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Original Research Article

Study of Culture Sensitivity of Semen in Infertility Cases – Retrospective Study

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Conflict of interest: Nil

Abstract:

Background: Male infertility is noted Up to 50% vitro studies revealed that, bacteria can negatively impact on sperm function. Hence the human microbe and dysbiosis have to be ruled out.

Method: 65 (sixty-five) infertile adults aged between 25 to 40 years were studied. Semen samples were collected in a sterile container by masturbation after the minimum obstinate of 3 days. Semen parameters included appearance, volume, pH, viscosity, liquefaction, motility, and morphology, which were analyzed microscopically.

Results: Out of 65, 55 (84.6%) were primary infertility, and 10 (15.3%) were secondary infertility. In a comparative study of semen parameters of bacteriological culture of positive and negative studies, mean volume, pH, sperm concentration, progressive motility, normal formation of sperm, had significant p-value ($p<0.001$). In comparison of PCR semen concentration, progressive motility morphology had a significant p-value ($p<0.01$).

Conclusion: Present bacteriological and PCR studies will help the clinician to rule out the etiology of abnormal semen parameters and to treat efficiently such infertile male cases.

Keywords: PCR, bacteriological, infertility, DNA extraction kit, semen analysis.

Introduction

Infertility affects 8 to 12% of couples globally. It is defined as the failure to conceive after 12 months of regular unprotected intercourse; male factor infertility is attributable in up to 50% of cases and potential cases [1].

Include urogenital tract infections, e.g., prostatitis, epididymitis. In vitro studies it is revealed that, the mechanism through which bacteria affect sperm function, including agglutation of motile sperm, induction of apoptosis, production of immobilisation factors and impairment of the acrosame reaction [2].However, the evidence for using empiric antibiotics in the clinical setting is controversial, as there are many conflicting data as to whether such pathogens cause abnormalities in semen parameters in vivo and whether treatment leads to an improvement in semen parameters and reproductive potential. Leucocytospermia has been posited as the pathogenesis for male factor infertility. It is associated with elevated oxygen species (ROS), which damage the DNA of spermatozoa [3].

The human microbiome is composed of the genetic material of microbial communities (e.g., bacteria, fungi, and viruses) and is more complex than the human genome [4]. Hence, an attempt is made to evaluate the bacteriological culture sensation and various parameters of semen.

Material and Methods: 65 adult males regularly visited the infertility section of the obstetrics and gynecology department of Dr. D. Y. Patil Medical College Hospital and Research Centre, Pimpri, Pune, Maharashtra-411018 were studied.

Inclusive Criteria: Male partners of couples attending the infertility center and given written consent for investigation were selected for the study.

Exclusion Criteria: Patients with congenital causes of infertility, such as anarchy, chromosomal disorders and patients without congenital causes of infertility who were immune compromised were excluded from the study.

Procedure: Semen samples were collected in a sterile container by masturbation after a minimum abstinence period of 3 days. None of the patients had taken prior antibiotics. Instructions were given regards semen sample collection about proper hand washing and sterile techniques. Semen parameters such as liquefaction, volume pH, viscosity, liquefaction, count, motility, and morphology were analyzed according to the WHO guidelines [5].

Microbiological evaluation included microscopic examination of Gram-stained smears and culture for bacteria. Inoculation was done on blood agar and MacConkey agar and incubated aerobically at 37°C for 48 hours.

Aerobic and facultative anaerobic bacteria isolates were identified by standard methods. A 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 the manufacturer's instructions. The extracted DNA material was tested for U. urealyticum and M. hominis by real-time polymerase chain reaction (PCR).

The duration of the study was from February 2012 to September 2015.

Statistical analysis: The demographic factors percentage, comparison of PCR positive and negative groups of semen parameters, and comparison of semen concentrations, motility, and morphology between groups I and II were studied with the t-test and the Mann-Whitney z-score, respectively. The statistical analysis was carried out in SPSS software.

Observation and Results

Table-1: Study of distribution of demographic factors –

- Types of infertility: 55 (84.6%) had primary infertility, 10 (15.3%) had secondary infertility
- Smokers: 6 (9.2%) were smokers, 59 (90.7%) were non-smokers
- Alcohol consumption: 11 (16.9%) were alcoholic, 54 (83%) were non-alcoholic

Table-2: Comparative of semen parameters between Bacteriological cultures in both groups –

- Mean volume ml/SD: 2.56 (± 0.33) in group-I, 1.96 (± 0.14) in group-II, t test 8.49 and $p<0.001$
- Mean PH: 8.12 (± 0.16) in group-I, 7.66 (± 0.18) in group-II, t test was 10.5 and $p<0.001$
- Median sperm concentration (millions.m1): 36.5 in group-I, 24 in group-II, Mann Whitney U=936 and $p<0.001$
- Median progress motility (%) 9.0 in group-I, 7.0 in group-II, Mann Whitney U=202, and $p<0.001$.
- Median % of Normal forms: 4.9 in group-I, 7.0 in group-II, Mann Whitney U=566 and $p<0.001$.

