Trichomonas vaginalis: Preliminary Characterization of a Sperm Motility Inhibiting Factor*

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ABSTRACT

This study determined the effects of Trichomonas vaginalis trophozoites, subcellular fractions, and medium from axenic T. vaginalis cultures on human sperm motility and viability. Spent medium (pH 7.0) caused complete cessation of sperm motility after 15 minutes incubation. Trophozoite soluble fraction or formalin-killed trophozoites caused a 50 percent reduction in sperm motility, compared to 25 percent reduction caused by the trophozoite particulate fraction or the sterile medium and three percent by saline (control). Spent medium from T. vaginalis cultures reaching stationary growth phase produced the greatest reduction in sperm motility, suggesting that potency was related to time in culture and trophozoites per ml. The T. vaginalis spermicidal activity was heat-stable, trypsin-sensitive, and had a molecular weight of 12–15,000 by gel filtration. This proteinaceous substance was present in and secreted by T. vaginalis trophozoites during normal growth in axenic culture. Since this T. vaginalis byproduct rapidly killed sperm in vitro, its effects in humans may contribute to infertility in infected couples.

Introduction

Trichomonas vaginalis, a sexually transmitted protozoan parasite, inhabits the genitourinary tract of both men and women. It is estimated that at least six million women and their partners in the United States are infected annually with this parasite, making trichomoniasis one of the most common sexually transmitted diseases. T. vaginalis is responsible for a spectrum of clinical manifestations ranging from asymptomatic infection to severe inflammation, cervical ulceration, and...
vaginal epithelial cell dysplasia. Various explanations for these differential effects have been hypothesized, including differences in parasite strain virulence or in host immune response or susceptibility. Direct contact with trophozoites causes cytolysis of host cells, while their production of extracellular toxins exerts indirect cytotoxic effects. Lushbaugh and co-workers have reported that trophozoite secretions or culture supernatants have sublethal-cytotoxic effects on baby hamster kidney cells in vitro. *Trichomonas vaginalis* infection has also been implicated as a possible cause of male sterility and human reproductive failure, although this is not a consistent observation in asymptomatic patients. Decreased sperm motility has resulted from exposure to live *Trichomonas vaginalis* trophozoites, crushed trophozoites, or supernatants from *Trichomonas vaginalis* cultures. Hynie et al hypothesized that toxic secretions of the parasites might contribute to sterility. Our study has characterized a sperm motility inhibiting factor isolated from pH neutralized, cell-free supernatants of axenic *Trichomonas vaginalis* cultures.

**Materials and Methods**

**Parasite Cultivation**

*Trichomonas vaginalis*, L. S. Diamond strain C-1 (ATCC # 30001), was obtained from the American Type Culture Collection (ATCC). Organisms were axenically cultured (37°C) in pH 6.5 Diamond’s trypti-case-yeast extract-maltose medium (TYM) supplemented with 10 percent heat-inactivated fetal calf serum and penicillin-streptomycin. 

**Preparation of Intact Killed Trophozoites**

Live trophozoites, for use in sperm motility assays, were centrifugally washed (three times) and washed (three times) in Earle’s balanced salt solution (EBSS), and counted in a hemocytometer. The pellet was resuspended in an equal volume of cold one percent formaldehyde and refrigerated overnight. Prior to use, killed trophozoites were centrifugally washed in EBSS (three times).

**Preparation of Parasite Fractions**

*Trichomonas vaginalis* trophozoites were removed from stationary-phase (three day old) cultures by centrifugation (900 × g, 5°C, 15 min). The decanted medium was adjusted to pH 7.0 with NaOH, sterilized by ultrafiltration (0.22 μm), and called “Spent Medium (SM).” The pellet was washed (three times) in EBSS, and resuspended to 10⁶ trophozoites per ml. Following freeze-thawing (three times), the preparation was sonicated on ice (Branson cell disrupter, five one-minute bursts at 30 MHz). Complete cellular disruption was verified by light microscopy. This preparation was centrifuged (11,100 × g, 5°C, 20 min); the decanted and ultrafiltered supernatant was called the “soluble fraction (SF),” and the pellet, resuspended to original volume, was called the “particulate fraction (PF).”

**Growth Curve Determination**

Cultures in TYM medium were seeded with 3 × 10⁶ *Trichomonas vaginalis* from a culture transferred twice after receipt from ATCC. Triplicate samples were tested for viability twice a day, beginning 18 hours after initiation of growth. Viability of trophozoites was assessed by their motility and trypan blue exclusion. Following determination of parasite density, Spent Medium was prepared and stored frozen (−70°C).

