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# Dual Energy Metabolism-Dependent Effect of *Ureaplasma urealyticum* Infection on Sperm Activity

MALKA REICHART,* HAYA LEVI,* ITZHAK KAHANE,†‡ AND BENJAMIN BARTOOV*

*From the *Male Fertility Laboratory, Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, Israel; and the †Department of Membrane and Ultrastructure Research, The Hebrew University Hadassah Medical School, Jerusalem, Israel.*

**ABSTRACT:** Genital *Ureaplasma urealyticum* infection is considered a sexually transmitted infection. It has long been debated whether the presence of *U urealyticum* in semen may be a possible cause of infertility. Long-term incubation (4 hours or overnight) of sperm cells with *U urealyticum* in vitro resulted in a significant inhibition of sperm motility and membrane alteration whereas a short incubation (45 minutes) of sperm cells with ureaplasmas resulted in an acceleration of sperm velocity. The aim of this study was to understand these contradictory reports of *U urealyticum* infection on sperm motility. Spermatozoa from fresh ejaculates of normozoospermic semen of men who were referred to the university Male Fertility Laboratory for semen analysis, with no history of genital tract infection, and from normal Assaf breed rams were infected in vitro with *U urealyticum* serotype 8, at different pHs and $O_2$ concentrations. Sperm viability and motility and changes in extracellular pH were evaluated. A significant (16%–43%) increase in sperm activity was observed upon infection at alkaline pH (7.8) under aerobic or hypoxic conditions, and a 58% increase was observed under anaerobic conditions and pH 7.2. When the infection was conducted under aerobic conditions and acidic pH (6.3), or under hypoxic conditions at neutral pH (7.2), an 8%–25% inhibition of sperm activity was observed. These results indicate that when sperm activity depends on mitochondrial oxidative phosphorylation, usually at low pHs, *U urealyticum* competes with mitochondrial energy production and therefore reduces sperm motility and viability. However, when sperm energy metabolism depends on glycolysis, usually at higher pHs, *U urealyticum* stimulates glycolysis and sperm activity.

Key words: Sperm energy metabolism, motility, viability, pH.
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*Ureaplasma urealyticum* is a self-replicating prokaryote belonging to the taxonomic class Mollicutes, which lack a cell wall (Styler and Shapiro, 1985). Genital *U urealyticum* infection is considered a sexually transmitted infection and therefore occurs more frequently during the fertile age. The bacterium was found to be involved in prostatitis (Weidner et al, 1985) and epididymitis (Jalil et al, 1988). In vivo studies have shown that the range of *U urealyticum* concentration in the semen of infertile males is between $10^2$ to $10^7$ colony-forming units per milliliter (CFU/mL; Busolo et al, 1984a; Kohn et al, 1998). *U urealyticum* was found to adhere to the sperm of infertile patients, especially to the sperm head in the postacrosomal region and to the middle piece (Gnarpe and Friberg, 1973; Fowlkes et al, 1975; Swenson and O'Leary, 1980; Xu et al, 1997). Invasion of the cytoplasmic droplet in the middle piece by *U urealyticum* has also been reported (Busolo et al, 1984a). Recently it was shown (Reichart et al, 2000) that under different conditions of infection (ie, aerobic, hypoxic, and anaerobic), the bacteria adhere to ram and human sperm head and tail and invade its cytoplasm.

It has long been debated whether the presence of *U urealyticum* in semen may be a possible cause of infertility (Hill et al, 1987; Montagut et al, 1991; Shalika et al, 1996; Kanakas et al, 1999). The changes in sperm parameters that could account for this effect are controversial, and contradictory effects of *U urealyticum* infection on sperm cell activity in vivo and in vitro have been reported. Some investigators were unable to correlate the presence of *U urealyticum* in semen with any alterations in semen characteristics (Bornman et al, 1990; de Jong et al, 1990; Eggert-Kruse et al, 1990). Others found a correlation with a reduction in sperm density (Upadhyaya et al, 1984; Naessens et al, 1986), motility (Busolo et al, 1984b; Naessens et al, 1986), and morphology (Busolo et al, 1984b; Sanchez et al, 1990; Xu et al, 1997).

Long-term incubation (4 hours or overnight) of sperm cells with *U urealyticum* species in vitro resulted in a significant inhibition of sperm motility and membrane alteration (Ross and Scott, 1994; Nunez-Calonge et al, 1998), whereas a short incubation (45 minutes) of sperm

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Correspondence to: Professor Benjamin Bartoov, Male Fertility Laboratory, Faculty of Life Sciences, Bar-Ilan University, Ramat Gan 52900, Israel (e-mail: bartoob@mail.biu.ac.il).

‡Deceased July 1997.

