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Impact of Coagulase-Negative *Staphylococci* and Other Germs on Sperm Forms

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ABSTRACT

Coagulase-negative Staphylococci (CoNS) is part of the microbiota of the male genitourinary tract, sometimes it has been considered as possible pathogenic microorganism. In the 5th version of sperm manual (WHO, 2010) sperm morphology criterion is very restricted to 4% of normal heads whereas David's criterion evaluates several spermatic forms. The abnormalities of sperm forms were evaluated according to criteria of spermatic morphology: WHO and David in semen samples with bacterial concentrations ≤ 10^3, 10^4 and ≥ 10^5 CFU/mL of CoNS as of other bacteria. Spermogram, sperm culture and antibodies anti-Chlamydia trachomatis IgA detection in 281 semen samples men were performed. CoNS was the most frequent germ isolated in pure culture (9.25%). Semen samples with CoNS showed higher round cells and microcephalus forms by means of David's criterion. CoNS in higher concentrations than 10^4 CFU/mL may have a negative impact on sperm cellularity, sperm head and probably on fertility.

Keywords: Coagulase-negative *Staphylococci*, Spermogram, *Chlamydia trachomatis*, Sperm culture

INTRODUCTION

Infections of the male genital tract have been associated with changes in sperm function and infertility [1]. These are often asymptomatic and chronic if they are not identified in a timely manner [2]. Microbiological culture has been a diagnostic tool that allows determining the susceptibility to antibiotics and to choose for the most effective therapy [3], while the polymerase chain reaction (PCR) allows the detection of the genome with greater sensitivity but does not determine the antimicrobial susceptibility [4].

Sperm cultures may be falsely negative due to the presence of bacteriostatic substances, which are produced in male accessory glands [5]. It´s necessary to decrease these substances by means of: centrifugation [6], dilution [7] or pre-incubation of sample before the inoculation into culture media to allow the bacterial growing [5].

Vilvanathan, et al., in seminal and urethral samples of infertile men found *Enterococcus faecalis* (30%), *Staphylococcus* coagulase negative (23.33%), *Staphylococcus aureus* (20%), *E. coli* (10%), *Klebsiella pneumoniae*, *Proteus* spp. (6.66%) and *Citrobacter* spp. (3.33%); these species were not associated with changes in seminal parameters [8]. Other similar study included *Chlamydia trachomatis* showing alterations on the markers of accessory glands and low integrity of the spermatic membrane, but not on the other seminal parameters [9]. It can be considered that several bacterial species found in the semen of infertile men are known as Coagulase Negative *Staphylococci* (CoNS). In turn, *Staphylococcus epidermidis* is the most common species of CoNS in human semen. Although *Staphylococcus epidermidis* had not been associated with alterations in the classic sperm parameters, high apoptosis and a low fertility index have been observed by mean of high resolution ultramicroscopy [10]. It is possible that CoNS under certain conditions behaves as a pathogenic microorganism. It has been suggested that in men with symptomatology of genital infection, germs considered microbiota of the male urethra have clinic important when their growth in cultures exceeds 10^5 colony forming units (CFU/mL), while for pathogenic microorganisms the value of clinical interest is 10^4 CFU/mL [11].

On the other hand, seminal leukocytes are inversely related to sperm concentration and motility [8]. For several years leukocytospermia has been suggestive of bacteriospermia, also aggregations, alterations of pH [12], hyper viscosity, premature acrosomal reaction [13], apoptosis and necrosis. These last two explain the reduced motility and vitality of spermatozoa in several cases [14]. Regarding the impact of bacteriospermia on spermatic forms the studies usually refer only to reduction in normal forms [12]. The latest WHO manual has an extensive abnormality range associated with low rate of fertilization [15]. WHO manual is a very restrictive criterion that does not emphasize the importance of other abnormalities on forms [16], whereas the criteria of David's differentiates up to 16 categories of spermatic forms in each one of parts of the spermatozoa and attributed a possible aetiology [17].

In this study, we compared the sperm morphology by means of the WHO manual and modified David in infertile men CoNS and other germs present in different concentrations.

MATERIALS AND METHODS

Semen samples of 281 infertile men were studied; these men were attended in the Diagnostic Center of Infertility and Gynecological Diseases "Dr. Giovanny Vivas-Acevedo" (CEDIEG), Faculty of Pharmacy and Bioanalysis, University of Los Andes. The patients signed the consent following the guidelines established in the Helsinki Declaration outlined in the Bioethics and Biosafety code of the National Fund for Science, Technology, and Innovation (FONACIT) for human research [18]. Samples of men with azoospermia, cryptozoospermia, hypogonadism and diabetes were excluded.

