CHAPTER V

SUMMARY

PART I

COMPARATIVE STUDY OF THE REPRODUCTIVE SYSTEM OF RAT AND GUINEA PIG:

A comparative histophysiological study of the reproductive organs of male albino rats and guinea pigs was carried out. Morphological differences in reproductive organs of both the animals were noted, which were very marked in epididymis and in the accessory glands. The epididymis of rat was dumb-bell shaped, whereas, in guinea pig, the caput epididymis was very small in comparison to the well developed cauda epididymis, which was divided into two distinct regions, viz., the proximal and distal cauda. The size and weights of cauda epididymis, seminal vesicle and ventral prostate of guinea pig were found to be more than of rat. The histological differences occurred in testis. In rat, the seminiferous tubular diameter and their germinal epithelial height were more than of guinea pig, although Leydig cell diameters was almost same. Epididymal histology also showed distinct differences in all the regions of both the animals. The tubular diameter of
caput epididymis was found to be least and of cauda
epididymis the highest, in both the animals. The epithelium
was pseudostratified throughout. Very tall columnar cells
of caput and corpus epididymides were observed in guinea
pig than in rat, which is species characteristic and known
to help in fluid resorption. Although the tubular size of
caput was variable in both animals, the difference between
extremely large and small tubules was very apparent in
guinea pig. The presence of sperm bundles were observed in
all three regions of epididymis, but guinea pig caput
epididymis had fewer spermatozoa than the caput of rat. The
epithelial cell height of seminal vesicle was found to be
greater in guinea pig than in rat and is correlated with
heigher fructose level. Similarly, the epithelial cell
height of ventral prostate of rat was greater in correlation
with the higher acid phosphatase activity therin.

Scanning electron microscopy of spermatozoa revealed
that the sickle and paddle shaped head of rat and guinea
pig respectively are characteristic features of the species.
Although the rat cauda epididymal spermatozoa appear
singly, those of guinea pig were observed in rouleaux
formation. The presence of a cytoplasmic droplet was
observed at the anterior part of the mid-piece region of
caput epididymal spermatozoa of both animals. The cauda
epididymal spermatozoa showed absence of kinoplasmic droplet and were more vigorously motile. The tail end-piece was tightly coiled, slightly looped or straight. The percent motility was greater in cauda epididymal spermatozoa of guinea pig than of rat, which has been correlated with their higher cholinesterase activity.

Biochemical differences were also noted with respect to levels of succinate dehydrogenase, protein, glutathione, cholesterol, ascorbic acid turnover pattern and distribution of metal ions in the various tissues of the male reproductive system of two animals. The testis and epididymis, being metabolically highly active organs, contained greater succinate dehydrogenase, glutathione and cholesterol as well as ascorbate turnover than the accessory organs. Although the overall ascorbic acid metabolism was higher in rat tissues but the storage of bound ascorbate was more in tissues of guinea pig, due to the lack of synthesis of the vitamin.

Histochemical localization of ascorbic acid (AA) revealed that in the germinal epithelial cells of rat testis, the nuclei were more darkly stained than the cytoplasm. The epididymis of rat had an intense localization of AA in nuclei of epithelial cells, stereocilia, spermatozoa and intertubular elements than in cytoplasm. Moreover, the cauda
epididymis of rat showed the highest content of AA than the other regions of epididymis. The staining pattern was similar in both animals, but rat reproductive tissues had higher ascorbic acid staining intensity than of guinea pig. The presence of α and β-adrenergic receptors were noted in isolated rat epididymis.