Table-3: Comparison of semen concentration, motility and morphology between group-I and group-II

- Semen concentration was normal in 22 group I, 11 in group II, 18 abnormal in group I, 14 in group II, chi-square=5.08and $p<0.05$ (highly significant)
- Progressive motility: 17 Normal in group I, 9 in group II, 23 abnormal in group I, 16 in group II and chi-square=4.8, and $p=0.001$ (p value is highly significant)
- Morphology of semen: 21 Normal in group I, 11 in group II, 19 abnormal group I, 14 in group II, chi-square=4.39 and $p<0.005$ (p value is highly significant)

Table 1: Distribution of demographic factors (Total No of patient: 65)

Factors	No. of Patients (65)	Percentage (%)
Types of infertility		
a) Primary infertility	55	84.6
b) Secondary infertility	10	15.3
Smoking		
a) Smoker	6	9.2
b) Non-smoker	59	90.7
Alcohol consumption		
a) Alcoholic	11	16.9
b) Non-Alcoholic	54	83

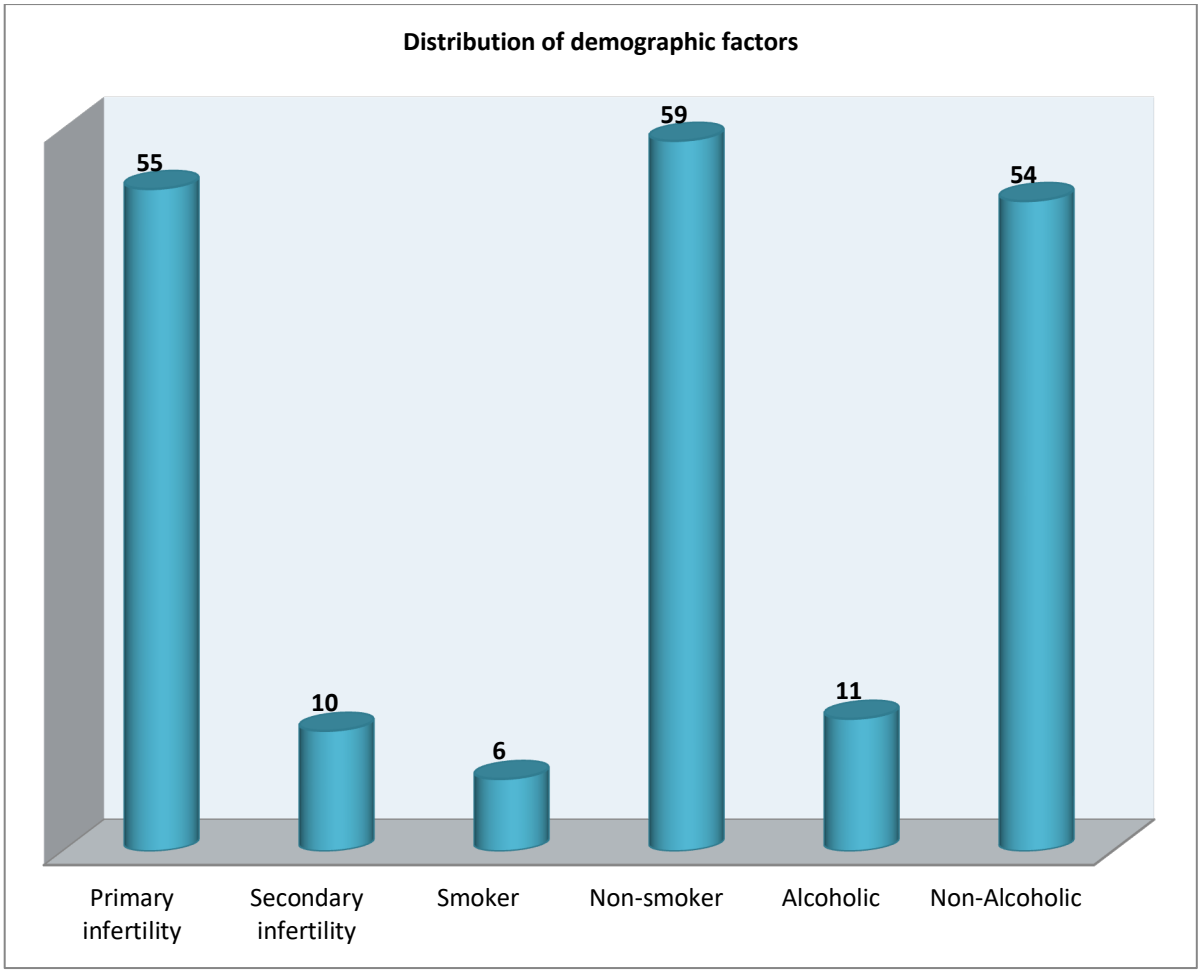

Figure 1: Distribution of demographic factors

Table 2: Comparison of Semen parameters between Bacteriological culture profile in Group I and Group II

Parameter	Group I (40) (positive)	Group II (25) (negative)	Test statistic	P Value
Mean volume (ml/SD)	2.56 (± 0.33)	1.96 (± 0.14)	t =8.48	P<0.01
Mean PH (SD)	8.12 (± 0.16)	7.66 (± 0.18)	t = 10.57	P<0.01
Median sperm concentration (millions/ ml)	36.5	24	Mann Whitney U=936 z score=12.50	P<0.01
Median progressive Motility (%)	9.0	7.0	Mann Whitney U=202 Z score=2.53	P<0.01
Median % of normal forms	4.9	7.0	Mann Whitney U=566 z score=7.48	P<0.01

From Above Tables: The Group I parameter values such as Mean volume (ml), Mean PH, Median sperm concentration (millions/ml) and Median progressive motility (%) are highly significantly more (p<0.01) than the Group II parameters. While median % of normal Norms of the Group II is significantly more than the Group I (p<0.01).