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cells with ureaplasmas resulted in an acceleration of sperm velocity (Talkington et al, 1991). To shed light on these contradictory results, we examined whether the effect of *U urealyticum* infection in vitro is related to sperm energy metabolism, which underlies sperm activity.

## Materials and Methods

### Sperm Preparation

Ram semen was collected from Assaf breed rams using a sterile artificial vagina after male stimulation by a rutted female. Human sperm cells were obtained from normozoospermic semen (World Health Organization, 1992) of men who were referred to the Bar-Ilan Male Fertility Laboratory for semen analysis, with no history of genital tract infection. The investigations of both study groups (ie, human and ram) were approved by the Ethics Committees of Bar-Ilan University. Because the study was prospective in nature, semen samples exhibiting positive cultures were rejected.

Small changes in human sperm quality are difficult to assess because the quality of human sperm cells, as expressed by motility and morphology, differs widely. Ram sperm cells were therefore used as an animal model. On the other hand, assessment of viability of ram sperm cells is difficult and not reliable. We therefore used both human and ram sperm cells to complement the study.

Semen samples from ram and humans were diluted 1:3 with Ringer glucose phosphate (RGP) buffer (100 mL of 0.9% [w/v] NaCl, 4 mL of 1.15% [w/v] KCl, 1 mL of 2.11% [w/v] $KH_2PO_4$, 1 mL of 3.82% [w/v] $MgSO_4 \cdot 7H_2O$, 2 mL of a 1.3% [w/v] solution of $NaHCO_3$, and 0.5 g glucose/100 mL and 20 mL of 0.25 M phosphate buffer pH 7.4 [Mann, 1964]). The diluted semen was centrifuged for 15 minutes at 400 $\times$ $g_{max}$ at 25°C. The sperm pellet was suspended in 3 mL RGP buffer and washed by centrifugation as above. The washed sperm pellet was suspended in RGP buffer and brought to a final concentration of 50 $\times$ $10^6$/mL.

### Preparation of U urealyticum Specimens

*U urealyticum* serotypes 8 were grown in 1-L batches, harvested, and washed as previously described (Saada et al, 1991). The final ureaplasmas pellet was resuspended in 5 mL Pleuropneumonia-Like Organism Broth (Difco, Detroit, Mich) pH 6.5, containing 5% dimethylsulfoxide (Sigma, St Louis, Mo). The colony-forming units titer was evaluated and found to be approximately $10^{10}$/mL. Half-milliliter aliquots were frozen in liquid nitrogen and stored at −70°C. For every experiment, 1–2 aliquots were thawed in a 37°C incubator for 30 minutes and then diluted 1:20 in RGP and centrifuged for 10 minutes at 26 890 $\times$ $g_{max}$ at 4°C. The bacterial pellet was suspended in 0.5–1.0 mL RGP buffer.

### Experimental Procedure

A 50 $\times$ $10^6$ sperm suspension was infected in vitro with *U urealyticum* at a multiplicity of infection (MOI) of 10 and 50 colony-forming units per sperm, at 37°C, under the different conditions as described herewith. Aliquots were taken at 30-minute intervals for viability (100 μL), motility (50 μL), glucose uptake (50 μL), lactate production (50 μL), and $O_2$ uptake (100 μL), for both the control uninfected and the infected sperm suspensions.

### Preparation of Different Incubation Conditions

The experiments were conducted in aliquots of 1-mL sperm suspension in RGP incubated at 37°C, and different pHs ranged between 5.75–8.25 in which sperm cells can survive (Peterson and Freund, 1976) and under the following oxygen concentrations:

*Aerobic*—Sperm suspensions were incubated in 40-well dishes (15 mm diameter, Nunclone, Roskilde, Denmark) under high moisture conditions.

*Hypoxic*—Sperm suspensions were incubated in 2-mL closed Eppendorf test tubes and were flushed with nitrogen gas just before the beginning of the experiment and whenever they were opened for sampling.

*Anaerobic*—Ram semen was collected and washed in anaerobically preprepared RGP buffer by degassing with argon. Sperm suspensions were incubated in 2-mL closed Eppendorf tubes that were continuously flushed with argon gas. For this purpose, 2 holes were made in the cover tube, one for the gas and one for releasing pressure and taking samples.

### Inhibition of Glycolysis

Sperm glycolysis was inhibited by a final concentration of 20 mM 2-deoxy-D-glucose (2-DOG; Sigma). The inhibitor was added to ram semen immediately after ejaculation as well as during the washing procedure. The 2-DOG stock was 500 mM in RGP, stored at 4°C.

### Inhibition of Urea Catabolism

Urea catabolism was inhibited by 0.1 mM flurofamide (Norwich Eaton Pharmaceuticals, Norwich, NY), which inhibits urease activity. The flurofamide stock solution was 10 mM in distilled water, stored at 4°C.

### Sperm Analyses

*Viability*—Sperm cell viability was assessed using eosin nigrosin staining as described previously (Glezerman and Bartoov, 1986).