PART II

STUDIES ON EFFECT OF ANDROGEN DEPRIVATION AND ANDROGEN REPLACEMENT:

1. EFFECTS OF ANDROGEN DEPRIVATION BY CYPROTERONE ACETATE (CA), ASCORBIC ACID (AA) THERAPY AND EFFECTS OF CA WITHDRAWAL:

The effects of androgen deprivation by cyproterone acetate administration to intact male albino rats for 7, 15, 30 and 60 days, combined CA + ascorbic acid feeding (AA, 100 mg/day/rat) for the same number of days and CA withdrawal treatments were investigated. CA caused reduction of percent motility of spermatozoa, their density and fertilizing ability. These changes are related to the changes in their morphology. CA manifested an antiandrogenic effect by lowering the serum testosterone level, and thereby caused reduction in a number of androgen sensitive parameters. The antiandrogenic effects of CA were coupled with its
antianabolic action, which helped in reducing the organ weights, protein concentrations and caused marked histological alterations in testis, epididymis, seminal vesicle and ventral prostate. CA manifested antigonadotrophic effects, since in initial stage (30 days) of CA treatment hypertrophy of Leydig cells and atrophy by 60 days of treatment were noted. The altered metal ion profile by CA may also contribute in changing the structural and physiological integrity of these organs, since a number of androgen dependent enzymes are zinc, manganese or copper metalloprotein.

The enhanced ascorbate turnover pattern was observed in testis and epididymis during CA administration. Combined treatment with CA + AA and CA withdrawal revealed that the antianabolic and antiandrogenic effects of CA were transient and almost reversible, without affecting its antifertility effects. The recovery in most of the androgenic parameters, serum testosterone levels, ascorbic acid turnover pattern, metal ion profile and the histological features of the various reproductive organs studied, were more pronounced by ascorbic acid feeding than by CA withdrawal treatment. Ascorbic acid feeding to CA treated rats maintained the redox milieu of the reproductive organs by potentiation of the anabolic action of endogenous testosterone since their levels are reduced due to treatment. On the other hand,
fertility was not restored by ascorbic acid feeding. Therefore, ascorbic acid has a beneficial effect in maintaining the structural and functional integrity of testis, epididymis and accessory organs in CA treated rats without interference with its contraceptive purpose. Considering the results of the withdrawal studies, it is evident that the duration after withdrawal of CA treatment needs to be extended further, till complete fertility is restored, since the fertility rate was 73% in the present study. The results of CA studies elucidate that it manifested antifertility, antigonadotropic, antianabolic and an androgenic effects in adult male rats. The latter two effects are transient and reversible especially by ascorbic acid feeding than by CA withdrawal treatment, without interfering with its antifertility effects.

2. EFFECTS OF ANDROGEN DEPRIVATION BY CASTRATION, REPLACEMENT THERAPY WITH ANDROGENS AND WITH ASCORBIC ACID ALONE; COMBINED TREATMENTS WITH ANDROGEN + ASCORBIC ACID:

a) EFFECTS OF CASTRATION, REPLACEMENT THERAPY WITH ASCORBIC ACID (AA) OR TESTOSTERONE (T) AND COMBINED T + AA TREATMENTS:

The effects of castration, replacement therapy with ascorbic acid or testosterone alone and in combination (T + AA) on histophysiology of epididymis, seminal vesicle and ventral
prostate of adult male albino rats were carried out. Castration for 10 days brought about diminished organ weights, depressed androgen sensitive parameters and altered significantly the histoarchitecture of epididymis, seminal vesicle and ventral prostate, suggesting that androgen is necessary for the maintenance of the structural and functional integrity of epididymis and accessory sex glands. Severe alterations in accessory sex glands were also observed. Castrated rats fed ascorbic acid alone for 10 days revealed significant recovery of several androgen sensitive parameters as compared to the castrated group. The histology of epididymis, seminal vesicle and ventral prostate were also found to be maintained to some extent by ascorbic acid feeding. The copper and manganese ion concentrations were recovered to control state, but those of zinc were elevated beyond control value. Thus ascorbic acid feeding to castrated rats maintained the structural and functional integrity of epididymis and accessory organs, by manifesting an anabolic effect. The testosterone treatment for 10 days revealed that ascorbic acid simulates the effect of testosterone in reproductive organs to a considerable extent. However, the recovery was more pronounced by T treatment in androgenic parameters, histology and metal ion profile (copper and manganese) of epididymis, seminal
vesicle and ventral prostate than by ascorbic acid alone. The combined treatment with testosterone + ascorbic acid for 10 days to the castrated rats manifested a synergistic effect for the recovery of organ weights, especially of corpus, cauda, seminal vesicle and ventral prostate and androgen dependent parameters of above tissues. The metal ion profile and histology of all the reproductive organs were also significantly restored by combined treatments, than by AA or T treatment, individually. This synergistic effect is manifested due to potentiation of the anabolic action of testosterone by ascorbic acid which itself is an anabolic compound and thereby a cumulative anabolic effect is produced. It is evident therefore, that ascorbic acid serves as an important source of electron energy for maintaining the structural integrity and functional milieu of the reproductive organs, in addition to the energy obtained by them, through the conventional breakdown of ATP.

