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IMPROVEMENT OF SEMEN QUALITY IN INFECTED ASYMPTOMATIC INFERTILE MALE AFTER BACTERIOLOGICAL CURE

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Abstract Bacteriological etiology was investigated in 29 infected asymptomatic infertile males. The localization of the infection and the effect of a long term antibiotic therapy on semen parameters were evaluated. The most frequent etiological agent isolated was *Enterococcus faecalis*. Positive bacteriological culture was obtained in prostatic fluid in 16 patients and in semen in 13. Bacteriological cure was achieved in 24 cases and it was associated with improved seminal parameters: sperm concentration, motility, viability and total motile sperm per ejaculate. In 5 patients without bacteriological cure there was no change in semen analysis after antibiotic therapy. In 45% of the infected patients there were less than 0.5 x 10^6/ml seminal polymorphonuclear leukocytes. In view of these findings granulocyte concentration seems to be a poor marker to predict infection.

Resumen ***Mejoría de la calidad del semen en pacientes infértiles infectados asintomáticos después de una cura bacteriológica.*** En 29 pacientes infértiles infectados asintomáticos fue estudiada la etiología de la infección, su localización y el efecto de la terapia antibiótica prolongada sobre los parámetros espermáticos. El agente etiológico más frecuente fue *Enterococcus faecalis:* 16 pacientes presentaron cultivo positivo en líquido prostático y 13 en semen. La cura bacteriológica se alcanzó en 24 pacientes y se asoció a mejoría de los parámetros seminales: concentración espermática, motilidad, viabilidad y número de espermatozoides totales móviles en el eyaculado. Luego de la terapia antibiótica, 5 pacientes no lograron cura bacteriológica y no presentaron mejoría en los parámetros seminales. El 45% de los pacientes infectados presentó una concentración seminal de leucocitos polimorfonucleares menor de 0,5 x 10^6/ml, por lo cual concluimos que la concentración de granulocitos no constituye un marcador confiable para predecir la presencia de infección.

Key words: male genital tract infection, sperm parameters, antibiotic therapy, sperm agglutinating antibodies, leukocytospermia

It is well known that certain bacterial infections such as those produced by *Neisseria gonorrhoeae, Mycobacterium tuberculosis* and *Chlamydia trachomatis*, are related to male infertility[1]. However, the phenomenom is less clear for other microorganisms that often colonize the anterior urethra and may become potential etiological agents of prostatitis and seminal infections. Moreover, little is known about how often microorganisms are the cause of leukocytospermia in infertile patients. Most papers reviewed mention no significant association between white blood cells (WBC) and microorganisms in semen. WBC are present in most human ejaculates, but abnormally high concentrations of seminal WBC may reflect an underlying pathological condition[2].

The absence of leukocytospermia does not exclude the possibility of infection. As infertile patients usually do not show symptoms of chronic genital tract infection the search for microorganisms in semen and genital tract secretions described by Stamey and Meares[3] should always be performed, regardless of the number of leukocytes in semen[4].

In order to evaluate the impact of male genital tract infections on semen quality in asymptomatic infertile patients we determined: 1) the microbiological etiology of infection; 2) the concentration of neutrophilic polymorphonuclear leukocytes (PMN) in semen as a marker of leukocytospermia; 3) the result of the administration of specific antibiotic therapy during a long period of time, according to the susceptibility pattern of microorganisms and the pharmacodynamics of the drugs.

Materials and Methods

Subjects

All couples included in this study had a history of infertility.

Patient evaluation: On the first visit patients were clinically evaluated and two consecutive semen analysis were per-

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formed according to WHO guidelines, with an interval of seven days. Four months later, sperm analysis were repeated. We found that among the 3 samples from each patient the coefficient of variation remained < 20% for sperm concentration, < 10% for sperm motility, < 5 % for sperm viability and < 10% for sperm morphology. After this, a new semen sample was obtained for microbiological cultures, antisperm antibodies, and evaluation of semen parameters. Those patients with positive microbiological cultures (MC+) received specific antibiotic therapy during 90 days. Two months after antibiotic therapy was completed, another microbiological study and semen evaluation were performed. A second semen sample was studied a week later, for sperm parameters only.

