The Professional Medical Journal
www.theprofesional.com

DOI: 10.17957/TPMJ/16.3445

ORIGINAL PROF-3445

TESTICULAR HISTOMORPHOLOGY;

EFFECTS OF RECOMBINANT HUMAN INTERFERON-α-2B IN ALBINO RAT MODEL

drsalmanakazi@gmail.com

Dr. Salman Ahmed Farsi Kazi[1], Dr. Mohammad Ghiasuddin Shah[2], Dr. Jameel Ahmed Gandahi[3], Dr. Shankar Lal Rathi[4]

1. MBBS, M.Phil
Postgraduate student
Department of Anatomy
Isra University Hyderabad
2. DVM, MSc, PhD
Professor and Chairman
Department of Anatomy and Histology
Faculty of Animal Husbandry and Veterinary Sciences
Sindh Agriculture University Tandojam
3. DVM, MSc, PhD
Assistant Professor
Department of Anatomy and Histology
Faculty of Animal Husbandry and Veterinary Sciences
Sindh Agriculture University Tandojam
4. MBBS, M.Phil
Professor of Pathology
Isra University Hyderabad

Correspondence Address:
Dr. Salman Ahmed Farsi Kazi,
M.Phil. Postgraduate Student
Department of Anatomy
Isra University
Hala Naka road Hyderabad
drsalmanakazi@gmail.com

Article received on:
12/05/2016
Accepted for publication:
16/08/2016
Received after proof reading:
07/10/2016

ABSTRACT… **Objectives:** The present study was conducted to investigate the effects of recombinant human interferon-α-2b (rh-INF-α-2b) on testicular histomorphology in adult rat model. **Study Design:** Experimental study. **Place & Duration:** Animal house, Sindh Agriculture University Tando Jam and Isra University Hyderabad from January to December 2014. **Methodology:** 80 adult albino rats were selected according to inclusion and exclusion criteria and divided into 4 groups. Group I: Control rats 0.9% saline, Group II: rhIFN α-2b (3MIU), Group III: rhIFN α-2b (5MIU) and Group IV received rhIFN α-2b (10MIU). The rhIFN α-2b was injected intra-peritoneal (i.p) three times a week for 3 weeks in doses of 3MIU, 5MIU and 10MIU. Animals were euthanized. Orchidectomy was performed and testicles were stored in 10% formaldehyde. 5μ thick tissue sections were stained by Hematoxylin & Eosin (H & E). **Results:** Atrophic seminiferous tubules with clumping of lining epithelia were noted. Germ cell maturation arrested was prominent; hypervascularity with reduced germ cells and sperm cells were noted in high dose rhIFN α-2b treated groups. Tubular desquamation and thick basement membrane were visible. The sertoli cells and interstitial cells of Leydig counts were increased. **Conclusion**: It is concluded that the recombinant human interferon-α-2b exerts serious adverse effects on testicular histomorphology.

Key words: Recombinant human interferon α-2b Testis Histology Rats

Article Citation: Kazi SAF, Shah MG, Gandahi JA, Rathi SL. Testicular histomorphology; Effects of recombinant human interferon-α-2b in albino rat model. Professional Med J 2016;23(10):1209-1213. **DOI:** 10.17957/TPMJ/16.3445

INTRODUCTION

Interferon's (IFNs) are a group of organically dynamic regular proteins, secreted by the immune cells. IFNs are created by immune cells because of viral and bacterial diseases and/or tumors. IFNs are isolated into different types. Notwithstanding, the most widely recognized are interferon-α, interferon-β and interferon-γ.[1] Interferon's (IFNs) are most strong natural cytokines of immune system, called immunomodulators. The IFNs display natural impacts like control of RNA (mRNA) formation, cell division and growth. They exert antiviral and antibacterial actions and intercede immune regulation.[2] The leukocyte IFNs are assigned as IFN-α and IFN-γ, while fibroblast releases IFN which are designed as IFN-ß.[3] It is accounted for that infusing IFN-γ in mice influences spermatogenesis and changes germinal epithelium.[4] Transgenic male mice presented to IFNs indicated bizarre changes in spermatogenesis and in the end got to be sterile. IFN-γ inhibits gonadal steroidogenesis in in-vitro and in-vivo conditions; however the basic components are not obviously illustrated.[5-7] In cultured cells, IFN-α is secreted by peritubular myoid cells and Sertoli cells, and additionally by germ cells. Interestingly, it is clear that the IFN-γ is secreted by early spermatids.[8,9] The IFN-α and IFN-γ receptors express cell membrane receptors on mammalian sperm cells within the seminiferous tubules during spermatogenesis. Receptor expression of IFN-α and IFN-γ demonstrate that the IFNs may be acting against anti-sperm vaccine contraception and infertility. In targeted gene mutation studies, it has been testified that the INF inhibits germ cell development within testes.[3,10]