*Motility*—Sperm motility was measured using the sperm motility analyzer (SPERMETER; Gamete Ltd, Moshav Hemmed, Israel; Bartoov et al, 1991). This instrument has a photoelectric cell that detects the variation in optical density (OD) caused by motility of sperm cells during 20 seconds. Detailed motility changes of the different sperm cell samples that occurred during the experiments were determined by the sperm motility index (SMI) measured on duplicate samples every 10–30 minutes. Recently, it was reported that a good correlation exists between the SMI values and concentration of progressively motile spermatozoa measured according to the recommendations of the World Health Organization ($r$ = 0.87; Martinez et al, 2000). For the sake of comparison between samples, the intensity of sperm motility was calculated using the sum of the SMI values obtained throughout the experiment until motility ceased. This value was defined as the integral of motility. In the ram sperm experiments, the integral of motility was defined as the sum of the SMI values

obtained throughout the experiment until motility ceased, whereas in the human experiments, it was defined as the sum of SMI values obtained at 30, 60, and 120 minutes. Measurement of sperm motility using the SPERMETER, especially in a multiple comparative study, has the advantages of objective and quick readout, and the total motility intensity during various experimental conditions can be easily quantified.

### Metabolism Outcome Measurements

*Glucose Uptake Rate*—Glucose levels were measured using the Sigma glucose kit (GTR-P, Sigma) for measuring the amount of $H_2O_2$ formed when glucose is oxidized by glucose oxidase. The rate of glucose uptake was calculated from the changes in the glucose levels that took place during the first 30 minutes of incubation.

*Lactate Formation Rate*—Lactate levels were measured using a lactic acid kit (826-A; Sigma) according to the method described by Freund and Peterson (1976). The rate of lactate formation was calculated from the changes in the lactate levels, which took place during the first 30 minutes of incubation.

*Oxygen Uptake*—The oxygen uptake of the sperm cells was estimated using a biological oxygen monitor (Yellow Springs Instruments Co), at 37°C. Under these conditions the amount of oxygen dissolved in 1 mL of RGP was 222.4 nM/mL. One hundred microliters of sperm cell suspension were estimated in 2.9 mL RGP at the appropriate pH and the $pO_2$ was measured for 60 seconds.

*pH Measurement*—The pH of the incubation mixture was measured at each sampling time with a digital pH meter (El Hama Instruments, Israel). ΔpH was defined as the change in the pH that took place during the entire experimental period.

### Statistical Analysis

Statistical analysis was performed on the raw values obtained for the different sperm parameters measured using the SPSS-X package (Norusis, 1985). Data were analyzed using the analysis of variance paired (control vs. experimental units) test. A $P$ value $< .05$ was considered statistically significant. We noted comparisons with a $P$ value $< .1$ in order to depict tendency. Means are presented with standard errors.

## Results

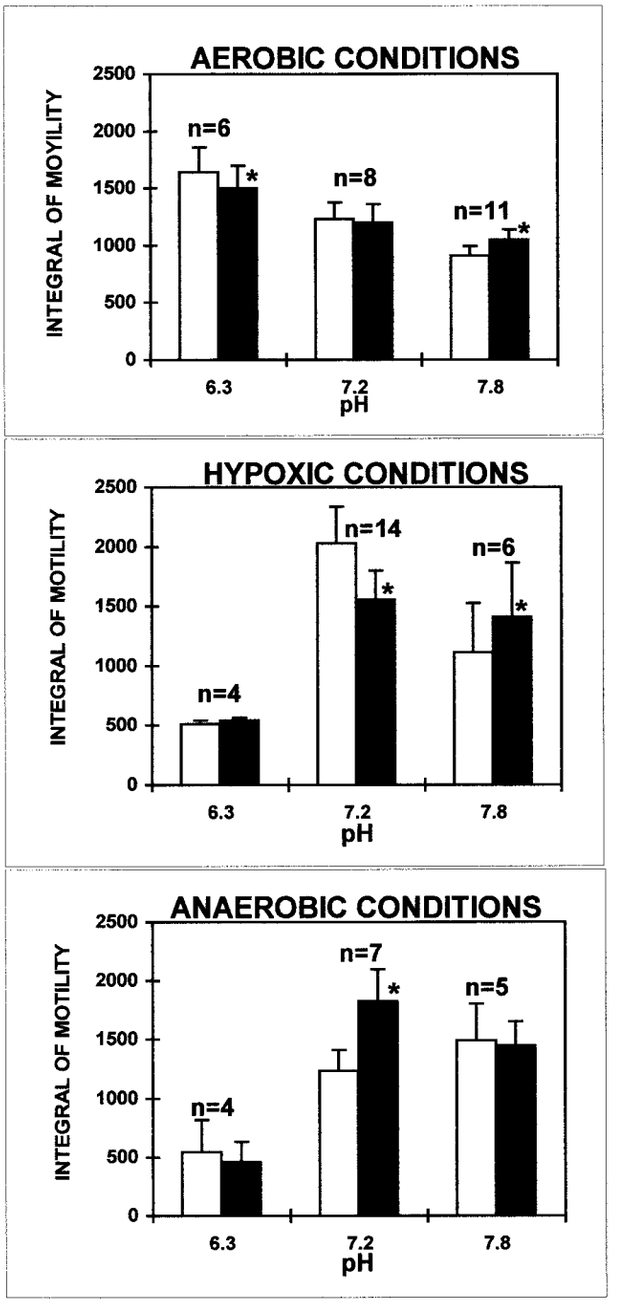
### Metabolism and Activity of Uninfected Ram and Human Sperm Cells

