Nigerian Journal of Clinical Practice
June 2007. Vol 10(2):169-173

GENITAL MYCOPLASMAS IN SEMEN SAMPLES OF MALES ATTENDING A TERTIARY CARE HOSPITAL IN NIGERIA: ANY ROLE IN SPERM COUNT REDUCTION?

***N. R. Agbakoba, **A. I. Adetosoye, ***J. I. Ikechebelu.**
*Department. of *Medical Microbiology and ***Obstetrics & Gynaecology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Nnewi, Nigeria, and **Dept. of Vet. Microbiology and Parasitology, University of Ibadan, Ibadan, Nigeria.*

ABSTRACT

Semen samples from 54 married men attending the outpatient clinics for problems of infertility and routine semen analysis were examined for the presence of genital mycoplasmas. The mean age of the men was 36.1 years with a range of 25 55 years. Majority of the men 57.4% (31 of 54) were in their fourth decade of life (30 39 years). This age group also had the highest percentage 57.2% (8 of 14) of positive isolates of genital mycoplasmas on semen culture.

A total of 21 organisms obtained from 14 (26.0%) positive samples were isolated. Mycoplasma and Ureaplasma spp. separately isolated from the samples yielded frequencies of 1 (1.9%) and 6 (11.1%) respectively and the remaining 7 (13.0%) samples were infected with both organisms. A breakdown of the mycoplasma species include 5 (23.8%) *M. hominis*, 2 (9.5%) *M. fermentans* and 1 (4.8%) *M. penetrans*. Apart from one isolate of *M. hominis* other Mycoplasma species were found in association with Ureaplasma species.

Fifteen (71.4%) of the 21 isolates [8 (53.3%) ureaplasmas and 7 (46.7%) mycoplasmas] were isolated from samples with sperm counts less than 20 million/ml while the remaining 6 (21.6%) isolates [5 (83.3%) ureaplasmas and 1 (16.7) mycoplasma] were from samples with counts greater than 20 million/ml. This finding could indicate a possible influence of genital mycoplasmas especially mycoplasmas species on sperm count.

Key words: Mycoplasma species; Ureaplasma species; Semen samples. (*Accepted 13 July 2006)*

INTRODUCTION

Infection in the reproductive tract of the male has long been recognized as a definite interference with sperm quality, transport and viability[1]. Some microorganisms that cause genital tract infections like urethritis, epididymitis and prostatitis have also been implicated in seminal fluid infections. Such seminal fluid infections can thus be traced back to sexually transmitted pathogens like *Neissaria gonorrhoeae*, *Chlamydia trachomatis*, *Ureaplasma urealyticum* and *Trichomonas vaginalis*[2].

Mycoplasma infections have been known to cause reproductive problems in some mammals[3,4]. This has therefore led to the suspicion that chronic asymptomatic genital tract colonization with mycoplasma may contribute to infertility[5]. The role of genital mycoplasmas particularly Ureaplasma species in infertility has been a controversial issue for long. Despite this, some workers have been able to establish the potential role for this organism in male infertility[6-8]. This is important in our environment where male infertility asthernospermia as major factors[9].

With the increasing reports of the possible role of Ureaplasma species in infertility, this investigation was carried out to establish the prevalence of genital mycoplasmas in semen samples and what possible effect they may have on sperm count.

Correspondence: N R Agbakoba
E-mail:nnerag@yahoo.com

MATERIALS AND METHODS

Patients: These comprise 54 men attending both the infertility clinic and the General Out-patients department of Nnamdi Azikiwe University teaching hospital, Nnewi, Nigeria. Each patient collected his semen sample by masturbation into sterile universal container and brought them to the laboratory within an hour of collection. All the men were married and there ages were obtained. Information on their sexual behavior (especially number of sexual partners) was not available for analysis.

Sperm Counts: The total sperm counts of each of the samples were determined using the automated semen

Quality analyzer and bases on this; the subjects were split into 2 groups - those with counts below 20 million/ml and those with counts above 20 million/ml.

