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Original Research Article

Study of Culture Sensitivity of Semen in Infertility Cases A: Retrospective Study)

Nikhila G.[1], Vishok M.[2]

[1]Assistant Professor, Department of Obstetrics and Gynaecology, Navodaya Medical College, hospital and research centre, Raichur, Karnataka, India

[2]Assistant Professor, Department of General Medicine, Surabhi Institute of Medical Sciences, Siddipeth, Telangana, India

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Corresponding author: Dr. Vishok M.

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Abstract

Background: Infertility is clinically defined as the failure of a couple to conceive after one year of regular Intercourse without protection and 6 months of trying if the woman age is above 35years.Despite advanced technologies and diagnostic methods, Diagnosing and treating male infertility has become a great challenge for gynaecologists and andrologist as well.

Method: 63 (sixty-three) infertile adults aged between 25 to 45 years were studied. Semen samples were collected in an plastic container by masturbation after the minimum abstinence period of 3 days. Semen parameters included appearance volume, PH, viscosity, liquefaction, motility and morphology were analysed microscopically.

Results: 53 (84.1%) had primary infertility, 10 (15.8%) had secondary infertility, 6 (9.5%) were smokers, and 11 (17.4%) were alcoholics. In comparison of semen parameters between bacterial cultures, Group I and Group II participants studied the mean volume of semen, pH value, median sperm concentrations, and median progressive motility, which had a significant p-value ($p < 0.001$). The comparison of semen concentration, motility, and morphology between PCR Group I and Group II had a significant p-value ($p < 0.001$).

Conclusion: Present morphological bacteriological studies will help the gynaecologist and andrologist to treat male infertility efficiently so that such patients can lead normal social life.

Keywords: Semen Parameter, Infertility, Polymerase Chain Reaction PCR, Bacteriological.

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Introduction

Infertility is clinically defined as failure of a couple to conceive after one year of regular intercourse without protection and after 6 months of trying if the woman age is above 35 years. An estimated 4-17% of couples seek medical treatment in order to rectify their infertility and it is reasonable to assume that there are many more cases of infertility that are unreported [1]. It has been observed that male factors are solely and partially implicated in 20-50% of cases of infertility. Despite advances in technologies and diagnostic methods in the field of andrology, there remains a significant subset of these subfertile men who are classified as having unexplained male infertility [2]. It includes urogenital tract infections, i.e., prostatitis and epididymitis. In vitro studies reveal that bacteria affect sperm function, including agglutination of motile sperm, induction of apoptosis, production of immobilization factors, and impairment of the acrosomal reaction [3]. There are also evidences for using empiric antibiotics in a clinical setting that is controversial, as there is much conflicting data as to whether such pathogens cause abnormalities in semen parameters [4]. Leucocystospermia has been pointed out as the pathogenesis for male infertility factors. Hence an attempt was made to evaluate the various parameters of semen analysis and the etiology of male infertility.

Material and Methods63 (sixty-three) adult males regularly visited the infertility section of the obstetrics and gynaecology department of Navodaya Medical College, Hospital and Research Centre, Raichur, Karnataka 584103, and were studied.

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

Exclusion Criteria: Patients with congenital causes of infertility, such as anarchy, and patients without congenital causes of infertility who were immunocompromised 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. Semen parameters such as liquefaction, volume, pH, viscosity liquefaction, count, motility, and morphology were analysed 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 inculcated aerobically at 37°C for 48 hours. Aerobic and facultative anaerobic bacterial 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 March 2024 to April 2025.

Statistical Analysis: The demographic factors percentage, comparison of PCR-positive(group 1) and negative ((group 2) 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: Distribution of demographic factors –

- 53 (84.1%) had primary infertility, 10 (15.8%) had secondary infertility
- Smokers – 6 (9.5%) were smokers, 57 (90.4%) were non-smokers
- Alcoholic – 11 (17.4%) were alcoholic, 52 (82.5%) were non-alcoholic

Table-2: Comparative study of positive (group I) and negative PCR (group II)

- The volume of semen 2.56 (± 0.32) in group-I, 1.98 (± 0.13) in group-II, t test 6.36 and $p<0.001$
- Mean PH of semen – 8.12 (± 0.15) in group-I, 7.65 (± 0.16) in group-II, t test was 9.93 and $p<0.001$
- Median sperm concentration (millions.m1) – 34.2 in group-I, 24 in group-II, Mann Whitney z score 5.36 and $p<0.001$
- Median progress motility (%) 8.2 in group-I, 7.3 in group-II, Mann Whitney z score 1.663 and $p<0.05$
- Median % of Normal forms – 4.6 in group-I, 7.1 in group-II, Mann Whitney z score 5.38 and $p<0.01$