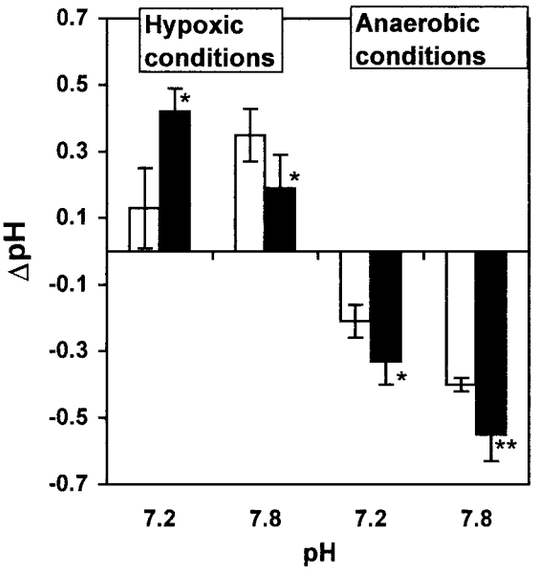
*Aerobic Conditions*—Washed ram spermatozoa under aerobic glycolysis exhibited 3 phases of metabolic activity, according to the $[H^+]$ concentrations: acidic phase, pH 5.75–6.25; neutral phase, pH 6.30–7.25; and basic phase, pH 7.30–8.25. Under aerobic conditions, equimolar concentrations of $O_2$ and glucose consumption and lactate formation were observed (Figure 1). Metabolic activity in the acidic phase was lower than in the neutral phase (1:2 times), and reached a maximal level at pH 7.25. This increase in metabolic activity may be related to the presence of exogenous glucose, because in the presence of 2-DOG, the maximal metabolic activity was observed in the acidic phase (Figure 2). Paradoxically to the above-mentioned metabolic activity, the maximum integral of ram sperm motility (approximately 2000) was obtained at pH 6.3. Increases in the medium pH yielded a linear decrease in motility (Figure 2).

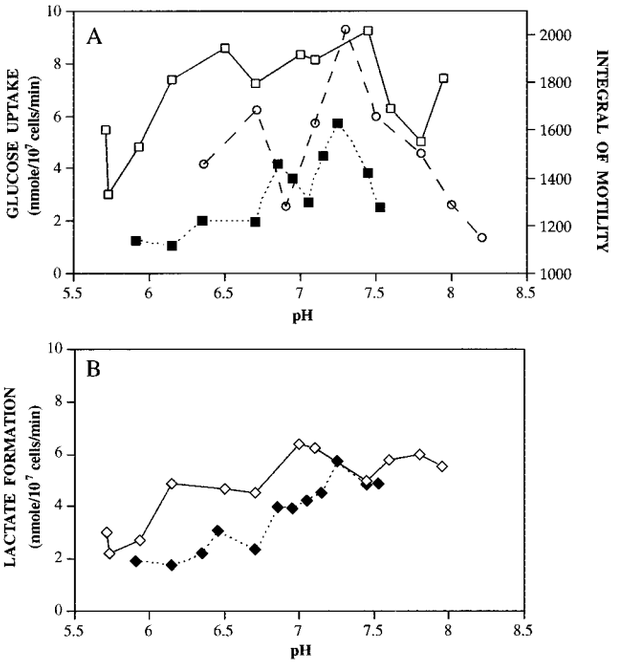
*Anaerobic Conditions*—Under anaerobic conditions a linear enhancement in the sperm integral of motility was observed upon elevation of the pH from 6.3 to 7.8, with the integral of motility reaching a maximum of 1500 (Figure 3).

Under these conditions a decrease in the ΔpH of the incubation medium in the uninfected sperm suspension was observed both in the neutral and in the basic phases (Figure 4).

