Potency of bacteriospermia and Sperm Quality in Leukocytospermic infertile males

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Abstract: Male factors are known to contribute to infertility problem. Semen samples were obtained from 50 Leukocytospermic infertile men with ($\geq 1x10^6$ peroxidase positive WBC's/ml) attending Hawaa fertility center, Benha, Egypt. Semen samples were categorizedinto two infertile male cohorts, based on bacteriological culture, Group1: positive bacterial culture (n=31) and Group 2: negative bacterial culture (n=19) and the seminological parameters of the two infertile male cohorts were compared with healthy controls (n=50). Positive bacterial culture has been defined by pathologically significant bacterial growth ($\geq 1x10^3$ bacteria/ml). Statistically significant deteriorated volume ($p<0.05$), viscosity ($p<0.05$), sperm concentration ($p<0.01$), vitality ($p<0.05$), progressive ($p<0.01$) and non-progressive motility ($p<0.05$) were detected in ejaculated samples of patients with positive bacterial culture in comparison to healthy controls. Statistically significant negative influence towards sperm reproductive potential has been revealed in case of *Escherichia coli* and *Staphylococcus aureus*. It would appear that the bacteria may be an additional negative factor influencing male fertility and worsening sperm quality.
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1. Introduction

Infections of the male genitourinary tract account for up to 15% of cases of male infertility (**Pellati *et al.*, 2008**). Recent studies have shown that acute and chronic infections and consequent inflammation in the male reproductive system may compromise the sperm cell function and the whole spermatogenetic process (**Urata *et al.*, 2001 and Sanocka *et al.*, 2005**) causing qualitative and quantitative sperm alterations.

Deterioration in spermatogenesis, obstruction of seminal tract and effect of spermatozoa function may be caused indirectly by activation of seminal plasma white blood cells or cellular reactions against microbial agents, as well as by direct influence of pathological bacterial strains on gametogenic cells (**Keck *et al.*, 1998**).

The bacteria responsible for semen contaminations generally originate from the urinary tract of patients or can be transmitted by the partner via sexual intercourse (**Purvis and Christiansen, 1993**). Many studies have examined the impact of genital tract infections on male fertility; however the effect of bacteriospermia on sperm quality is still controversial (**Haidl, 1990**).

The most frequently isolated microorganisms in male patients with genital tract infections or semen contamination is *Staphylococcus aureus* and *Escherichia coli*. The negative influence of this species on sperm quality is partially due to the following mechanisms: (a) Bacterial attachment to sperm and its effect on motility; (b) an immobilizing factor produced by some bacteria; (c) immune system recruitment, and (d) alteration of glandular function (**Diemer *et al.*, 2003 and Sanocka *et al.*, 2005**).

This study was conducted to evaluate the influence of different bacterial species on the sperm quality and semen characteristics of Leukocytospermic infertile men attending Hawaa fertility center in Benha City, Qaliubiya, Egypt.

2. Material and Methods

2.1. Study area

This study was carried out in Benha city, Qaliubiya, Egypt. Benha is the capital city and major commercial center of Qaliubiya Province. It is densely populated with men and women of all ages engaged in all walks of life. The semen samples used in this study were obtained from patients with infertility problems who were referred to Hawaa fertility center.

2.2. Selection of respondents

The study took place from June 2012 to April 2013.The Study population was made up of infertile males attending Hawaa fertility center. The inclusion criteria were as follows: (i) Participant must be a resident of Qaliubiya Province; (ii) Must have

been married for 1 year before inclusion into the study and were unable to achieve pregnancy; (iii) Must have not received an antibiotic treatment for the last 4 weeks prior to sampling; (iv) Aged 22-35 years old; (v) Showed leukocytospermia in their semen.

2.3. Sampling technique

The study samples were collected from patients who indicated willingness to participate in the study and have had 3 to 7 days of sexual abstinence from intercourse, using the masturbation method and ejaculated in wide mouthed plastic container as described by WHO (**WHO, 2010**). These patients were already confirmed with infertility problems by medical doctors and then referred to Hawaa fertility center for laboratory diagnosis.

These samples from healthy individuals who also indicated willingness to participate in the study as controls were collected in the same designated fertility center by the previously described method. All sample collection was performed at the center's sample collection room.

2.4. Seminological analysis

Altogether, 100 ejaculated semen samples from control individuals (n=50) and infertile males (n=50) were first analyzed for semen and sperm characteristics as diminished by WHO (**WHO, 2010**).

Samples were allowed to liquefy for 20 minutes and were examined for volume, PH, viscosity, spermatozoa count (using Neubauerhaemocytometer), Leukocyte detection and counting (using leukoscreen; Fertipro; Belgium), percentage of progressive motility and non-progressive motility, Sperm morphology (using spermac stain; fertipro; Belgium)and sperm vitality (using eosin-negrosin stain; fertipro; Belgium) .