Following this protocol, 70 asymptomatic male partners of infertile couples were evaluated during the last two years. Of these, 29 patients between 20 and 38 years old with MC+ completed all the steps described and their results are shown.

Laboratory procedures

Microbiological study: patients were asked to produce the samples in the laboratoy after three days abstinence and should have full bladder and desire to void. All specimens were obtained by a microbiologist and a physician for prostatic massage procedure. Microbiological cultures were performed in all patients from urethral swab (US), first voided urine (VB_1), midstream urine (VB_2), prostatic fluid (PF), voided urine after prostatic massage (VB_3), and semen (S), following the four specimen technique described by Stamey and Meares[3], with the addition of urethral swab and semen[5].

None of the subjects presented urethral discharge or urinary tract infections at the time of cultures. All samples were also assessed for normal genital tract microorganisms and sexually transmitted pathogens, including aerobic and anaerobic bacteria, fungus, yeasts and *Ureaplasma urealyticum* using standard procedures[6, 7, 8, 9].

The investigation of *Chlamydia trachomatis* was performed only in semen samples by direct immunofluorescence method (Syva trak Chlamydia direct IF kit).

Prostatic fluid or semen were considered infected if: 1) their colony counts were > 1 $\log_{10}$ with regard to VB_1, or 2) PF was not obtained, but VB_3 colony counts were > 1 $\log_{10}$ with regard to VB_1, or 3) a microorganism was not present in VB_1 or US, but was recovered from PF,S or VB_3 at any count.

Antibiotic therapy administered during 90 days consisted of: trimethoprim/sulfamethoxazole, ciprofloxacin, doxycyclin or minocyclin at usually doses according to the susceptibility pattern of the microorganisms.

Semen evaluation: semen samples were collected by masturbation into sterile containers after 3 days of sexual abstinence, and examined within 1 hour after ejaculation. After liquefaction, semen analysis included the determination of sperm concentration, motility and viability performed by two different experimented observers following WHO recommendations[4]. Sperm morphological assessment was performed on ethanol-ether fixed smears of fresh ejaculates stained by the Papanicolaou procedure recommended by WHO[4].

The concentration of PMN cells was measured by peroxidase stain method according to WHO[4] and adapted by Wolff[10].

The presence of sperm agglutinating antibodies in seminal plasma was investigated by the tray agglutination test. This method (TAT) is routinely applied in our laboratory using an antigen suspension composed of only motile spermatozoa obtained by swim up from semen with normal parameters, and was performed following WHO standard protocols[12].

Statistical Analysis

Friedman test, Wilcoxon signed rank test, and Spearman rank order correlation were used for statistical analysis of data. A probability value $p < 0.05$ was considered significant.

Results

Microorganisms isolated from prostatic fluid and semen are shown in Table 1. All patients were infected with only one microorganism, 16 (55%) had prostatic localization and the other 13 (45%) presented seminal infection.

The median concentration of PMN in semen of these infected males was 1×10^6/ml (0.05×10^6 to 3.5×10^6). There was no evident difference in PMN concentration between those who presented prostatic localization (median 0.61×10^6/ml, range 0.05×10^6 to 3.5×10^6), and those with seminal infection (median 1×10^6/ml, range 0.05×10^6 to 3.5×10^6), p: 0.53.

Bacteriological cure was achieved in 24 patients (83%). Four patients relapsed and one had a reinfection.

Sperm parameters before and after antibiotic therapy are shown in Table 2. For the patients who achieved bacteriological cure as there was not a significant variation on different samples during the 4 months preceding microbiological diagnosis, we considered each patient as his own control (A versus B, p: NS). Total sperm concentration, motility, viability, total motile sperm per ejaculate and PMN concentration improved after antibiotic therapy. Sperm morphology did not show significant differences.