*Hypoxic Conditions, Ram Sperm Cells*—Under hypoxic conditions the rates of glucose uptake and lactate formation were 2.43 and 1.31 times higher, respectively, than those observed under aerobic conditions (Figure 5). However, similarly to the aerobic conditions, metabolic activity was found to be dependent on pH. Maximal metabolic activity was measured during the neutral phase. Under this condition pH elevation in the incubation me-




dium throughout the experiment was observed both in the neutral (initially at pH 7.2) and the basic phases (initially at pH 7.8, Figure 4).

The pattern of the integral of uninfected ram sperm

Figure 3. Dual effect of *U urealyticum* on ram sperm motility under different oxygen and pH levels. (–□–) Control uninfected sperm cells, and (■) infected with *U urealyticum* at an MOI of 10 ureaplasmas per sperm. The integral of motility was the sum of the SMI values obtained every 30 minutes, from the beginning of the experiment until motility ceased. The results are mean ± SEM of the number of experiments mentioned at the top of the column. *Significantly different from control uninfected, $P < .05$.

Figure 4. Changes in pH levels, which took place during the experiment period until motility ceased, under hypoxic and anaerobic conditions (n = 8 and 4, respectively), in uninfected (–□–) and in *U urealyticum* infected ram spermatozoa (–■–) (n = 8 and 4, respectively). Sperm cells were infected with *U urealyticum* at an MOI of 10 ureaplasmas per sperm. *Significantly different from control uninfected, $P < .05$.

Figure 5. Effect of pH on the rate of ram sperm glycolysis and motility when incubated for 30 minutes in RGP. The rates of glucose uptake **(A)** and lactate formation **(B)** were measured under aerobic (–■– and –◆–, respectively) and hypoxic conditions (–□– and –◇–, respectively). Integral of motility was measured under hypoxic conditions (–○–).

Table 1. *Dual effect of* Ureaplasma urealyticum *on human sperm viability and motility**

| | Incubation Condition | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | pH 7.2 | | | | pH 7.8 | | | | | | | |
| | 30 min | | 60 min | | 30 min | | 60 min | | 120 min | | | |
| Cells | Control Uninfected | Infected | Control Uninfected | Infected | Control Uninfected | Infected | Control Uninfected | Infected | Control Uninfected | Infected |
| Live (%) | 19.6 ± 3.8 | 4.9 ± 1.0† | 16.3 ± 5.7 | 8.7 ± 4.8‡ | 31.2 ± 10.8 | 39.5 ± 7.6† | 20.3 ± 8.3 | 32.2 ± 7.9† | 15.0 ± 4.8 | 23.3 ± 7.1† |
| Dormant (%) | 38.3 ± 10.7 | 40.1 ± 5.1 | 37.5 ± 5.4 | 29.0 ± 5.5† | 34.3 ± 14.5 | 27.0 ± 10.0‡ | 36.1 ± 10.0 | 23.5 ± 7.4† | 43.3 ± 12.3 | 31.0 ± 11.8 |
| Dead (%) | 42.2 ± 6.7 | 55.3 ± 3.9† | 44.7 ± 7.0 | 61.8 ± 9.9† | 27.0 ± 3.1 | 28.3 ± 3.7 | 34.0 ± 5.7 | 31.7 ± 5.7 | 37.3 ± 9.4 | 40.7 ± 4.7 |
| Integral of motility | 1007 ± 475 | 862 ± 398† | 1378 ± 632 | 1089 ± 515† | 151 ± 51 | 196 ± 56† | 145 ± 54 | 241 ± 68† | 303 ± 26 | 566 ± 159‡ |

* Sperm cells were infected at a multiplicity of infection of 10 ureaplasmas per sperm under hypoxic conditions. Values are a mean ± SEM of 3 experiments, except for percentage of live cells at pH 7.8 that are a mean ± SEM of 6 experiments.
† Significantly different from control uninfected, $P < .05$.
‡ Significantly different from control uninfected, $P < .1$.

motility was complementary to the metabolic activity. The peak value of the integral of motility (= 2000) was observed at pH 7.2 (Figure 3).

*Hypoxic Conditions, Human Sperm Cells*—Under hypoxic condition the integral of motility of uninfected human sperm cells was higher at pH 7.2 than at pH 7.8 (Table 1).

The viability of uninfected human spermatozoa decreased spontaneously during 120 minutes of incubation under hypoxic conditions at pH 7.2 and 7.8 (Figure 6; Table 1). This necrotic effect was also pH-dependent (Figure 6; Table 1).

### *Metabolism and Activity of Infected Ram and Human Sperm Cells*

Because it was found that ram spermatozoa energy metabolism is regulated by 3 factors, oxygen concentration, pH, and exogenous hexose concentration, the effect of *U urealyticum* on the activity of ram and human sperm cells was therefore examined in a medium containing glucose, at different $[H^+]$ concentrations and under 3 $O_2$ concentration conditions, aerobic, hypoxic, and anaerobic.

