Original Article

Association of Semen Bacteriological Profile with Infertility:– A Cross-Sectional Study in a Tertiary Care Center

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Abstract

Context: Infections are an important cause of male infertility. The specific effects of infections on various semen parameters remain unexplored, especially within the Indian subcontinent. **Aim:** The aim of the study was to determine the bacteriologic profile of semen, and its effect on semen parameters, with particular emphasis given to *Ureaplasma urealyticum* and *Mycoplasma hominis* tested by semen polymerase chain reaction (PCR). **Study Setting and Design:** The research was a cross-sectionl analaytical study conducted in a tertiary care center in South India from March 2018 to November 2019, on 48 male partners of couples presenting with infertility. **Methodology:** After obtaining informed consent from the study participants, semen collection was done. The sample was subjected to standard semen analysis according to the WHO 2010 Manual, followed by bacteriological testing using routine culture methods. In addition, real-time PCR was done to test for *U. urealyticum* and *M. hominis*. **Statistical Analysis:** Demographic data, semen analysis parameters, bacteriological culture findings, and real-time PCR results were compared and analyzed using the software IBM® SPSS 19.0. **Results:** A significant difference in viscosity of semen, which was higher in the samples that were positive for real-time PCR of *M. hominis*, was found. Other than this, no other parameter had a statistically significant difference between culture or real-time PCR positive samples and negative samples. **Conclusion:** Our study, though limited by a small sample size, highlights the role played by seminal infections in the context of male infertility. Larger scale prospective studies in this area would be invaluable in deciding the management plans of male factor infertility.

Keywords: *Bacteriology, bacteriospermia, culture, infections, infertility, male infertility, mycoplasma, polymerase chain reaction, semen, seminal infections, ureaplasma*

Introduction

Infections constitute up to 15% of the causes of male infertility.[1] Seminal infection has been shown to affect semen parameters through various mechanisms such as breach of the blood–testis barrier and upregulation of inflammatory cytokines.[2,3] Infections have been shown to adversely affect semen parameters such as sperm concentration, motility, and DNA fragmentation.[4-6] In addition to the above, chronic infections can also cause direct mechanical damage to the semen pathway, and the inflammatory milieu can inhibit semen maturation.[7,8]

It has been noted that even a thorough evaluation fails to arrive at a cause for male infertility in 60% of men,[9] opening up possibilities on the role played by asymptomatic seminal infections and bacterial colonization. The presence of bacteria in the ejaculate

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may be because of infection, or even colonisation or contamination.[10] Various studies on bacteriospermia, over the years, have demonstrated a wide range not only in the prevalence of asymptomatic seminal infections but also in the species of organisms isolated.[11-14] There has also been a statistically significant association of bacteriospermia with the impairment of semen parameters.[6,15,16] While research has linked male infertility with sexually transmitted infections,[17] there is a paucity of data regarding the role played by asymptomatic bacteriospermia, especially in the setting of developing countries where infectious diseases are common. Our study was conducted with the aim to profile the bacteriology of semen and explore the association of bacteriospermia on semen parameters. Special emphasis was given to the role played by *Ureaplasma urealyticum* and *Mycoplasma hominis* as these pathogens have been shown by various studies conducted previously to have a significant adverse impact on semen parameters.[18-22]

Methodology

Our research was a cross-sectional analytical study, conducted in the Department of Obstetrics and Gynecology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, India. Clearance was obtained from the Institute Ethics Committee on March 15, 2018 (IEC NUMBER: JIP/IEC/2017/440), and the recruitment process was carried out from March 2018 to November 2019. Male partners of couples attending the infertility clinic were included. Participants with congenital causes of infertility such as anorchia, and absence of vas deferens, were excluded as the etiology of infertility is unlikely to be of infectious origin in such cases. Diagnosis of such conditions was made by a thorough physical examination including estimation of testicular volume, followed by imaging and hormonal profile if required. Adherence to the Helsinki Declaration (ethical principles for medical research) was ensured throughout the study process.

Procedure

Sample size

The sample size was calculated based on a review of the literature demonstrating a bacteriospermia range of 30% to 74%.[6,14,15,19] A sample size of 89 was estimated with a 5% level of significance and relative precision of 20%.

Recruitment

Convenient sampling method was used for recruitment. All male partners of infertile couples who visited the infertility clinic during the enrollement period were considered for the study. Among these, a number of participants were not willing to participate in the study or did not follow-up with semen analysis after the initial assessment [Figure 1]. A written informed consent was taken from all study participants.

