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## DOES THE IN VITRO FERTILIZATION PROCEDURE REQUIRE BACTERIOLOGICAL INVESTIGATION OF NORMOZOOSPERMIC EJACULATE?

### JE LI ZA OPLODNJU IZVAN TIJELA POTREBNO BAKTERIOLOŠKI ISPITATI SJEME U OSOBA S NORMOZOOSPERMIJOM?

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*Key words:* IVF, bacteriospermia, fertilization, antibiotic treatment

**SUMMARY.** *The aim* of our study was to compare the fertilization rate in IVF procedures in cases where the ejaculate was not subjected to bacteriologic investigation and in those where bacteriologic investigation of the ejaculate was performed and after which antibiotic therapy followed. **Material.** From among 241 normozoospermiaacs included in the IVF procedure, 56 cases were self-selected for bacteriologic investigation of ejaculate. The fertilization rate and the percentage of cycles with total fertilization failure following IVF was compared with the remaining 185 IVF cycles before which no bacteriologic investigation of ejaculate had been done and the men have received no antibiotic therapy. **Results.** Bacteria in the ejaculate were isolated in 95% (53/56). The culture remained sterile in 5% (3/56). The most frequently isolated bacteria were Peptostreptococci in 26%, Streptococcus viridans in 24%, Enterococci in 22%, alpha.hemolytic Streptococci in 20%, anaerobic gram-positive bacilli in 18%, anaerobic gram-negative bacilli in 13% and Diphtheroids in 10%. From among the less frequent bacteria, Staphylococcus epidermis and E. coli were assessed in 4%. In 22% of samples, only one bacterial species was isolated, in 38% two and in 40% three or more. There was no statistically significant difference between the groups with regard to the number of oocytes and embryos, the fertilization rate and the percentage of cycles without fertilization. **Conclusion.** According to our experience, bacteriologic investigation of normozoospermic ejaculate in the IVF procedure and subsequent antibiotic treatment of asymptomatic men with bacteria in the ejaculate bears no influence on the fertilization rate and pregnancy rate in IVF procedure.

*Izvorni članak*

*Ključne riječi:* IVF, bakteriospermija, fertilizacija, antibiotska terapija

**SAŽETAK.** *Cilj* studije bila je analiza uspješnosti oplodnje izvan tijela (IVF) u parova bez muškog čimbenika neplodnosti i normalnim nalazom ejakulata. Potencijalni utjecaj bakteriospermije na mehanizme oplodnje (fertilizaciju) i uspješnost samog postupka (stopu trudnoća na pokušaj) smo slijedili u dvije grupe pacijenata. Analizirali smo uspješnost IVF u grupi pacijenata u kojih nismo kontrolirali prisutnost bakterija u ejakulatu i u onih gdje smo analizirali prisutnost bakterija u ejakulatu i uveli terapiju po antibiogramu prije zahvata. **Metode.** U istraživanje smo uključili 241 par u kojeg je bio indiciran postupak oplodnje izvan tijela (IVF), a nalaz analize ejakulata smo ocijenili kao normozoospermiju. Pacijenti su samoselekcijom razvrstani u skupinu za bakteriološku analizu ejakulata (n=56) i skupinu bez bakteriološki kontroliranog ejakulata (n=185). U obje skupine smo pratili stopu oplodnje jajnih stanica, učestalost potpunog izostanka oplodnje u svih jajnih stanica iste pacijentice i stopu trudnoća. **Rezultati.** U 95% (53/56) svih analiziranih ejakulata smo našli bakterije. Najčešće izolirane bakterije bili su peptostreptokoki (26%), streptokokus viridans (24%), enterokoki (22%), alfa-hemolitični streptokok (20%), anaerobni gram-pozitivni bacili (18%), anaerobni gram-negativni bacili (13%) i difteroidi u 10%. Od manje učestalih bakterija izolirali smo Stafilokokus epidermidis i E. coli u 4%. U 22% analiziranih uzoraka izolirali smo samo jedan soj bakterija, u 38% dva, a u 40% tri ili više. Nismo našli statistički značajnih razlika u skupinama s obzirom na broj jajnih stanica i broj embrija, stopu oplodnje, stopu ciklusa bez oplođenih jajnih stanica i stopu trudnoća. **Zaključak.** Prema našem iskustvu, bakteriološka analiza ejakulata normozoospermika te terapija antibiotikom asimptomatskih bakteriospermija ne utječe na stopu oplodnje i stopu trudnoća u postupku izvanstanične oplodnje (IVF).

