

## Association of *Ureaplasma urealyticum* infection with Varicocele-related infertility

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### Abstract

**Background:** The role of seminal colonization of *Ureaplasma urealyticum* in varicocele-related infertility was investigated. **Methodology:** Semen samples were obtained from infertile patients with or without varicocele and healthy controls and were subjected to routine semen analysis and PCR. DNA was extracted by Cadieux method and analyzed by PCR protocol with species-specific primers for *U. urealyticum* (urease gene).

**Results:** *U. urealyticum* was detected by PCR in 23 of 146 (15.75%) semen specimens from infertile patients and in 3 of 100 (3%) healthy men ( $P < 0.001$ ). Infertile patients with varicocele had higher *U. urealyticum* colonization [17/81 (20.98%)] than those without varicocele [6/65 (9.23%),  $P = 0.086$ ] or healthy controls [3/100 (3%),  $P < 0.001$ ]. The percentage of sperm cells with motility, volume of semen fluid, concentration of sperm cells, and sperm cell with normal morphology were significantly decreased in infertile men ( $P < 0.001$ ). In the group of varicocele patients with PCR positive for *U. urealyticum* the volume, count and morphology of semen samples were lower than those in the varicocele patients with PCR negative results, but the differences were not significant ( $P > 0.05$ ).

**Conclusion:** Although the colonization of *U. urealyticum* does not affect the semen quality, the high prevalence of this microorganism in varicocele patients may be an additional negative factor affecting varicocele status and worsening reproductive potential.

**Key Words:** *Ureaplasma urealyticum*, infertility, varicocele, semen, PCR, spermatozoa.

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### Introduction

Varicocele is a physical abnormality present in 2% to 22% of the adult male population [1]. It is a collection of enlarged veins in the scrotum. A varicocele occurs next to and above one or both of the testicles. It is more common in men of infertile marriages, with a prevalence rate of about 15% in healthy men and 40% in men being treated in infertility clinics [2]. The exact association between reduced male fertility and varicocele is unknown, but analysis of World Health Organization (WHO) data clearly indicates that varicocele is related to semen abnormalities, decreased testicular volume, and decline in Leydig's cell function [3].

Genital tract infection is the most important cause of male infertility affecting not only sperm cell function, but also the whole spermatogenesis [4,5]. *Ureaplasma urealyticum* is a self-replicating prokaryote belonging to the taxonomic class Mollicutes, which lack a cell wall. Genital *U.*

*urealyticum* colonization has been found to be involved in non-gonococcal urethritis (NGU), prostatitis, epididymitis and infertility [6,7]. Some investigators reported that the presence of *U. urealyticum* in semen was related to a decrease in sperm density, motility and morphology [8,9,10,11]. Since male fertility and semen quality might likely be affected by both varicocele status and seminal colonization of *Ureaplasma urealyticum*, we decided to determine the prevalence of this microorganism in infertile patients with and without varicocele compared with healthy men and to investigate their negative effects on semen parameters.

### Materials and Methods

**Patients:** Data included in this study were selected from men who were consecutively admitted to the Royan Fertility Center, Tehran, Iran. The Investigation Committee of the Royan

Institute approved the study protocol, and all participants provided informed consent. All patients were examined clinically, and medical, sexual, and social histories were obtained before entrance into the program. From February to June 2005, 146 semen samples (81 samples from varicocele patients and 65 samples from individuals without varicocele) were taken from patients aged 21 to 50 (34.3±4.2) years with infertility of at least one-year duration.

Control subjects (n=100) were men aged 20 to 40 (31.2±4.1) years, who were attending for check-up, who were clinically asymptomatic (they were fertile and did not have varicocele). All semen specimens were obtained after 2 to 3 days' abstinence. After liquefaction at room temperature, semen samples were subjected to routine semen analysis (semen volume, sperm concentration, motility and morphology) [12] and for polymerase chain reaction (PCR) for *U. urealyticum* [13].

PCR: for PCR, samples were prepared as previously described [13]. Briefly, 1 ml of each sample was centrifuged at 12000 × g for 10 minutes. The pellet was washed in PBS and resuspended in 30µl of distilled water. After boiling for 10 minutes, an aliquot of 7µl was used directly in PCR experiments.

