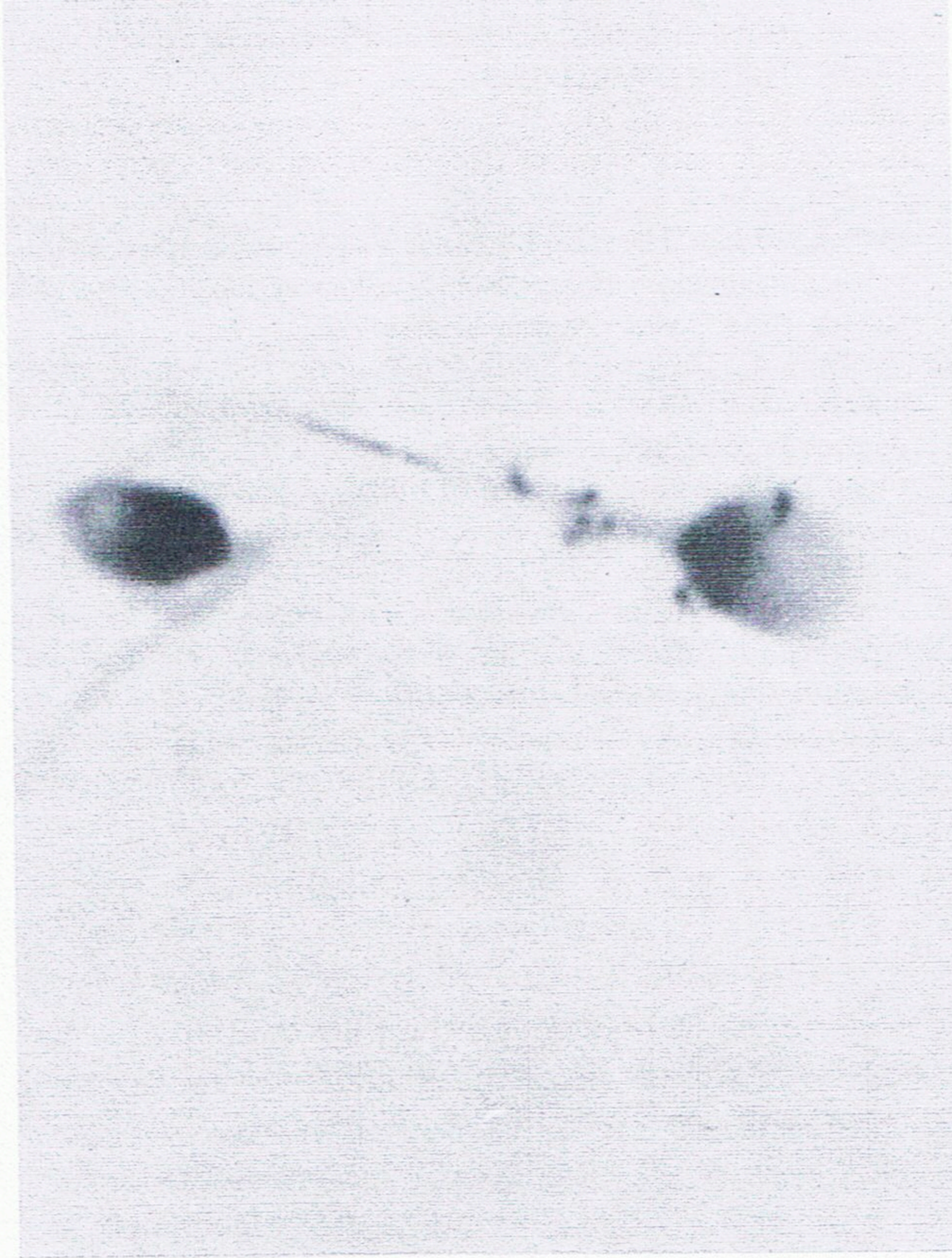

Figure 2. Photomicrograph of Gram-stained smear of spermatozoa showing round and rod-like particles over head, tail, and midpiece sections. *Veillonella parvula* and *Peptostreptococcus* were recovered through culture studies (× 1000).

occurred when gram negative aerobic organisms were added to sterile semen specimens. Such migration required 1 to 3 hours' incubation with a pool of spermatozoa before bacterial movement was noted and did not occur when spermatozoa were not present. These observations indicate that active spermatozoa facilitate the passage of bacteria through cervical mucus in an in vitro system.

These in vitro observations permit speculation on the role of spermatozoa in the etiology of salpingo-oophoritis in women. Barrier methods of contraception that either markedly diminish or usually totally prevent access of spermatozoa to the upper genital tract are associated with a lower-than-expected incidence of salpingitis.[9] In contrast, a number of case-control studies[10,11] have demonstrated an increased risk of salpingitis when an intrauterine device (IUD) has been the method of contraception. The use of the IUD does not prevent the passage of spermatozoa into the upper genital tract. No contradiction to the potentially important role of spermatozoa in increasing the risk of salpingitis is seen on review of case–control studies showing a lessened risk with oral contraceptives,[12,13] the use of which does not provide a mechanical barrier to spermatozoa. This lessened risk has been assumed largely due to increased antibacterial activity and the differences in the composition of cervical mucus. The present study, in addition, shows a marked decrease in sperm penetration of cervical mucus in women taking oral contraceptives. A clinical observation in the authors' laboratory has been that salpingitis in wives of several hundred azoospermic males who came for infertility consultation was a rare event. All these observations suggest a causal role for spermatozoa in the carriage of bacteria and the subsequent development of salpingo-oophoritis.

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