Comparing the treatments with testosterone or androstenedione alone to castrated rats, it is clear that T was more potent for recovery of androgen sensitive parameters. However, androstenedione replacement brought about more recovery in the activity of succinate dehydrogenase in all three regions of the epididymis and acid phosphatase in the cauda epididymis. On the whole, a more significant
synergistic effect for recovery of succinate dehydrogenase, acid phosphatase and seminal vesicle fructose were observed by androstenedione + AA than T + AA.

3. STUDIES ON TESTOSTERONE (T) TREATMENT TO INTACT MALE RATS:

The effects of injecting three different doses (1, 10 and 100 μg) of testosterone to intact rats for 5, 10 and 30 days were studied on their reproductive functions. The low dose of T suppressed spermatogenesis by probably decreasing the pituitary gonadotropin secretion, and thereby lowering the secretion of endogenous testosterone by the Leydig cells of the testis. The reduction of Leydig cell diameter and some important androgenic parameters of testis, epididymis and accessory organs occurred, suggesting that either the steroidogenesis process in testis or metabolism of endogenous testosterone are affected by the exogenous T administration which acts either directly on the testis or via the pituitary-gonadal axis. On the other hand, the exogenous testosterone in high dose (100 μg T) itself was sufficient for maintaining spermatogenesis, even though pituitary gonadotropin secretion was depressed. The epididymal histology was altered but those of seminal vesicle and ventral prostate were not much affected except that of seminal vesicle by 10 μg T treatment for 10 days. The percent
motility of spermatozoa and their density were diminished throughout the study period and the sluggishly motile sperms were unable to fertilize normal cyclic females. The reduction of fertility was more by 1 µg T for 10 days. Therefore it is clear that low dose of T for 10 days proved to be more effective for male contraception in rats. However, the feasibility of the use of testosterone or other androgens for male contraception in human beings depends on the establishment of proper dose and mode of administration to bring about antispermatogenic action without causing unwanted side effects such as on the general metabolism and without interference with libido.

4. EFFECTS OF ESTRADIOL BENZOATE (E₂B) TREATMENT TO INTACT MALE ALBINO RATS:

The effects of estradiol benzoate (50 µg/day/rat for 15 days) to healthy intact male albino rats of Holtzman strain were investigated. E₂B treatment caused significant reduction in androgen dependent parameters and the histology of androgen target tissues, viz., testis, epididymis, seminal vesicle and ventral prostate. The testicular changes involved arrest of spermatogenesis, reduction in tubular diameter and those of the Leydig cells. Severe histological alterations were noted in epididymis and accessory sex glands. The metal ion concentrations (copper, manganese, zinc) were found to
increase in epididymis which may contribute to the altered epididymal milieu and affect sperm maturation, rendering spermatozoa nonviable and non-fertilizable. It is evident that \( E_2 \) treatment to adult intact, male rats manifested antiandrogenic and antifertility effects. Severe changes in the testicular histology supports the finding that estrogens have direct action on testicular structure and functions.