IFN α or IFN-ß gene over expression retards the spermatogenesis and destroys the

spermatogonia eventually, has been proved in experimental transgenic mice model.[5,6] A previous study reported reduction in serum testosterone and total free androgen index in healthy male treated with IFN-α.[11,12] Deleterious effects of IFN–γ on testicular morphology have been reported. Reduced Sertoli cells, height of germinal epithelium and shrunken seminiferous tubules were reported.[3,10] However, the underlying mechanisms are poorly understood and remain ambiguous. Experimental animal studies have reported controversial findings.[11] The phenotypic impacts of the IFN drugs on testicular histology has seldom be addressed

As presently Pakistan has much prevalence of viral hepatitis for which perfect treatable medication being used is the recombinant human interferon-α-2b (rh-INF-α-2b), yet its impacts on testicular histomorphology are not assessed. Subsequently, the present was intended to investigate the phenotypic effects of rh-INF-α-2b on sperm and testicular morphology in adult rat model.

MATERIALS AND METHODS

The present experimental-Interventional study was conducted at Animal House, Sindh Agriculture University Tando Jam and Isra University Hyderabad, Sindh from January - December 2014. Eighty adult rats (n=80) were equally divided into 4 groups. Group I: Control rats 0.9% saline, Group II: Recombinant human interferon-α-2b (rhIFN α-2b) injection (3MIU), Group III: rhIFN α-2b injections (5MIU) and Group IV received rhIFN α-2b injections (10MIU). Albino rats were selected through non-probability purposive sampling in a systemic way according to inclusion and exclusion criteria. Adult male rats of 200- 250 grams were included and Sick rats, moribund and non feeding rats were excluded. Prior permission of ethical review committee (ECR) and Animal ethical clearance were taken from the institutes. Handling of rats was in accordance to NIH Guidelines for the Care and Use of Laboratory Animals. Stainless steel cages, equipped with feed containers and plastic drinker nozzles, were used for animal housing. Ventilation, humidity, water and feeding were provided as per standards. Rats were exposed to 12 hour light-dark cycles. The rhIFN α-2b was purchased from Isra University Hospital Pharmacy. It contained recombinant human IFN α-2b in water base. Cold chain and storage of rhIFN α-2b was ensured. The rhIFN α-2b was injected intra-peritoneal (i.p) three times a week for 3 weeks in doses of 3MIU, 5MIU and 10MIU. Rats were left for one week more. Animals were sacrificed for on 30th day post-interferon injection. Orchidectomy was performed and testicles were stored in 10% formaldehyde in deep freezers at -70^{0}C. The tissue was enclosed in a solid mass of paraplast. This was done with two L-shaped metal pieces. The cold L-shaped metal pieces were placed on a glass to produce squares of desired sizes. The enclosures were filled with melted paraplast. The tissue was placed in the squares in vertical position with the help of warm forceps. The blocks were labeled, allowed to cool, and the metal pieces were removed. The blocks were trimmed free of excess paraplast leaving some free margin around the embedded object. Testis samples were cut by microtome to 5 μ tissue section and stained by Hematoxylin & Eosin (H & E)[13] stain to make histological slides.