Semen samples were obtained by masturbation following the parameters established in the 5th WHO manual for semen analysis [15]. By means of David's morphological criteria the most frequent forms were evaluated [17]. To determine the presence of bacilli and coccus (aerobic and microaerophilic), followed the semen liquefaction, the sample was pre-incubated to reduce the bacteriostatic effect of seminal plasma according to the methodology described by Alo, et al. [5] modified. The ratio of semen to the thioglycolate broth was 1: 200, which was incubated at 37°C for 6 hours (5% CO_2), then the culture (100 µL) was performed in each of the following culture media: (5% CO_2), Thayer-Martin (5% CO_2), Saline Mannitol and Mac Conckey, incubated at 37°C for 48 hours. Subsequently the bacterial concentration in CFU/mL was determined [19]. Another aliquot of 200 µL of semen was mixed and centrifuged at 5.000 g for 10 minutes to obtain seminal plasma which was stored at -20°C for the measurement of IgA anti-*Chlamydia trachomatis* antibodies using a commercial kit (Sero ELISA; Savyon, Beer-Sheva, Israel). The procedure was performed according to the manufacturer's instructions.

RESULTS

Seminal characteristics: volume, sperm concentration, hypoosmotic test, round cell concentration and leukocyte concentration per millilitre were observed in the different groups with negative culture results, resident microbiota, and other microorganisms with concentrations ≥ 10^4 CFU/mL including CoNS as well as samples with anti-*Chlamydia trachomatis* antibodies - Increased round cells and leukocytes were observed in most of the groups with bacteriospermia (Table 1).

Table 1 Age and seminal characteristics in the groups of patients

Category (n)	Age (years)	Volume (mL)	Spermatozoa (× 10^6 mL)	Host%	Round cells (× 10^6 mL)	Leukocytes × 10^6/mL
Negative (149)	35.76 ± 9.22	4.00 ± 0.35	85.7 ± 78.9	60.01 ± 18.1	1.09 ± 0.7	0.30 ± 0.12
Resident Microbiota (13)	34.40 ± 6.71	3.66 ± 0.44	98.83 ± 80.47	59.24 ± 16.87	1.04 ± 0.8	0.21 ± 0.25
CoNS (26)	35.67 ± 7.70	4.01 ± 0.56	91.7 ± 76.2	57.69 ± 15.1*	1.78 ± 1.1**	0.57 ± 0.52*
C. trachomatis (17)	40.0 ± 6.88*	3.44 ± 1.0**	104.1 ± 88.3	54.1 ± 20.8*	2.87 ± 1.8***	0.56 ± 0.44*
E. coli (13)	36.50 ± 8.30	3.90 ± 1.44	109.8 ± 134.3	60.7 ± 21.1	3.21 ± 2.9**	1.07 ± 0.77**
Enterobacter spp. (12)	33.8 ± 7.4	4.07 ± 1.84	98.8 ± 67.4	59.4 ± 18.8	2.01 ± 2.0**	0.91 ± 0.82**
Enterococcus spp. (38)	34.40 ± 6.71	3.88 ± 2.81	88.2 ± 79.9	59.8 ± 17.8	4.41 ± 0.8**	0.97 ± 0.44**
S. group Viridans (11)	34.40 ± 6.13	3.68 ± 1.29	108.1 ± 99.5	59.6 ± 20.0	1.05 ± 0.9	0.040 ± 0.41

*P ≤ 0.05. **P ≤ 0.005. Average values ± standard error (EE). Comparison of patient age and semen characteristics (volume, sperm concentration / mL, hyposmolar test (HOST), round cell concentration / mL and leukocyte/mL concentration in semen samples with the most frequently identified germs: *p ≤ 0.05, **p ≤ 0.005, t-student

Table 2 shows the morphological characteristics by David and WHO criteria; the predominance of microcephalus is observed in almost all positive groups with bacteriospermia. The elongated heads are elevated in two positive groups of bacterial species and in the group microbiota. Tails defects are found in the groups CoNS, *C. trachomatis*, *Enterobacter* spp. and *Enterococcus* spp.

