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Original Article

The Effect of Asymptomatic Bacteriospermia on Semen Quality among Infertile Men Referred to Infertility Clinics in Kerman

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ABSTRACT

Background: Semen analysis is considered as an important tool in infertility assessment process. One of the reasons implicated for altered semen quality, is bacteriospermia. This study aimed to determine the prevalence of bacteriospermia in seminal fluid and its effect on semen characteristics of infertile men attending infertility clinics in Kerman.

Methods: Totally, 200 fertile and infertile men were investigated. Spermiogram, culture, isolation and identification were conducted according to WHO guideline and standard bacteriological methods.

Results: Semen parameters including sperm motility, sperm count, sperm morphology, sperm viability and the percentage of round cells in seminal fluid, showed significant difference between fertile and infertile groups. Also, in infertile men, abnormal sperm morphology was significantly higher among oligospermic specimens than normospermic ones.

Bacteriospermia was found in 10% of infertile men and *Staphylococcus aureus* was the most common organism which was mostly isolated from teratozoospermic and asthenozoospermic specimens, however this finding was not statistically significant. Totally, asymptomatic bacteriospermia did not show any association with either infertility or abnormal semen parameters.

Conclusion: Other bacterial/ non- bacterial agents or even factors other than infectious agents, may affect semen quality. However, to determine the exact role of bacteriospermia in infertility, further studies as well as evaluation of the outcome of bacteriospermia treatment, especially in those infected with *S. aureus*, are required.

Keywords: Male infertility, Bacteriospermia, Semen parameters, Seminal fluid

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Introduction

Infertility is a problem in about 10-15% of couples in reproductive age. As it can influence the social and psychological aspects of people's life, its importance can be more than the other health issues. Both genders are relatively equally involved, so that about 40-45% of infertility cases are related to males (1, 2). Approximately, the contribution of infectious agents in male infertility has been estimated at 15% (3, 4). However, as most of urogenital infections have no clinical symptoms, the necessity for treatment of infected individuals has not been proven. On the other hand, increasing evidences reveal that there is association between asymptomatic bacteriospermia (semen infections) and abnormalities in semen parameters. According to some studies, semen infections can affect fertility process through different mechanisms; spermatogenesis inhibition, abnormal decrease of spermatozoa, remarkable decrease in sperm motility, change in sperm morphology, formation of reactive oxygen species, development of anti-sperm antibodies and obstruction of genital tract (5, 6).

As the role of asymptomatic infections in infertility remains controversial, it is not clear if screening of these patients and subsequently their treatment can reduce the infertility rate. Therefore, this study was planned to determine the prevalence of bacterial infections and to investigate their impacts on semen characteristics among infertile men in comparison with the fertile ones.

Materials and Methods

In this cross- sectional study, from May to October 2019, simple random sampling was conducted to collect semen specimens from 100 infertile male partners referring to the infertility research-clinical centers in Kerman. Inclusion criteria for infertile group were as follows (7, 8): Male partner known as responsible for infertility, no abnormalities in reproductive organs (varicocele, hydrocele) or endocrine dysfunction, no sign of genitourinary tract infections, no antibiotic consumption within the last 2 weeks, no consumption of immunosuppressive drugs, sexual abstinence within the last week and having abnormal spermiogram.

Likewise, in the same period, fertile men in the same age range as infertile group, were sampled. Having normal spermiogram and history of normal pregnancy in their spouse were considered as inclusion criteria.

This research was approved by the ethical committee of Kerman University of Medical Sciences (IR.KMU.REC.1398.207). Written informed consent was obtained from all participants included in this study. After giving instruction to perform sampling, semen specimens through masturbation were collected in sterile containers.

Semen analysis including its volume, time of liquefaction, the presence of round cells as well as sperm concentration (number of sperm cells/ ml), sperm motility and morphology were performed by a technician at infertility center according to World Health Organization (WHO) guidelines (9).

Some of the semen specimens were transferred to the Microbiology Department of Kerman University of Medical Sciences and allowed to be liquefied at 37℃ for 30 minutes. Then, 50 µl of specimen was inoculated onto Blood agar and EMB plates and incubated overnight at 37℃. Isolated microorganisms (> 10^4 CFU/ ml) were identified by Gram staining and diagnostic biochemical reactions. Mixed cultures were considered as contamination and were excluded.