There was a low correlation between PMN concentration and total motile sperm per ejaculate pre and post

TABLE 1.– *Microorganisms isolated in prostatic fluid and semen in 29 infected patients*

Microorganisms	Prostatic Fluid	Semen
Enterococcus faecalis	6	7
Arcanobacterium haemolyticum	3	2
Escherichia coli	-	1
Staphylococcus aureus	1	-
Staphylococcus hominis	1	-
Staphylococcus warneri	-	1
Streptococcus pyogenes	2	-
Streptococcus pneumoniae	1	-
Streptococcus agalactiae	1	-
Gardnerella vaginalis	1	-
Ureaplasma urealyticum	-	1
Chlamydia trachomatis	-	1
Total	16 (55%)	13 (45%)

TABLE 2.- *Semen parameters in 24 patients before and after 90 days antibiotic therapy* ●

Parameter	Before therapy A	Before therapy B	After therapy C
Sperm concentration (10^6/ejaculate)	35.2 (0.017 to 415.4)	33.7 (0.016 to 378.4)	41.1 (0.47 to 548.5)∔
Sperm motility (%)	18.5 (0 to 67.0)	18.5 (0 to 65.0)	41.0 (4.0 to 75.0)∔
Total motile sperm (10^6/ejaculate)	3.37 (0 to 278.3)	2.98 (0 to 283.8)	8.1 (0.017 to 329.4)∔
Sperm viability (%)	69.0 (0 to 88.0)	70.0 (0 to 90.0)	72.0 (41.0 to 94.0)◉
Sperm morphology (% normal forms)	25.0 (5.0 to 67.0)	25.0 (3.0 to 70.0)	28.0 (5.0 to 70.0)†
Peroxidase-positive PMN (10^6/ml)	1.0 (0.050 to 3.5)	1.0 (0.050 to 3.5)	0.25 (0.045 to 1.3)†

The initial andrological status of the 24 patients (A and B) was: 4 normospermic, 4 oligoasthenozoospermic, 9 oligoasthenoteratozoospermic, 3 asthenozoospermic, 3 asthenoterazoospermic, 1 teratozoospermic.

● Values are medians from two consecutive semen analysis in A, B and C, with ranges in parentheses, for 24 patients

A: Five months before culture; B: At the time of bacteriological culture; C: Two months after finishing antibiotic therapy

In all cases p express the significance between each parameter before (A or B) versus C (after treatment) (Wilcoxon signed rank test)

∔ $p < 0.05$, ◉ p: 0.01, † p: 0.0001, ∔ p: NS

TABLE 3.- *Semen parameters before and after antibiotic therapy in 5 patients not achieving bacteriological cure* ●

Parameter	Before therapy A	Before therapy B	After therapy C	Difference between A, B, C
Sperm concentration (10^6/ejaculate)	37.0 (32.4 to 136.5)	37.5 (33.0 to 126.5)	36.9 (30.4 to 145.0)	NS∔
Sperm motility (%)	14.0 (8.0 to 16.0)	15.0(8.0 to 15.0)	14.0 (8.0 to 17.0)	NS∔
Total motile sperm (10^6/ejaculate)	5.1 (3.2 to 13.5)	5.0 (3.7 to 13.6)	5.8 (3.3 to 11.9)	NS∔
Sperm viability (%)	56.0 (15.0 to 65.0)	54.0 (15.0 to 65.0)	54.0 (16.0 to 65.0)	NS∔
Sperm morphology (%normal forms)	38.0 (30.0 to 50.0)	39.0 (31.0 to 50.0)	39.0 (30.0 to 52.0)	NS∔
Peroxidase-positive PMN (10^6/ml)	0.72 (0.12 to 4.0)	0.43 (0.15 to 1.35)	0.4 (0.14 to 1.3)	NS∔

● Values are medians from two consecutive semen analysis in A, B and C, with ranges in parentheses, for 5 patients

A: Five months before culture; B: At the time of bacteriological culture; C: Two months after finishing antibiotic therapy

∔ p: NS (Friedman test)

therapy or the percentage of normal forms after bacteriological cure (Spearman rank correlation coefficient, p: 0.387, p: 0.481 and p: 0.734 respectively).

The 5 patients without bacteriological cure (2 asthenozoospermic and 3 oligoasthenozoospermic) did not show changes in semen analysis before and after antibiotic therapy (Table 3).

Two patients showed antisperm antibodies in seminal plasma, with titers ranging from 1/64 to 1/128. They had a median of 1.5 x 10^6 PMN/ml at the moment of the infection (one patient with *Arcanobacterium haemolyticum* in PF, and one with *Ureaplasma urealyticum* in S). After antibiotic therapy both patients had negative antisperm antibody titers, and PMN concentration diminished to 0.25 x 10^6/ml.

