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Histological Study of Rat Testis After Withdrawal of Cimetidine Therapy

Sonia Bashir and Taquyya Sultana Abidi
Department of Anatomy, King Edward Medical, Lahore.

SUMMARY

Ninety six adult male rats were divided into four groups. They were treated with cimetidine 5.7 mg/kg body weight twice daily for 3,6 and 9 weeks intramuscularly. The testis showed decreased spermatogenesis in our research. Cimetidine if used for a period of three to six weeks does not alter the spermatogenic cycle. After a period of nine weeks of treatment with cimetidine prominent changes in the spermatogenic cycle were observed. The stage of cycle most effected is transformation of spermatids to spermatozoa. The testis were kept intact for two months after stopping the drug and histological study done at this stage showed recovery changes.

INTRODUCTION

Acid peptic disease in a common problem for which many patients receive cimetidine. Several adverse reactions have been reported. Thiel[1,2] and Fuentes[3] studied the various parameters on semen of patients treated with cimetidine. They reported decrease in sperm count. White4 and Porro[5] gave different reports regarding the sperm count. This study is carried out to seen the effects of cimetidine on testes and to see if the withdrawl of drug well result in any reversibility.

MATERIAL AND METHODS

Ninety six adult albino rats were taken for the experiment and divided into four groups. A, B, C and D, containing 24 animals in each. Group A acted as control and the rest as experimental groups. Cimetidine 5.7 mg/kg body weight was given I/M twice daily for three, six and nine weeks to group B, C and D respectively. One half of the animals were sacrificed. Their testes were removed, processed and microscopic assessment of the germinal epithelium was made. The other half of the animals were kept without drug for two months. After two months the animals were sacrificed and testis removed. The testis of all the groups were cut into small pieces, fixed in Zenker-Formalin for twenty four hours, processed, sectioned and stained by Haematoxylin and Eosin (H-E), and Periodic acid Schiff-Haematoxylin stain (PAS-H). A qualitative microscopic assessment of germinal epithelium was made. The diameter of the transversely cut seminiferous tubules was measured by an ocular micrometer.

RESULTS

Group A showed normal histological appearance (Figs. 1, 2). Group B and C on cimetidine therapy showed that the tubular diameter was decreased, unchanged basement membrane and Myoid cells were seen. All cells of spermatogenic series could be seen in the seminiferous tubules, decreased in group C as compared to control-group A and group B. The interstitial tissue was normal (Figs. 3, 4, 5).

After two months of stopping the drug in group B and C the size of tubules became normal, lumen was full of sperms, no change was observed in the basement membrane. All the cells of spermatogenic series could be seen. Replication process could be observed in some cells. Stromal architecture and Leydig cells were same as in control group (Figs. 8, 9, 10).

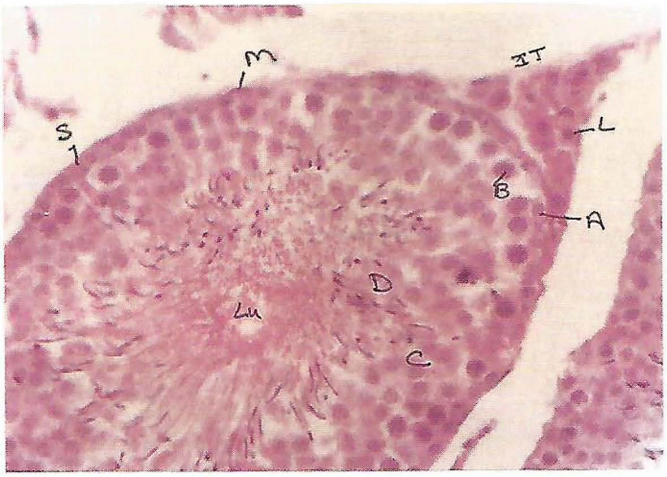

Fig. 1: **Photomicrograph of testis showing S, sertoli cell; M, myoid cells; L, leydig cells; A, spermatogonia; B, spermatocytes; C, spermatids; D, spermatozoa occupying the lumen; IT, interstial tissue (H&E stan) Group A.**

