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Research article

Immunization of Male Rabbits with *Pseudomonas aeruginosa* Increases Spermatogenesis

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ABSTRACT

The impact of immunity on spermatogenesis is documented in the literature. This study highlighted the influence of *Pseudomonas aeruginosa* hyper-immunization on male rabbit's testicular spermatogenesis as the effect of immune response on sex hormones changes. Morphometric criteria were used to evaluate spermatogenesis. The level of hormone was estimated by enzyme-linked immunosorbent assay (ELISA). We found that male rabbits vaccinated with the bacteria showed a highly significant ($P<0.001$) decreased in spermatogonia cell number (11.46±8.11 in test vs 45.19±10.76 in control). In addition there was a significant increase ($P<0.001$) in spermatocytes cell number represented by pachytene and zygotene cells in vaccinated animal as compared to control (88.5±8.11 in test vs 54.14±10.13 in control). In concomitance with these changes, there was a significant elevation in level of testosterone in immunized groups (3.55±3.86 in test vs 0.98±0.8 in control). While, no significant change was observed in progesterone (2.00±0.80 in test vs 1.83±0.79 in control) and estradiol (21.42±14.02 in test vs 19.31±3.72 in control) levels. The histopathological changes included detachment of spermatogonia in the test group was observed. The increase in spermatogenesis and the changes in hormones level as well as histopathological changes suggest that the immune mechanisms impact on spermatogenesis in males rabbits.

Keywords: *Pseudomonas aeruginosa*, **sex hormones, spermatogenesis.**

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INTRODUCTION

Male fertility requires the production by the testis of large numbers of normal spermatozoa through a complex process known as spermatogenesis [1]. It is a highly controlled process, being regulated by hypothalamic pituitary control as well as testicular somatic cells that regulates steroids production to ensure correct and coor-

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dinated process [2]. Follicle stimulating hormone (FSH) acts synergistically with testosterone to increase spermatogenesis efficiency [3]. Testosterone is essential to maintain spermatogenesis and male fertility. In the absence of testosterone stimulation, spermatogenesis does not proceed beyond the meiosis stage. After withdrawal of testosterone, germ cells that have progressed beyond meiosis detach from supporting Sertoli cells and die, whereas mature sperm cannot be released from Sertoli cells resulting in infertility [4]. In addition to the established role of gonadotropins and androgens, estrogens are now recognized as potential regulators of spermatogenesis in several species including humans [5]. The presence of estrogens in the male gonad is well documented and related to the presence of aromatase, an enzyme localized in most of the testicular cells [6].

Lipopolysaccharide (LPS) modulates production of immunological factor [7,8]. It is a component of Gram negative bacteria was shown effect on testicular histology in rats including inflammatory changes as well as decreased levels of serum testosterone (T), leutinizing hormone (LH) and follicle stimulating hormone (FSH) [9]. Microbial infections, localized as well as systemic, are known to cause transitive or permanent male infertility. However, the mechanisms of infection induced infertility are largely unknown. Earlier reports showed that steroidogenesis and spermatogenesis are affected during bacterial lipopolysaccharide (LPS)-induced acute inflammation [10]. Brecchia *et al.* [11] suggested that a sub-acute inflammation may cause infertility by compromising sperm membrane integrity, which decreased a month after LPS-treatment. In addition, the rabbit treated with LPS could be a useful model for studying the effect of an induced systemic inflammation on spermatogenesis [12]. In this communication the effect of hyperimmunization with *Pseudomonas aeruginosa* on male rabbits spermatogenesis and hormonal level were investigated to shed some light on the effect of chronic exposure to bacterial antigens on spermatogenesis.

MATERIALS AND METHODS

Animals

Outbread domestic male rabbits were used. The animals aged about 6 months. They were kept caged in a groups of four, and fed chow and water ad libitum.