## Introduction

In the male reproductive tract, the urethra excepted, aerobic bacteria are not normally present.<sup>1</sup> The presence of bacteria in ejaculate may be a sign of genital tract infection which may affect sperm number and function.<sup>2-4</sup> In such cases antibiotic therapy can be applied to improve ejaculate quality and consequently male fertility.<sup>3,5,6</sup> However, bacterial presence in ejaculate does not

always imply an infection, it can also be the consequence of ejaculate contamination with bacteria from the urethra, genital skin or the skin of the hands.<sup>5</sup> In such cases bacterial presence in ejaculate does not affect male fertility and antibiotic therapy is not required.<sup>7</sup>

In the IVF procedure, however, there is the possibility of bacteria from ejaculate contaminating and causing

infection of embryo culture, thus lowering the fertilization rate and causing the destruction of gamete or embryo. Such cases cannot be completely avoided, not even with special methods of ejaculate separation or addition of antibiotics to the media.<sup>8</sup> For these reasons antibiotic treatment is advisable prior to IVF procedure in men with bacteriospermia.<sup>9</sup> Several recent studies established that in these cases antibiotic therapy does not decrease the frequency of infections in culture media,<sup>10–12</sup> but according to some it rather increases the possibility of infection with resistant bacteria.<sup>5,13</sup> Due to such controversial standpoints, it was the aim of our study to establish whether bacteriologic investigation of ejaculate in normozoospermic asymptomatic men and antibiotic therapy in cases of bacteriospermia affect fertilization in IVF cycles.

## Subjects and methods

The study included 241 infertile couples treated in the same number of IVF cycles. The condition for inclusion was normozoospermia in the man without clinical signs of genital tract infection. Couples with previous IVF procedures without fertilization for noninfective reasons were not included. Those with cycles without follicle puncture or follicle puncture without oocyte recovery were excluded. All subjects gave informed consent to the procedure.

The average age of the women was 31.7±4.1. Indications for IVF procedure were: only tubal infertility in 149 (61.8%), unknown cause of infertility in 73 (30.3%) and other causes in 19 (7.9%) cycles. Ovarian stimulation was done with the follicle stimulating hormone (FSH) and gonadotropin-releasing hormone agonist (GnRH<sub>a</sub>). Follicular growth was followed ultrasonically by vaginal probe. The women received 10,000 IU human chorionic gonadotropin (hCG) when two or more follicles reached a diameter of ≥18 mm. Transvaginal oocyte puncture was done 36–37 hours after hCG application. On the evening prior to follicle puncture, the women inserted an antiseptic vaginal suppository. The ejaculate was prepared by centrifuging in media (Medi-Cult, Denmark) and by the swim-up technique. The oocytes were cultivated, inseminated and fertilization was assessed as described in the literature.<sup>14</sup> We used IVF media (Medi-Cult, Denmark) with an addition of penicillin and streptomycin.

