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Isolation of *Arcanobacterium haemolyticum* in semen of an infertile patient.

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Key words: *Arcanobacterium haemolyticum,* male infertility, seminal infection, sperm abnormalities.

Abstract. This is the report of a genital tract infection caused by *Arcanobacterium haemolyticum* in an infertile man from Venezuela. This 29 year-old patient was evaluated for primary infertility, without symptoms of seminal infection. Laboratory analysis showed leukocytospermia, low sperm count, motility and vitality, without abnormalities in hormonal profile. Sperm culture was positive for *A. haemolyticum*. After erythromycin therapy an improvement in some sperm parameters was observed. *A. haemolyticum* could be considered as a cause for silent seminal infection.

Aislamiento de *Arcanobacterium haemolyticum* en semen de un paciente infértil.

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Palabras clave: *Arcanobacterium haemolyticum*, infertilidad masculina, infección seminal, alteraciones espermáticas.

Resumen. Se describe un caso de infección del tracto genital causada por *Arcanobacterium haemolyticum* en un hombre infértil de Venezuela. El paciente de 29 años fue evaluado por infertilidad primaria, sin síntomas de infección de las vías seminales. Los análisis de laboratorio revelaron leucocitospermia, disminución del contaje, movilidad y vitalidad espermáticos, sin anormalidades en el perfil hormonal. El cultivo del semen fue positivo para *Arcanobacterium haemolyticum*. Después de tratamiento con eritromicina se observó mejoría en algunos de los parámetros espermáticos. *Arcanobacterium haemolyticum* puede ser considerado como un agente causal de infección seminal silente.

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INTRODUCTION

A. haemolyticum is a Gram positive bacillus that was initially described in USA as *Corynebacterium haemolyticum* and has been recognized worldwide as a causal agent for exudative pharyngitis and cutaneous infections (1-5). It is a facultative anaerobic organism that grows slowly, causes hemolysis and shows susceptibility to antibiotics like erythromycin, gentamicin, clindamycin and cephalosporins when it is isolated from human infections (6-9).

In teenagers and young people, *A. haemolyticum* has been pointed out as a causal agent of acute pharyngitis; clinically similar to streptococcic pharyngitis that can be associated with a cutaneous rash resembling that of scarlatina (2). Patients may show fever and lymphadenopathy, sometimes associated with a positive test for mononucleosis (3).

In the last decade, *A. haemolyticum* has been isolated in different organs and systems either in immuno-competent or immuno-compromised patients with high risk for infections (10-14).

A. haemolyticum seldom has been described as a causal agent for seminal infection in infertile patients. This is a case report and a brief review of the literature on male genital tract infections caused by *A. haemolyticum.*

CASE REPORT

A 29 years old healthy man was evaluated due to primary infertility. He never presented urethral secretion. General medical exam was normal, there was evidence neither of urethral discharge nor signs of seminal tract inflammation; testicular index was 12.45.

A previous semen analysis (6 months before) revealed oligoasthenoteratozoospermia, leukocytospermia and the sperm vitality, using the hyposmotic swelling test, was negative (Table I). Hormone profile was normal: LH: 3.11 mU/ mL; FSH: 3.81 mU/mL; testosterone: 497 ng/dL. Culture from a sample of seminal liquid obtained under strict antiseptic conditions was performed. Gram stain of semen smear showed Gram-positive bacilli. Seminal fluid was cultured in several selective media: Blood agar, urea-arginine broth (Lyo®, Bio-Merieux), Columbia agar-5% sheep blood, chocolate agar, Poly-Vitex, and gentamicin-chloramphenicol Sabouraud agar. Also, IgA anti-*Chlamydia trachomatis* (Immuno-

TABLE I
EVOLUTION OF SEMINAL PARAMETERS IN AN INFERTILE MAN WITH *Arcanobacterium haemolyticum* POSITIVE ISOLATE

Analysis	Semen		
	a	b	c
Volume (mL)	1	1.5	1.5
pH	8	7	8
Sperm concentration (10^6/mL)	12	13	25
Rapid Progression (% sperm)	5.88	7.96	12
Normal morphology (% sperm)	1	1	3
Leukocytes (10^6/mL)	1.5	1.3	0.8
HOST (% swollen sperm)	47	19	50

a: Before the positive seminal culture. b: Seminal culture positive for *A. haemolyticum*. c: After antibiotic therapy.

comb II *Chlamydia trachomatis*-monovalent IgA®; Orgenics) was determined in semen. After 48 hours of incubation in aerobic conditions a pure culture of small, transparent -hemolytic colonies (> 40 CFU/mL) was found. Gram stain from these colonies showed Gram-positive rods. For this isolate, reverse CAMP test was positive but catalase test was negative. Glucose and maltose fermentations were positive, while xylose and mannose fermentations were negative as well as urea hydrolysis. The results of these tests are compatible with the identification of a pure culture of *A. haemolyticum*. Standarized disk diffusion testing (NCCLS) showed susceptibility to cefuroxime, clindamycin, erythromycin and gentamicin, and due to the lack of accepted breakpoints for this microorganism, those indicated by the NCCLS for aerobically growing bacteria were used (15). Antibodies to *C. trachomatis* were not detected and not other bacteria were isolated from semen.