The study was conducted with the aid of intramural funding from the institute for postgraduate research projects. Although budgetary planning was done before the study, escalating costs of the testing kits resulted in capping of the number of kits that could be procured for the study.

Data collection included baseline demographic information, medical history, and details of comorbid conditions. History included a detailed sexual history including frequency of intercourse, sexual health issues, history of possible sexually transmitted diseases, and sexual contact with commercial sex workers. Participants were also subjected to a thorough physical examination. They were also subjected to routine blood investigations as part of workup for infertility, which included venereal disease research laboratory and viral serological markers for HIV and HBsAg. Following this, instructions were provided on semen collection according to the WHO guidelines.[23]

After semen collection, the sample was sent for both routine semen analysis and microbiological testing [Figure 2]. The microbiological evaluation included microscopic examination of Gram-stained smear and culture for bacteria. Inoculation was made in blood agar and MacConkey agar and incubated aerobically at 37°C for 48 h. Aerobic and facultative anaerobic bacterial isolates were identified by standard methods.

A part of the semen sample was also preserved at –80°C for further processing. DNA was extracted from the semen sample by the DNA extraction kit by *Helini Biomolecules*, as per manufacturer instructions. The extracted DNA material was tested for *U. urealyticum*

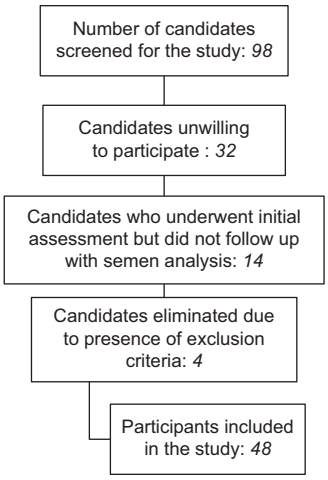

Figure 1: Recruitment process

and *M. hominis* by real-time polymerase chain reaction (PCR).

Statistical analysis

Demographic parameters and years of infertility were analysed for normality and described as mean/median with standard deviation/interquartile range (SD/IQR). Categorical variables such as type of infertility, alcohol consumption, and smoking have been described in proportions [Table 1]. Semen parameters such as semen volume, pH, sperm concentration, motility, and morphology were analysed for normality and their frequencies are described as mean/median with SD/IQR [Table 2].

Culture and PCR results were analysed as categorical variables and their frequencies have been described [Table 3]. Culture and PCR variables were compared with continuous variables of semen parameters using two sample *t*-test and the Mann–Whitney tests. Semen parameters were also analyzed as normal/abnormal motility/morphology and compared with categorized demographic variable using Chi-squared test and Fisher's exact test. Significance is described by assuming an alpha vale of 0.05. Statistical analyses have been done using the software IBM® SPSS 19.0.

Table 1: Distribution of demographic factors

Variable	Frequency (*n*=48), *n* (%)
Type of infertility	
Primary	41 (85)
Secondary	7 (15)
Smoking	
Smoker	4 (8)
Nonsmoker	44 (92)
Alcohol consumption	
Present	8 (17)
Absent	40 (83)