RESULT

Histomorphological examination of controls showed normal looking seminiferous tubules with intact basement membranes. Epithelial cells layers, sertoli cells and interstitial cells of Leydig, and tissue vascularity were found normal. While, experimental interferon treated rats showed reduction of lining epithelia of seminiferous tubules. The sertoli cells and interstitial cells of Leydig counts were increased. Clumping of lining epithelia, germ cell maturation arrest, thick basement membrane and defects of seminiferous tubules were observed in the lining epithelia. Figures 1–4 show significant differences in the histological findings of controls and interferon treated rats.

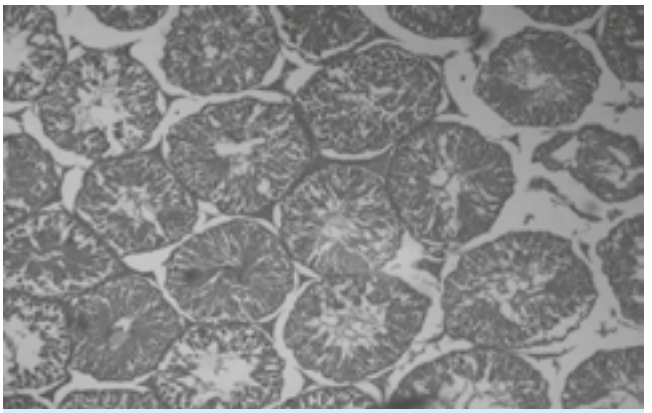

Figure-1. Group I. Controls- testicular tissue sections showed normal histological structure. H & E stain (x100)

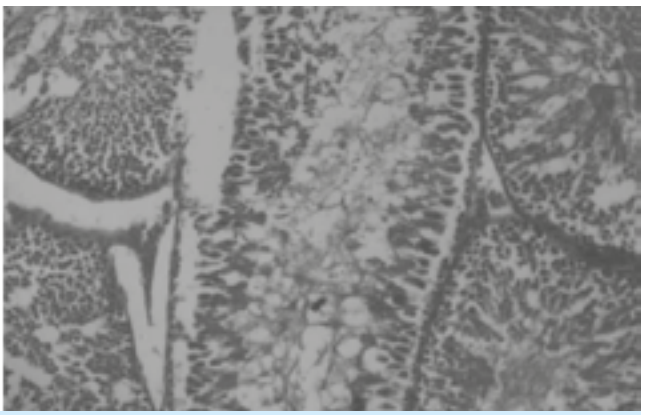

Figure-2. Group II rhIFN α-2b (3 MIU) – reduced sperms, germ cell maturation arrest and increased sertoli cells and interstitial cells. H & E (x100)

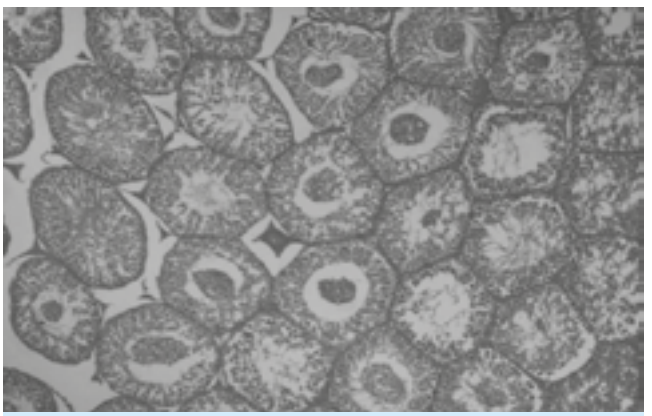

Figure-3. Group III rhIFN α-2b (5 MIU) Reduced sperm cells, increased sertoli cells, clumping of epithelial cells, thick basement membrane and maturation arrest. H & E stain (x100)

