Hiroshima Journal of Medical Sciences
Vol. 33, No. 1, 23~26, March, 1984
HIJM 33-4

Influence of *Ureaplasma urealyticum* and *Mycoplasma hominis* on the Human Spermatozoal Motility*)

Myung Woong CHANG[1], Tae Kyung CHOI[2], Yoshiyasu MATSUO[3] and Zensaku YOSHII[4]

1) *Department of Microbiology, Kosin Medical College, Busan 600, Korea*
2) *Department of Microbiology, College of Medicine, Chungnam National University, Daejeon 300, Korea*
3) *Department of Bacteriology, Hiroshima University School of Medicine, Hioshima 734, Japan*
4) *Department of Microbiology, Yamaguchi University School of Medicine, Ube-shi 755, Japan*

(Received November 29, 1983)

Key words: Ureaplasma urealyticum, Mycoplasma hominis, Spermatozoal motility

ABSTRACT

The human spermatozoal motility was significantly affected by the whole culture solution and slightly less significantly by the microbial cell suspension of *Ureaplasma urealyticum* but not the microbial metabolites (culture supernatant). The same phenomenon was observed with the preparations of *Mycoplasma hominis* in somewhat less degree.

INTRODACTION

Ureaplasma urealyticum and *Mycoplasma hominis* are frequently found in the human genitourinary tract[14]. Many investigators claimed the association of these microbes with nongonococcal urethritis[1], reproductive failure[3,12], intrauterine infection[4], placentitis[5] and chorioamnionitis[16]. However, the role of these organisms in diseases of genitourinary tract and reproductive failure is controversial. Folkes et al.[7] reported that aggregation of *U. urealyticum* with spermatozoa was a characteristic frequently observed with the semen infected with the organism and asserted that the association might contribute to the decreased motility of the spermatozoa. Yoshida[18] observed a positive correlation of the infection of *U. urealyticum* with human reproductive failure and found coiling of the tail of spermatozoa in the infected semen. Yun and Yun[19] reported a significant decrease of spermatozoal motility in the whole culture solution containing per ml 10^4 to 10^6 cells of *U. urealyticum* and *M. hominis*. Furness[8] found out the metabolic product of *U. urealyticum* which was inhibitory to the growth of the organism.

The present study was undertaken to see if these microbes influence the human spermatozoal motility.

MATERIALS AND METHODS

Organism. *U. urealyticum* strain T960 was supplied by Dr. J. A. Robertson, Department of Medical Microbiology, Medical Science Building, Edmonton, Alberta, Canada, and *M. hominis* strain PG21 by Dr. M. Nakamura, Department of Microbiology, Kurume University School of Medicine, Kurume, Japan.

Medium. A liquid medium 10-B[15] was used for the cultivation of *U. urealyticum* and Chanock broth[2] for *M. hominis*.

Sperm specimen. Each sperm specimen was collected in a sterilized tube from 25 healthy college students who were infected with neither *U. urealyticum* nor *M. hominis*.

Test for spermatozoal motility. *U. urealyticum* was cultivated for 12 to 14 hr in 50-ml 10-B medium and *M. hominis* for 96 to 100 hr in 50-ml Chanock broth. In both cases the microbial population reached 10^5 to 10^6 colony forming units (CFU) per ml. Each culture was

*) 張 明雄, 崔 大郷, 松尾吉恭, 吉井善作：ヒト精子運動に及ぼす *Ureaplasma urealyticum* 及び *Mycoplasma hominis* の影響

divided into two portions, one of which was referred to as the whole culture solution and stored at −80°C until use. Another portion was separated into the supernatant fluid and the pellet by centrifugation at 18,000 rpm for 30 min. The pellet was suspended in the original volume of the fresh medium and used as the microbial cell suspension. The supernatant was ultrasonicated for 10 min and referred to as the metabolites of the organism. A 0.2-ml each of the whole culture solutions, the microbial cell suspensions and the metabolites were transferred into a sterilized tube and mixed with the same amount of semen. Physiological saline and the fresh medium served as controls. The mixture was incubated at 37°C and the spermatozoal motility was observed microscopically at 4-hr interval up to 24 hr after incubation. The motility was enumerated by the Emmens motility scale[6] (Table 1).

