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Is there a role for *Serratia marcescens* in male infertility: An experimental study?

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ABSTRACT

Objective: Establishment of a male BALB/c mouse model to study the role of sperm impairing *S. marcescens* on mouse reproductive potential. The current study can add to use of reliable animal models to provide a noteworthy evidence for the microbial cause of infertility.
Methods: The mice in the test groups II, III, IV were intraperitoneally administered with different doses (10^4, 10^6 or 10^8 cfu) of *S. marcescens* whereas, group I serving as control, received PBS, for 10 consecutive days. The groups were evaluated for any change in body weight, tissue somatic index (%), seminal parameters and histology. Confirmation of *S. marcescens* from reproductive organs was done by reisolating the same by cultural characteristics and biochemical tests.
Results: The results showed that weight gain was evident only in mice receiving PBS (group I), whereas a decrease was recorded in the test groups (group II, III and IV). Only testes of test groups showed significant changes in TSI values whereas, no change in TSI was observed in any reproductive organ of any test group. Seminal parameters *viz.* sperm count, motility and viability were found to decrease in test groups II, III and IV as compared to control group I. Interestingly, the number of pus cells and percent decapitation was more prominent in test groups which received higher doses (i.e. group III and group IV). The histopathological examination revealed mild to dense inflammation in vas deferens and caudal epididymis in all test groups except hypospermatogenesis which was observed only in test group III and IV. However, in group I, neither adverse changes nor any sign of inflammation were observed.
Conclusion: Intraperitoneal inoculation of *S. marcescens* could lead to alteration of semen parameters, induction of decapitation in spermatozoa and histopathological changes, thereby decreasing the reproductive potential of male mice.

1. Introduction

Microbial infections in the male genitourinary tract account for 15% of male infertility cases [1]. The major classes of such pathogens include bacteria, viruses, fungi and parasites. Some of the most commonly isolated bacteria are *Chlamydia trachomatis*, *Ureaplasma urealyticum, Neisseria gonorrhoeae, Staphlyococcus* sp., members of the family *Enterobacteriaceae viz., Escherichia coli*, *Klebsiella* sp. and *Proteus* sp [2]. These pathogens can attack different sites of male reproductive tract such as testis, epididymis, vas deferens and male accessory sex glands, thereby, causing reproductive disturbances and hence, leading to infertility. This form of infertility, which arises as a result of infectious agents, is considered as a potentially correctable type of infertility and hence, can be treated by antibiotics [3]. However, a large number of patients remain untreated, because of lack of diagnosis of the infectious agent. The asymptomatic course of the disease and unspecific clinical signs allow the infection to remain unnoticed, thereby resulting into chronic illness [4]. This has brought into focus, the role of these asymptomatic colonizers in male infertility since, to date, there has been little knowledge about their absolute or relative contribution to male infertility. So far, most of the work has been accredited to organisms such as *C. trachomatis*, *Ureaplasma* sp., *N. gonorrhoeae* and Herpes simplex virus for their role in male infertility [5–8]. However, the data concerning the effect of other asymptomatic microorganisms on male infertility is negligible.

In an earlier work done in our laboratory, infertility in female BALB/c mice was observed upon vaginal colonization with sperm-

Abbreviations and Acronyms: PBS, phosphate buffered saline; TSI, Tissue Somatic Index.

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impairing *Serratia marcescens* and *Candida albicans* [9]. So, with an aim to assess the role of *S. marcescens* in male infertility, intraperitoneal infection in mouse model was established and the effects on the seminal parameters were studied.

2. Material and methods

2.1. Ethical note

All the experiments have been conducted in compliance with the protocols approved by Institutional Animal Ethics Committee, Panjab University (vide letter no. IAEC/504 dated 2 April 2014). All experiments were completed in agreement with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.2. Animals

Adult 5–6 weeks old male (25±2 g) BALB/c mice were used in the present study. Animals were individually housed in polypropylene cages in the animal room of Department of Microbiology, Panjab University, Chandigarh, India. The animals were fed with standard pellet food and water ad libitum and maintained in laboratory conditions (12:12, dark:light cycle).