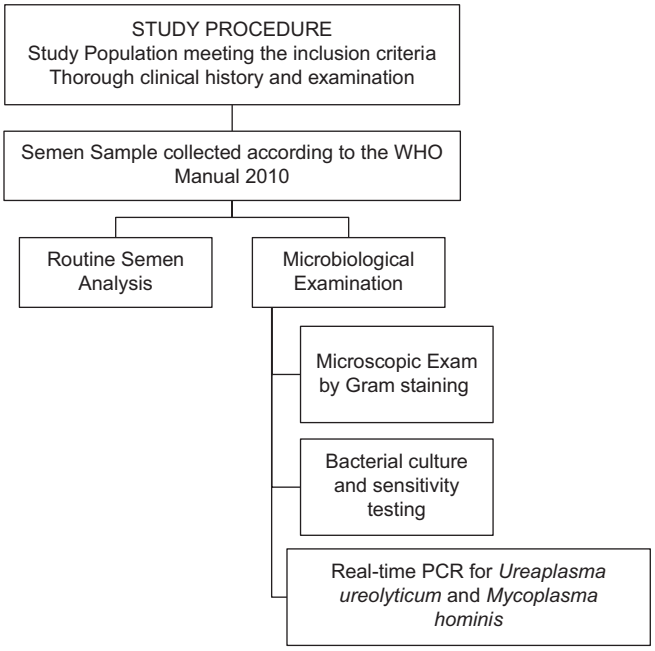

Figure 2: Study procedure

Table 2: Distribution of semen parameters

Semen parameter	Number of participants (*n*)	Measure of central tendency		Dispersion	Interpretation according to WHO manual[19] (%)
Volume	48	2.00 mL	Median	IQR: 1.65-3.00	
Sperm concentration		28.65 million	Median	IQR: 9.25-28.65	Normal: 32 (67) Reduced: 8 (16) Azoospermia: 8 (17)
Total motility	40 (excluding 8 cases of azoospermia)	31.04%	Mean	SD: 27.02	Normal motility: 18 (45)
Progressive motility		9.50%	Median	IQR: 0-37.00	Reduced motility: 22 (55)
Immotile sperms		50.33%	Mean	SD: 33.996	
Normal forms		5.80%	Median	IQR: 1.76-11.125	Normal morphology: 28 (70) Abnormal morphology: 12 (30)

The pH of all semen samples was found to be normal and ranged from 7.4 to 8.5. 31 of the 48 samples studied had a pH of 8.
IQR=Interquartile range, SD=Standard deviation

Table 3: Comparison of semen parameters between bacteriological culture positive and negative samples

Parameter	Number of samples (*n*=43)		Test	*P*
	Culture positive (*n*=24)	Culture negative (*n*=19)		
Mean volume (mL) (SD)	2.312 (0.976)	2.210 (0.871)	Two sample *t*-test	0.723
Mean pH (SD)	8.042 (0.251)	7.995 (0.299)	Two sample *t*-test	0.579
Median sperm concentration (millions/mL)	34.1	24	Mann-Whitney test	0.477
Median progressive motility (%)	7	7	Mann-Whitney test	0.99
Median percentage of normal forms (%)	4.65	6	Mann-Whitney test	0.284

SD=Standard deviation

Results

Demographic variables

The total number of participants recruited were 48. We were unable to reach the target sample size due to various limitations such as unwillingness to participate, difficulties in semen collection, and the cost of microbiological testing. The final sample size achieved was 48.

The median age of the 48 participants was found to be 35 years (IQR: 32–38.75). Their total number of years of formal education had a median of 10 years (IQR 8–12). The median number of years of infertility for the participants was 5.5 years (IQR 3–10.5). Among the 48 participants, 36 had no comorbidities. The distribution of comorbid conditions were as follows: Type 2 diabetes – 2, hypertension – 1, varicocoele – 4, two had a history of hernia repair, and one participant had erectile dysfunction. None of the participants had a history of sexual contact with commercial sex workers or history suggestive of sexually transmitted infections. Furthermore, none of the participants were positive for HIV or HBsAg.

Bacteriological profiling

Among the 48 participants, culture report could not be obtained for four participants, and the culture of one participant was reported as contaminated. Of the remaining 43 samples, 19 (44%) were negative for bacteriological culture. Among the 24 samples (56%) that were positive for culture, 2 were polymicrobial in nature. *Enterococcus fecalis* was the most common organism (8 samples) and *Staphylococcus hemolyticus* was the second most common organism (6 samples). The other bacteria grown were as follows: Two each of *Escherichia coli*,[2] Coagulase negative staphylococci other than *S. hemolyticus*,[2] and one each of *Staphylococcus aureus*, *Actinomyces urogenitalis*, *Enterobacter cloacae*, *Gardnerella vaginalis*, *Klebsiella pneumonia,* and *Morganella morganii*. It was difficult to differentiate between pathogenic bacteria and commensal bacteria/contaminated specimen. Among the isolates, only *E. fecalis*, *S. aureus,* and *G. vaginalis* can be regarded as pathogenic.

As most of the samples that tested positive for culture did not show any clinical signs of infections, the decision to start antibiotics were made based on an individual basis. Overall, two patients received a short course of antibiotics.

The first patient was a 34-year-old male with 3 years of primary infertility and no medical comorbid conditions who presented with a history of burning micturition, lower abdominal pain, and fever for one week. His semen culture as well as urine culture was positive for *E. fecalis* sensitive only to parenteral antibiotics. He was advised to receive a 5-day course of Amikacin IM on an outpatient basis and report subsequently. Unfortunately, this patient did not return for further follow-up. The other patient was a 37-year-old participant with two years of primary infertility who presented with on and off lower abdominal pain and brownish seminal discharge. His semen analysis did not reveal significant abnormalities, and semen culture was positive for *E coli.* He received a course of Ciproloxacin (oral) for 5 days based on the sensitivy pattern and reported relief of symptoms subsequently. However, he did not follow-up with a repeat semen analysis and culture as was requested.

Culture results and association with semen parameters

Culture-positive and culture-negative samples were compared to look for differences in semen parameters.