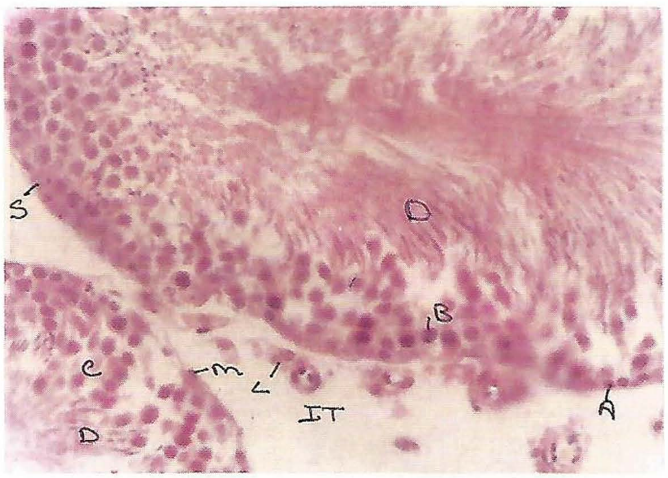

Fig. 3: **Photomicrograph of testis showing S, sertoli cell; M, myoid cells; L, leydig cells; A, spermatogonia; B, spermatocytes; C, spermatids; D, spermatozoa occupying the lumen; IT, interstial tissue (H&E stan) Group B.**

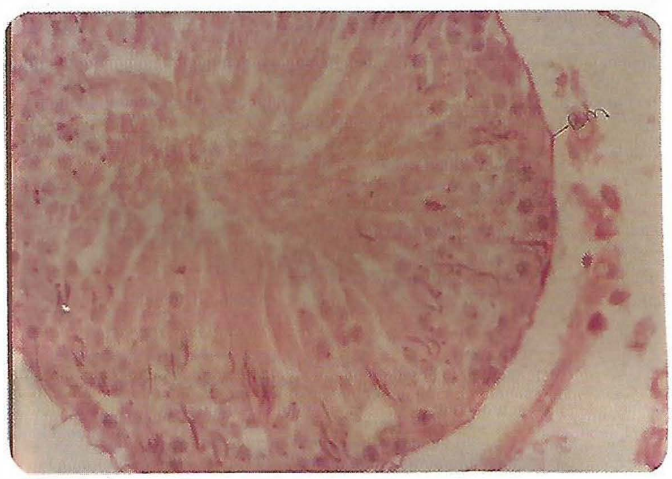

Fig. 2: **Photomicrograph of normal testis showing BM: Regular basement membrane, PAS-H stain in Group A rats.**

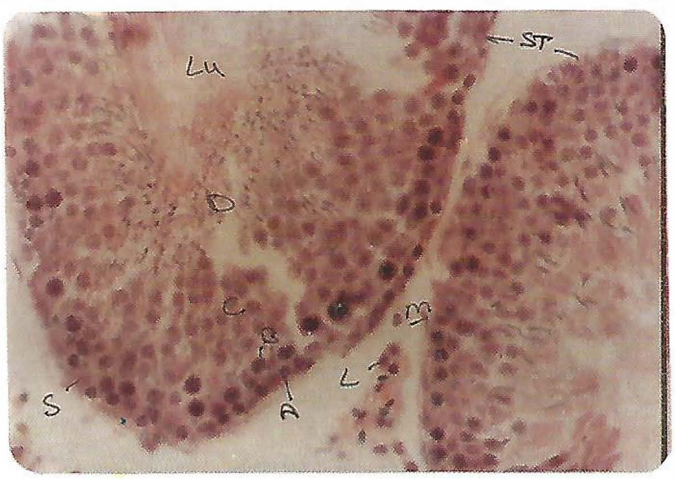

Fig. 4: **Photomicrograph of testis showing ST, seminiferous tubule; Lu, lumen; S, sertoli cells; M, myoid cells; L, leydig cells; A, spermatogonia; B, spermatocytes; C, spermatids lining the lumen of the tubules; D, mature spermatozoa advancing towards the lumen (H&E stan) Group C.**