Table-3: Comparison of semen concentration, motility and morphology between group-I (PCR positive) and group-II (PCR negative) groups

- Semen concentration study 44 normal, 19 abnormal chi-square 3.66, DF=1 and $p<0.05$ (highly significant)
- Progressive motility – 28 Normal, 35 abnormal, total 63 chi-square 0.476, DF 1 and $p=0.48$ (p value insignificant)
- Morphology of semen – Normal=42, abnormal=21, chi-square=4.303, DF=1, $p<0.03$ (p value is highly significant)

Table 1: Distribution of demographic factors (No. of Patients: 63)

Factors	No. of Patients	Percentage (%)
Types of infertility		
a) Primary infertility	53	84.1
b) Secondary infertility	10	15.8
Smoking		
a) Smoker	6	9.5
b) Non-smoker	57	90.4
Alcohol consumption		
a) Alcoholic	11	17.4
b) Non-Alcoholic	52	82.5

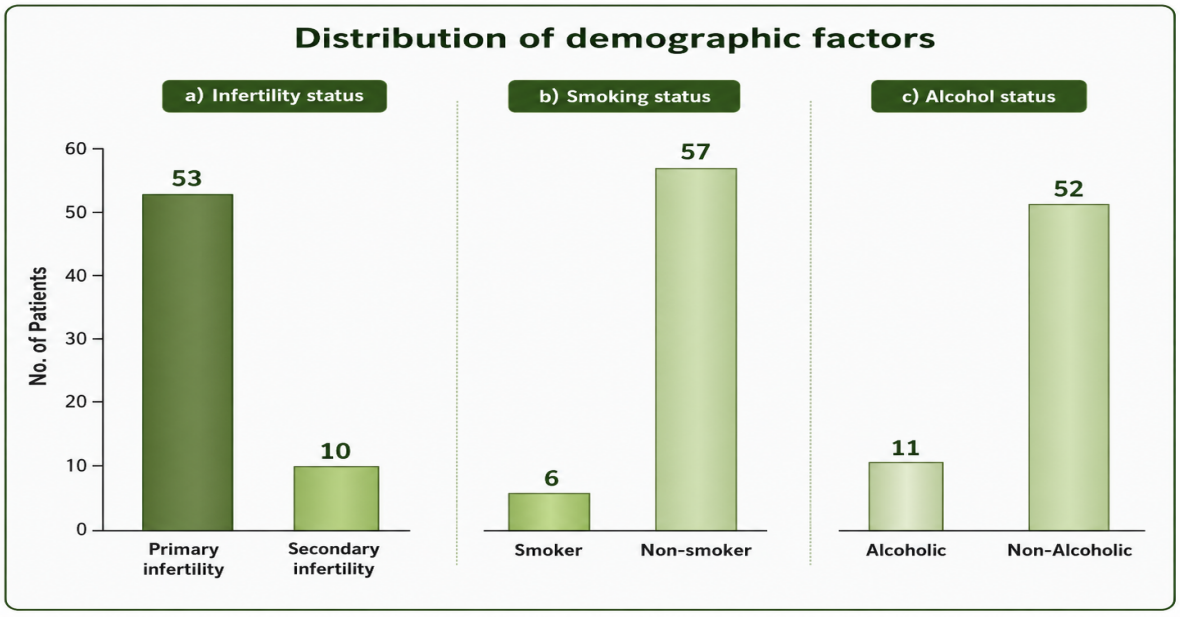

Figure 1: Distribution of demographic factors

Table 2: Comparison of Semen parameters between Bacteriological culture Group I and Group II participants (No. of Patients: 63)

Parameter	Group I (50) (positive)	Group II (13) (negative)	Test statistic	P Value
Mean volume (ml/SD)	2.56 (± 0.32)	1.98 (± 0.13)	t =6.36	P<0.01
Mean PH (SD)	8.12 (± 0.15)	7.65 (± 0.16)	t = 9.93	P<0.01
Median sperm concentration (millions/ ml)	34.2	24	Mann Whitney Z score=5.3644	P<0.01
Median progressive Motility (%)	8.2	7.3	Mann Whitney Z score=1.6636	P<0.05
Median % of normal forms	4.6	7.1	Mann Whitney Z score=5.3848	P<0.01

Statistically there is significant difference observed in Volume, PH, Sperm concentration, Progressive motility and Normal forms between Group I and Group II participants.