Immunization

P. aeruginosa was grown onto tryptose soy agar for 18 h at 37°C. The growth was harvested with sterile saline and washed three times with saline bye centrifugation at 6000 rpm for 20 min. The cells were killed by boiling and bacterial number was adjusted to 5×10^{10} cells/ml. Groups of rabbits (five animals in each group) were immunized. Rabbits received six subcutaneous injections of bacterial suspension (0.25, 0.5, 1.0, 1.5, 2.0, and 2.0 ml) (without adjuvant), at different time intervals (zero time, 2, 4, 6, 8, 10 days) [13]. The route of subcutaneous immunization in the back of the neck region was followed. The animals were rested for 14 days after the last injection. Blood was drawn by heart puncture while the rabbits were under Ktamine anesthesia. Sera were separated and stored at -20°C.

Serum hormone concentrations

Rabbit enzyme-linked immunosorbent assay kits were used to measure Progesterone, Testosterone and Estradiol in sera of test and control rabbit groups as per the manufacturer's instructions (Monobind Inc, CA, USA).

Histological analysis

Testes were obtained and cut into two half and fixed using 10% formaldehyde. Testes specimens were processed and embedded in paraffin wax. Paraffin section of testes of test and control groups were cut at 5 µm thick and stained with hematoxylin eosin staining protocol [14]. Slides were enumerated for spermatogonia, meiotic dividing spermatocytes including pachytene and zygotene cells. Sections were examined for any abnormality in the spermatogenic process.

Statistical

All values were taken as the mean value and standard deviation calculated. The differences were analyzed by using Student's t test. A value of $P<0.05$ was considered to be statistically significant.

RESELTS AND DISCUTION

Table 1 showed the hyperimmunization of male rabbits with heat killed *P. aeruginosa* stimulated spermato-genesis. It was found that an increase in pachytene and zygotene meiotic cells. The percentage of meiotic spermatocytes was 88.53 ± 8.11 in test animals vs 54.5 ± 10.13 in control ($P < 0.001$).

Table 1 Number of Sprmatogensis cells in testes of animals immunized with *P. aeruginosa* vaccinated male rabbit and control.

Cell type (%)	Control (n, 5)	Test (n, 5)	P- value
Spermatogonia	45.19 ± 10.76	64.54 ± 10.13	P < 0.001
Spermatocytes	11.56 ± 8.11	88.53 ± 8.11	P < 0.001

In concomitant with this the significant decrease in sprmatogonia 11.46 ± 8.11 in test animals was found as compared to 45.19 ± 10.76 in control ($P < 0.001$). These finding indicated that a positive shift in spermatogenesis was observed. Comparing with control the profile of sex hormones in immunized group of rabbits showed significant elevation in level of testosterone (**table 2**). On

the other hand no, there was no significant change in progesterone and estradiol in immunized groups (table 2) the basis of this finding is unclear at this time.

Table 2. Sex hormone levels of *Pseudomonas aeruginosa* immunized male rabbit and control.

Sex hormone	Control (n, 5)	Test (n, 5)	P- value
Testosterone (ng/ml)	0.98 ± 0.8	3.55 ± 3.86	0.04
Estradiol (pg/ml)	19.31 ± 3.7	21.42 ± 14.02	0.72
Progesterone (ng/ml)	1.83 ± 0.79	2.00 ± 0.8	0.71

Histological finding revealed accumulation of spermatogonia in the lumen of seminiferous tubule. This change was accompanied with disruption of cells contact or loss of arranged spermatogonia in the basal layer of the tubular (**fig. 1, fig. 2**).

