CHAPTER VI

CONCLUSIONS

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

1. Hypospermatogenesis occurs with advancing age.

2. Higher ascorbate turnover is correlated with concomitantly higher sperm density and motility.

3. The differences in semen characteristics could be related to the variations in androgen sensitive parameters and ascorbate metabolism.

4. Accessory gland secretion is not significantly altered with age in each semen type.

5. The variations in each parameter was observed to be more in younger age group in comparison to the older one in all semen types investigated.

6. The establishment of normal profile of semen in each category is necessary for a better understanding of population studies on Indian men.

7. Vasectomy had no adverse effects on semen biochemical profile in comparison to normal control.

8. Vasectomy did not affect the accessory gland function.
9. Serum testosterone levels of normospermic, oligospermic, azoospermic and vasectomized men were within the normal range (3-12 ng/ml).

10. Higher levels of free ascorbate and fructose were associated with the semen of azoospermic and vasectomized men.

11. Copper and manganese levels in semen, seminal fluid were lowered by vasectomy.

12. Cu$^{++}$ and Mn$^{++}$ levels were correlated with sperm concentration and motility.

13. The Mn$^{++}$ free radical spectra of semen of normo-, oligo-, azoospermia and vasectomized men were the same.

14. Zn$^{++}$, Mg$^{++}$, Na$^+$, K$^+$ and Na$^+$/K$^+$ ratios were not altered in seminal fluid component by vasectomy in comparison to other semen types. Their levels in spermatozoa of normo- and oligospermic subjects were similar.

15. Ascorbate turnover in sperm pellet of normospermic men was higher than in oligospermia.

16. Ascorbic acid, acid phosphatase, succinate dehydrogenase, cholinesterase, sorbitol dehydrogenase, proteins and ribonucleic acid in semen were more in seminal fluid component.
17. Cholinesterase and succinate dehydrogenase activities are related to sperm concentration and motility.

18. RNA in seminal fluid increases with age but not DNA whose concentration is associated with sperm density.

19. RNA was not present in spermatozoa, whereas, DNA was not present in seminal fluid component.

20. The frequency of occurrence of abnormal forms were observed to be more in oligospermic individuals in comparison to normospermic men with proven fertility.

21. The anomalies were mostly associated with head and middle piece regions. Tail deformities were comparatively rare.

22. These abnormalities include irregular shape of head viz., angular, amorphous, round, elongated, tapered and microcephalic forms as well as immature forms. The midpiece was elongated with undifferentiated gyres and the tail end piece was seldom coiled.

23. These abnormalities are probably due to numerous factors.

24. HCG-antiserum treatment manifests androgen deprivation of target organs which resulted in gross alterations in sperm morphology, motility, loss of their fertilizability and reduction in testicular sperm concentration as a consequence of changes in testicular and epididymal milieu.
25. HCG-antiserum treatment also manifested anti-androgenic and antifertility effects in rats.

26. An increase in ascorbate metabolism occurred on day 45 with the onset of puberty in rats.

27. This change was accompanied by a decrease in cholesterol level in target organs, i.e., testis and adrenal and a parallel increase in circulating testosterone levels corresponding with the increasing age in rats.

28. A definite relationship exists inbetween ascorbate content and testosterone levels during pubertal development.

29. The Leydig cell function also increases with onset of puberty, probably due to increase in the activities of 3\(\beta\) and 17\(\beta\) -HSDS concomitantly with increased blood testosterone levels.

30. A dose dependent AA and cholesterol depletions occurred in immature rat testis by LH stimulation. So, it is suggested that immature rat testicular AA and cholesterol depletion (TAAD/TCH) could be taken as indices for steroidogenesis by LH stimulation, in addition to Parlow's OAAD method.

31. E\(_2\)B treatment for 15 days was more effective in adult rats than in the younger ones.
32. The mechanism of action is direct and affects steroidogenesis in Leydig cells of testis and probably the cortical cells of adrenal, and thereby manifested decline in androgen levels leading to androgen deprivation of target organs.

33. Circulating testosterone levels were decreased significantly in both groups with $E_2B$ treatment but LH and FSH levels were not altered.

34. The treatment also brought about a decline in organ weights, in ascorbate metabolism, steroidogenesis, cholesterol, protein levels, and glutathione in both organs of all treated groups.