The primers published by Blanchard *et al.* [14] were used for identification of *U. urealyticum*: primers U5 (5'-CAATCTGCTCGTGAAGTATTAC-3') and U4 (5'-ACGACGTCCATAAGCA ACT-3'). The PCR assay was performed in 50µl of reaction mixture containing 10µl of 10× PCR buffer; 2.5 mM MgCl<sub>2</sub>; 200µM dNTP; 1.25 units of Taq polymerase; 20pmol of each primer; and 7µl of sample DNA. The reaction mixtures were placed in a thermal cycler (Eppendorf, USA). The thermal profile involved an initial denaturation step at 94°C for 3 minutes followed by 30 cycles of denaturation at 94°C for 1 minute, primer annealing at 52°C for 1 minute, and primer elongation at 72°C for 1 minute. The cycling was followed by a final extension step at 72°C for 10 minutes. Aliquots of amplified samples (10µl) were analyzed by electrophoresis on a 1% agarose gel and stained with ethidium bromide.

Statistical analysis: Results are presented as mean values with standard deviation (SD). The statistical significance was assessed using Chi-Square (X<sup>2</sup>) test and Mann-Whitney test.

## Results

PCR results: *U. urealyticum* was detected by PCR in 23 of 146 (15.75%) semen specimens from infertile patients and in 3 of 100 (3%) healthy men (P<0.001).

As shown in Table 1, in the group of infertile patients, the prevalence of *U. urealyticum* in patients with varicocele was higher [17 of 81 (20.98%)] than in those without varicocele [6 of 65 (9.23%)], although this difference was not significant (P=0.086). The *U. urealyticum* was detected in both groups of infertile patients higher than that of the healthy controls, but in the group of varicocele patients, this difference was found to be statistically significant (P<0.001).

**Table 1.** Detection of *U. urealyticum* from infertile patients and healthy men by PCR.

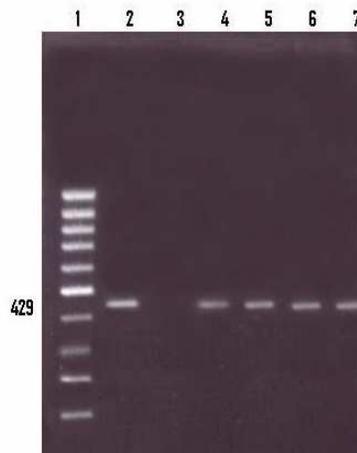
	Patients with Varicocele	Patients without Varicocele	Healthy men
No. of sample	81	65	100
No. of PCR positive	17(20.98%)*	6(9.23%)	3(3%)

\*P<0.001.

A photograph of electrophoresis based on bromide-stained agarose gel for PCR-amplified products from the *Ureaplasma* strains is presented in figure 1. A 429bp fragment of the urease gene was amplified for identification of *U. urealyticum*. They have been shown previously to be highly specific for *U. urealyticum* and under optimal conditions, to allow detection of <10CFU of each serotype of the organism [14].

**Figure 1.** Electrophoretic analysis of PCR products for *U. urealyticum* from semen samples.

Lane 1, 100bp size marker; lane 2, standard strain (429bp); lane 3 negative control (distilled water); lane 4, 5, 6, 7) positive patient samples.



Semen parameters: Volume of semen, percentage of sperm cells with motility, concentration of sperm cells and percentage of normal sperm cells in infertile patients (with or without varicocele and *U. urealyticum*) were significantly lower than those in healthy men (Table 2,  $P < 0.001$ ).