Table 1. Numerical motility scale of spermatozoa devised by Emmens

4.0	Full activity
3.5	Detectable dampening of activity
3.0	Sluggish activity
2.5	Many cells stationary with tails vibrating
2.0	Most cells stationary with tails vibrating
1.5	Many motionless, none actively progressing
1.0	Only tails moving
0.5	Only a few tails moving
0.0	No activity

RESULTS

Influence of the whole culture solution, the microbial cell suspension and the metabolites of *U. urealyticum* on the human spermatozoal motility is shown in Table 2. The spermatozoal motility decreased significantly within 8 hr of exposure to the whole culture solution ($p<0.01$) and completely disappeared after 20 hr of incubation. The motility was also affected by the microbial cell suspension ($p<0.01$), but slightly less significantly than by the whole culture solution. The metabolites of the organism were slightly inhibitory to the motility only at 8 hr of exposure ($p<0.05$).

Influence of the same preparations of *M. hominis* on the spermatozoal motility was similar to that of those of *U. urealyticum*, but the intensity was slightly milder in general (Table 3).

DISCUSSION

Gnarpe and Friberg[9] postulated a positive correlation of ureaplasma infection with human

Table 2. Numerical scale of spermatozoal motility under the influence of *Ureaplasma urealyticum* (N=20)

Exposure hr	0	4	8	12	16	20	24
Saline	4.0	3.7±0.41	3.2±0.33	2.3±0.31	1.4±0.52	0.8±0.35	0.2±0.25
Ureaplasma broth	4.0	3.4±0.52	3.1±0.46	2.3±0.63	1.5±0.50	0.8±0.55	0.2±0.25
Whole broth culture	4.0	3.4±0.30	2.2±0.40**	1.1±0.34**	0.4±0.44**	0.0**	0.0
Metabolites[1]	4.0	3.5±0.52	2.7±0.45*	1.8±0.51	1.3±0.47	0.7±0.53	0.0
Cell suspension[2]	4.0	3.4±0.39	2.2±0.33**	1.5±0.60**	0.9±0.51**	0.2±0.25**	0.0

*: significant ($p<0.05$) **: very significant ($p<0.01$)
1) supernatant of broth culture, 2) cell pellet suspended in fresh broth

Table 3. Numerical scale of spermatozoal motility under the influence of *Mycoplasma hominis* (N=20)

Exposure hr	0	4	8	12	16	20	24
Saline	4.0	3.7±0.41	3.2±0.33	2.3±0.31	1.4±0.52	0.8±0.35	0.2±0.25
Mycoplasma broth	4.0	3.5±0.50	2.9±0.45	2.1±0.48	1.4±0.51	0.8±0.53	0.2±0.25
Whole broth culture	4.0	3.3±0.25	2.5±0.55*	1.6±0.41**	0.9±0.36**	0.3±0.22**	0.0
Metabolites	4.0	3.4±0.49	2.6±0.34*	2.2±0.56	1.7±0.54	1.0±0.40	0.5±0.21
Cell suspension	4.0	3.6±0.25	2.5±0.35*	1.5±0.52**	1.1±0.34*	0.4±0.34*	0.0

See Legend in Table 2.

reproductive failure. They[10] further reported a neuraminidase-like substance produced by T-mycoplasma, and assumed that the substance might interfere with fertilization or development of fertilized egg. Matthews et al.[13] found little difference in the isolation rates of *U. urealyticum* from fertile, infertile and pregnant women. Fowlkes et al.[7] and Klainer and Pollack[11] detected particles attached to spermatozoa by electronmicroscopy and stated that the particles were suggestive of *Ureaplasma*. Toth et al.[17] reported strange shapes of spermatozoa having coiled tails with granular appearance which were observed in 70% of the specimens infected with *U. urealyticum*. Yoshida[18] reported that the spermatozoa infected with *U. urealyticum* showed coiling of the tail and thought that this phenomenon would be attributable to one of the causes of human reproductive failure.

In the present study, the motility of spermatozoa was significantly affected by the whole culture solution and the microbial cell suspension of either *U. urealyticum* or *M. hominis*. No or little effect was observed with the microbial metabolites. These results are in accord with the earlier findings of Yun and Yun[19], where the spermatozoal motility decreased significantly after 4 hr of exposure to the whole culture solution of *U. urealyticum* and *M. hominis*. The results suggest that the microbial cell suspensions of *U. urealyticum* and *M. hominis* interfere with the motility of spermatozoa but the metablites do not.

