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Survival of *Trichomonas vaginalis* in human semen

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SUMMARY Although exposure of *Trichomonas vaginalis* to human semen is of short duration, any effect that this fluid may have on the urogenital protozoon could affect its transmission, especially if only few trichomonads are present. Small numbers of parasites (about 2500/ml semen) incubated in semen from different donors at 37°C, were found to survive or grow for up to 12 hours in all samples and for up to 24 hours in most. Survival and growth of *T vaginalis* in semen most resembled that found in Diamond's trypticase, yeast extract, and maltose (TYM) medium without serum supplement, rather than in complete TYM medium and phosphate buffered saline. Contrary to previous reports, semen did not inhibit the survival of *T vaginalis*, and the presence of trichomonads did not alter motility or numbers of spermatozoa up to 24 hours. The data suggest that semen provides a favourable milieu for transmitting trichomonads.

The role of men in transmitting *Trichomonas vaginalis* to women is not understood well. Male sexual consorts are generally accepted as being the primary sources of infection in women, but the extent to which these parasites exist in men is not clear.[1] Three possibilities are usually suggested; (1) that *T vaginalis* is only transient in men, (2) that the organism usually exists in such small numbers that it is difficult to detect and the host is symptomless, and (3) that in a few men certain symptomatology can occur, for reasons not yet clear, accompanied by a detectable number of parasites.[2,3] Whatever the case, the vehicle for transmission from a man to an uninfected woman must be semen. For the trichomonads in semen to infect successfully they must remain alive in this transfer milieu long enough to establish themselves in a new host environment. Trichomonads have only a short exposure time to semen itself, from ejaculation to cervical transfer. After ejaculation, however, semen forms part of the material in which the parasites will begin to establish themselves in the vaginal vault. Inhibitory activity by semen could reduce the infectivity of trichomonads, especially if only few are present. The effects of semen could also play an important part in the survival of *T vaginalis* in fresh or frozen (cryopreserved) semen used for artificial insemination.

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This study set out to assess the effect of semen on the ability of *T vaginalis* to survive, and thus whether semen contributes to or inhibits successful transmission of trichomonads.

Patients, materials, and methods

CULTURES

Isolates of *T vaginalis* used in these experiments were obtained from patients at the University Hospital, Little Rock, Organisms were maintained in Diamond's trypticase, yeast extract, and maltose (TYM)[4] medium supplemented with 5% inactivated human serum, incubated at 37°C, and subcultured every 48 hours.

HUMAN SEMEN

Human semen was obtained from donors selected through the University Semen Cryobank. All but one donor, who was 52, were in their 20s and were students. All semen samples were used fresh with no cryoprotectant added.

EFFECT OF HUMAN SEMEN ON SURVIVAL OF *T VAGINALIS*

Inoculation and incubation

Late log and early stationary phase cultures of *T vaginalis* were centrifuged and washed twice with phosphate buffered saline (PBS) at pH 7·2. Cells were resuspended in PBS, the suspension was adjusted to 40 to 100 trichomonads/μl PBS, and 10 μl suspension

were added to 0·2 ml aliquots of semen in 2 ml volume test tubes. For anaerobic incubation, these tubes were placed unstoppered in Gas Pak jars and carbon dioxide, and hydrogen generators were employed; the jars were then closed and incubated at 37°C. For aerobic incubation, stoppered tubes were incubated directly in a 37°C incubator. In all experiments, tubes of inoculated semen were removed from incubation after 6, 12, and 24 hours, and the contents were gently stirred with a bacteriology inoculating loop. Four samples of 10 μl each were removed and inoculated into modified TYM medium previously maintained as liquid at 40°C. The modified TYM medium used contained 0·45% Noble agar, 5% inactivated human serum, and antibiotics as described by Ivey.[5]

Controls

Tubes containing 0·2 ml complete TYM medium were used as growth controls, TYM medium minus the serum additive as incomplete growth controls, and PBS as no growth controls. These tubes were also inoculated with 10 μl trichomonad suspension and were incubated and sampled in the same way as the inoculated semen.

Colony counts

The colony count technique was used in these studies to assess population viability of trichomonads inoculated into semen and control media. This allowed the detection of small numbers of trichomonads in semen as well as the use of small aliquot volumes of individual semen specimens. Small inoculum sizes also approximated to the population densities in transient conditions and in low grade infections in men. Preparation of inoculated agar plates and colony count techniques followed the methods of Ivey,[5] and Matthews and Daly.[6] Inoculated liquefied TYM medium was poured into plastic petri dishes, allowed to harden, placed in Gas Pak jars containing carbon dioxide and hydrogen, and incubated for four to five days at 37°C. Incubated plates were then counted for the number of trichomonad colonies present. As different inoculum sizes were used in each experiment, the data were expressed as percentages above the initial inocula of 100%.

EFFECTS OF TRICHOMONADS ON SPERMATOZOA

Pairs of semen samples (semen with and semen without trichomonads) were compared by phase microscopy for spermatozoal motility and by haemocytometer counts for numbers of spermatozoa at 0, 6, 12, and 24 hours after incubation at 37°C.