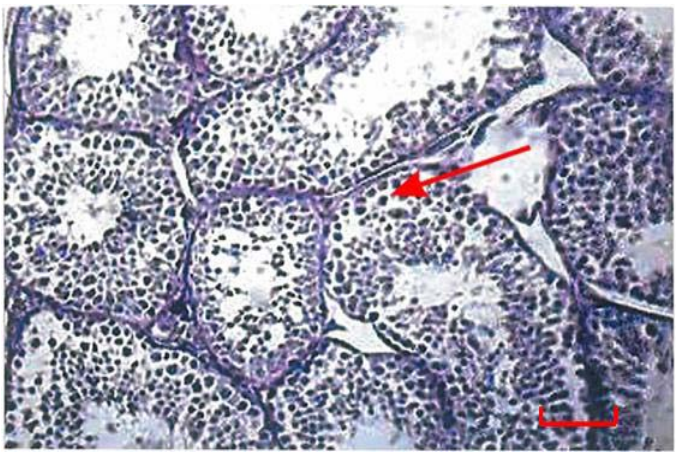

Fig. 1 Section of rabbit testes showing stage of spermatogenesis in test group. It was seen increase mitosis spermatogenic cells, arrow points at spermatogonia (bar, 100 µl).

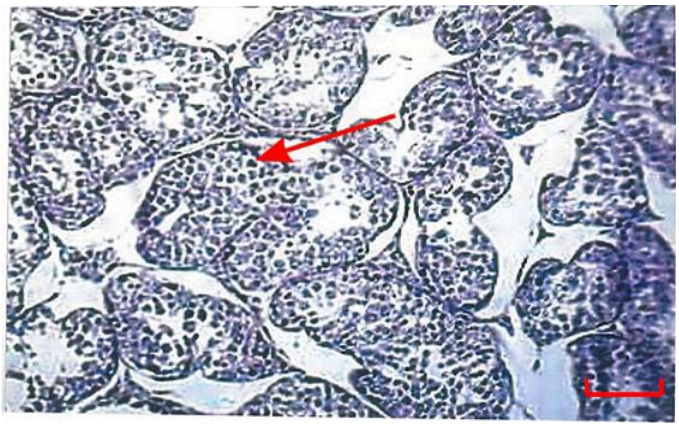

Fig. 2 Cross section in rabbit testes showing stage of spermatogenesis in control groups. Arrow points at spermatogonia (bar, 100 µl).

Similar finding was demonstrated in rat test treated with lipopolysaccharide of *Escherichia coli* serotype 026:B6 [15]. LPS affects on testicular histology in rats including inflammatory changes as well as decreases levels of serum Testosterone, LH and FSH [9, 16, 17]. In our study, hyperimmunization with *P. aeruginosa* might induce a similar effect.

Intensive hyperimmunization of rabbit, reminiscent of chronic infection might affect on testicular function at the level of gamete and endocrine compartments. Reddy, *et al*. [10] demonstrated that bacterial LPS induced oxidative stress and impaired steroidogenesis and spermatogenesis in rat model. In association with the effect of lipopolysaccharide on testes a study done by O'Bryan *et al*. [18] indicated that pathological change in spermatogenic function during severe inflammation were similar to direct effects of inflammatory mediators on the seminiferous epithelium or testicular vasculature, rather than inhibition of the brain-pituitary-Leydig cell axis.

Many studies demonstrated negative affect of LPS on testes [19-21]. However, in present study LPS stimulated spermatogenesis the basis of this stimulation effect might be at the level of endocrine compartment involving mainly Leydig cells which is the source of testosterone (**table, 2**). The effect of LPS, which is the main component of *P. aeruginosa* used as immunogen in this study, LPS stimulated toll like receptor (TLR) that crosstalk with a G- protein coupled receptor (GPCR) [22]. It was shown that LPS affect on cellular signaling and activation through G-protein coupled receptors by the mediator cyclic adenosine monophosphate (cAMP) and protein kinase (PKC) [22, 23]. Similarly signaling cascade of LPS and estrogen was also demonstrated [24] and testosterone effect mediates through G-protein coupled receptor [25]. Within GPCR stimulated by the increased testosterone seen in our results is the factor involved in stimulating the spermatocytes or other mechanism that required additional studies. The effect of distorted spermatogonia as well as stimulating the meiotic spermatocytes (Table 1) could be increased testosterone level as well as may increase others paracrine and autocrine factor, which need further investigation. Overall, the results presented in this report might point-out a link between LPS in the immunizing bacterial antigen, GPCR activation, and increased spermatogenesis. Additional studies are required to address such links.

Conflict of interest

The author declares that he has no conflict of interests.

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