2.3. Microorganism

The standard strain of *S. marcescens* (MTCC 7641), used in the present study, was procured from the Microbial Type Culture Collection, Institute of Microbial Technology, Sector 39, Chandigarh, India. The strain was grown in Brain Heart Infusion (BHI) broth and maintained as glycerol stocks at −80 °C. The strain was found to cause sperm impairment by agglutination *in vitro*.

2.4. Inoculum

Serratia marcescens strain was cultivated in BHI broth, incubated under shaking conditions (150 rpm) for 24 h at 37 °C and then centrifuged at 10,000 rpm for 20 min. The pellet obtained was washed twice with PBS (50 mM, pH 7.2). The cells were resuspended in the same buffer to get a cell count of 10^4, 10^6, and 10^8 cfu/20 μl.

2.5. Inoculation procedure

Male BALB/c mice were divided into 4 groups with 3 mice in each group. Group I, inoculated intraperitoneally with 20 μl of PBS alone for 10 consecutive days, served as a control. The remaining three test groups (Group II-IV) were intraperitoneally administered with 10^4, 10^6 or 10^8 cfu of *S. marcescens* per mouse in 20 μl PBS respectively for the same time period. Weight profile of all the mice groups was evaluated. The mice were sacrificed on day 12 and the parameters which were evaluated include TSI (%), seminal parameters and histopathological changes. The experiment was repeated twice for consistency of the results.

2.6. Weight responses and tissue somatic indices TSI (%)

Initial body weight of mice from each group was taken on the 1st day of experiment and final weight on the last day of experiment. On day 12, mice from each group were sacrificed by cervical dislocation and the various reproductive organs (*viz.* testis, caudal epididymis and vas deferens) were removed aseptically. The organs were grossly examined and weighed. The TSI (percent tissue/organ weight in relation to body weight) was evaluated [10].

2.7. Determination of seminal parameters

2.7.1. Sperm count and sperm morphology

Mice from each group were sacrificed by cervical dislocation on day 12 and were dissected. The vas deferens was pulled out and placed in freshly prepared 500 μl of PBS buffer (50 mM, pH 7.2). Gentle teasing was done to enable the spermatozoa to swim out into the buffer in a glass plate. A fixed volume of 10 μl of the sample was placed on a glass slide and examined under light microscope at 400× magnification. Around six to eight fields were scanned and mean number of spermatozoa in all the fields was multiplied by 10^6. The slides were also assessed for the morphology of spermatozoa in each field to evaluate the respective abnormalities [11].

2.8. Sperm motility assay

Motility of the sperms extracted from the sacrificed mice was determined by the method of Emmens [12].

2.9. Sperm viability assay

In order to estimate the percentage of viable sperms, an equal volume of mouse spermatozoa was mixed with 0.5% eosin and examined under the light microscope at 400× magnification.

2.10. Reisolation of S. marcescens from the various reproductive organs on day 12

The various reproductive organs were homogenized manually in sterile PBS (50 mM, pH 7.2) to form a uniform homogenate. 100 μl of serially diluted tissue homogenate was plated on BHI agar plates and incubated overnight at 37 °C. The bacterial load in terms cfu/g of tissue was calculated. The reisolated bacteria from various organs were confirmed as *S. marcescens* by culture characteristics and biochemical tests. Further the isolates were also checked for spermagglutinating activity *in vitro*.

2.11. Histopathological studies

Histopathological analyses of the reproductive organs (testis, caudal epididymis and vas deferens) of mice from each group sacrificed on day 12 were carried out. The various reproductive organs were harvested, fixed in 10% formaldehyde for 24 h and then embedded in paraffin according to standard histological methods. Serial paraffin sections of 4 mm were stained with hematoxylin-eosin and observed at 400× magnification for any significant changes in reproductive organs.

2.12. Statistical analysis of data

The results of all the experiments in this study were analysed using Microsoft Word Excel software. The statistical significance of differences between control and test groups was evaluated by Student's t-test. Results are expressed as mean± standard deviation. Differences were considered to be statistically significant when p value < 0.05.

3. Results

The impact of *S. marcescens* on body weight, TSI (%) of various reproductive organs, seminal parameters and histological changes in mice belonging to each group was evaluated.