Table 2 Characteristics of the spermatic morphology with David and WHO strict criteria

Category (n)	Morphologic David´s criterion							WHO Strict criterion
	Normal	Abnormal postacrosome region	Microcephalous head	Elongated head	Thin head	Tail: bend, absent, coiled, short, multiple, irregular	Abnormal residual cytoplasm	
Negative (149)	14.9 ± 8.34	31.8 ± 16.2	28.7 ± 14.6	8.2 ± 6.1	0.5 ± 0.4	2.36 ± 2.3	3.1 ± 3.8	7.5 ± 2.7
Resident Microbiota (13)	8.8 ± 0.63	11.06 ± 0.91	9.90 ± 0.12	5.96 ± 0.44*	0.8 ± 0.10	4.70 ± 0.87	3.40 ± 0.16	7.2 ± 0.66
CoNS (26)	12.8 ± 7.16	37.0 ± 21.3	34.7 ± 9.61*	7.4 ± 5.8	0.5 ± 0.8	3.90 ± 2.7*	4.2 ± 3.4	7.1 ± 3.7
C. trachomatis (17)	13.2 ± 8.56	36.47 ± 19.7	37.8 ± 12.1*	5.20 ± 5.8*	0.0 ± 0.8	4.07 ± 2.6*	3.20 ± 3.4	7.42 ± 3.1
E. coli (13)	16.1 ± 12.61	35.9 ± 21.4	34.4 ± 18.4*	5.1 ± 5.8*	1.6 ± 0.8	2.7 ± 2.7*	4.2 ± 3.4	7.1 ± 3.8
Enterobacter spp. (12)	13.7 ± 10.13	32.0 ± 18.2	29.2 ± 18.3	7.3 ± 5.8	0.6 ± 0.8	3.01 ± 2.6*	3.6 ± 3.4	7.1 ± 2.9
Enterococcus spp. (38)	13.4 ± 11.11	34.4 ± 17.4	35.0 ± 18.3*	8.7 ± 5.8	0.5 ± 0.8	4.2 ± 2.7	3.8 ± 3.4	7.5 ± 3.3
S. group *Viridans* (11)	14.1 ± 9.22	36.3 ± 19.9	33.1 ± 18.0**	8.3 ± 5.8	0.8 ± 0.8	3.40 ± 2.7	4.2 ± 3.4	6.1 ± 2.9

*p ≤ 0.05 **p<0.005. Average values ± standard error (SE). Comparison of normal spermatic forms according to David' and WHO criteria in semen samples with the most frequently microorganisms: *p ≤ 0.05 **p<0.005 t-student.

Sperm characteristics were analysed in relation to the bacterial concentration expressed in CFU/mL for all positive cultures independently of the found species. Four groups were categorized: Negative, ≤ 10^3 (Resident Microbiota), 10^4 and ≥ 10^5 CFU/mL. The high values of round cells were found in samples with bacterial concentrations equal to or greater than 10^4 CFU/mL ($p \leq 0.005$) (Table 3).

Table 3 Seminal characteristics in samples with different bacterial concentrations

Category (n)	Age (years)	Volume (mL)	Espermatozoa (× 10^6 CFU/mL)	HOST%	Round cells × 10^6 CFU/mL	Leukocytes × 10^6 CFU/mL
Negative (119)	35.76 ± 9.22	4.00 ± 0.35	85.7 ± 78.9	60.01 ± 18.1	1.59 ± 0.7	0.40 ± 0.12
≤10^3 CFU/mL (30)	35.51 ± 19.88	3.97 ± 2.22	98.8 ± 55.32	57.7 ± 32.31	2.01 ± 1.68	0.41 ± 0.22
10^4 CFU/mL (55)	37.21 ± 20.83	3.90 ± 2.18	102.8 ± 79.95	57.8.4 ± 32.36	4.21 ± 2.35**	1.01 ± 0.56**
≥10^5 CFU/mL (77)	38.80 ± 25.99	3.44 ± 2.30	96.3 ± 64.52.37	55.5 ± 38.52*	5.3 ± 1.92**	1.44 ± 0.96**

* P ≤ 0.05. **P ≤ 0.005 Comparison of patient age and seminal characteristics (volume. sperm concentration/mL. hyposmolar test (HOST%). round cell/mL concentration. and leukocyte concentration/mL) in samples of positive semen with criteria from ≤ 10^3 CFU/mL – 10^4 and 10^5 CFU/mL. It does not include samples with *C. trachomatis.*

Table 4 shows the characteristics of sperm morphology in the groups with ≤ 10^3 CFU/mL, 10^4 CFU/mL and ≥ 10^5 CFU/mL compared to the group negative. Using David's morphological criterion, microcephalus increases when bacterial concentrations are above 10^4 CFU/mL independently of the bacterial species ($p \leq 0.05$). Main alterations were observed in abnormal flagellum ($p \leq 0.05$), Tail defects: bend, absent, coiled, short, multiple ($p \leq 0.05$), irregular and abnormal residual cytoplasm ($p \leq 0.05$) when the bacterial concentrations were over 10^5 CFU/mL.