Statistical analysis

Chi square test (or Fisher exact test) was employed to assess the association between bacteriospermia and semen parameters. A *p*-value ≤ 0.05 was regarded as significant.

Results

In this study, analysis was conducted on a total of 200 semen specimens. Infertile males ranged 21-60 years of age (35.23± 7.38) and fertile men were from 17-60 years old (34.29 ± 7.3).

The most prevalent abnormality in semen specimen in infertile group was sperm viability (76%), followed by asthenozoospermia (total motility < 40%), teratozoospermia (typical morphology <4%), abnormal sperm count and increase of round cells which were observed in 66%, 37%, 26% and 19% of specimens, respectively.

Among infertile group, 74% of specimens showed normospermia (spermatozoa concentration $\geq 15x10^6$/ml), while in 24 specimens (24%), oligospermia (sperm counts < $15x\ 10^6$/ml) and in 2 specimens, azoospermia (no sperm cells) were detected. In comparison, in

fertile group, only 7 specimens (7%) showed oligospermia and the remained ones had normal sperm count. Totally, there were significant differences between two groups, in terms of motility, morphology, concentration and viability of sperm cells, as well as the percentage of round cells in seminal fluid (Table 1). Also, spermiogram analysis revealed that the mean percent of sperm motility (both progressive and non-progressive motility) was significantly lower in infertile group. In thesame way, the rate of immotile sperms were significantly higher in this group (Table 1).

Table 1. The frequency of semen parameters in fertile and infertile men referring to the infertility clinics in kerman

Semen parameters	Fertile group		Infertile group*		P-value
	Normal	Abnormal	Normal	Abnormal	
Total sperm motility	100	0	33	65	0.000
Sperm concentration	93	7	74	26	0.001
Sperm viability	98	2	24	74	0.000
Color	100	0	94	4	0.5
Liquifaction time	99	1	98	0	0.5
Sperm morphology	99	1	62	36	0.000
Round cells	94	6	79	19	0.004
Volume	86	14	86	12	0.4
Progressive sperm motility (%)	45.81± 15.05		16.93± 11.5		<0.0001
Non-progressive sperm motility (%)	17.11± 6.98		13.55± 9.88		0.003
Immotile sperm (%)	37.32± 12.55		68.30± 18.45		<0.0001

* In two specimens, some information of semen analysis was not available.
Normal range of measured parameters: total sperm motility: sum of progressive and non-progressive motility of sperms >40%, sperm concentration>15 x 106 / ml, sperm viability % >58%, normal color of semen: gray to milky, liquifaction time <60 minutes, sperms with normal morphology >4%, round cells <5 x106 / ml

Multiple abnormalities such as oligotratozoospermia, oligoasthenozoospermia, asthenoteratozoospermiaand oligoasthenoteratozoospermia were respectively detected in 18, 19, 30 and 17 specimens of infertile group.

Seminal fluid analysis within the infertile group showed that the presence of tehratozoospermia among oligospermic specimens was significantly higher compared to normospermic ones. Also, abnormalities in viability and motility of sperm cells among oligospermic specimens were higher than those in normospermic ones, but these differences were not significant (Table 2).

Table 2. Frequency (%) of abnormal semen parameters in normo/ oligospermic specimens in infertile group

Abnormal Semen parameters	Oligospermia	Normospermia	P-value
Sperm morphology	75	24	0.000
Total sperm motility	79	62	0.1
Sperm viability	83	73	0.4

Normal range of measured parameters: total sperm motility: sum of progressive and non-progressive motility of sperms >40%, sperm viability % >58%, sperms with normal morphology >4%
Normospermia: sperm concentration >15x 106 / ml, Oligospermia <15 x 106/ ml

Totally, bacteriospermia was observed in 10% (n=10) and 15% (n= 15) of infertile and fertile specimens, respectively. The most prevalent microorganisms in infected infertile specimens were *Staphylococcus aureus* (50%, n= 5), followed by *Escherichia coli* (20%, n=2), and *S. epidermidis*, *S. haemolyticus* and *Enterococcus faecium* (each one 10%, n=1); whereas, in infected fertile specimens, *S. epidermidis* (40%, n= 6) and *E. coli* (33.3%, n=5), *E. faecalis* (13.3%, n=2), *E. faecium* and *S. aureus* (each one 6.6%, n= 1) were the most prevalent ones. No significant association was found between sperm parameters and bacterospermia (Table 3). Also, abnormal parameters of semen specimen did not show any association with bacterial infection.