PART III

1. STUDIES ON VASECTOMY AND VASECTOMY + ASCORBIC ACID (AA)

FEEDING:

Effect of vasectomy for 30, 60 and 90 days were studied on male reproductive tissues of rat. To another group of rats ascorbic acid (100 mg/day/rat) was fed for the same number of days. Vasectomy (30, 60 and 90 days) resulted in an increase in organ weights, but percent motility and density of cauda epididymal spermatozoa were declined and fertility rate was negative. The androgen-sensitive parameters were reduced as a result of vasectomy, but the acid phosphatase activity of caput epididymis increased in the initial stages of vasectomy, which may be due to the lysosomal activity and phagocytosis in the lumen. The decrease in androgen dependent parameters suggest that circulating testosterone levels might be lowered by vasectomy in
initial stage and this decreased serum testosterone level might have affected the histoarchitecture of testis, epididymis, seminal vesicle and ventral prostate. The changes in testis involved complete degeneration of many seminiferous tubules, arrest of spermatogenesis and atrophy of Leydig cell diameter during initial stages. These alterations elucidate that steroidogenesis might be affected. In caput epididymis, diminished tubular diameter and their epithelium, nuclear pycnosis, disintegration and degeneration of tubules were observed by long term vasectomy, but not much changes were observed in corpus epididymis. The cauda epididymis was most affected, wherein, extensive increase in interstitium, reduction in tubular diameter and their epithelium was observed. Sperm granulomas occurred at the site of vasectomy and also in the cauda epididymis. Vasectomy also caused diminished mucosal height of the accessory glands. It is evident from the results that long term vasectomy brought about recovery in histological features of epididymis and seminal vesicle, suggesting that vasectomy imposed transitory structural change in reproductive tissues but the functional and secretory activity are not recovered in long-term vasectomy in comparison to control. Similarly the concentrations of copper and manganese were also restored to control value by 90 days post vasectomy. However, zinc concentrations
were increased and is attributed to depressed sperm motility and non-viable spermatozoa which are present in the epididymis. Moreover, the metabolic pattern of ascorbic acid was enhanced in initial period of vasectomy, concurrent with its active mobilization and was probably responsible for the restoration of histology of epididymis and accessory sex glands during the later stages.

Ascorbic acid feeding to vasectomized rats brought about recovery in activities of succinate dehydrogenase, acid phosphatase and cholinesterase, ascorbic acid turnover pattern, metal ion profile especially in the testis, epididymis and restored the histoarchitecture of most of the reproductive tissues studied, as well as helped in elevation of organ weights beyond control values. Therefore, ascorbic acid has a beneficial role in maintaining and restoring the structural and physiological integrity of testis, epididymis and accessory sex glands, probably by potentiation of the anabolic action of endogenous testosterone during vasectomy. It is therefore, suggested that ascorbic acid and its mechanism of action will have important implications in prophylactic treatment during and following vasectomy in human males. The results of the present study are significant and ought to be extended to clinical trials in human males, since Alexander and Clarkson (1978)
reported that vasectomy increased the severity of diet-induced atherosclerosis in Macaca fascicularis than in sham vasectomized control monkeys fed the diet. Since ascorbic acid is known to oxidise the cholesterol and decrease its levels in blood, it is evident that ascorbic acid feeding to vasectomized animals/men will have definite beneficial effect in reducing the severity of the occurrence of atherosclerosis.

2. STUDIES ON VASOCCLUSION BY ETHANOL AND ASCORBIC ACID THERAPY:

A single injection of 95% ethanol (50 μl) into the distal vas deferens of fertile albino rats was found to cause severe changes in the histophysiology of testis, epididymis and accessory sex glands after 30 days post injection. The testis histology showed arrest of spermatogenesis, reduced seminiferous tubular diameter and their epithelium. The Leydig cell diameter was also reduced, suggesting that alcohol treatment has direct action on testicular functions. A sperm granuloma at the site of injection and within the epididymis was observed, resulting in almost complete occlusion. The present study revealed that permanent sterility within 30 days after single ethanol injection was achieved, concurrent with reduced sperm motility and density of cauda epididymal spermatozoa. The
decrease in androgen-sensitive parameters were observed in testis, epididymis, seminal vesicle and ventral prostate, which can be attributed to the decrease in plasma testosterone levels. The metal ion profile of the above tissues showed an increase.