There were three samples with increased viscosity, and all of them were culture positive with three different organisms (*E. fecalis, M. morganii,* and *S. hemolyticus*). Further, as *E. fecalis* was the most common organism found, we analysed the effect of this organism on semen parameters interpreted as normal or abnormal based on the WHO 2010 manual.[23] The data from *E. fecalis* positive and negative groups were compared using the Chi-square test. This analysis too did not reveal any statistically significant difference among the two groups, with *P* values of 2.22, 0.086, and 0.09 for sperm concentration, motility, and morphology, respectively. One sample tested positive for *S. aureus,* in which both motility and percentage normal forms were grossly reduced. However, our sample size was insufficient to prove any association of culture positivity with reduced semen parameters.

Real-time PCR for *Ureaplasma urealyticum* and *Mycoplasma hominis*

All 48 samples were analysed through PCR for *U. urealyticum* and *M. hominis.*

There were no instances of coinfection with both pathogens. Eight samples tested positive for *U. urealyticum* and three samples tested positive for *M. hominis*.

Semen parameters were compared with *U. urealyticum* PCR positive and PCR negative samples. There was no statistically significant difference. The results are given in the following Table 4.

Semen parameters were compared with *M. homins* PCR-positive and PCR-negative samples and the results are described in Table 5.

The difference in viscosity between the two groups was *statistically significant* with a *P* value of *0.045*.

Comparison of semen parameters between samples positive for polymerase chain reaction of either *Ureaplasma urealyticum* or *Mycoplasma hominis* and polymerase chain reaction negative samples

Semen samples were interpreted as normal or abnormal[23] and compared between two groups:

Group 1: Samples positive for PCR of either *U. urealyticum* or *M. hominis* (11 in total)

Group 2: Samples negative for both (37 in total).

The results are given in the Table 6 below.

In the above data set, although we noted difference in semen concentration, differences between the two groups did not meet conventional levels of statistical significance.

Discussion

There is a paucity of studies on the role played by infections on male infertility, especially within the Indian subcontinent. Species such as *E.coli*, *Ureaplasma urealyticum* and *Mycoplasma hominis* have been shown previously to derange semen parameters.[24] To the best of our knowledge, ours is the only study from India to focus on *Ureaplasma uralyticum* and *Mycoplasma hominis* through real-time PCR of semen samples. Here, we have aimed to interpret our results, especially in the context of data from other studies with a similar focus.

Demography

Our study included male partners of couples seeking infertility treatment at a tertiary center in South India. The median age of the participants was 35 years. The mean age for a similar study in India published in 2011 was 33.09 years.[14] A similar study in France published in 2018 had a mean age of 40.4 years.[19] The overall demographic profile was comparable to that of Jajoo and Kalyani,[22] although the percentage of smokers and men taking alcohol were lower in our study.

Semen parameters

Semen volume

The mean semen volume in our study (2 ml) was lesser compared to Jajoo and Kalyani[22] in which a majority of men had semen volume was between 2 and 4 ml.

Sperm concentration

Thirty-three percent had normal sperm concentration in our study that is of a lesser incidence compared to the results obtained by Vilvanathan *et al*.[13] and comparable to Jajoo and Kalyani[22]

Motility

The median progressive motility in our study was 9.5%, with 53% of the participants having asthenozoospermia.

Table 4: Comparison of semen parameters between *Ureaplasma urealyticum* polymerase chain reaction positive and negative samples

Parameter	Number of samples (*n*=48)		Test	*P*
	PCR positive (*n*=8)	PCR negative (*n*=40)		
Mean volume (mL) (SD)	2.31 (1.307)	2.25 (0.940)	Two sample *t*-test	0.873
Median sperm concentration (millions/mL)	28.65	28.75	Mann-Whitney test	0.813
Median progressive motility (%)	21.5	8.5	Mann-Whitney test	0.391
Median percentage of normal forms (%)	2.5	6.1	Mann-Whitney test	0.388
Viscosity				
Normal	8	37	Chi-square test	0.424
Increased	0	3		

SD=Standard deviation, PCR=Polymerase chain reaction

Table 5: Comparison of semen parameters between *Mycoplasma hominis* polymerase chain reaction positive and negative samples

Parameter	Number of samples (*n*=48)		Test	*P*
	PCR positive (*n*=3)	PCR negative (*n*=45)		
Mean volume (mL) (SD)	2.16 (1.040)	2.66 (1.003)	Two sample *t*-test	0.868
Median sperm concentration (millions/mL)	17	30.7	Mann-Whitney test	0.286
Median progressive motility (%)	10	8	Mann-Whitney test	0.779
Median percentage of normal forms (%)	2.3	6	Mann-Whitney test	0.764
Viscosity				
Normal	2	43	Chi-square test	0.045
Increased	1	2		