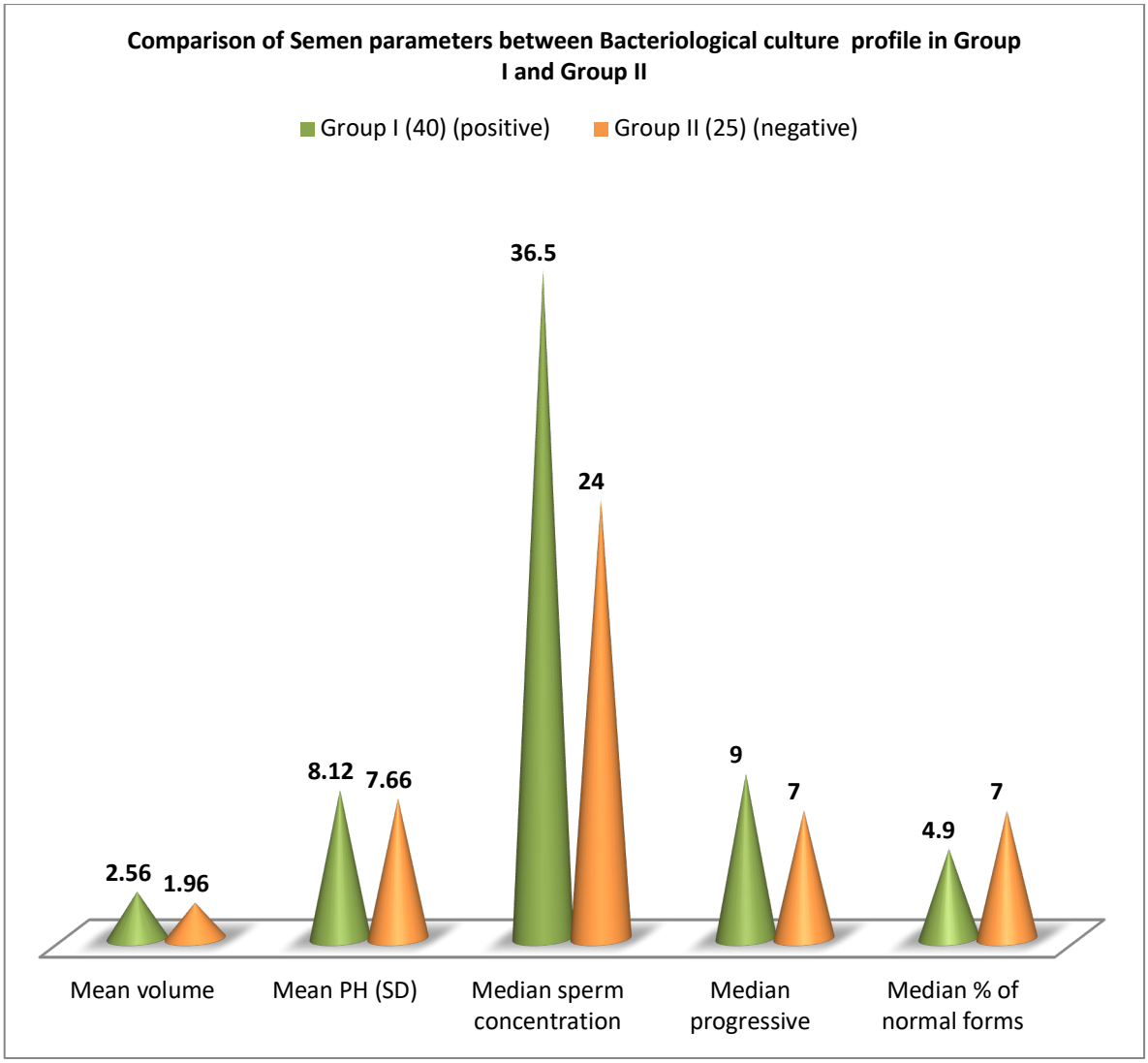

Figure 2: Comparison of Semen parameters between Bacteriological culture profile in Group I and Group II

Table 3: Comparison of Semen concentration, Motility and Morphology between PCR positive group and PCR negative group

Parameter	Interpretation	Group I (40) (PCR positive)	Group II (25) (PCR negative)	Test statistic
Semen concentration	Normal	22	11	Chi-square = 5.08 DF=1, p<0.05 Significant
	Abnormal	18	14	
	Total	40	25	
Progressive Motility	Normal	17	09	Chi-square = 4.68, DF=1, p<0.05 Significant
	Abnormal	23	16	
	Total	40	25	
Morphology	Normal	21	11	Chi-square = 4.39 DF=1, p<0.05 Significant
	Abnormal	19	14	
	Total	40	25	

To assess association between PCR positivity and parameters i.e., Semen concentration, progressive motility and morphology, statistical Chi-square test is applied. There is highly significant association observed in all parameters ($p<0.001$). PCR = Semen polymerase chain Reaction