Discussion

We consider the infection of the male genital tract as an important morbidity factor. It is known that it may affect seminal quality through a direct action on spermatozoa or their environment, including local inflammatory reaction and composition of seminal plasma[13-16].

We have been involved in the diagnosis of seminal infection in infertile men since 1979[17, 18]. We followed the technique described by Stamey and Meares that allows for the localization of the infection in prostatic gland or in other sites of the male genital tract different from the prostate (excluding urethra). The culture of semen alone may lead to controversial results when compared to those obtained by the Stamey and Meares protocol that we have used in the present study. We have previously shown a prevalence of infection of about 46% in two large populations of infertile men. *Enterococcus faecalis* was the most frequent etiological agent followed by *Ureaplasma urealyticum*[17, 18].

After specific antibiotic therapy we waited 2 months to control sperm parameters since the entire process of spermatogenesis takes approximately 70 days[19] and requires between 4.3 to 4.7 cycles of the seminiferous epithelium[20]. Until microbiological cure was achieved, we advised the couples to use condom during sexual intercourse to prevent reinfections and to avoid harmful effects of antibiotics on semen parameters[21].

Males have been treated during three months according to Meares[22, 5], who showed that in patients with long-term therapy the cure rates have been 32% to 71%, more than twice those noted with short-term therapy (two weeks or less). In spite of a long term antibiotic therapy, 17% of patients in this study did not achieve bacteriological cure, and did not modify their seminal parameters (including PMN concentration).

In this group of patients we have found *Enterococcus faecalis* as the main etiological agent in PF and S. Most of the etiological agents isolated such as *Enterococcus faecalis*, *Enterobacteriaceae*, coagulase-negative staphylococci *and Streptococcus agalactiae* (Table 1) are constituents of the normal enteric flora that often colonize the anterior urethra and that may also cause urinary tract infections[23-25]. Other microorganisms such as *Ureaplasma urealyticum, Gardnerella vaginalis* and *Chlamydia trachomatis*, are well recognised etiological agents of sexually transmitted diseases[26-28]. The quantification of all of them is extremely important to determine their clinical significance in each localization, and to avoid confusion with the normal flora of skin and mucous membranes.

The finding of *Chlamydia trachomatis* in semen samples by immunofluorescence does not allow differential counts, therefore patients must be treated even if the infection site cannot be determined.

The World Health Organization has defined leukocytospermia as the finding of > 10^6 WBC/ml in semen. Granulocytes are the predominating WBC in semen, and were reported to represent about 50 to 60% of all WBC in ejaculates from fertile and infertile men[29]. To identify PMN granulocytes in semen routine analysis, we used the peroxidase test recommended by WHO because it is fast, specific and cost-effective. It is based on the visualization of peroxidase activity to identify and quantify PMN. In agreement with previous reports by Endtz[30] and Yanushpolsky[2] we defined the threshold for leukocytospermia as a concentration of PMN equal to 0.5 x 10^6/ml.

Thirteen infected patients had a concentration of PMN in semen less than 0.5 x 10^6/ml (45% of the total infected population). For this reason we believe that leukocy-tospermia is a poor marker of infection. On the other hand, most leukocytospermic patients (9 of 16, 56%) resolved it after antibiotic therapy.

In accordance with Yanushpolsky[31], we consider that a single positive finding of leukocytospermia does not require antibiotic therapy without further evidence of a specific genital tract bacterial infection. For this reason, it is important to conduct a complete microbiological study such as the one described here.

Our results show that after microbiological cure, total sperm concentration, viability, motility and total motile sperm clearly improved.

The percentage of teratozoospermic patients did not change after antibiotic therapy, and those with normal morphology values remained unchanged.

In two patients with antisperm antibodies, the antibody titer became negative after bacteriological cure. Genital tract infections in males may trigger immunoresponse and elicit the formation of antisperm antibodies[32]. A possible mechanism was reported by Kurpisz & Alexander[33] regarding common antigenic determinants between infectious organisms and components of the reproductive system.

In conclusion, in this study we have shown that sperm parameters improve after bacteriological cure in infected asymptomatic infertile patients. These results stress the importance of proper microbiological studies and specific antibiotic therapy in individuals suspected of having genital tract infections.

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