2.5. Bacteriological analysis

Thereafter, bacteriological semen analysis was performed on a solid-phase using standard bacteriological culture techniques as described previously (**McGowan *et al.*, 1981**). Within 1 h of collection, the seminal fluids of all sample group were cultured using Nutrient Agar, Blood Agar (BA), Chocolate Agar and MacConkey Agar (Oxoid, cairo, Egypt). The culture plates were incubated aerobically at 37°C for 48 hours.

After incubation period, the number of bacteria in 1ml of semen was estimated using standard plate count method as described previously (**Reynolds and Farinha, 2005**).

The isolation and identification of bacterial isolates were carried out in accordance with Bergey's Manual of Determinative Bacteriology (**Buchanan and Gibbons., 1978**) as Following:

Colonial characteristics, gram stain, catalase, Dase tests and haemolysis on BA medium, lactose fermentation and other biochemical tests as indole production, citrate utilization, triple sugar iron agar test, gas and hydrogen sulphide production, urease and oxidase test were conducted.

2.6. Statistical analysis

The collected data were tabulated and analyzed using IBM SPSS version 19 software. Categorical data were presented as frequency and percentages while quantitative data were expressed as mean ± standard deviation. The collected data were represented graphically using Microsoft Excel 2010 software. Categorical data were represented by Pie chart, while quantitative data were expressed by Bar chart.

The accepted level of significance in this work was stated at 0.05 ($P < 0.05$ was considered significant, $P < 0.01$ was highly significant and P value > 0.05 was insignificant).

3. Results

3.1. Semen parameters

Normozoospermic healthy individuals with volume > 1.5ml, viscosity <2Cm, sperm count $>15\times10^6$/ml, progressive motility >32%, normal morphology > 4%, vital sperms > 56% constituted the control group "n=50" (**WHO, 2010**).

Leukocytospermic infertile males with ($\geq1x10^6$ peroxidase positive WBCs/ml) were subdivided into two infertile cohorts, positive "n=31" ($\geq1x10^3$ bacteria/ml) and negative bacterial culture "n=19" ($<1x10^3$ bacteria/ml).

Volume of semen samples in infertile cohorts with positive bacterial culture (PBC) was significantly lower than in healthy controls ($P<0.05$; see table 1). In the group of patients with negative bacterial culture (NBC) the volume of ejaculate was also diminished, although it was statistically not significant ($p>0.05$; see table 1).

Viscosity of semen samples in infertile cohorts with positive bacterial culture was significantly lower than in healthy controls ($P<0.05$; see table 1). In the group of patients with negative bacterial culture the Viscosity of ejaculate was also diminished to be statistically significant but with lower significant than in infertile cohorts with positive bacterial culture ($p<0.05$; see table 1).

PH of semen ejaculates also diminished, although it was not statistically significant in both infertile patients' cohorts.

The concentration of sperm cells and it's ability for rabid and slow progression was

significantly diminished in both cohorts of infertile patients with leukocytospermia but in the group of bacteriological positive infertile patients the detrimental influence of bacterial infection have been demonstrated at higher statistical significance ($p<0.01$; see table 1).

Table 1. Seminological analysis of ejaculates from healthy men and infertile patients with positive and negative bacterial culture

Semen Parameter		Control Group (Peroxidase negative<10^6/ml) N=50	Study Group (Peroxidase negative<10^6/ml) N=50			
			Patients with PBC n=31	p-value	Patients with NBC n=19	p-value
Macroscopic Parameters	Volume (ml)	2.466 ± 0.59	02.06 ± 0.079 *	0.013	02.61± 00.46	0.077
	PH	07.27 ± 00.09	07.27 ± 0.088	0.698	07.30 ± 0.091	0.169
	Viscosity (Cm)	0.164 ± 0.083	01.03 ± 01.30 *	0.021	01.08 ± 01.16 *	0.04
Microscopic parameters	Leukocyte Count (10^6/ml)	0.036 ± 0.041	04.55 ± 00.95 ***	< 0.001	03.06 ± 00.85 ***	< 0.001
	Sperm Concentration (10^6/ml)	43.80 ± 15.20	33.48 ± 15.88 **	0.008	34.10 ± 14.35 *	0.043
	Motility (%motile)	70.54 ± 10.26	68.10 ± 13.80	0.726	68.40 ± 11.60	0.61
	Progressive motility (%)	49.90 ± 07.26	42.70 ± 15.50 **	0.009	42.80 ± 15.80 *	0.026
	Non-progressive motility (%)	20.64 ± 08.55	25.40 ± 08.90 *	0.027	25.60 ± 09.70 *	0.048
	Vitality (%vital)	90.50 ± 03.53	88.80 ± 03.90 *	0.042	91.00 ± 02.40	0.866
	Morphology (% abnormal)	79.00 ± 04.35	81.20 ± 05.90	0.083	82.20 ± 06.30	0.056

Non-progressive motility of sperm cells in both cohorts of infertile male patients was significantly different from the values observed in ejaculates of healthy men but in the group of bacteriological positive patients with a little higher statistical significance ($P<0.05$; see table 1).