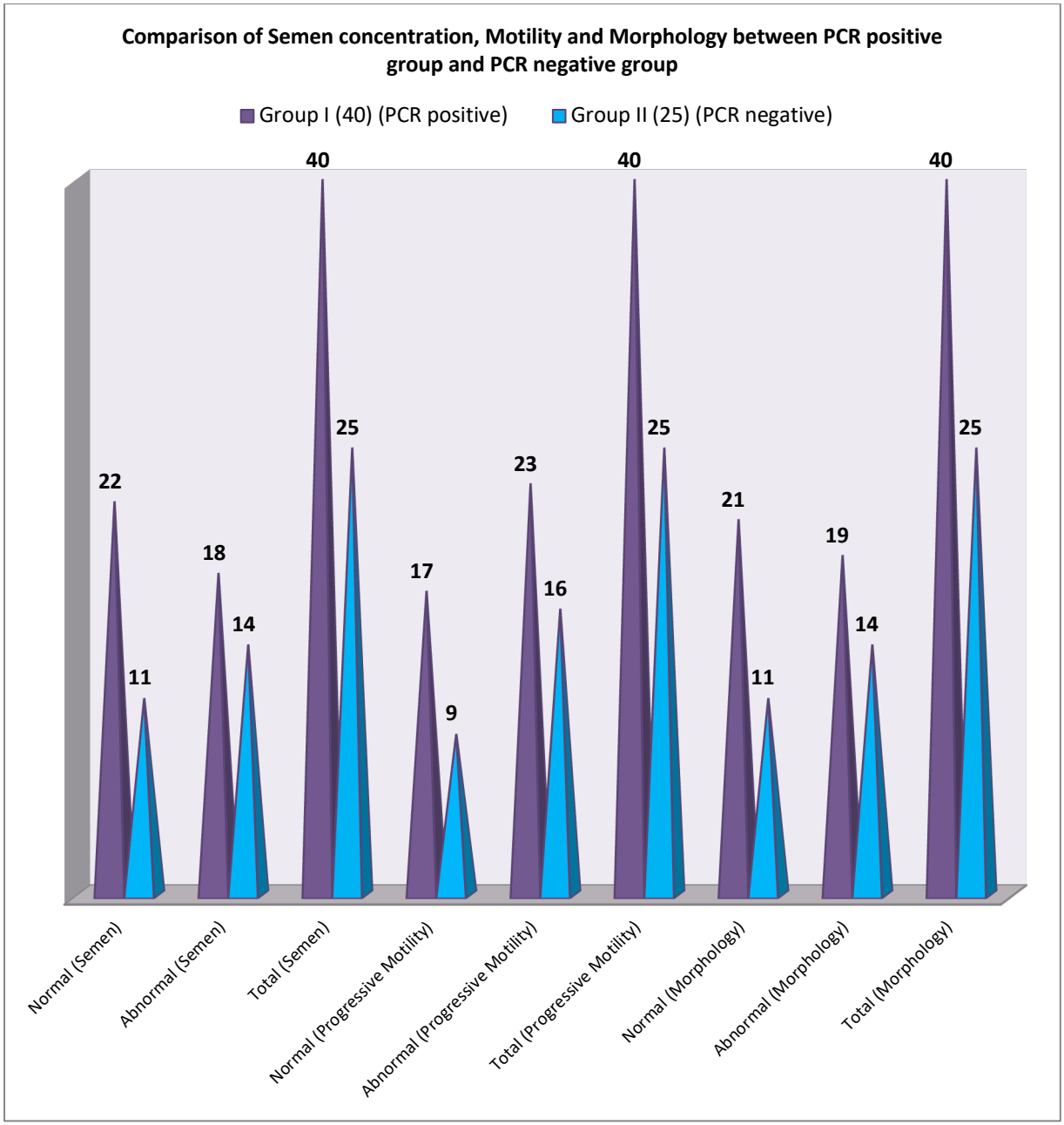

Figure 3: Comparison of Semen concentration, Motility and Morphology between PCR positive group and PCR negative group

Discussion

In the present study of culture sensitivity of semen in infertility cases in Maharashtra. 55 (84.6%) had primary infertility, 10 (15.3%) had secondary infertility, 6 (9.2%) were smokers, 59 (90.7%) were non-smokers, 11 (16.9%) were alcoholics, and 54 (83%) were non-alcoholic (Table 1). In a comparative study of parameters between the bacteriological culture profile in group I and group II, the mean volume of semen, pH, median sperm concentration, progressive motility, and normal forms of sperm had significant p-values ($p<0.001$) (Table 2). In a comparative study of semen concentration mortality, the morphological study had a significant p-value ($p<0.001$). These findings are more or less in agreement with previous studies [6,7,8].

Male genital tract infections are difficult to detect as they are asymptomatic and often remain undiagnosed unless the patient seeks treatment for specific symptoms. Infections are potentially treatable causes of male infertility, and resistance to common antibiotics and poor compliance may impede the efficacy of antibiotics in resolving complicated UTIs or restoring fertility [9]. It is reported that leukocytes frequently appear in ejaculates, even in fertile men. Leukocytes are powerful producers of reactive oxygen species (ROS) and may have detrimental effects on sperm function and DNA integrity. It is pointed out that asymptomatic leuko-