Table 4 Characteristics of sperm morphology according to David and WHO criteria in seminal cultures

Category (n)	Morphologic David´s criterion							Oms
	Normal	Abnormal postacrosome region	Microcephalous head	Elongated head	Thin head	Tail: bend. absent. coiled. short. multiple. irregular	Abnormal residual cytoplasm	Strict criterion
Negative (119)	14.9 ± 8.34	31.8 ± 16.2	28.7 ± 14.6	8.2 ± 6.1	0.57 ± 0.4	2.36 ± 2.3	3.1 ± 3.8	4.5 ± 2.7
$\leq 10^3$ CFU/mL (30)	15.5 ± 8.62	36.1 ± 10.3	31.7 ± 19.1	7.4 ± 5.9	0.40 ± 0.31	2.60 ± 2.2	4.6 ± 3.4	4.4 ± 2.9
10^4 CFU/mL (55)	16.7 ± 11.11	35.2 ± 16.7	37.1 ± 18.3*	8.0 ± 5.8	0.70 ± 0.8	3.3 ± 2.1	4.0 ± 3.4	4.1 ± 3.7
10^5 CFU/mL (77)	10.6 ± 7.77	31.6 ± 12.9	39.6 ± 15.1*	7.2 ± 5.8	0.70 ± 0.8	5.9 ± 2.7*	6.5 ± 3.4*	4.1 ± 3.7

*P ≤ 0.05 Average values ± standard error (SE). Comparison of morphologic changes in semen samples with negative culture. microbiota and positive cultures 10^4 and 10^5 CFU/mL t-student.

DISCUSSION

The impact of bacteriospermia on sperm morphology was demonstrated by mean of David morphological criterion for an increase of small heads ($p \leq 0.05$) except in *Enterobacter* spp. infection ($p \leq 0.05$). No differences were observed in normal head under strict criteria of WHO manual ($p \leq 0.05$) neither middle segment, flagellum, or residual cytoplasm (data not included in this study). The increase of the small heads may be attributed to immature chromatin [17], oxidative stress and fragmentation of the sperm DNA [20], which allows assume that there is an oxidative effect in sperm chromatin when CoNS is present in high concentrations, being deleterious in sperm forms as well as other pathogens. In the samples with Ab. Anti-*Chlamydia trachomatis*, CoNS, *E. coli*, and *Enterobacter* spp. alterations were observed in the flagellum ($p \leq 0.05$). Díaz-García and Nuñez-Calonge have found association between two types of alterations of the spermatic tail, finding folding in presence of *M. hominis* [21], and absence of the same in presence of *U. ureayticum* [22]. With respect to short tails only is known that is a finding that is a more frequent finding in populations of South American men [23]. WHO sperm morphology values didn´t show significant differences.

Round cells were increased in some cases. A study showed that increased round cells were inversely associated with sperm count/mL, motility, and normal forms [24]. Round cells are not often leukocytes, they may originate from the germinal or glandular epithelium and are indicative of other possible lesions of the reproductive tract, prostate, seminal vesicles, urethra [25] or epididymis [26,27]. Higher leukocytospermia was observed in the groups with $\geq 10^4$ CFU/mL. Gdoura has recommended a range of leukocytospermia $\geq 0.3 \times 10^6$ CFU/mL as a parameter suggestive of bacteriospermia [28]. The results of this study suggest that the concentration of round cells over 4×10^6 CFU/mL with or without leukocytospermia could be an important indicator of bacteriospermia.

Sperm cultures allows to quantify bacterial concentration and selecting specific therapy by antibiogram. Although CoNS has been considered normal microbiota of the genital tract, counts of them $\geq 10^4$ CFU/mL in presence of signs of infection should be taken into account. The increase in the number of round cells $\geq 10^4$ CFU/mL and small heads $\geq 37\%$ could be suggestive data of seminal infection even in the absence of leukocytospermia. To choose treatment for infection by CoNS. it is important to corroborate the bacterial concentration in a second semen sample under strict asepsis conditions.

CONCLUSION

The predominance of an abnormal form should be reported in seminal evaluations even with normal forms $\geq 4\%$. The high concentration of CoNS in semen can have a negative impact on cellularity, sperm morphology and probably on fertility. In the study of infertile man's semen, it would be very useful to describe the morphological alterations of the spermatozoon head as indicated by David's morphological criterion as additional data to identify the cause of infertility.

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