The cycles were divided in two groups. *Group A* comprised 56 cycles which were self-selected from entire group as those whose woman started their stimulation protocol on the same day of the week after synchronization with oral contraceptives. Seven days prior to the beginning of controlled ovarian hyperstimulation in the women a bacteriological investigation of the partner's ejaculate was performed. Before that the subjects received written instructions regarding the procedure of ejaculate collection. After a 2–5 days abstinence, the semen samples were given by masturbation into a sterile cup after urination and washing of genitals and hands. The semen volume, the number, vitality and motility of

sperm was evaluated on the basis of WHO criteria. Following liquefaction, part of the sample was transferred under sterile conditions to a transport medium and sent to the microbiology laboratory for dilution with physiological solution in a 1:1 ratio and cultivated on the medium on the same day. The aerobes were cultivated for 48–72 hours on blood agar, blood agar with 5–10% CO<sub>2</sub> and thioglycolate liquid medium. The anaerobes, however, were cultivated for 48 hours in anaerobic conditions on blood and Schaudler agar. Bacterial growth was marked as significant if more than 1000 colonies of unimorphic appearance grew per ml of diluted ejaculate. The bacteria were identified and sensitivity to various antibiotics was assessed by disk diffusion test. The men with bacteriospermia received an antibiotic therapy based on the antibiogram for at least seven days and also on the day of ultrasonographic follicle puncture in the female partner.

*Group B* comprised 158 cycles in which no bacteriologic investigation of ejaculate was carried out and the men had no antibiotic therapy during the last two months. Groups A and B were compared as regards the woman's age, cause of infertility, stimulation protocol, number of oocytes and embryos, percentage of cycles without fertilization and fertilization rate using the chi-square and t-test. P values <0.05 were considered statistically significant.

## Results

Potentially pathogenic microorganisms were isolated from 94.6% (n=53) ejaculates sent for bacteriologic investigation (n=56). The most frequently isolated microorganisms were Peptostreptococci in 26.8% (n=15), viridans Streptococci in 23.2% (n=13), Enterococci in 21.4% (n=12), alpha-hemolytic Streptococci in 19.6% (n=11), anaerobic gram-positive bacilli in 17.9% (n=10),

Table 1. The outcome of IVF cycles with and without examination of ejaculate

Tablica 1. Ishod IVF ciklusa s bakteriološkom pretragom sjemena i nje

	Group A Bacteriological examination and antibiotic treatment	Group B Without bacteriological examination	Statistical significance
Number of cycles	56	185	/
No. of ejaculates with bacterial presence	53	/	/
No. of patients receiving antibiotic treatment	53	/	/
Average no. of oocytes retrieved ±SD	4.62±3.34	4.28±3.24	NS
Average no. of embryos ±SD	3.39±2.76	3.04±2.73	NS
No. of cycles without fertilization (%)	3 (5.4)	12 (6.5)	NS
Fertilization rate	41 (73.4)	131 (71.0)	NS
Pregnancy rate	19 (33.9)	65 (35.1)	NS

NS = non significant

other anaerobic gram-negative bacilli in 12.5% (n=7) and Diphtheroids in 10.7% (n=6). From among the less frequent bacteria, *E. coli*, *Staphylococcus epidermis* and *Staphylococcus aureus* were assessed in 3.6% (n=2). In 22.6% (n=12) of samples, only one bacterial species was isolated, in 37.7% (n=20) two and in 39.6% (n=21) three or more. On the basis of the antibiogram 41.5% (n=22) received doxycycline 100 mg/12 h, 45.3% (n=24) ciprofloxacin 250 mg/12 h and 13.2% (n=7) other antibiotics or antibiotic combinations.

No statistically significant differences were found between Groups A and B as regards the woman's age, indications for IVF and the stimulation protocol. The comparison of Groups A and B with regard to oocyte number, number of embryos, number of cycles without fertilization and the fertilization rate can be seen in *Table 1*.

In all cycles without fertilization, infection of culture medium was excluded as a possible cause.