The patient was treated with erythromycin 500 mg TID during 7 days. Two months later an improvement in progressive motility and sperm count was observed; sperm function returned to a previous value, but teratozoospermia still persisted. Leukocyte count declined to less than 10^6/mL after therapy (Table I). A control sperm culture was negative.

DISCUSSION

This is a report of *A. haemolyticum* isolation in semen from an infertile patient with disorders in sperm count, motility, vitality and morphology but without any clinical sign or symptom of infection.

In a Medline (1996-2005), Index Medicus and LILACS (Latin American and Caribbean Health Sciences Information System) databases review we only found a study that referred 5 cases of *A. haemolyticum* infection of seminal tract, in infertile patients otherwise asymptomatic. *A. haemolyticum* was isolated from semen in 2 patients and from prostatic fluid in 3 patients that in one case was associated to antisperm antibodies that became negative after antibiotic therapy (16).

In the present case, there were features that agree with some microbiological criteria described by Funke *et al*. (17), such as the results of Gram stain, both in semen smear and colony samples, leukocyte reaction in semen, pure growth of the microorganism, positive biochemical tests and the isolation from a sterile site. These findings suggest the possible association of *A. haemolyticum* with seminal tract infection.

Natural habitat of this bacteria is not completely known. However, it has been isolated from usually sterile sites and it has not been shown to be a commensal of human flora. For these reasons its role in human infections has been suggested (4, 12). Furthermore, genital tract infection with other corynebacteria like *Corynebacterium seminale* has been described (18).

Bacteria can exert deleterious effects on male fertility by direct and indirect mechanisms. Direct action depends on the microorganism presence in semen causing sperm lesions by adherence or intracellular colonization. Indirect mechanisms could affect male fertility as a consequence of inflammation and cicatrisation of seminal tract and adnexa glands or induced immunological response mediated by antibodies, cytokines or oxygen reactive substances, that may have adverse effects on sperm function (19-22).

Some bacteria can adhere to spermatozoa affecting some seminal parameters like sperm count, morphology, motility and capacitation. Also, bacteria can penetrate into spermatozoa and transform them in active agents for infection transmission to the female genital tract (20, 23, 24). Eventhough controversial, it has been suggested

that bacteria can share similar epitopes of spermatozoa eliciting the production of antibodies directed against flagellar structures, setting thus an autoimmune response occurring locally in male genital tract and that affects motility and capacitation of the gamete (25, 26).

A. haemolyticum infection can induce a humoral immunological response in patients with pharyngotonsillitis during the acute phase of the disease and also during convalescence, from 2 weeks to 3 months post- infection (27). Also *A. haemolyticum* is able to synthesize extracellular enzymes like neuroaminidase and phospholipase C, which cause damage to mammalian cell membranes (4). Hyposmotic swelling test, a measure of sperm membrane function, was clearly abnormal during infection in this case patient and after antibiotic treatment an improvement in this parameter was observed. It is possible that *A. haemolyticum* can contribute to sperm damage by several ways. As other bacteria, it could induce damage by means of an immune response, generating antibodies against spermatozoa, and may be associated with a failure in reproductive function, as it has been pointed out (16). A toxic mechanism could be another possibility involving extracellular action of bacterial enzymes on membranes or other sperm structures. Further studies, i.e. evaluation of spermatozoa changes induced by co-incubation with that bacteria, should be interesting and are required to confirm these features.

The presence of *A. haemolyticum* in the male genital tract may evidence its adaptation to the seminal tract without producing symptoms of infection, but being able to cause some seminal abnormalities like oligozoospermia and sperm membrane damage. *A. haemolyticum* has been shown colonizing the female genital tract and provoking damage to ovaries and fallopian tubes (28), therefore it could be sexually transmitted by infected males.

We think there is a need of an adequate infection screening of asymptomatic infertile men and to be aware of searching *A. haemolyticum* in order to stablish an association to human infertility, since it could be considered as an emergent organism causative of seminal infection, and a risk for exposed females.

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