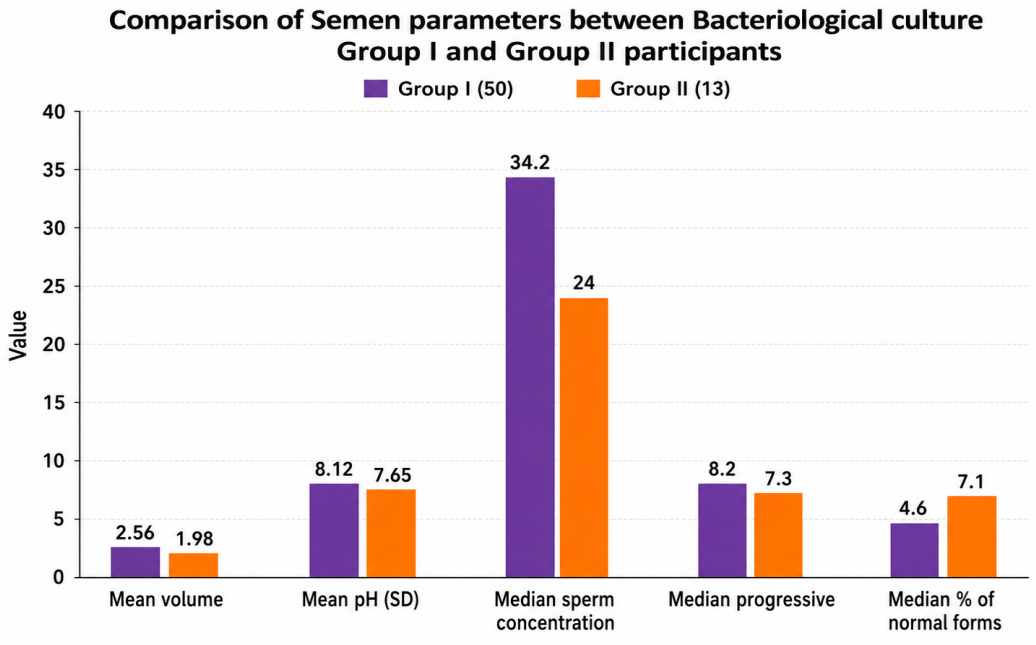

Figure 2: Comparison of Semen parameters between Bacteriological culture Group I and Group II participants

Table 3: Comparison of Semen concentration, Motility and Morphology between PCR Group I and Group II (Total of patients: 63)

Parameter	Interpretation	Group I (50) (PCR positive)	Group II (13) (PCR negative)	Total	Test statistic
Semen concentration	Normal	38	06	44	Chi-square = 3.66 DF=1, p=0.05*
	Abnormal	12	07	19	
	Total	50	13	63	
Progressive otility	Normal	22	06	28	Chi-square = 0.476 DF=1, p=0.4893
	Abnormal	30	05	35	
	Total	52	11	63	
Morphology	Normal	37	05	42	Chi-square = 4.303 DF=1, p=0.03*
	Abnormal	15	06	21	
	Total	52	11	63	

***indicates significant difference, PCR = Semen polymerase chain Reaction**

Statistically there is significant difference in count observed between Group I and Group II participants in semen concentration and Morphology but no significant difference observed in progressive motility count of group I and group II participants.