PCR=Polymerase chain reaction, SD=Standard deviation

Table 6: Comparison of semen parameters between samples positive for polymerase chain reaction of either *Ureaplasma urealyticum* or *Mycoplasma hominis* and polymerase chain reaction negative samples

Parameter	Interpretation	Group 1 (PCR positive)	Group 2 (PCR negative)	Total	*P* value of Chi-square test
Semen concentration	Normal	25	7	32	0.06
	Abnormal	12	4	16	
	Total	37	11	48	
Motility	Normal	13	5	18	0.523
	Abnormal (excluding 8 cases of azoospermia)	18	4	22	
	Total	31	9	40	
Morphology	Normal	24	4	28	3.612
	Abnormal (excluding 8 cases of azoospermia)	7	5	12	
	Total	31	9	40	

PCR=Polymerase chain reaction

This excludes the 8 (23%) participants having azoospermia. Vilvanathan *et al*.[13] had a comparable asthenozoospermia rate of 28.23%. Although Jajoo and Kalyani[22] did not specify the type of motility, 30% had sperm motility of <50%.

Morphology

The study by Vilvanathan *et al*.[13] had a teratozoospermia rate of 81.17%, which is far higher than the rate of 30% found in our study. The study by Jajoo and Kalyani also found an abnormal morphology rate of 69%. The reason for these relatively lower rates of abnormal morphology is unknown.

Rate of bacteriospermia

Our study had a bacteriospermia (culture positive) rate of 56%, which is higher than the 35.29% bacteriospermia rate detected by Vilvanathan *et al*.[13] A large-scale Italian study by Moretti *et al*.[6] put forth a bacteriospermia rate of 33.2%, which is lesser than the rate obtained from our study. However, a similar study in Nigeria[14] had a very high bacteriospermia rate of 74.9%. This demonstrates the geographical variation in the prevalence of semen samples testing positive for bacteriological culture among different countries, as economically backward regions are likely to have a higher proportion of infectious diseases.

Organisms grown

The most common organism grown in our study was *E. fecalis*, followed by *S. hemolyticus*. *E. fecalis* was also the most common organism isolated by Vilvanathan *et al*.[13] and Moretti *et al*.[6] *G. vaginalis* was the most common organism isolated in a similar study in France by Virecoulon *et al*.[25] The above findings demonstrate that *E. fecalis* continues to be the most common pathogen isolated in semen across studies conducted in different areas.

Detection of microorganism by real-time polymerase chain reaction

The rate of samples testing positive through PCR for *U. urealyticum* in our study was 16.67%, as opposed to 7.2% detected by Virecoulon *et al*.[25] A study in China by Zhou *et al*.[19] published in 2018 demonstrated a prevalence of 39.6% of *Ureaplasma* species, with 14.5% of those being *U. urealyticum*.

There were more cases of *U. urealyticum* in our study population, but our sample size was insufficient to demonstrate an association with infertility. The rate of *M. hominis* isolated in our study is 6.25%, whereas a study by Gdoura *et al*.[26] concluded that the prevalence of *M. hominis* was 10.8%.

Association of semen parameters with positive microorganism by Real-time PCR

Apart from the increase in viscosity noted in the presence of *M.* homins by PCR, our study could not demonstrate a statistically significant association of abnormalities in semen parameters such as sperm concentration, motility, and morphology with the presence or absence of bacteria in the semen. This can be attributed to the smaller sample size in our study. In our study, there was also one sample each that tested positive for *S. aureus* and *G. vaginalis,* respectively, and in both cases, the motility was found to be grossly reduced.

These findings demonstrate the need for more focused research in this area with studies equipped with a higher sample size.

Conclusion

Our study is one of the first to focus on the role of infections in male infertility within Indian Subcontinent. We were able to demonstrate a significant difference in viscosity, which was higher in the samples that were positive for real-time PCR of *M.hominis*. However, our study was limited by a small sample size and we

could not arrive at a statistically significant difference among most of the parameters compared. Larger-scale prospective studies in this area would be invaluable in deciding the management plans of male factor infertility. If the specific role played by certain organisms are identified and treatment options including antibiotics are explored, it may result in a significant improvement in pregnancy rates and also the economic burden of fertility care.

Data availability statement

The data sets used for this study are available with the corresponding author on request.

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The study was conducted with the aid of funding from the institute for postgraduate research projects as a JIPMER Intramural research grant.

Conflicts of interest

There are no conflicts of interest.

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