Culture: The culture media used for the cultivation of genital mycoplasma is the modification of Hayflick modified medium[10]. The substrates glucose and arginine were not incorporated into the mycoplasma media and this is to minimize the proliferation of commensal organisms that may be present in the semen. Urea was however incorporated into the ureaplasma medium. The liquid-to-solid culture technique was used whereby the semen samples were first inoculated into mycoplasma and ureaplasma broths before being subcultured onto their corresponding agar. The broth media were incubated in air at 37C and while the ureaplasma broths were incubated for 24 hours before being subcultured, the mycoplasma broths were incubated for up to 3 days before being subcultured. Incubation of the agar plates was in candle jar at 37C.

Identification: Using the dissecting microscope the ureaplasma agar plates were examined after 24/48 hours while the mycoplasma plates were examined as from the third day and daily for up to 10 days after which negative plates were discarded. Typical mycoplasma/ureaplasma colonies showing "fried-egg" appearance were noted and characterized using biochemical and serological analysis[11].

Data Analysis: The data were presented in tables and figures compared with percentages and Chi square used for test for significance.

RESULTS

The mean age of the men was 36.1 years with a range of 25 55 years. Majority of the men 57.4% (31 of 54) were in their fourth decade of life (30 39 years). This age group also had the highest percentage 57.2% (8 of 14) of positive isolates of genital mycoplasmas on semen culture.

The overall prevalence rate of genital mycoplasmas from this study is 26%. Mycoplasma and Ureaplasma species separately isolated from the samples yielded frequencies of 1(1.9%) and 6 (11.1%) respectively while the remaining 7 (13.0%) samples were infected with both organisms (Table 2).

The distribution of the genital mycoplasmas by semen count shows that 11(32.3%) of the positive samples had sperm counts less than 20 million/ml and 3 (15.0%) had counts greater than 20 million/ml ($p < 0.05$). A breakdown of the 21 isolates into their various species is as shown in Table 3.

Ureaplasma species were more predominant With 13 (61.9%) isolates and is followed by *M. hominis, M. Fermentans* and *M. penetrans* with 5 (23.8%), 2 (9.5%) and 1 (4.8%) prevalence rates respectively. It further shows the distribution of the Ureaplasma species and different species of mycoplasma according to the sperm counts of the patients. Of the 21 isolates, 15 (71.4%) were found in samples with counts less than 20 million/ml and 6 (28.6%) in samples with counts greater than 20 million/ml.

Table 1: **Age distribution of the according to the sperm counts and presence of genital mycoplasmas.**

Age Total	*TSC<20x10⁶		*TSC<20x10⁶	
	Positive Isolate	Negative Isolate	Positive Isolate	Negative Isolate
25-29	1(9.1%)	5(21.7%)	1(33.3%)	2(11.8%) 9(16.7%)
30-34	2(18.2%)	5(21.7%)	0(0.0%)	7(41.2%) 14(25.9%)
35-39	5(45.5%)	8(34.8%)	1(33.3%)	3(17.6%) 17(31.5%)
40-44	3(27.3%)	2(8.7%)	0(0.0%)	3(17.6%) 8(14.8%)
45-49	0(0.0%)	2(8.7%)	1(33.3%)	1(5.9%) 4(7.4%)
50and above	0(0.0%)	1(4.4%)	0(0.0%)	1(5.9%) 2(3.7%)
Total	**1(32.4%)**	**23(67.6%)**	**3(15.0%)**	**17(85.0%) 54(100%)**
		34(63.0%)		**20(37.0%)**

*TSC = Total sperm count (cells/ml)

Table 2: **Overall prevalence of the genital mycoplamas and their distribution according to the sperm counts. (=54)**