**Sperm Motility Inhibition Assay**

Semen obtained from healthy volunteers was used within two hours of col-
lection. Initial motility (sample diluted 1:2 in Dulbecco’s phosphate buffered saline* [PBS]) was determined by counting sperm using light microscopy and a $40 \times$ objective. The percentage of sperm rated as motile (actively moving), sluggish (non-directional movement or twitching of flagellum), or nonmotile (no movement of sperm or flagellum) was determined by assessing at least 100 sperm. All assays were performed by the same individual to enhance reproducibility.

Inhibition of sperm motility was assayed by incubating 200 µl of sample (SM or parasite fraction) with 200 µl of semen (as in table I) or one ml of sample with 100 µl of semen (table II) for 15 minutes (25°C) before microscopic evaluation. The percentage of sperm motility inhibition was calculated by the formula: 
\[
\frac{\text{number of non-motile sperm}}{\text{total number of sperm counted}} \times 100
\]

**GEL FILTRATION CHROMATOGRAPHY**

One ml samples of SM were layered onto a 1.5 × 120 cm column of S200 Sephacryl† and eluted with PBS (5°C). Eluate absorbance was monitored at 280 nm, and an aliquot of each one ml fraction assayed for inhibition of sperm motility. The molecular weight of eluate fractions with activity to inhibit sperm motility was estimated from a calibration curve obtained by elution of proteins of known molecular weight† (molecular weights 6,500 to 66,000).

**TRYPSIN INHIBITION OF ACTIVITY**

Trypsin and trypsin inhibitor were obtained as lyophilized preparations† and reconstituted in PBS, at a concentration of 0.01 mg per ml. Spent Medium was incubated five minutes

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* Gibco Laboratories, Grand Island, NY.
† Sigma Chemical Company, St. Louis, MO.

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**TABLE I**

| Effect of *Trichomonas Vaginalis* Trophozoites, Fractions and Culture Medium on Sperm Motility |
|------------------------------------------------------|------------------|------------------|
| Group                        | Motile | Sluggish | Non-motile |
| PBS                          | 96     | 11      | 3          |
| Killed Trophozoites          | 14     | 34      | 51         |
| Soluble Fraction             | 26     | 21      | 52         |
| Particulate Fraction         | 31     | 25      | 28         |
| Clean Media                  | 36     | 40      | 24         |
| Spent Media                  | 0      | 0       | 100        |

Fresh semen was incubated 15 minutes at 25°C with an equal volume of killed *T. vaginalis* trophozoites (5 × 10⁴/ml) or fractions. Aliquots were observed and counted microscopically to determine sperm motility. Data given are an average of two trials.

**TABLE II**

<table>
<thead>
<tr>
<th>Inhibition of Sperm Motility As a Function of Culture Age</th>
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</thead>
<tbody>
<tr>
<td>Sample</td>
</tr>
<tr>
<td>PBS</td>
</tr>
<tr>
<td>CM</td>
</tr>
<tr>
<td>1 Day SM</td>
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<tr>
<td>2 Day SM</td>
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<tr>
<td>3 Day SM</td>
</tr>
<tr>
<td>4 Day SM</td>
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<tr>
<td>3 Day SM + 56°C</td>
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<tr>
<td>3 Day SM + T</td>
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<tr>
<td>3 Day SM + T &amp; TI</td>
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<td>3 Day SM + TI</td>
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One hundred microliters of semen were incubated in one ml of sample for 15 minutes prior to microscopic evaluation of sperm motility. PBS = Dulbecco’s phosphate buffered saline. CM = clean medium.

1 Day SM = Spent medium from a one day old culture.
2 Day SM = Spent medium from a 2 day old culture, etc.
3 Day SM + 56°C was heated for one hour.
3 Day SM + T was preincubated with trypsin prior to assay.
3 Day SM + T & TI was preincubated with trypsin plus trypsin inhibitor prior to assay.
HEAT INACTIVATION

Spent medium was incubated one hour in a 56°C water bath prior to assay for inhibition of sperm motility.

Results

EFFECT OF PARASITE FRACTIONS AND CULTURE MEDIUM ON SPERM MOTILITY

The effects of incubating semen with killed *T. vaginalis* trophozoites, soluble or particulate *T. vaginalis* fractions or SM are shown in table I. Controls were sperm incubated in PBS or in sterile clean culture medium (table I). Sperm incubated in clean medium (CM) lost motility somewhat faster than those incubated in PBS. The decrease in motility observed after 15 minutes incubation of semen with killed intact trichomonads or their soluble fraction was twice that of CM controls. However, motility of sperm incubated with the particulate fraction was not much different from control. Spent medium caused cessation of sperm motility and loss of trypan blue exclusion (death) within 15 minutes.