*Aerobic Conditions*—Under these conditions, *U urealyticum* infection in vitro (by an MOI of 10 ureaplasmas per sperm cell) caused a significant dual effect on the ram sperm integral of motility: Inhibition at pH 6.3 (−8.0% ± 1.2%; Figure 3) and acceleration at pH 7.8 (+16.6% ± 3.3%; Figure 3; Table 2).

*Anaerobic Conditions*—Infection with *U urealyticum* resulted only in unidirectional acceleration of ram sperm motility at both pH 7.2 and pH 7.8 (58.4% ± 21.7% and 14.0% ± 22.4%, respectively; Table 2).

An additional, significant decrease in the pH (higher ΔpH) was observed upon infection of ram spermatozoa at both initial pHs, 7.2 and 7.8 (Figure 5).

Table 2. *Summary of the dual effect of* Ureaplasma urealyticum *on human and ram sperm cells activity**

| | pH | | |
|---|---|---|---|
| Incubation condition | 6.3 | 7.2 | 7.8 |
| (a) | | | |
| Aerobic | −8.0 ± 1.2† (n = 6) | −1.1 ± 8.8 (n = 8) | +16.6 ± 3.3† (n = 11) |
| Hypoxic | +5.4 ± 3.0 (n = 4) | −23.8 ± 3.9† (n = 14) | +42.7 ± 12.1† (n = 6) |
| Hypoxic + urease inhibitor | … | −11.5 ± 8.7† (n = 5) | +41.0 ± 18.6† (n = 5) |
| Anaerobic | −3.7 ± 80.7 (n = 4) | +58.4 ± 21.7† (n = 7) | +14.0 ± 22.4 (n = 5) |
| (b) | | | |
| Hypoxic | … | −14.4 ± 2.7† (n = 3) | +29.8 ± 10.6† (n = 3) |
| (c) | | | |
| Hypoxic | … | −75.0 ± 0.7† (n = 3) | +26.6 ± 7.6† (n = 6) |

* Sperm cells were infected at a multiplicity of infection of 10 ureaplasmas per sperm. The integral of motility in ram sperm obtained throughout the experiment until motility ceased (a) and in human sperm cells after 30 minutes of infection (b). The viability of human sperm cells was determined after 30 minutes of infection (c). The values are expressed by mean percent of inhibition (−)/acceleration (+)± SEM.
† Significant inhibition or acceleration, $P < 0.05$.

*Hypoxic Conditions, Ram Sperm Cells*—Infection of ram sperm cells with *U urealyticum* had a dual effect on the ΔpH compared with uninfected sperm cells. A significantly higher ΔpH was measured in experiments with an initial pH of 7.2, whereas a significant decrease in ΔpH was found in experiments with an initial pH of 7.8 (Figure 4).

A significant dual effect of *U urealyticum* infection on ram sperm motility was observed. At an MOI of 10 ureaplasmas per sperm cell, inhibition of the integral of ram sperm motility was observed at pH 7.2 (−23.8% ± 3.9%), whereas a +42.7% ± 12.1% acceleration of sperm motility was observed at pH 7.8 (Figure 3; Table 2).

Inhibition of urea catabolism by 0.1 mM flurofamide did not result in any alteration of the dual effect on ram sperm motility by *U urealyticum* (Table 2; n = 5).

*Hypoxic Conditions, Human Sperm Cells*—A similar significant dual effect of infected human sperm cells was found on human sperm motility compared with uninfected sperm cells. Inhibition at pH 7.2 (−14.4% ± 2.7%, after a short infection time [30 minutes] at an MOI of 10 ureaplasmas per sperm cell) and acceleration at pH 7.8 (+29.8% ± 10.6% and +86.8% ± 33.4%, after a short infection time [30 minutes] and a long infection time [120 minutes], respectively, at an MOI of 10 ureaplasmas per sperm cell; Table 1).

*U urealyticum* infection also caused a dual effect on the reduction of sperm viability during incubation. Upon a short infection time (30 minutes) at an MOI of 10 per sperm cell and pH 7.2, a significant decrease in the viability was observed as expressed by a 75.0% ± 0.7% decrease in the percentage of live spermatozoa accompanied by an elevation in the percentage of dormant and dead spermatozoa. In contradistinction, a 26.6% ± 7.6% increase in the percentage of live spermatozoa was observed at pH 7.8, accompanied by a decrease in the percentage of dormant spermatozoa (Figure 6; Table 1). The viability enhancement effect of *U urealyticum* was found to be dose- and time-dependent (Figure 6).