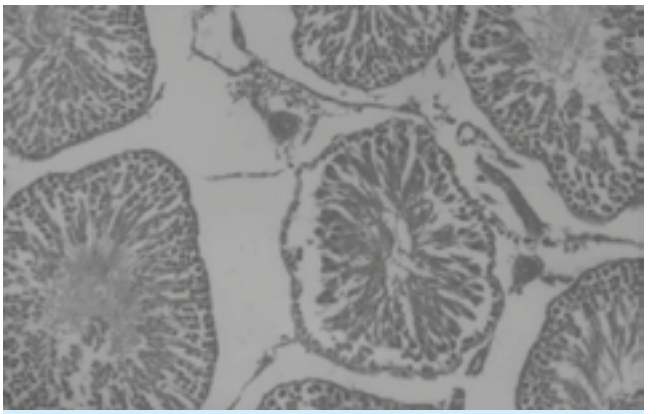

Figure-4. Group IV rhIFN α-2b (10 MIU) Arrested maturation of germ cells, increased sertoli cells and Leydig cell, hyper-vascularity and thick basement membrane. H & E stain (x200)

DISCUSSION

Currently, much interest has grown in the research of inter relationship of cytokines and human gonadal functioning because the rhINFα-2b affect the fertility pathways at different levels.[14] In present study, effects of rhINFα-2b on the testicular histomorphology were researched in a rat model. The present study observed maturation arrest and tubular desquamation in the male gonad of rats treated with rhINFα-2b. Histology showed thickened basement membrane, Hypervascularity and edema of interstices prominently in rhINFα-2b treated rats. Interstitial cells of Leydig and Sertoli cells were increased in number in the experimental rats. Testicular histomorphology was noted at different doses of rhINFα-2b such as 3 MIU, 5 MIU and 10 MIU used in the present study. The findings of present study are in agreement with previous studies.[15-18] On the contrary, other studies reported none of such adverse effects. Those previous studies reported no adverse effects of rhINFα-2b on the spermatogenesis.[19,20] The findings of above studies are in contrast to present and previously research studies.[21,22] The previous studies[23,24] investigated the effects of IFN-γ on germinal epithelium in experimental mice model. Both above studies reported adverse changes in the germinal epithelium and spermatogenesis was found impaired.[23,24] The findings of above studies are in agreement to the present research work.

Our findings are also consistent to previously reported studies.[25,26] They had reported serious adverse effects on the germ cells with complete sterility in the rhINFα-2b treated rats. Natwar et al[24] reported seriously deleterious changes exerted by INF on the mice testicles. They reported collapsed seminiferous tubules, reduced germ epithelium height and desquamation of the germinal epithelium; these findings are consistent to the present study. However, Natwar et al[24] reported reduced sertoli cell counts which is in contradistinction to present study as increased sertoli cells were noted. Hibi et al[27] also reported results contrary to present study, as they reported increased spermatogenesis. The findings of Natwar et al[24] and Hibi et al[27] are in contrast to the present and previous studies.[21,22] The present research observed that the rh-IFN-α has serious implications on the testicular histomorphology. Leydig`s cells were increased in rh-IFN-α treated experimental rats in the present study. Above findings are in agreement to previous studies.[28,29] A recent study[22] administered rhIFN-α (5 mIU and 10 mIU) daily for thirty days and reported no adverse effects on testicular histology. Findings of above study are in contrast to present study. Findings of above study[22] are also in contrast to other previous studies.[21,23] Such controversies might be due to different strains of rats used, different drug quality, different drug dosing schedule, and duration and research bias. The results obtained by present study are of clinical significance and it is concluded that sufficient information is obtained from the experimental study.

CONCLUSION

Recombinant human interferon-α-2b (rhINFα-2b) exerts serious adverse effects on the testicular histomorphology. Interferon treated rats showed atrophic seminiferous tubules with desquamation. Sertoli cells and interstitial cells of Leydig were increased. Clumping of lining epithelia, germ cell maturation arrest, thickening of basement membrane and defects of seminiferous tubules were noted.

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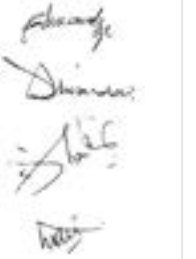
AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Dr. Salman Ahmed Farsi Kazi	Study conception and design Acquisition of data, Drafting of manuscript	
2	Dr. M. Ghiasuddin Shah	Drafting of manuscript, Critical revision, data analysis, final approval	
3	Dr. Jameel Ahmed Gandahi	Drafting of manuscript, Plagiarism check, Analysis and interpretation of data	
4	Dr. Shankar Lal Rathi	Study of microscopic slides and interpretation	