**Table 2.** Seminological analysis from infertile patients and healthy men.

Patients with Varicocele		
Semen parameters	PCR	
	PCR positive	PCR negative
Volume (ml)	2.56±0.70	3.47±0.70
Motility (%)	24.16±7.64	20.57±3.22
Sperm count ( $1 \times 10^6$ /ml)	13.43±10.55	29.12±6.56
Morphology (%)	8.40±3.55	9.26±1.48
Patients without Varicocele		
Semen parameters	PCR	
	PCR positive	PCR negative
Volume (ml)	2.05±0.61	2.98±0.24
Motility (%)	10.83±4.90	17.46±1.87
Sperm count ( $1 \times 10^6$ /ml)	6.0±3.26	38.21±9.08
Morphology (%)	5.17±2.37	8.31±0.38
Healthy men		
Semen parameters	PCR	
	Total	
Volume (ml)	3.47±0.14	
Motility (%)	39.01±0.39	
Sperm count ( $1 \times 10^6$ /ml)	92.50±6.62	
Morphology (%)	40.48±0.41	

In the group of infertile-varicocele patients with PCR positive for *U. urealyticum*, the concentration of sperm cells, volume of semen, and percentage of normal sperm cells were lower than those in infertile-varicocele patients with PCR negative results, although they were not statistically significant (Table 2).

The percentage of normal sperm cells, volume of semen, percentage of sperm cells with motility and concentration of sperm cells in the group of infertile patients without varicocele, but with PCR positive for *U. urealyticum* were lower than those in patients with PCR negative results, although these differences were not found to be statistically significant (Table 2).

## Discussion

Many studies have shown that there is a higher rate of infertility in men with varicocele compared to those who did not have a varicocele. Varicocele

is thought to cause spermatogenic defects by raising the intratesticular temperature in both the affected and contralateral testes [15]. On the other hand, most studies have compared the incidence of *U. urealyticum* in semen with semen parameters and the implication of fertility treatment [8-11,16,17,18]. These findings raise a major question: Is there any role for *U. urealyticum* in pathophysiology of varicocele-induced damage? *U. urealyticum* has been found to be involved in prostatitis, epididymitis, and infertility [6-10]. The mechanisms by which *U. urealyticum* affects sperm quality has not been elucidated. Some investigators reported that the presence of *U. urealyticum* in semen was related to a decrease in semen volume, count, motility, and morphology [16-19], but the others were unable to correlate the presence of *U. urealyticum* with any alteration in semen characteristics [20,21]. Herein we have shown that *U. urealyticum* colonization in varicocele patients was significantly higher than that in healthy men ( $P < 0.001$ ). In this study we have also shown that the prevalence of *U. urealyticum* in patients with varicocele was higher than in those without varicocele, although this difference was not significant ( $P = 0.086$ ). The volume, count and normal morphology of semen samples in varicocele patients with PCR positive for *U. urealyticum* were lower than those in the varicocele patients with PCR negative results, although they were not statistically significant. These results indicate that *U. urealyticum* colonization may be an additional negative factor influencing varicocele status and worsening the reproductive potential. However, although our study demonstrated a significant increase of seminal colonization of *U. urealyticum* in infertile men with varicocele, we did not find that it caused adverse effects on semen quality.

*U. urealyticum* lacks a cell wall; it can adhere to the sperm membrane, thereby potentially causing gamete dysfunction [22]. 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) [23]. Potts, et al. [11] 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.

In conclusion, this study has shown a high prevalence of *Ureaplasma urealyticum* in varicocele patients. Although this colonization apparently does not cause adverse effects on semen quality, further studies are necessary to investigate the potential pathophysiological role of *Ureaplasma urealyticum* in varicocele..

