CHAPTER V.

SUMMARY AND CONCLUSION

In the present study, the contraceptive efficacy of a progestin (MPA) and a combination of a progestin and an androgen (MPA + TE) has been evaluated. The contraceptive efficacy of these treatments was assessed in relation to the biochemical and morphological alterations in the residual spermatozoa of the caput and cauda epididymides in adult rats treated for 60 days. The side effects of these hormonal treatments on vital organs and recovery status of these treatments were also studied.

The parameters studied were the fertility rate, whole body and organ weights, the cauda epididymal sperm profile like motility, count, viability, morphology, sperm nuclear and acrosomal integrity. The biochemical parameters of sperm viz., succinate dehydrogenase (SDH), adenosine triphosphatase (ATPase), protein, sialic acids, hyaluronidase, acrosin, superoxide dismutase (SOD) were estimated in order to assess their metabolism and function. Vas deferens SDH, ATPase, total proteins and sialic acid acids were also quantified. Electrophysiological changes in the vas deferens, haematological parameters viz., haemoglobin content, blood cell counts, blood SOD and serum levels of testosterone, cholesterol, protein and transaminases were also analyzed. In addition, adrenal catecholamines, liver glycogen and phosphorylase and the histology of various reproductive as well as vital organs were also carried out in normal, treated and withdrawal groups of animals.
MPA treatment and its recovery

Intramuscular injections of MPA (5 mg/week/Animal) for 60 days were found to exert an antispermatogenic effects by reducing the epididymal sperm count. It indicated the suppression of spermatogenic activity in testes, acting probably, indirectly by inhibiting gonadotropin secretion and directly by blocking Leydig cell function. The treatment also brought about a significant decline in body growth of these animals. The weights of the testis, epididymis and vas deferens showed the same trend. The decrease in the weight of these organs reflects on the androgen antagonistic effect of this treatment as these are androgen sensitive parameters. Sperm motility by MPA injections was also suppressed in the present study indicating the influence of the treatment on post-testicular site ie., the epididymis. The viability of the sperm were also affected due to loss of their membrane permeability. The changes in the sperm viability was correlated with alterations in their morphology. The sperm anomalies like decapitation and accumulation of cytoplasmic droplets on mid-piece and tail regions, acrosomal loss and other head defects were also noted in the cauda epididymal sperm of MPA treated rats. These observations suggest that sperm maturation and fertilizing ability were altered in the rat epididymis as a result of MPA treatment. However, the nuclear integrity of the sperm was not changed by the treatment, as all the sperm had green fluorescence like those of the control animals.

The biochemical parameters of the caput epididymal sperm viz; ATPase, SDH, sialic acid, protein were also reduced in MPA treated rats as the epididymal function is affected due to androgen deficiency induced by MPA injections. However, these parameters in the cauda epididymal sperm showed an increment
due to their altered metabolism and epididymal maturation, exerted by the treatment as the serum testosterone levels reduced significantly in this study. The acrosomal membrane bound enzymes i.e. hyaluronidase and acrosin activities were also declined significantly in the epididymal sperm of the treated rats. Alteration in these enzyme levels indicated further loss of fertilizing capacity of the sperm in treated rats. Similarly the sperm SOD levels also reduced revealing their altered membrane permeability.

The mating rate also reduced along with a loss of fertility rate of the MPA treated rats. The histology of the testes, caput and cauda epididymides and vas deferens revealed degenerative and involutory changes due to the anti-androgenic effect of MPA treatment. Metabolic functions of the vas deferens were also affected since, its androgen sensitive parameters viz., SDH, protein and sialic acid were reduced. Similarly, the contractility pattern of the vas deferens to various doses of nor-adrenalin were also affected which probably reveals the physio-pharmacological alteration of the vas deferens occurred during the treatment. The adrenal catecholamine levels also altered in the MPA treated animals and it is correlated with hypertrophy of chromaffin cells in the adrenal medulla. The liver glycogen increased with a concomitant reduction in its phosphorylase activity. The histology of the liver also revealed alterations.

The data on clinical chemistry reflected on increased levels of transaminases as result of abnormal function of the liver. Similarly, alteration of serum cholesterol levels were also observed. Heamatological data showed no significant changes in haemoglobin content and RBC counts. However, an increase in WBC counts were observed indicating changes in immune function of these rats.
Discontinuation of the treatment for 90 and 120 days, restored these induced effects on sperm gradually, and the fertility rate of these animals also returned towards normal levels. The vas deferens and liver functions appeared to be normal. Haematology and serum parameters also seemed to be comparable to those of normal animals. However, the recovery of these parameters was slow as compared to that of MPA + TE rats, probably due to slow elimination rate of MPA and its high dose in the present study.

**MPA + TE COMBINATION**

Weekly intramuscular injections of MPA (3 mg) along with TE (2 mg) for sixty days, were found to have no significant alterations in the whole body and organ weights. The data revealed a depletion in sperm reserves of the cauda epididymis as result of this treatment, in addition to the loss of sperm motility in cauda epididymis. Morphological defects are observed by this combination due to its effects on the target organs. The sperm nuclear integrity study revealed that the double stranded DNA of the sperm is intact since, all the sperm exhibited green fluorescence during experimental periods.