The results also support the findings of Fowlkes et al.[7], Yoshida[18], Klainer and Pollack[11] and Toth et al.[17]. The morphology of spermatozoa infected with these microbes is under investigation.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Robertson and Dr. Nakamura for providing us with the strains of *Mycoplasma*.

REFERENCES

1. **Bowie, W. R., Wang, S.-P., Alexander, E. R. and Holmes, K. K.** 1977. Etiology of nongonococcal urethritis, p. 19-29. *In* D. Hobson and K. K. Holmes (ed.), Nongonococcal urethritis and related infections. American Society for Microbiology, Washington, D. C.
2. **Chanock, R. M., Hayflick, L. and Barile, M. F.** 1962. Growth on artificial medium of an agent associated with atypical pneumonia and its identification as a plueropneumonia-like organism. Proc. Natl. Acad. Sci. USA. **48** : 41-49.
3. **Desai, S., Cohen, M. S., Khatamee, M. and Leiter, E.** 1980. *Ureaplasma urealyticum* (T-mycoplasma) infection: does it have a role in male infertility? J. Urol. **124** : 469-471.
4. **Dische, M. R., Quinn, P. A., Czegledy-Nagy, E. and Sturgess, J. M.** 1979. Genital mycoplasma infection. Intrauterine infection: pathogenic study of the fetus and placenta. Amer. J. Clin. Pathol. **72** : 167-174.
5. **Embree, J. E., Krause, V. W., Embil, J. A. and MacDonald, S.** 1980. Placental infection with *Mycoplasma hominis* and *Ureaplasma urealyticum*: clinical correlation. Obstet. Gynecol. **56** : 475-481.
6. **Emmems, C. W.** 1947. The motility and viability of rabbit spermatozoa at different hydrogen ion concentration. J. Physiol. (London) **106** : 471-481.
7. **Fowlkes, D. M., Dooher, G. B. and O'Leary, W. M.** 1975. Evidence by scanning electron microscopy for an association between spermatozoa and T-mycoplasmas in men of infertile marriage. Fertil. Steril. **26** : 1203-1211.
8. **Furness, G.** 1973. T-mycoplasmas: their growth and production of a toxic substance in broth. J. Infect. Dis. **127** : 9-16.
9. **Gnarpe, H. and Friberg, J.** 1972. Mycoplasma and human reproductive failure. Amer. J. Obst. Gynec. **114** : 727-731.
10. **Gnarpe, H. and Friberg, J.** 1973. T-mycoplasmas on spermatozoa and infertility. Nature **245** : 97-98.
11. **Klainer, A. S. and Pollack, J. D.** 1973. Scanning electron microscopy techniques in the study of the surface structure of mycoplasmas. Ann. N. Y. Acad. Sci. **225** : 236-245.
12. **Kundsin, R. B.** 1970. The role of mycoplasmas in human reproductive failure. Ann. N. Y. Acad. Sci. **174** : 794-797.
13. **Matthews, C. D., Elmslie, R. G., Clapp, K. H. and Svigos, J. M.** 1975. The frequency of genital mycoplasma infection in human fertility. Fertil. Steril. **26** : 988-991.
14. **McCormack, W. M., Braun, P., Lee, Y.-H., Klein, J. O. and Kass, E. H.** 1973. The genital mycoplasmas. New Engl. J. Med. **288** : 78-89.
15. **Shepard, M. C.** 1967. Cultivation and properties of T-strains of mycoplasma associated with nongonococcal urethritis. Ann. N. Y. Acad. Sci. **143** : 505-514.
16. **Shurin, P. A., Alpert, S. and Rosner, B.** 1975. Chorioamnionitis and colonization of the newborn infant with genital mycoplasmas. New Engl. J. Med. **293** : 5-8.
17. **Toth, A., Swenson, C. E. and O'Leary, W. M.** 1978. Light microscopy as an aid in predicting

ureaplasma infection in human semen. Fertil. Steril. **30** : 586-591.

18. **Yoshida, S.** 1977. Infertility and infectious diseases. Clin. Gynecol. Obstet. **31** : 867-871 (in Japanese).

19. **Yun, Y. R. and Yun, N. S.** 1983. A study of the effect of sperm motility by mycoplasma. Korean J. Urol. **24** : 255-260 (in Korean).