An enhanced ascorbate turnover pattern was observed in testis and epididymis as a result of greater rate of mobilization of bound ascorbate to the free form. On the whole, ascorbic acid feeding to ethanol treated rats caused recovery in organ weights, activities of succinate dehydrogenase, acid phosphatase and restoration of ascorbic acid turnover pattern. However, fertility rate was negative. The ethanol treatment caused antiandrogenic and antifertility effects wherein, former was reversible by ascorbic acid feeding. Therefore, it is suggested that during ethanol treatment, if animals are fed with ascorbic acid, it will have a beneficial effect in maintaining the structural and functional integrity of the reproductive tissues without interference with its contraceptive purpose. As vasocclusion is a non-surgical procedure, consumes lesser time and efforts to achieve 100% antifertility effects, therefore, it could be useful as an important male contraceptive technique.
PART IV

EFFECTS OF COPPER AND PROSTAGLANDINS ON MALE REPRODUCTIVE FUNCTIONS:

1. EFFECTS OF COPPER:

The effects of an Intra-scrotal copper device (ISCD) and Intra-epididymal copper device (IECD) implantation (20 days) on male reproductive functions of albino rats were studied. The results showed reduction in percent motility and density of cauda epididymal spermatozoa. The fertility rate was found to be 100% negative in IECD treated rats, but was 67% negative in ISCD bearing rats. This reduced fertility is related to the anomalies in sperm morphology involving swelling of acrosome, decapitation and mid-piece as well as tail abnormalities by both copper devices and are attributed to the spermicidal action of cuprous ions released by oxidation of metallic copper. Moreover, pus formation was observed at the site of IECD implantation only. The androgenic parameters were reduced by both copper devices. The decrease in the various parameters was differential in the different organs and also variable under the two treatments. However, accumulation of cholesterol was noted in corpus and cauda epididymides of IECD bearing rats. The decrease in androgenic parameters of testis,
epididymis and accessory sex glands and changes in their histology might be due to the local toxic effects of the accumulated copper in these organs of IECD and ISCD bearing rats. The unaltered Leydig cell diameter, may be due to the direct action of copper on testis, independent from the pituitary gonadotropin. Although the intra-epididymal copper implantation was found to be a more effective male contraceptive device than the ISCD, further work is necessary in terms of reversibility of the effects and easy removal of the device.

2. STUDIES ON PROSTAGLANDINS (PGs):

1) SUBCUTANEOUS INJECTION OF PG:

The effects of subcutaneous injections of prostaglandins F2α and E1 (PGF2α and E1) were studied on the histophysiology of the male reproductive organs of mature albino rats and their fertility rate. Although most of the androgen sensitive biochemical parameters were reduced by PG treatment, apparently normal histology of Leydig cells, the levels of cholesterol and activities of 3β- and 17β-hydroxy steroid dehydrogenases were not significantly altered in the testis. These results indicate a probable decline in target organs response to androgen and/or in conversion of testosterone to its metabolites. The reduction in fertility
rate of prostaglandin treated male rats has been correlated with reduced motility and density of cauda epididymal spermatozoa as well as their altered morphology. It is also suggested that the altered copper manganese and zinc levels in epididymis might also render the epididymal milieu hostile for sperm survival leading to their decapitation and damage.

The weights of testis and epididymis were significantly reduced but those of seminal vesicle (SV) and ventral prostate (VP) were increased by PG treatment. The seminiferous tubular diameter and their epithelium were reduced but Leydig cell diameter was not affected. Severe degenerative changes were observed in the epididymis involving increase in interstitium and pycnotic degeneration of epithelium. However, the changes were most pronounced in cauda than in other regions of epididymis by both PG treatments. The reduced fructose in seminal vesicle and the corresponding increase in its weight indicates that it undergoes hypertrophy but no hyperplasia. On the other hand, in ventral prostate there probably occur both hypertrophy and hyperplasia. It is evident from the results that PGF2α and E1 exert a definite growth promoting effect, particularly in seminal vesicle and ventral prostate together with the antiandrogenic and partial antifertility effects. The antifertility effects
could probably have been more pronounced if a higher dose of PG been administered.