Cimetidine treated group D showed a considerable decrease in the diameter of seminiferous tubules, the tubular lumen showed only a few mature spermatozoa, the spermatids were lining the lumen. A thickening of the basement membrane was seen and the Myoid cells were prominent. The tubules showed small spermatogonia lining the basement membrane. Few spermatocytes could be seen at some places. Their was no evidence of cell division. Some tubules showed no spermatozoa, in the other tubules very few spermatozoa could be appreciated and those even appeared to be small in

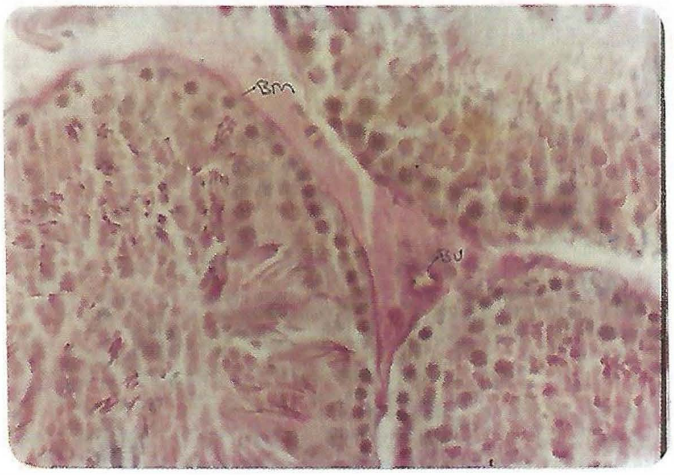

Fig. 5: **Photomicrograph of testis showing BM, regular basement membrane; BV, patent blood vessel. PAS-H stain Group B and C.**

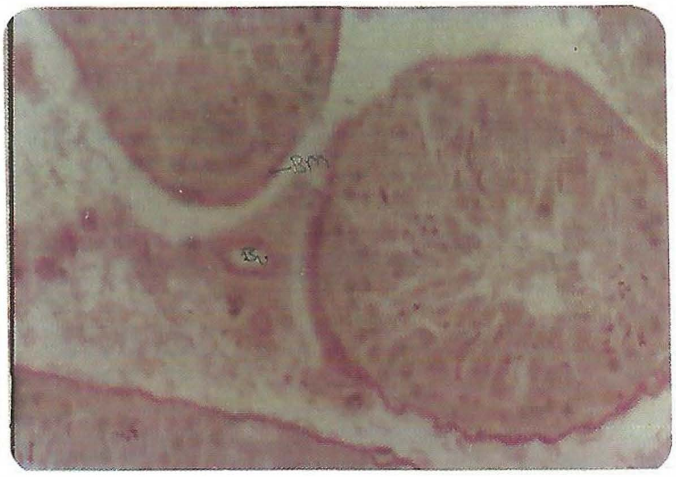

Fig. 7: **Photomicrographs of testis showing BM, thickening of basement membrane; BV, blood vessels with patent lumen but with thick walls PAS stain Group D.**