35. In Vitro study revealed that AA is involved in testicular testosterone production.

36. The production of testosterone by testis and adrenal was significantly reduced by $E_2B$ administration.

37. The mechanism of action of AA is via the formation of its free radical and probably complexing with steroids, in addition to AA-C-AMP system in steroidogenic organs.

38. Thus, the metabolism of the tissues and spermatozoa are energized not only by high energy $\sim P$, but also via the paramagnetic electron flow from MDHA.

Based on the work embodied in the present thesis the following investigations could be carried out to
evaluate further the base line values of semen for developing a suitable male contraceptive especially at sperm level; the causative factors of infertility; the beneficial role of ascorbic acid in antifertility treatments of human beings and its involvement in steroidogenesis:

1. Effect of age on semen characteristics and its biochemical profile ought to be carried out in subjects older than 50 years.

2. Improvements of methods for measurement of sperm motility and establishment of base line values of semen of men in different population groups will be worked out as recommended by WHO 9th Annual Report, 1980.

3. The establishment of normal profile of other semen parameters of each type will also be required for better understanding of the population studies on Indian men. These would be useful in evaluation of their fertility and sterility.

4. Effects of vasectomy on other biochemical parameters and hormonal profiles of semen are urgently needed for understanding the consequences of vasectomy.
5. Clinical studies on feeding of AA to human vasectomized volunteers will be initiated for evaluating its prophylactic effect during and after vasectomy, as worked out in rodents. These studies are relevant in the light of N.J. Alexander's report (Alexander and Clarkson, 1978) that vasectomized monkeys had an increased tendency to develop atherosclerosis in comparison to control. Therefore, AA feeding would be beneficial as AA is known to decrease the serum cholesterol level (Ginter et al., 1970, 1971, 1972).

6. Further intensive studies will be needed to elucidate changes in hypothalamo-pituitary-gonadal/adrenal axis after long-term vasectomy in man as there are numerous conflicting data.

7. Evaluation of metal ion distribution in semen, seminal fluid, spermatozoa of normo-, oligo-, azoospermia and vasectomized men will also be investigated in order to elucidate their precise role in sperm metabolism and motility.

8. The composition of elements in head, mid-piece and tail regions of spermatozoa in fertile and infertile human volunteers need to be evaluated and their correlation with sperm count and motility should be studied. This type of study would be helpful to develop newer contraceptives in male.
9. Transmission Electron Microscopic (TEM) studies on the anatomical differences of spermatozoa from normal, fertile and sterile oligospermic individuals need to be studied in addition to the SEM study presented.

10. TEM study on spermatozoa of rats treated with HCG-antiserum and \( E_2 \)-B will be studied to evaluate their consequences on their internal structures.

11. TEM studies on the histoarchitecture of testis, epididymis, vas deferens, and accessory glands of rats, other mammals and human beings of different ages under normal and altered physiological conditions need to be studied for elucidating the effects exerted by the respective treatment and age.

12. The histochemical localizations of Vitamin C and other steroid dehydrogenases of testis, adrenal, epididymis and vas deferens during these treatments will need to be investigated.

13. The effects of estrogens on other steroidal hormones and gonadotrophins will be studied in order to understand their mechanism of action in reproductive tissues.

14. The contraceptive efficacy of estrogens and their combinations with androgen/and AA will be studied in
view of their synergistic effects. This data will be important from the clinical point of view as smaller doses of contraceptive steroids would be needed.

15. Estrogens and hCG-AS effects on more biochemical parameters in target organs need to be investigated.

16. The acetylcholine-acetylcholinesterase and the choline acetyl transferase system of spermatozoa need to be studied in detail in order to develop a potential male contraceptive at the sperm level.

17. Ascorbic acid turnover in relation to the onset of puberty in testis and adrenal also needs further study.

18. Its relationship with steroids in steroidogenic organs and AA synthesizing tissue should be extensively studied.

19. The AA depletion by IH will be further extended to isolated Leydig cell cultures in vitro.

20. In vitro cultures of isolated organs viz., testis and adrenal under normal and altered physiological conditions will be undertaken, by addition of steroid precursors like cholesterol, and progesterone etc., with and without supplementation of ascorbic acid, to study the precise role of ascorbate during steroidogenesis.
21. Similar studies will also be carried out using the isolated Leydig cells of rodents under normal and altered experimental conditions.

22. *In vitro* studies on adrenal cortico-steroidogenesis under normal and experimental conditions and the effects of ascorbic acid supplementation will also be investigated.