## Discussion

Ram spermatozoa, suspended in RGP, utilize equimolar amounts of glucose and oxygen. Therefore, they do not exhibit the Pasteur effect. Inhibition of glycolysis by 2-DOG revealed that during the acidic phase (pH 5.75–6.25), endogenous substrates are used for energy production. During the neutral phase (pH 6.30–7.25), a linear increase in the rate of aerobic glycolysis was found up to a pH of 7.2. This increase was dependent on external hexose utilization. In contradistinction, during the alkaline phase (pH 7.30–8.25), a decrease in glucose uptake was observed, probably due to feedback regulation by elevated lactate formation, which could be expressed by lack of cytoplasmic $NAD^+$. A decrease in the glycolytic rate at alkaline pHs was also reported for human spermatozoa by Peterson and Freund (1976). An inverse relationship was found between the intensity of sperm metabolism and motility under normal $O_2$ levels. This indicates that the energy produced by endogenous substrate respiration is utilized much more efficiently for sperm

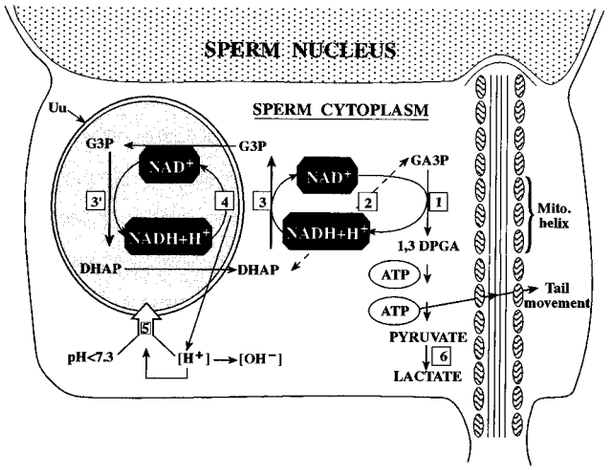



Figure 7. A model for metabolic interaction and cooperation between sperm cells and *U urealyticum* for energy production via the glycerol phosphate shuttle. DHAP, dihydroxyacetone-phosphate; DPGA, diphos-

motility than the energy produced via the glycolytic pathway. These results confirm our previous findings on ram sperm collective motility in the presence of 2-DOG (Bartoov et al, 1980). Under hypoxic conditions and low pHs, energy production via endogenous substrates is probably reduced, and motility therefore decreases. The maximal intensity of motility was observed at pH 7.2. The integral of motility was also low during the alkaline phase, during which, as mentioned before, glycolysis is inhibited. A linear correlation was found between sperm motility enhancement and pH elevation under anaerobic conditions.

Because sperm energy metabolism and motility are pH-dependent and because *U urealyticum* energy metabolism is based on proton-dependent adenosine 5′-phosphatase (ATPase), which is also pH-dependent (Romano et al, 1980), it is not surprising that *U urealyticum* exerts a dual effect on both ram and human sperm activity, which depends on the pH of the medium and the oxygen concentration. When spermatozoa are infected under alkaline conditions, *U urealyticum* cannot efficiently utilize its ATPase, which exhibits optimal activity at pH 7.3 (Romano et al, 1980). Alternatively, it can utilize the NADH produced by the glycerol phosphate shuttle via glycerol phosphate dehydrogenase and NADH dehydrogenase, which are found in abundance in the bacteria (Cocks et al, 1985; De Silva and Quinn, 1991). As in mitochondria, the protons produced by NADH oxidation are released outside the bacterial membrane and create an electrochemical gradient that supplies the driving force for the bacterial ATPase to create adenosine 5′-triphosphate (ATP; Figure 7). The glycerol phosphate shuttle will increase the $NAD^+$ level in the sperm cytoplasm, which in turn, will reduce glycolysis inhibition and therefore enhance sperm activity, as mentioned above. Indeed, in our experiment it was found that *U urealyticum* infection under alkaline conditions resulted in a decrease in the pH levels, indicating a further increase in lactic acid production.

On the other hand, under acidic pHs, the bacterial proton, ATPase, can compete with the sperm mitochondrial ATPase for the proton concentration, thereby inhibiting sperm activity. Indeed, under this condition we demonstrated that *U urealyticum* infection resulted in an increase in the pH of the medium compared with uninfected spermatozoa. Under hypoxic conditions and neutral pH, maximum sperm metabolism activity was found at pH 7.2. At this pH, the bacterial ATPase is also maximally activated. Therefore, it is not surprising that maximal inhibition of sperm activation takes place under these conditions. The dual effect of *U urealyticum* infection on sperm motility was not related to urea catabolism, because inhibition of urease activity by a specific inhibitor had no effect on this phenomenon. Under complete anaerobic conditions, when the Embden-Meyerhof pathway is the only pathway available for sperm energy production, the bacteria can only use the glycerol phosphate shuttle described above and increase cytoplasmic $NAD^+$ levels (Figure 7). This will increase sperm glycolysis, indicated by a decrease in the $\Delta$pH, and therefore exert only a stimulatory effect on sperm activity.