INHIBITION OF SPERM MOTILITY AS A FUNCTION OF CULTURE AGE

The effect of SM on sperm motility was related to the age of the *T. vaginalis* culture from which it was prepared (table II). The percentage of motile sperm present following incubation with SM from a one day old culture (day 1 SM) was not significantly different from that of sperm exposed to clean media or PBS. Incubation of sperm with day 2 SM decreased their motility to 44 percent. Day three or four SM caused a decline in sperm motility to 7 percent.

The sperm motility inhibiting activity of three day SM was heat stable and trypsin sensitive (table II). A greater percentage of sperm treated with trypsin-inactivated-SM were motile than those treated with native SM. Spent medium preincubated with either trypsin inhibitor or with trypsin inhibitor-plus-trypsin had slightly more sperm motility inhibitory activity than untreated SM.

INHIBITION OF SPERM MOTILITY AS A FUNCTION OF PARASITE GROWTH

Density of *T. vaginalis* trophozoites in culture was correlated with the activity of corresponding culture supernatants (SM) to inhibit sperm motility (figure 1). Since pH of SM was adjusted to 7.0 prior to assay, any effects of medium pH changes owing to normal *T. vaginalis* growth were minimized. Inhibition of sperm motility by SM increased rapidly with length of culture and trophozoites

![Figure 1](image-url)
per ml, reaching a maximum at 48 to 66 hours. After 48 hours in culture, the trophozoites were in the beginning stationary phase of growth as demonstrated by their slowed doubling still accompanied by a high degree of viability (figure 1).

**Gel Filtration of SM**

Elution of three day SM from the S 200 column isolated a single peak [four (one ml) fractions] that decreased sperm motility by 92 to 100 percent. Its elution volume corresponded to a molecular weight of approximately 12,000 to 15,000.

**Discussion**

Although *Tritrichomonas foetus* has been identified as a cause of abortion and sterility in cattle, a similar role for the human vaginal Trichomonad has yet to be shown. Hynie et al.\(^1\) demonstrated that crushed trophozoites or supernatant from *T. vaginalis* cultures impaired motility of sperm after two hours of exposure. They postulated that toxic products secreted by the parasite may be a cause of sterility, especially when the infection is a heavy one. Other organisms, notably *Candida albicans*, *Escherichia coli*, and *Ureaplasma urealyticum*, have also been demonstrated to cause decreased sperm motility\(^2\) or have been associated with infertility in humans.\(^2\,7\)

The mechanisms responsible for host cell damage and cytotoxicity in *T. vaginalis* infection still remain to be elucidated; toxic byproducts have been suggested but not identified or characterized.\(^1\,6\,8\,9\,10\,11\,13\,15\,17\,20\) Exposure of a variety of cell cultures to live trichomonads has resulted in the disruption and destruction of these monolayers through contact-related target cell cytolysis.\(^1\,5\,8\,10\,13\,14\) Culture supernatants\(^5\) and *T. vaginalis* conditioned medium\(^15\,17\) have been reported to produce lethal or sublethal-reversible morphologic changes in target cells, implying that release of toxins or metabolites may contribute to production of some cytopathogenic changes.

This study showed that direct contact with *T. vaginalis* trophozoites is not necessary to inhibit sperm motility or cause their death. Although killed trophozoites and fractions resulted in some decrease in sperm motility, supernatants from *T. vaginalis* cultures quickly immobilized or killed the sperm. This spermicidal activity in supernatants was produced in culture by viable, dividing trophozoites. Its elaboration appeared not to be the result of death or disintegration of the parasites. Trypsin treatment reduced the spermicidal activity of preparations of SM indicating susceptibility of the factor to enzymatic digestion. However, addition of trypsin inhibitor to preparations of SM enhanced their spermicidal activity; perhaps trypsin inhibitor itself has some toxicity for sperm. The heat stability, trypsin sensitivity, and low molecular weight (12,000 to 15,000 daltons) of the *T. vaginalis* sperm motility inhibiting factor make it likely that the substance is an enzyme or metabolite produced as a normal byproduct of *T. vaginalis* growth in axenic culture. A neuraminidase, isolated from *T. foetus*, has been implicated in that organism’s pathogenicity;\(^9\) various other cellular products associated with growth and metabolism of other protozoan parasites\(^3\,16\) have been reported to affect mammalian target cells and sperm motility or activity.\(^12\)

The possibility that a sperm motility-inhibiting factor may be elaborated by *T. vaginalis* as a result of normal growth and metabolism is especially important when considering causes of human infertility. *T. vaginalis* infections are often asymptomatic, particularly in males, and occult trichomoniasis should perhaps be considered as a cause of reproductive dysfunction in infertile couples. The role played by *T. vaginalis* in sterility has not
been clinically evaluated. Further characterization of the sperm motility inhibiting factor from *T. vaginalis* cultures is planned.

**Acknowledgments**

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**References**