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Effect of *Escherichia coli* on Semen Quality of Infertile Human Male

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Research Article

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Abstract

Male genital tract infections are one of the most major causes of infertility worldwide. It is expected that about 15% of male infertility is due to genital tract infections. These infections may contribute to deterioration of various sperm parameters, especially in infertile men. The negative effects of many bacteria on human spermatozoa have been widely reported. The aim of this study was to investigate the effect of *Escherichia coli* on the semen of infertile men and their effect on sperm quality. Sixty seven semen samples of infertile males were investigated by standard bacterial culture methods. WHO guidelines were followed to perform standard semen analysis. We found that *E. coli* was present in infertile males and there was a relation between *E. coli* infection and semen quality.

Keywords: *E. coli*; Infection; Infertility; Semen

Abbreviations: GTI: Genital Tract Infections; CPX: Ciprofloxacin; GEN: Gentamycin; CTX: Cefotaxime; STR: Streptomycin; TET: Tetracyclin; AMP: Ampicillin; IPM: Imipenem.

Introduction

Infertility is a complex human health situation which particularly alters the quality of life in couples facing the condition. Infertility results from multiple factors that are responsible for impairments of reproductive function in humans. Male urogenital tract infection is one of the most major causes of infertility worldwide. It is estimated that about 15% of male infertility is related to genital tract infections (GTI) [1]. GTI and consequent inflammation in male reproductive system may comprise sperm cell function and whole spermatogenic process. *Escherichia coli* is the most frequently isolated and studied genital organism in its regard to male infertility. In an investigation the mechanism of immobilization of spermatozoa by *E. coli*; they reported a soluble, dialyzable, heat stable, spermatoxic factor which immobilizes spermatozoa without agglutinating it [2]. *E. coli* is also the principle microorganism causing prostatitis and epididymitis [3,4].

The negative effect of *E. coli* on semen quality is due to its effect on motility and due to impaired acrosomal function. In 1996 a two-step mechanism was suggested for showing the negative impact of *E. coli* on the spermatozoa; firstly by the interaction by adhesion and later on the destruction of sperm membrane [5]. In another report reported the agglutination of spermatozoa due to adhesion to *E. coli* leading to morphological alterations in sperm involving plasma membrane and degeneration of acrosome [6].

Microorganisms might affect the male reproductive function causing the alterations in cell morphology,

reducing ability for the acrosome reaction and also causing the agglutination of motile sperm [7,8]. The aim of this study was to investigate the semen sample of infertile men and to analyse the influence of bacterial pathogens on the sperm quality.

Materials and Methods

Study Population

The study population consisted of sixty seven men with fertility disorders who contacted the Fertility Clinics of Kurukshetra. The average age of men was 35.07 years. The study was conducted in the Department of Microbiology, Kurukshetra University, Haryana state of India from January 2018 to December 2018. The samples were protected from extremes of temperature i.e. not less than 20°C and not more than 40°C during transport to the work area. Experiments were performed within 1h of obtaining samples. The samples showing normal semen parameters according to WHO were used as control [9].

Sample Collection

Semen samples of 67 infertile men attending to infertility clinics were collected in the clinic. The patients have not taken any antibiotic from one week before collecting a semen sample. Before collecting the sample, patients must wash their hands and genital area with soap and water and the patients were instructed to ensure that all the ejaculate, especially the first sperm rich portion were included. Samples were collected in sterile plastic containers used for collecting of semen sample. Then, all of the samples were rapidly transferred to microbiology laboratory.

Ethical Clearance

The protocols for the present study were approved by the Institutional Ethics Committee of Kurukshetra University, Kurukshetra.

Semen Parameter Analysis

The semen samples were allowed to liquefy at 37oC for 30 minutes before examination. After that the samples were analysed for sperm parameters such as appearance, volume, pH, viscosity, count, motility, morphology, presence of other cells like epithelial cell or round cell, and sperm agglutination were recorded according to the WHO guidelines.

All the procedures above were carried out in duplicate and the results were compared for maximum acceptable differences between replicate results in order to achieve low sampling error according to WHO specification [9].

Microbiological Analysis

Standard bacterial culture method (on blood agar, Mac conkey agar) was performed for each semen sample to detect microbial agents. Cultures were incubated at 37°C for 24-48 h. Each plate was examined for evidence of growth and the isolates identified by standard biochemical tests [10].

Antimicrobial Sensitivity Testing

The antimicrobial sensitivity testing was done on each of the isolates by the Kirby-Bauer disk diffusion method [10].

Statistical Analysis

The relationships between GTIs and semen parameters were analyzed using Chi-square ($\chi 2$) test.

Results

Semen Parameters

During our study, 67 semen samples were received from the patients with fertility disorders who contacted the Fertility Clinics of Kurukshetra. The mean age of the study patients was 35.07 years. The 23 (34.33%) number of patients were below the age group of 30 years and 44 (65.67%) patients were belonging to the age group of more than 30 years.