## Discussion

When investigating infertile couples, pathogenic microorganisms are frequently found in the ejaculate of asymptomatic men. In the literature the percentage of men with presence of bacteria in ejaculate is between 41% and 100%.<sup>2,6,7,15,16</sup> In our study, bacteria were isolated in 95% of ejaculates, which is among the highest rates mentioned in the literature. The incidence of bacteriospermia depends on the time elapsing between the collection and bacterial investigation of ejaculate, the method of collection and isolation of microorganisms. Liversedge et al.<sup>5</sup> find that in ejaculates where transport to the laboratory was  $\geq 2$  days the percentage of bacteria was significantly higher, particularly gram-negative bacilli and enterococci. They believe that with time the activity of bactericide and bacteriostatic substances from the prostate and seminal fluid is decreased.<sup>5</sup> This cannot be the reason for the low percentage of our subjects with sterile ejaculate since the semen in our study was sent to the laboratory on the same day. More likely our results were not affected by the method of ejaculate collection. Namely, Boucher et al.<sup>16</sup> established that the percentage of sterile cultures increases from 23.1% to 59.6% when the patients receive verbal beside written instruction regarding ejaculate collection. All our patients received written and verbal instructions. Another possible reason may be the particular attention turned also to the isolation of anaerobic bacteria. Most studies mentioned in the literature focused merely on the cultivation of aerobes. Similar to us, Egert-Krusse et al.<sup>7</sup> focused on isolation and identification of anaerobes and established bacterial presence in as many as 99% of ejaculates. Therefore the high percentage of anaerobes found in our study is no surprise. However, a relatively small percentage of commensal bacteria such as *Staphylococcus epidermis* was noted.

Due to the frequent presence of potentially pathogenic bacteria in ejaculate there is the risk in IVF procedures of these bacteria infecting embryo cultures and affecting fertilization.<sup>9</sup> On account of this danger numerous IVF

centers carry out microbiological investigation of semen prior to the procedure and in case of bacterial presence introduce an antibiotic therapy.<sup>17</sup> Some noteworthy studies state that these procedures are unnecessary and do not affect the outcome of the IVF cycle.<sup>10–12</sup> Shalika et al.<sup>18</sup> believe that antibiotic therapy is only reasonable in cases when *E. coli*, *Staph. aureus* or *Ureaplasma urealyticum* are isolated in ejaculate as they all have a negative effect on the outcome of the cycle, while *Enterococcus* spp. has no such effect and its presence does not require antibiotic treatment. Liversedge et al.<sup>5</sup> believe that antibiotic treatment of asymptomatic bacteriospermia may even increase the risk of embryo culture infection. This is explained by the secretion of antibiotics into the ejaculate which is transferred to the vagina during intercourse, causing the growth of resistant bacterial flora which can be transferred to the culture medium during follicle puncture.

Our results confirm the opinion that bacteriological investigation of ejaculate and antibiotics therapy do not affect fertilization in IVF procedures. In our study even in the 185 cycles prior to which the ejaculate was not investigated bacteriologically and the men received no antibiotic therapy, no infection of embryo culture was noted. Like other authors,<sup>10</sup> we also believe that the majority of bacteria can be removed by adequate preparation of semen while the growth of others in embryo culture can be prevented by using IVF media with the addition of antibiotics. Our results and the superfluousness of antibiotic therapy in this study can also be explained by the assessments of Shalika et al.<sup>18</sup> It is namely very rarely that *E. coli*, *Staph. aureus* or *Ureaplasma urealyticum* were isolated and they are presumably the only ones able to affect the outcome of IVF cycles. The group with the presence of these bacteria was too small to allow us to establish whether antibiotic therapy was justified at least in this case. In our study we also did not notice antibiotic therapy increasing the risk of embryo culture infection. It is possible that by adequate preparation of the vagina before follicle puncture we prevented the contamination of embryo cultures with resistant microorganisms from the vagina. The study reinforced our conviction that prior to the IVF procedure bacteriological investigation of ejaculate and antibiotic treatment of bacteriospermia in normozoospermia is unnecessary and can be omitted, thus simplifying and cheapening the IVF procedure.

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