The altered levels of SDH and ATPase in the sperm were correlative of their metabolic changes in these animals received hormonal combination. The changes in the total proteins and sialic acids in sperm also explained the effect of MPA + TE combination on the micro-environment of the epididymis. The elevated frequency of non-viable sperm and reduction in the SOD levels implied alterations in their membrane permeability. The sperm acrosin system and hyaluronidase levels in treated rats decreased and are related to loss of their fertilizing capacity.
Hence the sperm were unable to fertilize the ovum, thereby impairing the fertility rate of the treated animals.

Normal mating rate in MPA + TE treated animals were observed. However, the fertility rate was declined with reduced litter size in these treated animals. Similarly, the testosterone was not altered appreciably revealing normal libido of these animals. The testicular histology indicated spermatogenic arrest in MPA + TE treated rats. However, the histology of the caput and cauda epididymides did not seem to be affected adversely. But the tubular lumen had less number of sperm due to this hormonal regimen. The vas deferens physiology also was not altered significantly. No significant alterations were noted in its biochemical parameters studied. Similarly, contractility pattern of the vas deferens to different doses of nor-adrenalin was also not affected. Serum testosterone levels were within the normal range.

The observed side effects overall in the present study on reproductive organs except the spermatogenic arrest were minimum. Moreover, no significant changes were detectable in haemoglobin levels and blood cell counts. Clinical parameters were within the normal range. However, an insignificant elevation in transaminase levels were observed probably due to an elevation in androgen levels. Similarly, serum cholesterol levels was also increased in this study. The liver glycogen content elevated in MPA + TE combined treatment, followed by a decline in phosphorylase activity due to an altered carbohydrate metabolism. However, much changes were not observed with the histo-architecture of the liver. Similarly, adrenal morphology with respect to the cortex was unaffected. However, medullary region had increased spaces filled with secretory materials,
between the chromaffin cells during the treatment. Subsequently the levels of catecholamines were also found to be increased stating their accumulation as a result of these hormonal injections.

The recovery data after discontinuation of the hormonal regimen for 60 and 90 days revealed gradual restoration of all the affected parameters viz., sperm concentration, sperm motility, sper viability and morphology in the cauda and caput epididymides. The fertility rate of these animals also restored after withdrawing the injections. Similarly, normal function of vital and reproductive organs were also observed during the recovery period of this treatment. Serum chemistry and haematological parameters were also within the normal levels. Hence, the results suggest that the observed side effects seem to be transient and are completely reversible upon the withdrawal of the treatment.

In conclusion, it may be mentioned that MPA and MPA+TE induced contraceptive effect by generating oligospermic state with altered sperm function in rats. The side effects of MPA were related to androgen deprivation effect. MPA also caused alteration in the physio-metabolic state and regressive changes in target organs.

As MPA causes these side effects, a combination of MPA + TE would be feasible, in steroid contraception rather than using MPA alone. The body and organ weights, morphology and physiology of the vas deferens and other organs were not much affected during MPA + TE injections. The side effects induced on vital organs appear to be comparatively less by the combined treatment. The libido was maintained as serum androgen were within the normal range. The required hormonal doses are at minimum and side effects are also limited incase.
of MPA + TE for a longer period of administration in animals in comparison to administration of either of these hormones separately.
FUTURE LINE OF WORK

Based on the work embodied in the present thesis the following investigations could be carried out to ascertain the contraceptive efficacy of the steroid hormones.

1. The exact hormonal pill dose is to be decided so as to produce severe oligospermic condition, with free of side effects.

2. The detailed electrophoretic pattern of residual sperm proteins need to be studied.

3. The phospholipid contents of the sperm membrane have to be assessed to understand membrane permeability changes.

4. The sperm free radical contents will also be analyzed as these are related to sperm membrane functions.

5. The quantification of sperm glycoproteins would be necessary by using plant lectins and flow cytometry as these proteins are very much essential for sperm maturation and fertilizability.

6. Sperm ultrastructural studies need to be carried out using electron microscope.

7. Sperm fertility potential could be further assessed using rat/mouse oocyte penetration assay (ROP/MOP) Systems.

8. Sperm elemental analysis could also be done.

9. Epididymal carnitine, glyceryl phosphoryl choline and glyco-proteins could be estimated to know its micro-environment.

10. Pharmacological effects of epididymal tubules to different doses of noradrenalin would also further studied to understand the effect of MPA and
other androgens.

11. Serum protein hormones, estradiol, testosterone ratio and other hormones are to be estimated.

12. Lipid and proteins profiles in serum need to be measured to evaluate the feasibility of hormonal doses.

13. Accessory sex gland function has also to be evaluated.

14. The duration of hormonal treatment would also be extended, more than 2 months and the reversibility studies will be accordingly planned.

15. Testicular spermatokinetics studies are needed to prove the effects of steroids at specific cellular levels.

16. Testicular steroidogenic pathway is to be done for assessing Leydig cell function during this hormonal treatment.

17. Long acting androgen like 20 Aet 1 with MPA combination would be used in order to avoid supra physiological androgen levels during the treatment period.

18. The structure and function of hypothalamus and pituitary are to be evaluated to understand the details of these organ functions in treatment conditions.

19. Effect on vital organ such as liver, kidney, brain and other tissue will be evaluated.

20. Chromosomal aberration in testis and bone marrow will also be done.

21. Micronucleus studies to assess the toxic effect of these hormonal combination will be called for.