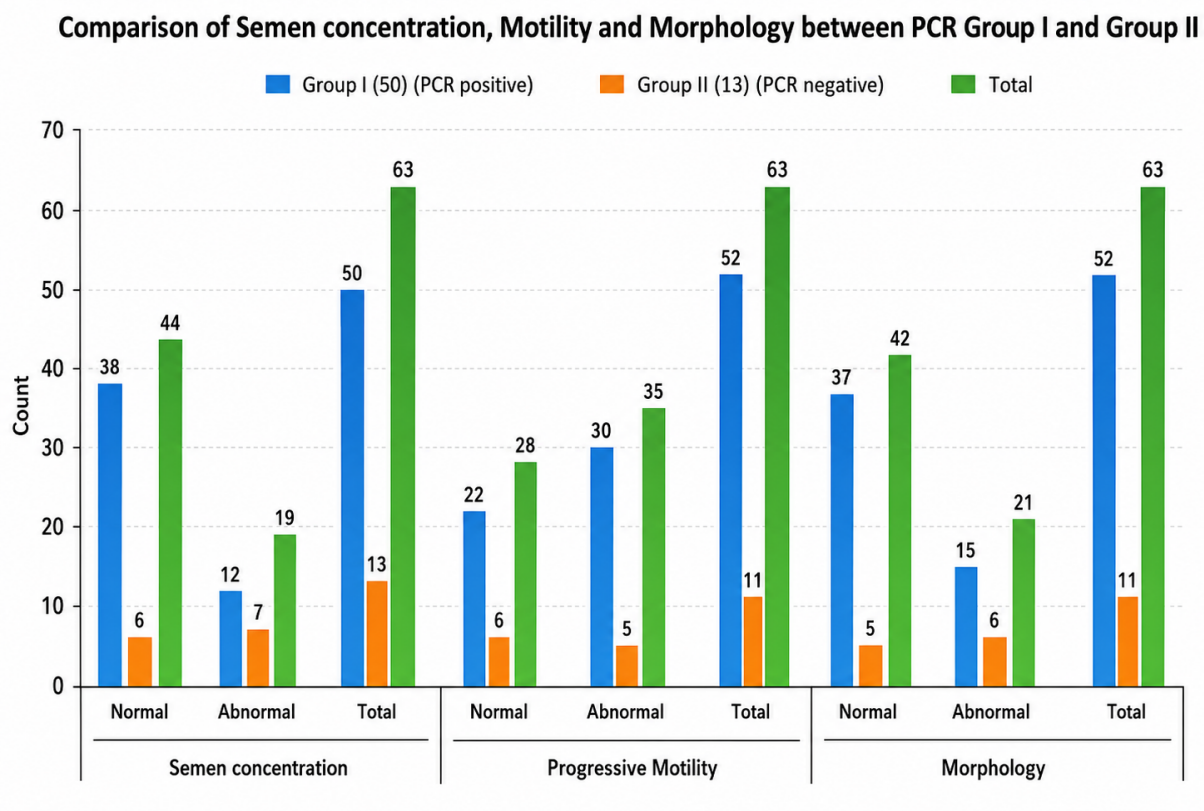

Figure 3: Comparison of Semen concentration, Motility and Morphology between PCR Group I and Group II

Discussion

The present study of culture and the sensitiveness of semen in infertility cases in the North Karnataka population. In the demography profile study, 53 (84%) had primary infertility, 10 (15.8%) had secondary infertility, 6 (9.5%) were smokers, and 11 (17.4%) were alcoholics (Table 1). In the comparative study of bacteriological culture, the mean volume of semen, the mean pH of semen, the median sperm concentration, the median progressive motility, and the median percentage of normal forms had significant p values ($p < 0.001$) (Table 2). In comparison of PCR groups I and II, semen concentration had a chi-square of 3.66, DF = 1, and $p < 0.001$. I found progressive motility of sperm, chi-square 0.473, DF = 1, and $p < 0.48$ (p value is insignificant) because motility in both groups was more or less the same. Morphology of sperm chi-square 4.303 was highly significant ($p < 0.003$) (Table 3). 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 leukospermia 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 UTIs include C. trachomatis, Ureaplasma, Ureaplasma urealyticum, gonorrhea, and Mycoplasma hominis, with the exception of E., considered the most common cause of non-sexually transmitted urogenital tract infection, particularly in epididymoorchitis or prostatis. In addition, a host of viral infections, including mumps, human papilloma virus, 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 [12]. Sperm chromosomal abnormalities are most often seen in men with decreased sperm cell count (oligozoospermia), decreased motility (asthenospermia), or a high percentage of morphologically abnormal sperm (teratozoospermia). Moreover, gene mutations and polymorphisms have also been recognized in infertile men with normal spermiograms.

Summary and Conclusion

Present study of the culture and sensitiveness of semen in the north Karnataka population. 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 because proper understanding of the in vivo process of human fertilization and sperm egg interaction in vitro is the key to envisage the sperm functional in alterations with tremendous influence on diagnosis and treatment of male sub fertility.

Limitation of study – Owing to the remote location of the research centre, the small number of patients, and the lack of the latest techniques, we have limited findings and results.

This research paper was approved by Ethical committee of Navodaya Medical College, hospital and research centre, Raichur, Karnataka-584103.

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