Table 3. Comparison of semen parameters of the fertile and infertile men within the infectious and non-infectious groups

Group	Semen parameters	Infectious	Non- infectious	P-value
Infertile	Normospermia	7	67	0.71
	Oligospermia	3	23	
	Normal sperm motility	3	30	1.00
	Abnormal sperm motility	7	58	
	Normal sperm morphology	5	57	0.49
	Abnormal sperm morphology	5	31	
Fertile	Normospermia	14	79	1.00
	Oligospermia	1	6	
	Normal sperm motility	15	85	ND
	Abnormal sperm motility	0	0	
	Normal sperm morphology	15	84	1.00
	Abnormal sperm morphology	0	1	

Normospermia: sperm concentration >15x 106 / ml, Oligospermia <15 x 106/ ml
Normal range of measured parameters: Total sperm motility: sum of progressive and non-progressive motility of sperms >40%, sperms with normal morphology >4%
ND: not determined

Discussion

Semen analysis involves some descriptive parameters of seminal fluid and spermatozoa which helps to estimate semen quality (10). In this study, asthenozoospermia was the most common abnormal characteristic, following sperm viability, whereas in reports of Owolabi *et al* (11) and Vilvanathan *et al* (12), teratozoospermia was the most common one among infertile male. Regarding multiple abnormalities, asthenoteratozoospermia was the most prevalent within our specimens in comparison to oligoteratozoospermia reported by Vilvanathan *et al* (12). Similar to some studies (11, 12), teratozoospermia and asthenozoospermia were more prevalent among oligospermic specimens than normospermic ones, however for the latter one, this association was not significant.

The incidence of infertility increases with age, both in males and females. For various reasons, the age of marriage and subsequently the age of the first pregnancy is increasing (13, 14). Our study showed that most of the participants who were under infertility treatment (78.4%) were older than 30 years old.

Frequency of bacteriospermia was 10% which is somehow similar to Domes *et al* study (15). Although some studies have reported higher frequencies of bacteriospermia (13, 15, 17).

The role of bacterial infection in male infertility and subsequently the importance of asymptomatic bacteriospermia is controversial. Some researchers consider bacteriospermia as contamination (18), while others have demonstrated that bacterial infection can affect male reproduction function either directly by impaired sperm motility, morphology and sperm agglutination or indirectly by oxidative stress (5-6, 17). This situation underscores the treatment of asymptomatic bacteriospermia.

Staphylococcus aureus was the dominant bacterial agent in infertile group which is similar to the previous report of Owolabi *et al* (12). Although in some studies *Enterococcus faecalis* (13,15) and in some others, *S. epidermidis* were reported as the most prevalent ones (19).

Totally, in agreement with Vilvanathan *et al* (12) semen parameters were not affected by bacterial infections. In this study, *S. aureus* as the most prevalent cause of bacteriospermia in infertile group, was mostly isolated from teratozoospermic and asthenozoospermic specimens, however this finding was not statistically significant. In this context, some studies have shown that *S. aureus* has the most negative effect on morphology and motility of sperms (20, 21). A possible explanation that can be proposed is that this was probably a new infection and under this condition the proximity of bacteria was not enough to damage sperm cells. Also, it can be supposed that seminal fluid in fertile men is able to inhibit mechanisms through which bacteria may damage sperm cells.

In spite of many efforts to establish standard definition for bacteriospermia, the line between contamination and infection is not clear yet. This subject may also justify the absence of bacteriospermia effect on semen parameters.

Conclusion

In conclusion, no association between asymptomatic bacteriospermia and infertility was found. However, as in this study all bacterial infections such as mycoplasma and chlamydia were not examined, considering all these agents together, may help to reach a more appropriate

conclusion. Furthermore, evaluation the treatment of asymptomatic bacteriospermia particularly *S. aureus* infections, will be helpful.

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