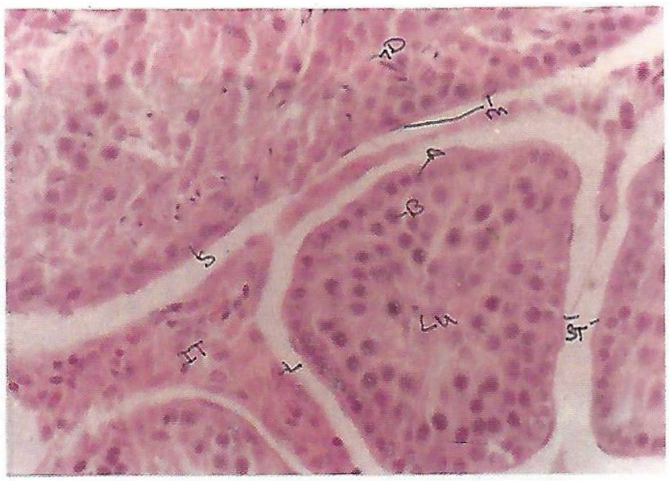

Fig. 6: **Photomicrograph of testis showing ST, small size seminiferous tubule; Lu, lumen occupied by few spermatids (C), M, myoid cells; S, decrease in number of sertoli cells; A, spermatogonia small sized; B, undividing spermatocytes; D, very few spermatozoa in between the spermatogenic cells; IT, increased interstitial tissue; L, very few scattered leydig cells (H&E stan) Group D.**

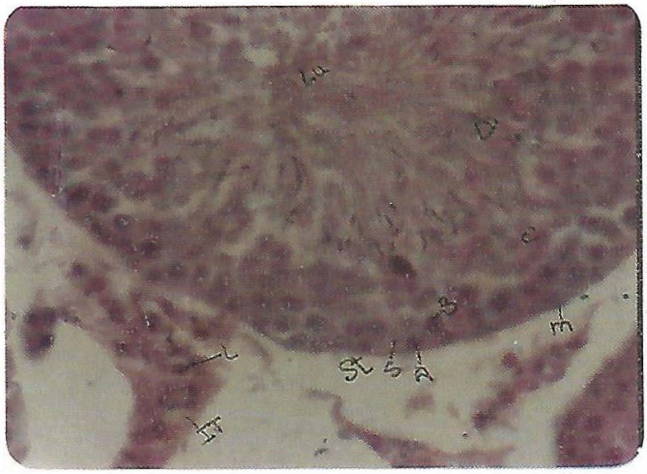

Fig. 8: **Photomicrograph of testis showing Lu, lumen occupied by spermatozoa; S, sertoli cells; M, myoid cells; L, leding cells; A, spermatogonia; B, spermatocyte; C, spermatids (H&E stain). Group B.**

size as compared to control. Sertoli cell number was also reduced. There was an increase in the interstial stroma thus dispersing the Leydig cells. The walls of the blood vessels appeared to have thickened. Leyding cells were decreased and dispersed due to increased interstitial stroma (Figs. 6, 7).

In group D after stopping the drug for two months showed improvement in the diameter of the seminiferous tubules. Lumen showed few matured sperms and these even absent in some tubules. Thick basement membranes and number of Myoid cells was less than group B and C. Spermatogonia and primary spermatocytes at many sites were actively replicating thus depicting active

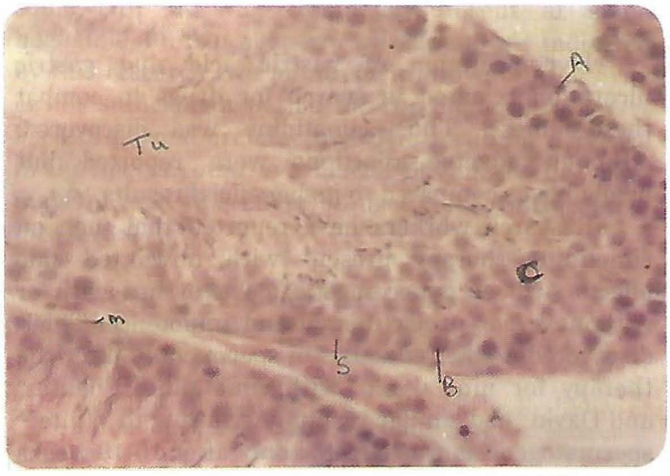

Fig. 9: Photomicrograph of testis showing near normal tubular size Tu, tubular lumen streaming with mature spermatozoa; A, spermatogonia; B, prominent spermatocytes; C, dividing spermatids; S, sertoli cells (H&E stain) Group C.

