CHAPTER VI

CONCLUSIONS

From the work embodied in this thesis the following overall conclusions could be drawn:

1. Ascorbic acid can be localized in reproductive tissues by a specific staining method.

2. Higher ascorbic acid localization was observed in dividing cells such as those of the germinal epithelium, and those possessing a high tempo of metabolism such as the spermatozoa.

3. Steroidogenic tissues also contained a rich deposition of ascorbic acid.

4. Rat epididymal spermatozoa possessed a higher turnover of ascorbic acid than the tissue component and luminal plasma probably due to their high energy requirements for motility and metabolism.

5. The mobilization and utilization of the vitamin by the testicular and epididymal spermatozoa takes place via the ascorbic acid charge transfer complex formation as well as the enzymic pathway.
resulting in the formation of its free radical monodehydroascorbic acid (MDHA) which participates in the metabolism of the spermatozoa as a source of electron energy by virtue of possessing an unpaired electron. Hence MDHA supplements the energy obtained by the spermatozoa via the conventional breakdown of ATP.

6. Short term vasectomy was found to decrease some androgenic parameters of testicular and epididymal spermatozoa but recovery was obtained by 20 days post vasectomy or else by ascorbic acid feeding.

7. The ascorbic acid turnover in the tissue components of testis and epididymis was enhanced due to the probable stress imposed by vasectomy. The metabolism in the luminal plasma was reduced. Vasectomy produced no significant adverse effects on the metabolism of testis and epididymis since ascorbic acid has a beneficial influence without affecting the contraceptive purpose of vasectomy.

8. Ascorbic acid and its mechanism of action have important implications in the prophylactic treatment of human volunteers during and following vasectomy.
9. Cyproterone acetate manifested marked antifertility effects in male rats probably due to the loss of motility, alterations in the morphology of the epididymal sperms and the hostile epididymal milieu for sperm survival caused by the treatment.

10. Withdrawal of CA treatment (30 days) was not adequate to restore normal morphology and 100% fertility in rats.

11. Administration of ascorbic acid to CA treated rats did not improve the motility and fertilizability of epididymal spermatozoa.

12. Cadmium chloride treatment caused adverse changes in the metabolism of ascorbic acid in the rat testicular and epididymal spermatozoa but specially of the former.

13. The antifertility effects of cadmium chloride have been correlated with abnormalities in the head and tail regions of sperms and their decapitation.

14. The feeding of ascorbic acid along with cadmium chloride treatment manifested a beneficial
influence in maintaining the redox milieu in the testis and epididymal spermatozoa.

15. The in vitro decrease in rat epididymal sperm motility was brought about by different concentrations of copper sulphate solutions. The decrease was more rapid with increasing concentrations of the solution. The addition of ascorbic acid to copper sulphate solution inhibited the motility much more rapidly than copper sulphate solution alone.

16. Some androgenic parameters of the testis and epididymal spermatozoa decreased in intra-epididymal and intrascrotal copper device (IECD, ISCD) bearing rats.

17. Accumulation of copper occurred in reproductive organs of the copper device bearing animals. But the levels were restored to almost control value after feeding ascorbic acid to the copper implanted rats.

18. Ascorbic acid had beneficial effects in copper toxicity i.e. in animals bearing the device.
19. The IECD was found to be more effective male contraceptive device than the ISCD.

20. Prostaglandin F2a and E1 reduced the fertility, percent motility and density of epididymal spermatozoa.

21. Loss of fertility and motility after PG treatment have been related to gross alterations in the sperm morphology and to the changes in the milieu and/or adrenergic response of the epididymis itself.

22. A single injection of ethanol directly into the vas deferens of fertile albino rats caused adverse changes in the structure and metabolism of testicular and epididymal spermatozoa.

23. The vasal lumen was occluded due to sperm granuloma formation which also occurred in the epididymis.

24. The alcohol treatment caused loss of fertility, alteration in sperm morphology, reduced activity of some spermatozoal enzymes as well as decline in their redox milieu.
25. The administration of ascorbic acid to ethanol injected rats brought about a recovery in total ascorbic acid and increase in reduced ascorbic acid but the motility and fertilizability of the spermatozoa were not affected.