Out of 67 samples analysed based on the guidelines of WHO, 29 (43.28%) had normal sperm count, 33 (49.25%) oligozoospermia and 5 (7.46%) had azoospermia (Figure 1).

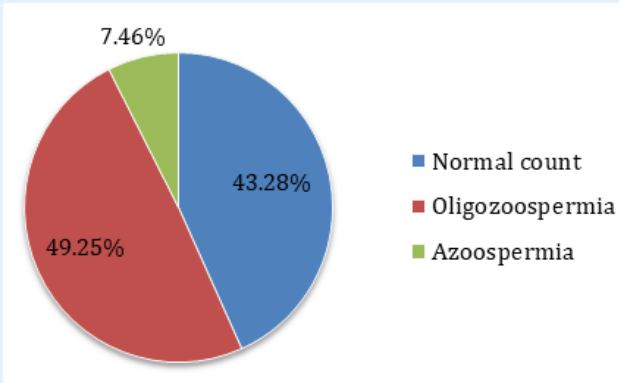

Figure 1: Density of semen in study population.

Semen parameters of infertile males with genital tract infections were significantly different than in healthy individuals used as control. Volume of semen and number of sperm cells was lower in infected individuals. Also the motility and vitality was reduced as compared to healthy individuals, and this difference was found to be statistically significant ($p<0.05$; Table 1).

Semen parameters	Healthy controls	Infertile individuals without GTI	Infertile individuals with GTI
Volume (mL)	2.8±0.5	2.2±0.5	1.9±0.7
Concentration of sperm cells ($1x10^6$/mL)	61±52	20±9	17±5
Rapid and slow progression of sperm cells (%)	67±11	29±10	11±4
Immobilized sperm cells (%)	29±9	53±12	89±16
Morphology (%)	41±6	15±7	12±5
Vitality (%)	75±10	63±11	61±10

Table 1: Analysis of semen ejaculated from healthy men and infertile patients with and without genital tract infection.

Microbiological Analysis

Among the 67 samples 42 (62.68%) were found to be infected with various organisms. *E. coli* (64.28%) was the most common microorganism isolated from the patients. About 80% of the azoospermic samples were found to be infected; 51.51% of the oligospermic samples were infected while 48.27% of those with normal concentration were infected.

Antimicrobial Susceptibility Testing

When the isolated *E. coli* were tested against the commonly used antimicrobial drugs, showed that 17 isolates were susceptible to ciprofloxacin, 12 to gentamycin, 18 to cefotaxime, 3 to streptomycin, 5 to tetracyclin, 22 to ampicillin and 25 to imipenem. Some of them are resistance to various antibiotics (Table 2).

Organism	*Escherichia coli* (n=27)
CPX	17 (62.9)
GEN	12 (44.4)
CTX	18 (66.7)
STR	3 (11.1)
TET	5 (18.5)
AMP	22 (81.5)
IMP	25 (92.6)

Table 2: Antibiotic susceptibility profile of *E. coli*. CPX-ciprofloxacin; GEN-gentamycin; CTX-cefotaxime; STR-streptomycin; TET-tetracyclin; AMP-ampicillin; IPM-imipenem.

Discussion

Infertility in the male partner contributes to approximately half of all cases. Infertility can be defined as a failure to conceive in a couple trying to reproduce for a period of 2 years of constant unprotected sex. Bacterial GTIs may contribute to deterioration of various sperm parameters, especially in infertile men. It is well known that these parameters are important in the fertility potential of a male. Several studies have examined the role GTIs on the parameters of sperm for the fertility potential but it is still controversial [11,12]. In our study we have shown that the bacterial genital tract infections may have the negative effect on the infertile males and based on the density of sperms in the samples nearly half of the population was oligospermic (49.25%). The changes in the morphology and motility are more commonly observed in the oligospermic individuals. Similar findings have been reported in the Jajoo and Kalyani [13] and Owolabi, et al. [14].

The presence of bacteria in the studied population is 62.68%. The similar results were obtained in the Isaiah, et al. [15] and Merino et al. [16]. Lower rates of were also reported in Golshani, et al. [17] and Cottell, et al. [18]. *Escherichia coli* (64.28%) has dominated in this study as compared to other studies where Staphylococcus aureus and S. epidermidis has been isolated [14,19]. In some cases E. faecalis has been the main isolate [20,21].

The negative effect of *E. coli* on sperm motility and number has been reported by Diemer, et al. [22] and Prabha, et al. [23]. Similar negative effects of other bacteria like E. faecalis, U. urealyticum, and M. morganii shown by Moretti, et al. [24].

Conclusion

In conclusion, the role of bacterial genital tract infections should be taken into consideration as they may

have additional negative effect on the sperm quality and motility in infertile males. Microbiological analysis must be carried out in the diagnosis of male infertility and suitable antibiotic should be given to control the infection.

Conflict of Interest

The authors declare that they have no conflict of interests.

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