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Detection of *Ureaplasma urealyticum* in Semen of Infertile Men by PCR

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Abstract: *Ureaplasma urealyticum* is a causative agent of non-gonococcal urethritis, prostatitis, epididymitis and infertility. The organism is more common in partners of infertile than fertile marriages. *U. urealyticum* infections not only jeopardize fertility but also pose a risk for infertility treatment and resulting pregnancies. The purpose of this study was to determine the prevalence of *U. urealyticum* in semen of infertile and healthy men by Polymerase Chain Reaction (PCR). Semen samples were obtained from infertile patients and healthy control and were subjected to the routine andrological analysis and PCR. DNA was extracted by Cadieux method and analyzed by PCR protocol with species-specific primers for *U. urealyticum* (urease gene). *U. urealyticum* was detected significantly by PCR in 12 of 100 (12%) semen specimens from infertile patients and in 3 of 100 (3%) healthy men. The volume of semen fluid, concentration of sperm cells and sperm cell with normal morphology were significantly decreased in infertile men. In the group of infertile patients with PCR positive for *U. urealyticum* the volume, count and morphology of semen samples were lower than in the infertile patients with PCR negative results.

Key words: *Ureaplasma urealyticum*, semen, infertility, sperm, PCR

INTRODUCTION

Mycoplasmas as a group are the smallest recognizable free-living bacteria. Their genomes are approximately 500-800 kbp in contrast with the genome of *E. coli*, which is approximately 4600 kbp. In addition to their small size, these bacteria lack cell walls and are hence deformable. Mycoplasmas are widespread in nature as parasites of humans, mammals, reptiles, fish, arthropods and plants. The primary habitats of human mycoplasmas are the mucosal surfaces of the respiratory and urogenital tracts (Razin *et al*., 1998).

The genus ureaplasma, a genus within the family Mycoplasmataceae, class Mollicutes, has been defined by its ability to hydrolyze urea. Subdivisions within this genus have been based largely on the host species and antigenic heterogeneity. *Ureaplasma urealyticum* is the designation for all ureaplasmas isolated from humans (Harassawa *et al*., 1991; Roberston *et al*., 1993).

U. urealyticum was discovered in 1954 when it was isolated from the urethra of men with non-gonococcal urethritis (Shepared *et al*., 1974). In men, it has been implicated as the causative organisms in cases of urethritis, prostatitis, epididymitis and infertility (Salan and Kanmi, 2003; Badalyan *et al*., 2003; Jalil *et al*., 1988; Gnarpe and Friberrgy, 1972). In women, an increased incidence of obstetrical and postpartum complications has been associated with *U. urealyticum* (Chua *et al*., 1998; Abele-Horn *et al*., 1996; Andrew *et al*., 1995; Yoon *et al*., 2000).

The role of *U. urealyticum* in male infertility has been controversial. Some investigators (Fenkci *et al*., 2002; Kanokas *et al*., 1999; Schleger *et al*., 1991) have failed to show any significant difference in the fertilizing ability of the sperm with a positive culture or any influence on pregnancy rates or outcome. Other investigators believe that *U. urealyticum* infection may alter various parameters of the semen, such as concentration, motility and morphology, thereby influencing fertilization or pregnancy rates (Nunez-Calonge *et al*., 1998; Malka *et al*., 2000; Shalika *et al*., 1996; Xu *et al*., 1997).

The main method of detecting *U. urealyticum* is by culture, but the organism is difficult to isolate and requires special culture media. Polymerase Chain Reaction (PCR) is revolutionizing the diagnosis of many infectious diseases, particularly those caused by organisms that are difficult to cultivate. However there are only a few reports of the detection of *U. urealyticum* by PCR. Thus, the aims of this study were to determine the prevalence of

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U. urealyticum infection in infertile men and healthy controls by PCR and to analyze the influence of *U. urealyticum* on semen quality.