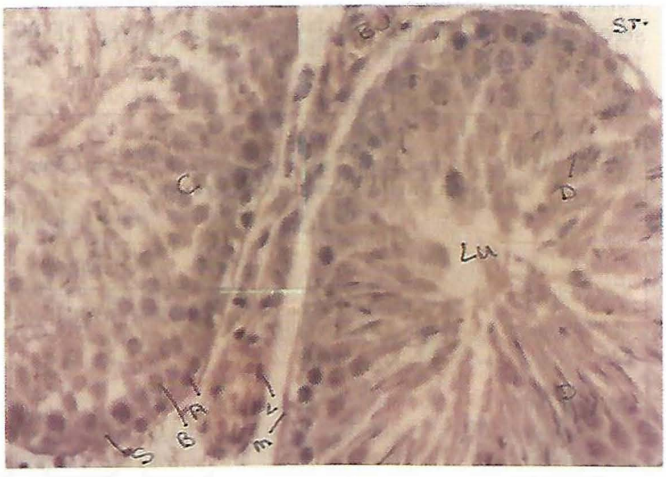

Fig. 11: Photomicrograph of testis showing ST; increase in tubular diameters; Lu, lumen; M, myoid cells; S, sertoli cells; A, spermatogonia; B, prominent spermatocytes; C, dividing spermatids showing replication; D, spermatozoa advancing towards the lumen; L, leyding cells; BV, blood vessel with patent lumen (H&E stain). Group D.

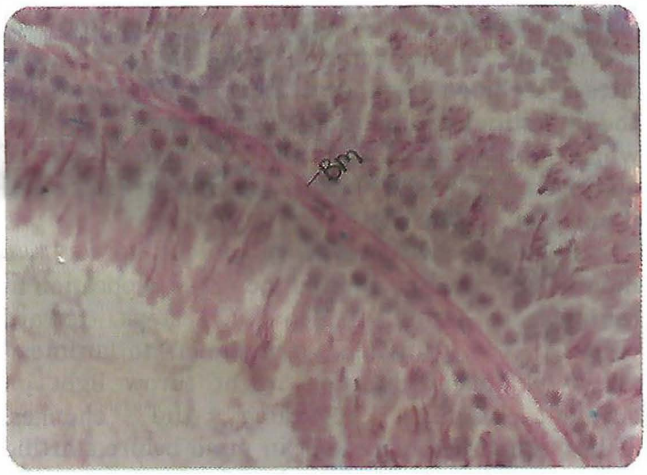

Fig. 10: Photomicrograph of testis showing BM, near normal basement membrane. (PAS-H stain). Group B and C.

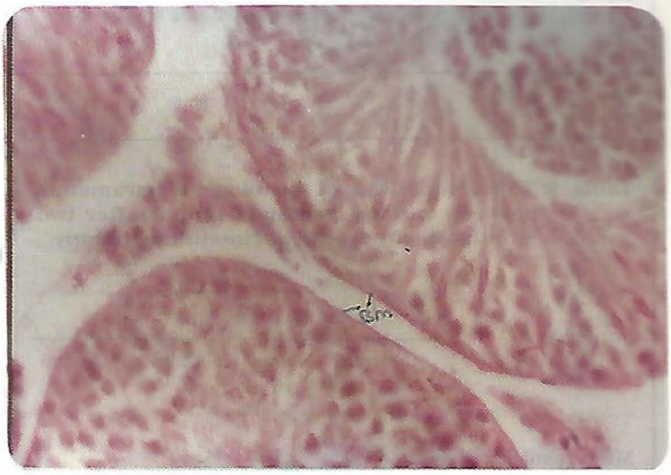

Fig. 12: Photomicrograph of testis showing BM, basement membrane normal at some sites and slightly thickened at some (PAS-H stain). Group D.

proliferation. Quite and number of spermatids were also seen multiplying and some could be seen transforming to spermatozoa stage. Mature spermatozoa were approaching the lumen and some had already lined the lumen of the tubules. The mature spermatozoa had improved in number but still do no seemed to have reached the control level. Signs of reversal towards normal structure were definitely there but still normal sperm count was not achieved. Sertoli cell count reversal toward normal was still in progress. Leyding cells showed normal

pattern but some areas still showed increased stroma and Leydig cells scattered apart (Figs. 11, 12).