N

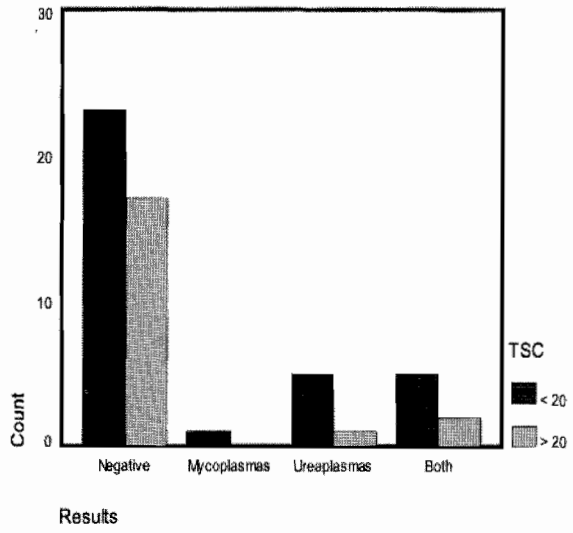
Organisms	Total sperm count (cells/ml)		Total
	$<20x10^6$	$>20x10^6$	
Mycoplasma spp.alone	1(2.9%)	0(0.0%)	1(1.9%)
Ureaplasma spp. alone	5(14.7%)	1(5.0%)	6(11.1%)
Both species	5(14.7%)	2(10.0%)	7(13.0%)
Negative	23(67.7%)	17(83.0%)	40(74.0%)
Total	**34(63.0%)**	**20(37.05**	**54(100.0%)**

$X^2 = 2.383; df = 3; p < 0.05$

Table 3: **Breakdown of the total isolates into different species and their distribution according to the sperm count N=21.**

Organisms	No.isolated	Total sperm count (cells/ml)	
		$<20x10^6$	$>20x10^6$
Mycoplasma hominis	5(23.8%)	4(26.7%)	1(16.7%)
Mycoplasma fermentans	(4.5%)	2(13.3%)	0(0.0%)
Mycoplasma penetrans	1(4.8%)	1(6.7%)	0(0.0%)
Ureaplasma spp	13(61.9%)	8(53.3%	5(83.3%)
Total	**21*(100.0%)**	**15(71.4%)**	**6(28.6%)**

*More than one species was isolated from some



DISCUSSION

The ureaplasma isolates in this study are referred to as Ureaplasma species. Recent studies have separated the only human ureaplasma, *U. urealyticum,* into 2 species -*U. parvum* and *U. urealyticum* [12]. Separation was only possible by molecular studies but these were not available at the time of this study. Waites[13] reported that separating infections caused by the 2 species of Ureaplasma is not possible or necessary and so they are considered clinically and in the diagnostic laboratory as Ureaplasma species. The mycoplasma isolates were however characterized[11] and three separate species were seen in this study. Both the ureaplasma and mycoplasma isolates found in urogenital tracts are generally referred to as 'genital mycoplasmas'.

Fourteen (26.0%) of the 54 semen samples were positive for genital mycoplasmas and a total of 21 organisms were isolated from them. The prevalence of the Ureaplasma and Mycoplasma species are 13 (27.8%) and 8 (14.8%) respectively. These values were comparable with those obtained by Hill[14] who reported 29.0% for *U.urealyticum* and 12.0% for *M. hominis*; and those of AndradeRocha[15] who reported 28.2% for *U. urealyticum* and 24.8% for *M. hominis* for one study group and 20.5% for *U. urealyticum* and 13.3% for *M. hominis* in another study group. In this investigation, mycoplasma species were found predominantly in association with ureaplasma species (87.5%). This finding supports that of Ladipo and Osoba[6] whose mycoplasma isolates from semen samples were found only in association with ureaplasmas. The significance of this finding is Uncertain. However, most of the genital mycoplasmas were isolated from patients with semen counts less than 20 million/ml and in their thirties. This is the age of maximal sexual activity and experimentation in men. History of the number of sexual partners of these men may have shade more light on the source of the infection but was not obtained in the course of the study.

Counts less than 20 million/ml and in their thirties. This is the age of maximal sexual activity and experimentation in men. History of the number of sexual partners of these men may have shade more light on the source of the infection but was not obtained in the course of the study.