Results

Populations of *T vaginalis* were found not only to survive in human semen at 37°C but also showed some growth. Five different semen specimens were examined comparing aerobic and anaerobic incubation using only the complete TYM as a control. Table 1 shows results of paired experiments on growth or survival of *T vaginalis* in semen or complete medium incubated under aerobic compared with anaerobic conditions. Growth or survival were poorer in semen and medium under aerobic conditions than under anaerobiosis. Four other semen samples were examined for survival or growth under anaerobic conditions only, and we used complete TYM medium, TYM without serum, and PBS as control media. Table 2 shows results of paired experiments using the three growth controls. Few organisms survived in PBS after incubation for six hours. Growth in semen was most closely comparable with that in incomplete TYM medium. Although the trend at 24 hours was towards greater growth in semen, the difference was not significant (310% *v* 116%). As expected, growth was excellent in the complete medium, but the difference between it and in semen was evident only at 24 hours (820% *v* 310%, $p = 0{\cdot}005$). Combining the data from all nine paired semen samples incubated anaerobically, survival of *T vaginalis* at 24 hours ranged from 2% to 535% of the initial inoculum with a mean (SE) of 251 (70)%.

No differences were found in the degree and quality of spermatozoal motility in the presence or absence of trichomonads. The means of double blind triplicate

Table 1 *Survival or growth of Trichomonas vaginalis in human semen or trypticase, yeast extract, and maltose (TYM) medium under anaerobic (carbon dioxide and hydrogen) or aerobic conditions at 37°C. (Figures represent mean (SE) percent of organisms (relative to initial populations of 100%) in five experiments)*

	TYM medium		*Semen*	
Hours	*Aerobic*	*Anaerobic*	*Aerobic*	*Anaerobic*
6	51 (63)	94 (29)	96 (67)	168 (53)
12	29 (49)	205 (109)	44 (37)	159 (62)
24	41 (59)	450 (156)	9 (18)	182 (112)

Table 2 *Survival or growth of Trichomonas vaginalis in human semen, complete trypticase, yeast extract, and maltose (TYM) growth medium, incomplete medium (TYM minus serum), and phosphate buffered saline (PBS) incubated under anaerobic (carbon dioxide and hydrogen) conditions at 37°C. (Figures represent mean (SE) percent of organisms (relative to initial populations of 100%) in four experiments)*

Medium	*6 hours*	*12 hours*	*24 hours*
PBS	8 (16)	6 (11)	0·3 (0·5)
Incomplete TYM	141 (81)	171 (98)	116 (214)
Semen	195 (117)	213 (106)	310 (175)
Complete TYM	221 (127)	253 (131)	820 (200)

motility readings in aliquots of semen with *v* without trichomonads were 51 *v* 52% initially, 44 *v* 43% after 6 hours, 22 *v* 21% after 12 hours, and 2 *v* 2% after 24 hours. The degree and quality of motility were indistinguishable between media at the same observation periods, apart from heightened spermatozoal agglutination initially in one sample with parasites. Spermatozoal microscopy rarely showed motile trichomonads initially (because only small numbers were present), but they were easily found in the 24 hour samples. Duplicate and mean measurements of the total numbers or concentration (millions/ml) of spermatozoa during incubation for 24 hours showed no change. We found no evidence, therefore, for phagocytosis of spermatozoa by trichomonads in incubated infected semen, in contrast to the spermatozoal phagocytosis by trichomonads reported for vaginal secretions.[7]

Discussion

Published reports indicate that certain components of seminal fluid may inhibit the survival of *T vaginalis*. Canine and human prostatic fluids have been found to inhibit trichomonal survival.[8 9] This was related to the presence of zinc in other experiments in which the trichomonads were directly exposed to this ion.[8-10]

Previous investigators used phase microscopy to detect viable trichomonads in microtitre plates, but failed to find *T vaginalis* surviving in seminal fluid after incubation for six hours at 30°C.[8] In contrast to these findings, we found excellent survival of *T vaginalis* in semen from different donors after incubation for up to 24 hours at 37°C. The failure of other workers to find viable *T vaginalis* in seminal fluid incubated for six hours may have been due to the large size of their initial inoculum (40 000 trichomonads/ml seminal fluid) or the different incubation temperatures, or both. In the study reported here the colony formation technique allowed us to use a small inoculum (about 2500 cells/ml semen), which would more approximate to populations of trichomonads seen in infected men.

As the data presented herein indicated that semen acts as an incomplete medium, it follows that the limiting factor(s) for growth would shorten the growth curve considerably in the presence of a large inoculum. Using colony counts as a measurement of viabilty, the stationary phase of *T vaginalis* in growth medium has been shown to be short and the death or decline phase pronounced.[11] The trichomonads in seminal fluid were possibly too far into the death phase to remain viable or to retain active motilty. We are currently examining the effect of different inoculum sizes on trichomonad survival in semen.

A factor other than zinc may also be responsible for the inability of trichomonads to thrive in the urogenital tracts of men.[8 10] The work published here suggests that one factor could be nutritional, as the growth response indicated that semen was an incomplete medium for *T vaginalis*. Poor survival in prostatic fluid may be due to the lack of carbohydrate in the form of fructose, which is found in seminal fluid.[12] Another possibility (yet to be tested) to explain the results in the present report, is that the presence of spermatozoa may enhance trichomonad survival. Our finding of no effect by trichomonads on spermatozoal motility does not support a report by Tuttle *et al* that a striking decrease in motility occurs during incubation with these parasites at 37°C for six hours.[13] The high concentration of trichomonads that they used (about five times our "normal" numbers) were possibly the causative variable.

In conclusion, the survival data in the present report support the view that semen can serve as a good vehicle for transmiting trichomonads.

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