spermia may be indicative of early or silent genital tract infections [10]. It is estimated that more than one million sexually transmitted infections are acquired every day throughout the world.

The most prevalent sexually transmitted pathogens in uncomplicated UTI include C-trachomatis, Urea plasma, Urealtticum gonorrhoea, and Mycoplasma homins, with the exception of E.coli, considered the most common cause of non-sexually transmitted uro-genital tract infection, particularly in epididymoorchitis or prostatis.

In addition, a host of viral infections, including mumps, human papillomavirus, herpes simplex virus, and HIV virus, also contribute to increasing leukocyte concentrations [11].

Apart from broad-spectrum antibiotic therapy, antioxidants can manage ROS, and E. coli is aerobically treated with gram-negative antibiotics.

Summary and Conclusion

In Present study of the culture and sensitiveness of semen in infertility patients, there were significant results in PCR and the bacteriological profile. It will be helpful to clinicians to treat infertile men, but such clinical trials must be conducted on a large number of patients in a Hi-tech hospital where the latest techniques are available to confirm these significant results.

Limitation of study: Owing to the tertiary location of the research center, the small number of patients, and the lack of the latest techniques, we have limited findings and research.

This research paper was approved by the ethical committee of Dr. D. Y. Patil Medical College Hospital and Research Centre Pimpri, Pune, Maharashtra-411018.

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