26. Feeding of protein free diet to albino rats resulted in decrease in sperm metabolism, lack of mating behaviour, loss of libido, severe abnormalities particularly in the acrosome and middle piece region of the epididymal spermatozoa and the animals were 100% infertile.

27. The feeding of ascorbic acid to the protein deficient rats caused a marked recovery in sperm metabolism without restoring its motility and fertilizability to normal level. Therefore, ascorbic acid has a beneficial influence for the maintenance of sperm metabolism in acute protein deficiency although it does not interfere with its antifertility effects.

28. Vitamin C deficiency in guinea pigs was accompanied by a reduction in the tissue retention of ascorbic acid, but the level of glutathione
was increased specially in the ESS. The redox milieu of the tissue is maintained by increase in reduced ascorbic acid and the transport of ascorbate from storage organs via blood to sites of active metabolism.

29. Vitamin C deficiency caused reduction in fertility and changes in the epididymal spermatozoa particularly involving the acrosome.

30. The above mentioned results elucidate the importance of ascorbic acid and its free radical monodehydroascorbic acid (MDHA) in the metabolism of testicular and epididymal spermatozoa and indicate that the metabolic turnover of the sperms is energized not only by the conventional breakdown of ATP but also via the paramagnetic electron flow from MDHA, in agreement with the earlier work of Chinoy and her associates.

31. The results also suggest that intensive studies on vasectomy, cyproterone acetate, prostaglandins, vas occlusion, copper devices and nutritional deficiencies should be undertaken in order to develop a simple, reversible and widely
applicable male contraceptive technique. Some suggestions for undertaking future work are given below.

Based on the work presented in this thesis the following investigations could be undertaken to further evaluate the efficacy of contraceptive techniques and the repercussions of nutritional deficiencies on reproductive functions.

1. Blood levels of androgens after vasectomy.

2. Effect of vasectomy on the histology of reproductive organs.

3. Effectiveness of vasovasostomy and patency of vas deferens thereafter.

4. Structure and functions of nonendocrine organs after vasectomy.

5. Studies on CA treatment with more emphasis on the ultrastructure of spermatozoa.


7. Effect of combined treatments of CA and testosterone to intact animals.

9. Studies on the effects of CA treatment on the structure and function of vas deferens.


13. In view of the use of cadmium chloride in animal husbandary as a chemical castrating agent, specially in cattle and poultry, detailed studies on the combined treatment with cadmium chloride and ascorbic acid on the histology and ultrastructure of reproductive organs.

14. Studies on the contraceptive feasibility of intrascrotal copper device (ISCD) using copper devices of different sizes and copper wire of different diameters inorder to find out the most suitably sized device for inducing reversible infertility.
15. Hormonal status of blood after ISCD implantation.
16. Ceruloplasmin levels in the blood after ISCD implantation.
17. Studies on the reversibility of ISCD.
18. Further studies on the beneficial role of ascorbate in copper device bearing animals.
19. Studies on the reversibility of effects of prostaglandins on male reproductive functions.
20. Studies on prostaglandins in relation to ascorbic acid metabolism in spermatozoa.
22. Effects of long term treatments with different doses of PGF2α and PGE1.
23. Effects of long term silastic PVP implants of prostaglandin E1 and F2α on the ultrastructure of spermatozoa.
25. Androgen levels in blood after vas occlusion by ethanol.

27. Comparative studies on the effects of protein free diet and protein deficiency on reproductive functions.

28. Studies on the structure and function of endocrine and non endocrine tissues under the above two conditions.

29. Studies on the structure of spermatozoa, their viability and survival after feeding protein free and protein deficient diets.

30. Studies on the recovery from the effects of vitamin C deficiency either by feeding vitamin C fortified normal diet or by feeding ascorbic acid along with the deficient diet, with special reference to sperm structure and function.

31. Effects of vitamin C deficiency on other endocrine organs, specially adrenal.

32. Studies on hormone levels in blood after vitamin C deficiency.

33. Studies on the plasma and leucocyte levels of ascorbic acid after vitamin C deficiency.