Unlike the investigators that isolated only *M. hominis* from their studies, other Mycoplasma species apart from *M. hominis* were isolated in this study and they include *M. fermentans* and *M. penetrans.* Though with low prevalence, both species were isolated from semen samples with counts less than 20 million cells per ml. These 2 species of mycoplasmas are fastidious and have been reported to be difficult to isolate culturally[16]. This fact might have been responsible for their low isolation rates. Their role in semen bacteriology needs further investigation especially as several workers have isolated them from the urine and urethral samples of HIV positive patients[17-19]. *M. hominis* was more prevalent (23.8%) than the other two species and this is because they are less fastidious and easier to grow culturally. They have been reported to grow on ordinary blood agar and on some routine blood culture media[20].

The low isolation rate of genital mycoplasmas (26.0%) obtained in this study notwithstanding; they were isolated more from patients with counts less than 20 million per ml as against those with counts greater than 20 million per ml. Bacterial infections are frequently found in semen samples from asymptomatic infertile patients than those from fertile men[21]. In their own study, Kerc et al[22] reported that infectious processes might lead to deterioration of spermatogenesis, impairment of sperm function and/or obstruction of the seminal tract. The finding in this investigation could therefore indicate a possible influence of genital mycoplasma on sperm count. However, the presence of these organisms in samples with counts greater than 20 million/ml may be as mere colonizers of the genital tract and thus may not be pathogenic as to have effect on the sperm count. Shepard and Lunceford[23] reported that not all serotypes of ureaplasmas are pathogenic while Kerc et al[22] also reported that detection of bacteria in semen does not necessarily signify infection since bacteriospermia may represent contamination,

colonization or infection. It is significant to note that mycoplasma species were more prevalent in samples with counts less than 20million/ml (oligospermia) than ureaplasma species. This indicates that mycoplasma species rather than ureaplasmas has greater influence on sperm count.

In conclusion, the prevalence of genital mycoplasmas in the study population was low. Their presence in oligospermic samples was significantly higher than in those with normal sperm count especially tory role for them in sperm reduction possible. It is therefore necessary to study the effect of these organisms on semen quality in order to establish a firm role for them in sperm reduction.
For mycoplasma species thus making a contributory role for them in sperm reduction possible. It is therefore necessary to study the effect of these organisms on semen quality in order to establish a firm role for them in sperm reduction.

ACKNOWLEDGEMENTS

We thank Prof. O.O. Mbonu of the Dept. of Surgery, Nnamdi Azikiwe University, Nnewi, for reading this manuscript and the Medical Laboratory Scientists of the Dept. of Medical Microbiology Nnamdi Azikiwe University Teaching Hospital, Nnewi, who assisted in the semen collection.

REFERENCES

1. **Dalberg B**. Asymptomatic Bacteriospermia:Cause of infertility in men. Urology. 1976: 8: 563-566.they were isolated more from patients with counts

2. **Ludwig M, Kummel C, Diemer T, Ringert RH**. Infections of ejaculate by sexually Rtransmissible pathogens. Urologe A. 1994: 33: 203 - 210.

3. **Chima JC, Ojo MO, Molokwu TU, Okewole PA**. Characterization of mycoplasmas isolated from genital tract infections of sheep in Nigeria. Review of Science Technology. 1995: 14: 865-871.

4 **Spergser J, Aurich C, Aurich J, Rosengarten R**. High prevalence of mycoplasmas in the genital tract of asymptomatic stallions in Austria. Veterinary. Microbiology 2002: 87: 119-129.

5 **Abdulrazzak AA, Bakr SS.** Role of Mycoplasma in male Infertility. Eastern Mediterranean Health Journal. 2000: 6: 1 - 6.

Screening for *Cylamydia trachomatis* and Ureaplasma *urealyticum* infection in semen from asymptomatic male partners of infertile couples prior to in vitro fertilization. International Journal of Andrology. 1999: 22:113 118.

6 **Ladipo OM, Osoba AO**. Ureaplasma urealyticum (T-mycoplasma) and male infertility in tropical countries. African Journal of Medical Sciences. 1978: 7:187-190.

7 **Xu C, Sun GF, Zhu Wang YF**. The correlation of Ureaplasma urealyticum infection with infertility. Andrologia. 1997: 29:219-226.