The percentage of abnormal sperm cells was increased in both cohorts of infertile male patients in comparison to healthy controls, but it was not statistically significant in both leukocytospermic infertile patients' cohorts ($P>0.05$: see table 1).

Vitality of spermatozoa in ejaculates of bacteriological positive infertile patients was also diminished as comparing to controls, and this difference was found to be statistically significant ($P<0.05$; See table 1). In the group of bacteriological negative leukocytospermic patients, the vitality of spermatozoa was also diminished, although it was not statistically significant ($p>0.05$; see table 1).

3.2. Semen bacteriology

3.2.1. Identification of bacterial isolates

Pathological bacterial isolates present in semen were identified according to Bergey's Manual of Determinative Bacteriology (**Buchanan and Gibbons., 1978**).

From the data showed in table (2) it was concluded that groups of pathological bacterial

isolates were respectively: *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus epidermidis*, *klebsiella pneumonia*, *Streptococcus pyogenes* (See table 3).

3.2.2. The Frequency of isolated pathological bacterial isolates

The prevalence of bacterial agents isolated from the bacteriological positive infertile males were in order of appearance: *Staphylococcus aureus* (32.26%); *Escherichia coli* (25.81%); *Staphylococcus epidermidis* (19.35%); *klebsiella pneumonia* (12.9%); *Streptococcus pyogenes* (9.68%) (See table 3).

3.2.3. Role of specific organisms

In ejaculated samples from infertile patients with genital tract infection it was observed that there were statistical significant correlations between *E. coli* and *S. aureus* against selected semen parameters and those correlations were of negative nature except for morphology it was of positive nature because here in the present study morphology represents (% abnormal forms). The presences of *E. coli* and *S. aureus* were associated with the lower sperm concentration (10^6/ml ejaculate) and diminished progressive motility with negative correlation (See table 4). Additionally, *S. aureus* had been positively correlated with sperm morphology and this positive because morphology represents abnormal forms percent (Table 4).

4. Discussion

The prevalent opinion is that leukocytospermia is always concomitant with bacteriospermia; however the lack of leukocytospermia does not preclude the development of genitourinary symptoms of disease (**Potts *et al.*, 2000**). This study did confirm this opinion as 62% of leukocytospermic male infertile patients showed linked bacteriospermia.

Many studies examined the impact of male reproductive potential; however, the effect of bacteria on sperm quality is still controversial **(Merino *et al.*, 1995 and Sanocka *et al.*, 2004**).

In this study, it was reported that, the frequency of the bacterial isolates, particularly the genera *Staphylococcus* and *Escherichia* were the most predominantly common bacterial isolates associated with the semen of men complaining of infertility. This report finding was supported by earlier reports by **Khalili and Yazdi (2001)** and **Ekhaise and Richard (2011)**.

Among the bacteria isolates, the highest count was recorded for genera *Staphylococcus* (51.16%) and this finding strongly correlates with previously published reports which found 68%, 41.5% and 77.7% of infertile male patients have Staphylococcal infections (**Merino *et al.*, 1995; Khalili and Yazdi., 2001 and Ekhaise and Richard., 2011**) respectively and this variations may be due to the difference of study area and distribution of this bacterial strain among infertile couples.

The presence of the microorganisms is an indication of microbial infection. **Gomez *et al.* (1979)** reported that microbial infections of the semen are major causes of male infertility. In the study, high percentage of the isolates was recovered from semen samples with poor semen data as well as the profound effect of micro-organisms on the sperm progressive motility and concentration.

The viability and structural integrity of the semen lies on its characteristic feature of mobility as it was reported by **Stephen *et al.* (1989)**. The results of this study confirmed the fact that the microorganisms have negative influence towards sperm reproductive potential in cases of infection with *E. coli* and *Staph. aureus* amongst other microorganisms **(Sanocka *et al.*, 2005**).

The obtained results of this study showed great agreement with studies carried out by **Auroux *et al.* (1991)** which indicated that it was probable that the presence of *E. coli* in semen decreases sperm motility, as it showed here a significance value ($p<0.01$).

The findings of this study showed that the simple presence of bacteria might alter the sperm quality and seminal characteristics in volume, viscosity, concentration, progressive and non-progressive motility and viability within the infertile males.