It can be concluded that upon invasion of sperm cells, *U urealyticum* behave similarly to sperm mitochondria and use proton ATPase for their energy metabolism. When sperm activity depends on mitochondrial oxidative phosphorylation, usually at low pHs, *U urealyticum* will compete with mitochondrial activity and will therefore reduce sperm motility and viability. However, when sperm energy metabolism depends on glycolysis, *U urealyticum* will activate the glycerol phosphate shuttle, which can take place at higher pHs, and will thereby stimulate glycolysis and sperm activity in parallel to its own energy metabolism (Figure 7). Sperm mitochondria apparently cannot utilize their lactate dehydrogenase and glycerol phosphate dehydrogenase shuttles at alkaline pHs.

The contradictory results reported in the literature of *U urealyticum* infection in vitro on sperm cell activity (Talkington et al, 1991; Ross and Scott, 1994; Nunez-Calonge et al, 1998) may be explained by the above model for the interaction between *U urealyticum* and sperm energy metabolism mechanisms. Thus, the overnight incubation in Ham's F-10 at an initial pH of 7.6 described by Ross and Scott (1994), or 4 hours of incubation in menezo-B2 at an initial pH of 7.4, described by Nunez-Calonge et al (1998), would result in an excess lactic acid accumulation and pH reduction. As mentioned above, under these con-

phoglycerate; G3P, glycerol-3-phosphate; Mito, mitochondria; Uu, *U urealyticum,* 1, glyceraldehyde-3-phosphate dehydrogenase; 2, trios-phosphate isomerase; 3,3′, glycerol-3-phosphate dehydrogenase of sperm and *U urealyticum,* respectively; 4, NADH dehydrogenase; 5, ATPase; 6, lactate dehydrogenase.

ditions, *U urealyticum* competes with the mitochondria for the $H^+$ concentration and causes inhibition of sperm activity. On the other hand, the short, 45-minute incubation in Ham's F-10 at an initial pH of 7.6, described by Talkington et al (1991), will maintain a high pH during incubation and will therefore result in an enhancement of sperm glycolytic rate and activity.

It can be argued that the concentration of ureaplasmas used in this in vitro study ($50 \times 10^7$/mL) is higher than the CFU/mL found in semen of infertile men. However, there is one report claiming up to $10^7$ CFU/mL in human semen, which may be an underestimation of the true *U urealyticum* concentration because 1 CFU in vivo can represent an agglutination of *U urealyticum,* containing a large number of bacteria. Furthermore, the aim of this study was to explain the contradictory results reported in the in vivo studies of Ross and Scott (1994), Talkington et al (1991), and Nunez-Calonge et al (1998), which used similar *U urealyticum* concentrations. However, the relevance of the specific concentration of *U urealyticum* undertaken in this study to the pathophysiology of *U urealyticum* infection in vivo still needs to be studied using a wide range of dose responses of *U urealyticum* concentrations.

It should be noted that the implication of the dual effect of *U urealyticum* infection on sperm energy metabolism and activity found in this study, to the in vivo *U urealyticum* infection, is indirect because the in vitro experiments were done under strict environmental conditions that do not necessarily exist in vivo.

However, we can speculate that the ramification of these in vitro studies on sperm physiology are as follows: Because epididymal spermatozoa maintain their metabolism mainly on mitochondrial oxidative phosphorylation at a pH of about 6.8 (Mann and Lutwak-Mann, 1981), the presence of *U urealyticum* in the epididymis may inhibit their activity. On the other hand, ejaculated sperm cells maintain their energy metabolism mainly on aerobic glycolysis (Peterson and Freund, 1976), as explained by a high concentration of fructose and a pH above 7.5 in the seminal plasma. Therefore, under such conditions, *U urealyticum* infection will probably enhance their activity. Because the pH of the seminal plasma is dictated by relative excretions of the accessory glands (seminal vesicle/prostate), prostatitis caused by *U urealyticum* infection may result in acidic ejaculate, which may cause inhibition of sperm motility. Upon entry of the ejaculate into the female reproductive tract, the pH of the vagina and the frontal cervical mucus immediately becomes 7.2 (Harper, 1988). Therefore, *U urealyticum* infection at this site will result in inhibition of sperm motility, which could impair sperm penetration through the cervical mucus. On the other hand, when infection takes place in the ampullae of the oviduct, where the pH is 7.8 (Hamner, 1973), sperm motility will be accelerated. This will improve sperm penetration through the zona pellucida into the ovum, and will increase the ovum fertilization rate with sperm infected by *U urealyticum,* thereby endangering normal embryonic development (Reichart et al, 2000).

In conclusion, the dual effect of *U urealyticum* on sperm activity represents 2 different sperm–bacteria relationships, either symbiotic, when sperm metabolism occurs via the glycolytic pathway, or parasitic, when it occurs via respiration.

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