8 **Levy RR, Layani-Milon MP, Giscard D'Estaing S, Najioullah F, Lornage J, Aymard M, Lina B**. g for *Cylamydia trachomatis* and Ureaplasma *urealyticum* infection in semen from asymptomatic male partners of infertile couples prior to in vitro fertilization. International Journal of Andrology. 1999: 22:113 118.

9 **Ikechebelu JI, Adinma JIB, Orie EF, Ikegwuonu SO**. High prevalence of male infertility in southeastern Nigeria. Journal of Obstetrics and Gynaecology 2003: 23 (6): 657 659.

10 **Freundt EA**. Culture media and classic mycoplasmas. *In*: Methods in Mycoplasmology Vol.1 Mycoplasma Characterization. (Razin, S. and Tully, J.G.). Academic Press New York USA. 1983: Pp 127-135.

11 **Agbakoba NR, Adetosoye AI, Adewole IF.** Biochemical and Serological identification of Mycoplasma species from the genital tracts of humans in Nigeria. African Journal of Medicine and Medical Science. 2006. (In Press)

12 **Robertson JA, Stemke GW, Davis JW Jr, Harasawa R, Thirkell D, Kong F, Shepard MC, Ford DK.** Proposal of *Ureaplasma parvum* sp. nov. and emended describtion of *Ureaplasma urealyticum* (Shepard et al. 1974) Robertson et al. 2001. International Journal of Systematic and Evolutionary Microbiology. 2002: 52: 587-597.

13 **Waites KB**. Ureaplasma infection. Retrieved 6 October,2004from http://www.emedicine.com/Med/topic 2340.htm

Disease .Journal of clinical Laboratory Analysis 2000: 14 246-255.

14. **Hill AC**. Mycoplasmas, a review of surveys examining human genital infections and experimental infections in mice with special reference to in vitro fertilization. Lijec Vjesn. 1990. 112 (11-12): 358-360.

15 **Andrade Rocha FT.** *Ureaplasma urealyticum* and *Mycoplasma hominis* in men attending for routine semen analysis. Prevalence, incidence by age and clinical settings, influence on sperm characteristics, relationship with the leucocyte count and clinical value. Urology International. 2003: 71 (4): 377-381.

16. **Waites KB, Beaber CM, Robertson JA, Takington DF, Kenny GE.** Cumitech 34, Laboratory diagnosis of mycoplasmal infections. Coordinating ed., F.S. Nolte. American Society for Microbiology, Washington, D.C. 2001

17 **Grau O, Slizeweez B, Tuppin P, Launay V, Bourgeois E, Sagot N.** Association of Mycoplasama penetranas with human immunodeficiency virus infection.Journal of Clinical Microbiology.1995:172:43-45

18. **Kovacic R, Launay V, Tuppin P, Lafeuillade A, Feuillie V, Montagnier L, Grau O.** Search for the presence of six Mycoplasma species in peripheral blood mononuclear cells of subjects sero-positive and sero-negative for human immunodeficiency virus. Journal of Clinical Microbiology. 1996:34: 1808-1810.

19 **Cardova CM,Blanchard A, Cuntia RA.** Higher prevalence of urogenial mycoplasma in human immunodeficiency virus-positive patients as compared patients with other sexually transmitted diseases. Journal of Clinical Laboratory Analysis. 2000:14:246-253

20 **Taylor-Robinson D, McCormack W.M.** The genital mycoplasmas. The New England Journal of Medicine. 1980b: 302: 1063-1067.

21 **Kohn FM, Erdmann I, OedaT, el Mulla KF, Schiefer HG, Schill WB**. Influence of urogenital infections on sperm functions. Andrologia. 1998: 30: 73-80.

22 **Kerc C, Gerber-Schafer C, Clad A, Wilhelm C, Breckwoldt M.**_Seminal tract infections: impact on male fertility and treatment options. Human Reproduction Update. 1998: 4 (6): 891-903.

23 **Shepard MC, Lunceford CD.** Serological Typing of *Ureaplasma urealyticum* isolates from urethritis patients by agar growth inhibition method. Journal of Clinical Microbiology. 1978: 8: 566-574.