11) STUDIES ON INTRAVASAL INJECTION OF PROSTAGLANDINS:

The effects of a single intravasal injection of PGE1 and PGF2a (50 μg/μl) on histophysics of reproductive organs of male rats was studied. The postinjection period was 30 days. The PGE1 and PGF2a treatment manifested antiandrogenic and antifertility effects as is evident from the reduction in several androgenic parameters, density of cauda epididymal spermatozoa, their percent motility as well as the acrosomal, mid-piece and tail abnormalities of spermatozoa in treated rats. The treatment also caused decapitation of sperms. Both PGs also bring about an antianabolic effect by affecting the histology of the reproductive tissues and reduction in protein concentrations. The ascorbate turnover was however, increased to overcome the stress conditions induced by PG treatment. The data reveals that intravasal injection of PGs brought about definite antiandrogenic, antianabolic and antifertility effects. Moreover, PGE1 showed growth promoting effect on seminal vesicle and ventral prostate.

Comparing the effects of subcutaneous and the intravasal PG treatments, it is clear, that although the major effects
were similar on male reproductive tissues, the latter treatment is better to follow up, as it is easy, less time consuming and manifested greater antifertility effects. In intravasal treatment, PGF2α was more effective than PGE1 as the former treatment caused greater reduction in fertility rate (67%).

This data also suggests that effect of PG on reproductive organs are dependent on several factors, viz., the mode of administration, the close, age and strain of animals used. The mechanism of action of PG is direct on testicular steroidogenesis, but its effect via the pituitary-gonadal axis cannot be ruled out.

PART V

EFFECTS OF NUTRITIONAL DEFICIENCY:

VITAMIN C DEFICIENCY IN GUINEA PIG:

A scurvy condition was created in guinea pigs by feeding them a vitamin C deficient diet for 21 days. The effects of vitamin C deficiency were studied on the physiology of testis, epididymis and accessory sex glands and emphasis has been laid in investigating ascorbic acid metabolism and its role in reproduction. The organ weights were diminished, the percent motility of spermatozoa and their density also declined, suggesting that vitamin C deficiency imposed antifertility effects in guinea pigs. The
antifertility effect is also attributed to spermatozoa anomalies, wherein, decapitation, acrosomal abnormalities, uncoiling of tail and removal acrosomal cap was observed. The alterations in sperm morphology are also attributed to the changes in the metal ions in epididymis by vitamin C deficiency. The depressed sperm count of testis also suggests that the scorbutic condition affects spermatogenesis. The antiandrogenic effects were evident due to loss of organ weights, reduced androgenic biochemical parameters and these effects were coupled with the antianabolic effects, wherein, atrophy of testis, alterations in its histology and those of epididymis, and those of SV and VP were observed. The protein concentrations also declined along with reduced organ weights. However, protein increased during experimental condition in caput and corpus epididymides. The above data suggests that vitamin C deficiency probably causes reduction in circulating androgen levels. Scorbutic condition was ascertained by measuring the total ascorbic acid content in circulating blood, wherein, it was diminished significantly as compared to control. The reduced ascorbate level in blood as well as in reproductive tissues suggests that active mobilization of bound ascorbic acid occurred for maintaining the high tempo of metabolism in reproductive organs. Ascorbic acid plays a role in several oxido-reduction reactions and helps in maintaining the internal milieu and
functional status quo of testis, epididymis and accessory glands.

These results elucidate that vitamin C deficiency manifested antiandrogenic, antianabolic and partial antifertility effects in guinea pigs. The primary action seems to be at testis level wherein, steroidogenesis is affected and antianabolic as well as androgen deprivation effects are secondary manifestations as consequences of the primary effects.