MATERIALS AND METHODS

Patients: Semen samples were obtained from infertile patients (n = 100) and healthy control (n = 100). After liquefaction at room temperature, semen samples were subjected to the routine andrological analysis (semen volume, sperm density, motility and morphology) and for Polymerase Chain Reaction (PCR) for *U. urealyticum*.

PCR: For PCR, samples were prepared as previously described (Cadieux *et al*., 1993). Briefly, 1 mL of each sample was centrifuged at 12000 x g for 10 min. The pellet washed in PBS and resuspended in 30 μL of distilled water. After boiling for 10 min, an aliquot of 7 μL was used directly in PCR experiments. The primers published by Blanchard *et al*. (1993) were used for identification of *U. urealyticum*: Primers U5 (5'-CAATCTGCTCGTGAAGTATTAC-3) and U4 (5'-ACGACGTCCATAAGCA ACT-3). The PCR assay was performed in 50 μL of reaction mixture containing 10 μL of 10 × PCR buffer, 2.5 mM $MgCl_2$, 200 μM dNTP, 1.25 units of Taq polymerase, 20 pmol of each primer and 7 μL of sample DNA. The reaction mixtures were placed in thermal cycler (Eppendorf, USA). The thermal profile involved an initial denaturation step at 94°C for 3 min followed by 30 cycles of denaturation at 94°C for 1 min, primer annealing at 52°C for 1 min and primer elongation at 72°C for 1 min. The cycling was followed by a final extension step at 72°C for 10 min. Aliquots of amplified samples (10 μL) were analyzed by electrophoresis on a 1% agarose gel stained with ethidium bromide.

Statistical analysis: Chi-square (χ^2) test was used for the generation of $p<0.05$ values.

RESULTS

PCR results: *U. urealyticum* was detected by PCR in 12 of 100 (12%) semen specimens from infertile patients and in 3 of 100 (3%) healthy men. There were significant differences in *U. urealyticum* loads between infertile patients and healthy men ($p<0.05$). A photograph of electrophoresis based on bromide-stained agarose gel for PCR-amplified products from the ureaplasma strains is presented in Fig. 1. A 429 bp fragment of the urease gene was amplified for identification of *U. urealyticum*. They have been shown previously to be highly specific for

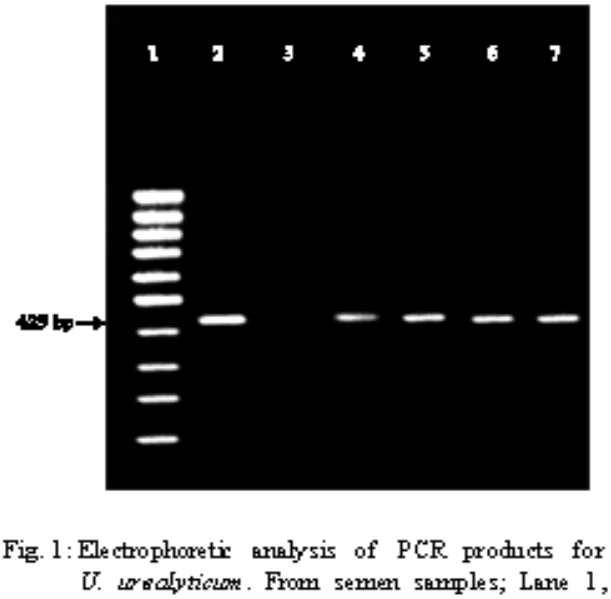

Fig. 1: Electrophoretic analysis of PCR products for *U. urealyticum*. From semen samples; Lane 1, 100 bp size marker: Lane 2, standard strain (429 bp); lane 3 negative control (distilled water), lane 4,5,6,7) positive patient samples

U. urealyticum and under optimal conditions, to allow detection of <10 CFU of each serotype the organism (Blanchard *et al*., 1993).