## References

- Kursh ED (1987) What is the incidence of varicocele in a fertile population? *Fertil Steril* 48:510-511.
- Chehval MJ, Purcell MH (1992) Deterioration of semen parameters over time in men with untreated varicocele: evidence of progressive testicular damage. *Fertil Steril* 57:174-77.
- World Health Organization (1992) The influence of varicocele on parameters of fertility in a large group of men presenting to infertility clinics. *Fertil Steril* 57: 1289-93.
- Henkel R, Schill WB (1998) Sperm separation in patients with urogenital infections. *Andrologia* 30:91-97.
- Sanocka D, Fręczek M, Jedrzejczak P, Szumala-Kakol A, Kurpisz M (2004) Male genital tract infection: an influence of leukocytes and bacteria on semen. *J Reprod Immunol* 62:111-24.
- Badalyan RR, Fanariyan SV, Aghajanyan IG (2003) Chlamydial and ureaplasma infections in patients with nonbacterial chronic prostatitis. *Andrologia* 35:263-5.
- Kilic D, Basar MM, Kaygusuz S, Yilmaz E, Basar H, Batislam E (2004) Prevalence and treatment of Chlamydia trachomatis, *Ureaplasma urealyticum*, and *Mycoplasma hominis* in patients with Non-Gonococcal Urethritis. *Jpn J Infect Dis* 57:17-20.
- Han XD, Wang Y, Chen JX (2003) A comparative study on interrelations among microelements, infection of *Ureaplasma urealyticum*, and male infertility. *Arch Androl* 49:265-9.
- Shalika S, Dugan K, Smith RD, Padilla SL (1996) The effect of positive semen bacterial and *Ureaplasma* cultures on *in vitro* fertilization success. *Hum Reprod* 11:2789-92.
- Potts JM, Sharma R, Pasqualotto F, Nelson D, Hall G, Agarwal A (2000) Association of *Ureaplasma urealyticum* with abnormal reactive oxygen species levels and absence of leukocytospermia. *J Urol* 163:1775-78.
- Reichert M, Levi H, Kahane I, Bartoov B (2001) Dual energy metabolism-dependent effect of *Ureaplasma urealyticum* infection on sperm activity. *J Androl* 22:404-12.
- World Health organization (1992) Laboratory manual for the examination of human semen and semen-cervical mucus interaction. 3rd ed. New York: Cambridge University Press.
- Cadieux N, Lebel P, Brousseau R (1993) Use of a triplex polymerase chain reaction for the detection and differentiation of *Mycoplasma pneumoniae* and *Mycoplasma genitalium* in the presence of human DNA. *J Gen Microbiol* 139:2431-2437.
- Blanchard A, Henstehel J, Duffy L, Baldus K, Cassell GH (1993) Detection of *Ureaplasma urealyticum* by polymerase chain reaction in the urogenital tract of adults, in amniotic fluid, and in the respiratory tract of newborns. *Clin Infect Dis* 17(suppl 1):S 148-53.
- Sandlow JL (2004) Do varicoceles really affect male fertility? *Sexuality, Reproduction & Menopause* 2:219-21.
- Malka R, Kahane I and Bartov B (2000) *In vitro* and *In vivo* impairment of human and Ram sperm nuclear chromatin integrity by sexually transmitted *Ureaplasma urealyticum* infection. *Biol reprod* 63: 1041-1048.
- Nunez-Calonge R, Caballero P, Redondo C, Baquero F, Martinez-Ferrer M, Meseguer MA (1998) *Ureaplasma urealyticum* reduces motility and induces membrane alterations in human spermatozoa. *Hum Reprod* 13:2756-61.
- Kohn FM, Erdmann I, Oeda T, El Mulla KF, Sehiefer HG, Sehill WB (1998) Influence of urogenital infections on sperm functions. *Andrologia* 30(Suppl 1): 73-80.
- Xu C, Sun GF, Zhu YF, Wang YF (1997) The correlation of *Ureaplasma urealyticum* infection with infertility. *Andrologia* 29:219-26.
- Upadhyaya M, Hibbard BM, Walker SM (1984) The effect of *Ureaplasma urealyticum* on semen characteristics. *Fertil Steril* 41:304-308
- Andrade-Rocha FT (2003) *Ureaplasma urealyticum* and *Mycoplasma hominis* in men attending for routine semen analysis. Prevalence, incidence by age and clinical setting, influence on sperm characteristics, relationship with the leukocyte count and clinical value. *Urol Int* 71:377-81.
- Fowlkes DM, Doohar GB, O'Leary WM (1975) Evidence by scanning electron microscopy for an association between spermatozoa and T-mycoplasmas in men of infertile marriage. *Fertil Steril* 26:1203-1207.
- Meier B, Habermehl G (1990) Evidence for superoxide dismutase and catalase in mollicutes and release of oxygen species. *Arch. Biochem Biophys* 277:74-79.

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**Conflict of interest:** No conflict of interest is declared.