Table 1: The comparison of histological parameters between various treatment groups.

Parameters	*Groups*			
	Control A	*3 weeks* B	*6 weeks* C	*9 weeks* D
Mean tubular diameter (UM)	208.5	208.1	204.9	148.1
Basement membrane	Normal	Normal	Normal	Normal
No. of spermatogonia	+ + +	+ + +	+ +	+
No. of spermatocytes	+ + +	+ + +	+ +	+
No. of spermatods	+ + +	+ + +	+ +	+
No. of spermatozoa	+ + +	+ + +	+ +	+
No. of seroli cells	+ + +	+ + +	+ +	+
No. of leydig cells	+ +	+ +	+ +	+

Table 2: The comparison of histological parameters between various treatment groups after two months of withdrawal of cimetidine therapy.

Parameters	*Groups*			
	Control A	*3 weeks* B	*6 weeks* C	*9 weeks* D
Mean tubular diameter (UM)	210	211.1	207.1	162.8
Basement membrane	Normal	Normal	Normal	Normal
No. of spermatogonia	+ + +	+ + +	+ +	+ +
No. of spermatocytes	+ + +	+ + +	+ +	+ +
No. of spermatods	+ + +	+ + +	+ +	+ +
No. of spermatozoa	+ + +	+ + +	+ +	+ +
No. of seroli cells	+ + +	+ + +	+ +	+
No. of leydig cells	+ +	+ +	+ +	+

DISCUSSION

Hypersecretion of gastric acid and gastric ulceration resulted in search for drugs to combat these illness. Thus cimetidine was discovered, although adverse reactions were reported but negligible as compared to its beneficial results.

Research workers have reported that men on cimetidine therapy present with unwanted side effects such as gynecomastia, galactorrhea with or without hyper prolactinemia and impotence. Thiel[6] discovered a 43% reduction in sperm count after the therapy for nine weeks with cimetidine. Fuentes[3] and David[7] had similar results. According to White[4,8] spermatogenesis was effected only after 10-13 weeks of cimetidine treatment.

Much information is not available regarding the mechanism responsible for the reduction in sperm count. It may be due direct toxic effect on spermatogonia or on some other stage of spermatogenesis, or some other mechanism.

In our research, cimetidine therapy for 3-6 weeks did not cause much damage to testis, whereas therapy for 9 weeks definitely affected the spermatogenic cycle. The transformation of spermatids to spermatozoa is decreased, at some sites it is completely absent. There is an increase in the interstitial stroma thus effecting the Leydig cells which are decreased. It is thought that atrophy of androgen responsive tissues results in decreased number of sperms[2].

Regarding withdrawal of cimetidine for two months our research showed increased replication in seminiferous tubules depicting steps toward recovery. These findings were similar to lardinois[9] Work for longer duration might throw light on different aspects regarding the changes. Oligospermia has to be kept in mind before starting with cimetidine for longer duration and especially those who are still in the process of completing their family. Reversal of effects of the drug is there but chances cannot be taken regarding the fertility of such patients.

CONCLUSION

Cimetidine if used for a period of 3-6 weeks does not alter the spermatogenic cycle. After nine weeks of treatment with cimetidine, prominent changes in the spermatogenic cycle were observed. The stage of the cycle most effected was

transformation of spermatids to spermatozoa. At some sites it was completely absent at some negligible number of sperm were found. As indicated by this research two months withdrawal of the drug is not sufficient a period for the changes to revert back to normal architecture. The resultant decrease in the sperm count is a matter of concern in young males. Could this effect be used as a contraceptive measure.

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The Authors:

Sonia Bashir
Assistant Professor
Department of Anatomy,
King Edward Medical,
Lahore.

Taquyya Sultana Abidi
Department of Anatomy,
King Edward Medical,
Lahore.

Address for Correspondence:

Sonia Bashir
Assistant Professor
Department of Anatomy,
King Edward Medical,
Lahore.