The mean sperm concentration in the group of individuals positive for bacteria was significantly lower than that observed in controls ($p<0.01$), however, it was $> 20 \times 10^6$ sperm/ml, value considered normal for WHO (**WHO, 2010**).

The negative influence of bacteria on sperm motility is well known (**Sanocka *et al.*, 2005; Fraczek *et al.*, 2007**). In our study, motility was significantly reduced in groups infected by *E. coli* and *S. aureus* ($p<0.01$; $p<0.001$) respectively.

The decrease in sperm motility may be due to immobilization as demonstrated by **Paulsson and Polakoski (1977)** or death of spermatozoa due to the action of bacterial toxins (**Delporto *et al.*, 1975**).

Previous studies have also shown that a decrease in sperm morphology may be due to bacterial disintegration (**Gopalkrishnan *et al.*, 1988**), but there was no significant difference in this characteristic in the present study.

Table 2. Characterization and identification of bacterial isolates from leukocytospermic semen samples

Culture Characterization	Case Morphology/Gram staining	catalase Test	DNAse Test	Citrate Test	Motility Test	MR Test	VP Test	Indole Test	Urease Test	Oxidase Test	Trible Sugar Test	Acid Production	Gas Production	Probable Organisms
Purple colonies on macconkey agar plates.	**Gram-negative / rods / single and few in pairs**	+	-	+	-	-	+	-	+	-	+	+	+	***Klebsilla pneumonia***
Pink colonies om macconkey agar plates.	**Gram-negative / short rods / coccobacillus scattered and in signals**	+	-	-	+	+	-	+	-	-	+	+	+	***Escherchia coli***
Golden yellow colonies on nutrient agar plates.	**Gram-Positive /cocci / clusters and pairs form irregular clusters.**	+	+	-	-	-	+	-	+	-	+	+	-	***Staphylococcus aureus***
White colonies on blood agar plates.	**Gram-Positive /cocci / clusters and/or pairs form irregular clusters.**	+	-	-	-	-	+	-	+	-	+	+	-	***Staphylococcus epidermidis***
White colonies produced large zones of beta-haemolysis on blood agar plates.	**Gram- positive / Cocci / arranged in short or tall chains.**	-	-	-	-		-	-	-	-	+	+	-	***Streptococcus pyogenes***

Table 3. Percentage of pathological bacterial isolates from semen samples of leukocytospermic infertile patients with PBC

Bacterial Strain	Proportion (%)
Staphylococcus aureus	32.26
Escherchia coli	25.81
Staphylococcus epidermidis	19.35
Klebsilla pneumonia	12.9
Streptococcus pyogenes	9.68

Table 4. Spearman rank order correlation between the semen parameters and pathological bacterial isolates

Correlation	Spearman rank (r)	Statistical significance (p)
Escherchia coli vs Sperm concentration ↓	-0.444	0.011
Escherchia coli vs Progressive motility ↓	-0.376	0.004
Staphylococcus aureus vs Sperm concentration ↓	-0.465	0.001
Staphylococcus aureus vs Progressive motility ↓	-0.496	0.0002
Staphylococcus aureus vs Sperm morphology ↑	0.538	0.001

The results of this study oppose some authors who find no difference between semen characteristics from groups of infected and non-infected infertile men (**Lewis *et al.*, 1981; Makler *et al.*, 1981**).

Hillier *et al.* (1990) found no difference in semen parameter according to the number of different types of microorganisms, Conversely, in a comparison of infertile and fertile men having a positive semen culture, **Jacques *et al.* (1980)** found a lower percentage of motile spermatozoa (27 vs. 35%, $p < 0.00l$) and this study showed agreement results with **Jacques *et al.* (1980)**.

Herein, we have confirmed the past findings that some pathogens: *E. coli* and *S. aureus* may have direct negative influence on semen quality of infertile males (**Sanocka *et al.*, 2005**).

One explanation for the wide variability of results might be differences in patients' recruitment, as well as different methods of semen collection and asepsis.

5. Conclusion

In conclusion, the results obtained from this study indicate that:

a) Pathological bacterial isolates that contaminate semen samples may directly deteriorate the sperm quality and negatively affect sperm cells.
b) The genital tract infection may be an additional negative factor influencing male fertility and worsening reproductive potential.
c) Microbiological screening should be always performed when investigating male infertility.

Although our findings shown that contamination of sperm samples by some species is more closely associated with infertility, however, this is only part of the problem. As in this study, we only dealt with aerobic and facultative anaerobic bacteria. The clinical significance of strict anaerobes in sperm samples is a subject of dispute.

Anaerobic bacteria are not routinely sought in sperm samples, because they are fastidious to cultivate and may be damaged by the contact with oxygen for the duration of transportation.

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