Semen parameters: Volume of semen samples in infertile patients with PCR positive for *U. urealyticum* was significantly lower than in healthy men ($p<0.001$, Table 1). The percentage of sperm cells with motility in both of infertile patients was significantly different from the values observed in healthy controls ($p<0.001$, Table 1). The concentration of sperm cells was significantly diminished in both of infertile men ($p<0.001$, Table 1). In the group of infertile patients with PCR positive for *U. urealyticum* the concentration of sperm cells was also diminished, although it was not statistically significant. The percentage of abnormal sperm cells was increased in both of infertile patients in comparison to healthy men and this difference was found to be statistically significant ($p<0.001$, Table 1).

Table 1: Seminological analysis from infertile patients and healthy men

	Infertile patients		Healthy men
PCR Semen parameters	PCR positive	PCR negative	Parameters of semen
Volume (mL)	2.30±0.40**	3.09±0.19	3.47±0.14
Motility (%)	17.99±4.37*	17.86±1.58*	39.01±0.39
Sperm count (1×10^6/mL)	29.83±19.61*	36.15±6.50*	92.50±6.62
Morphology (%)	8.33±1.88*	8.64±0.72*	40.48±0.41

*$p<0.001$; **$p<0.05$

Table 2: Detection of *U. urealyticum* from patients according to age

		Age			
Groups	PCR results	21-30	31-40	41-51	Total
Healthy men	PCR negative	35	52	10	97
	PCR positive	2	1	0	3
Infertile men	PCR negative	30	46	12	88
	PCR positive	4	6	2	12

The age of the patients from who were PCR positive varied from 21- 41 years. Distribution of the *U. urealyticum* in accordance to patient's age is presented in Table 2. No significant difference was found between the age of patients whose sample was PCR positive (positive group) and that of the other men (negative group).

DISCUSSION

The relationship among *U. urealyticum* infection and infertility is unclear. However, the prevalence of *U. urealyticum* is higher among infertile (42 to 95%) than fertile (23 to 26%) couples (Potts *et al*., 2000; Fowlkes *et al*., 1975). Several abnormal semen characteristics have been observed in the presence of *U. urealyticum*, such as midpiece tail defect, agglutination and impaired oocyte penetrations (Malka *et al*., 2000; Kalugan *et al*., 1996). Because the organism lacks a cell wall, it can adhere to the sperm membrane, thereby potentially causing the gamete dysfunction (Fowlkes *et al*., 1975). Herein we have shown that *U. urealyticum* infection in infertile patients was significantly higher than in healthy men ($p<0.05$) and semen quality (volume, count, motility and morphology) was significantly affected in infertile men. In this study we have also shown that the volume, count and normal morphology of semen samples in infertile patients with PCR positive for *U. urealyticum* were lower than in the infertile patients with PCR negative results, although they were not statistically significant.

Adherence of *U. urealyticum* to the sperm membrane may also enhance the adverse effects of superoxide and hydrogen peroxide produced by the organism, with subsequent spermatozoan hyper production of Reactive Oxygen Species (ROS) (Meier *et al*., 1990). Potts *et al*. (2000) reported that the seminal ROS are elevated among patients with *U. urealyticum*. These investigators suggested that the ROS induces lipid peroxidation, which reduces membrane fluidity and sperm fertilization capability and may be the mechanism by which *U. urealyticum* impairs sperm function.

Although the precise role of *U. urealyticum* in human infertility has not firmly established, there is strong support in the literature for its causal role in several sexually transmitted diseases, reproductive failure and neonatal morbidity and mortality (Gnarpe *et al*., 1972; Chua *et al*., 1998; Abele-Horn *et al*., 1996; Andrew *et al*., 1995; Yoon *et al*., 2000; Nunez-Calonge *et al*., 1998; Blanchard *et al*., 1993). Thus, the identification and treating *U. urealyticum* in infertile couples could be important and necessary. PCR is a more sensitive and reliable means of detecting *U. urealyticum* in the clinical specimens; its results can be available within a day, compared with 2-5 days for culture.

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