CHAPTER V

SUMMARY AND CONCLUSIONS

PART I

SECTION I (ENDOCRINE STUDIES)

The present study was carried out in 122 patients with various complaints of male infertility. These males included adults of reproductive ages as well as pre-adolescent age. The cases investigated were classified into four major groups based on clinical diagnosis.

GROUP I

Group I comprised of 35 normal males of proven fertility in the age group of 20-35 years. Endocrine investigations carried out in these males revealed normal ranges and therefore this group was considered as control group and all other groups were compared to group I.

GROUP II

Group II comprised of males referred for primary sterility, azoospermia with certain chromosomal anomalies viz. Klinefelter syndrome in some cases. The investigation of the 24 azoospermic cases revealed significant alteration in the hormonal profile. In 62.5% cases, primary gonadal failure was observed with
significantly elevated FSH, LH and low T. Out of the 24 azoospermic cases, 8 males were confirmed to have Klinefelter’s syndrome as evident from their karyotype. These patients also showed elevated gonadotropins with subnormal T levels. In 20.8% cases of the azoospermic patients normal hormonal profile was seen indicating obstructive disorders as a cause. In 4.16% cases of the azoospermic patients elevated FSH with normal LH and T were found due to primary spermatogenic failure. Elevated LH levels were seen in 8.3% cases suggesting Leydig cell failure.

Among the 16 primary sterility cases, 62.5% showed normal hormonal profile. Primary testicular failure and Leydig cell failure were also responsible in a few cases. Rather than endocrine alterations, seminal characteristics showed significant alteration thereby contributing to reduced fertility.

GROUP III

This group comprised of males diagnosed as having oligozoospermia, hypogonadism, gynaecomastia and varicocele.

Among the 11 cases of hypogonadism, 36.36% cases showed normal endocrine profile. Here again significant alterations were seen in semen characteristics.

Among the 9 cases of gynaecomastia, significantly elevated E$_2$ levels were found in association with low T values resulting in a decline in the T/E$_2$ ratio which was responsible for gynaecomastia in these cases.

The oligozoospermic males and varicocele cases (4 cases) revealed normal
hormonal profile. Here again endocrine alteration was not found to be the cause of infertility.

GROUP IV

This group comprised the miscellaneous group consisting of various sexual anomalies viz. delayed development, intersex, ambiguous genitalia, hermaphrodite, precocious puberty, abnormal sexual development etc.

A total of 18 males were categorised in this group of which 11 were adults and 7 were pre-adolescent males. In the 18 adult males, significant alterations in the hormonal profile was observed. However, the pre-adolescent males showed low basal levels of hormones in all the cases investigated.

SECTION I B (HAEMATOLOGICAL STUDIES)

The haemoglobin, blood glucose and serum cholesterol levels assayed in all the patients were unaltered suggesting that rather than endocrine and metabolic factors, semen characteristics play an important role in contributing to poor fertility.

SECTION II (SEmen ANALYSIS)

Detailed semen analysis was carried out both manually as well as by computerised automated semen analyser (CASA) to probe into the possible causative factor leading to infertility in the various cases referred for investigation.

In group I males, a significant reduction was obtained in the volume
suggesting reduced accessory gland function which correlates well with the low T levels assayed in these cases. The pH and viscosity did not show any alteration in any of the groups studied. Similarly no significant alteration was seen in the sperm mitochondrial activity index (SMAI), percent green fluorescing sperms and percent abnormal sperms as seen by the Acridine Orange and Silver Nitrate staining respectively suggesting that the sperm metabolic and energy generating processes were not altered and the nuclear integrity was maintained.

Among the 16 primary sterility cases, the patients were classified into two sub-groups (A & B) on the basis of their sperm count. The manual and automated analysis both revealed a significant reduction in sperm count in sub-group A while sub-group B has counts comparable to normal. Sperm motility evaluated by both the methods showed a significant reduction in the two groups studied. The CASA system provided vital information regarding the sperm forward progressive motility. In the present study, it was seen that despite a normal sperm density in individuals of sub-group B, the forward progressive motility of spermatozoa was significantly lower than normal which resulted in impaired movement of sperm along the reproductive tract and subsequently reduced fertility. Correlated with this a lower percentage of live sperms were seen in sub-group B patients indicating that poor sperm viability and impaired forward progressive motility contributed to lower semen quality standards in these individuals. Group III individuals comprising of oligozoospermic males also showed a similar trend. The computer assisted semen analysis in particular was important therefore in giving details of sperm motion characteristics and in the present investigation revealed that poor forward
progressive motion of the spermatozoa could be one of the causative factors leading to sub-fertility in an otherwise normal sample.

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

SECTION I A

ENDOCRINE STUDY

1) Hormonal imbalance was the major cause in Azoospermic males.

2) Significantly elevated gonadotropins with correspondingly low T levels indicating primary testicular failure was observed in the vast majority of azoospermic cases.

3) In 33.3% cases azoospermia was related to cytogenetic anomalies (Klinefelter's syndrome) where significantly elevated gonadotropins with low T levels were also observed.

4) In azoospermic patients with normal hormonal levels, obstruction of the ejaculatory system was probably the causative factor.

5) Primary spermatogenic failure without associated Leydig cell damage was seen in a few azoospermic males.

6) Leydig cell failure was also found to be a causative factor in some azoospermic males.

7) Among the primary sterility cases, majority revealed normal hormonal profile, however their seminal characters showed significant alterations.
8) In the remaining cases of primary sterility isolated spermatogenic failure, primary testicular failure and Leydig cell failure was found to cause infertility.

9) Normal hormonal profile was seen in most of the cases referred for hypogonadism. The oilgozoospermic males also showed hormonal profile comparable to normal and therefore infertility in these men were related to poor semen quality standards.

10) Imbalance of T/E2 ratio was observed in males with gynaecomastia.

11) In the miscellaneous group hormonal imbalance was seen in almost all the cases studied. However, in the preadolescent males of this group low basal hormonal levels were observed.

SECTION I A

HAEMATOLOGICAL STUDIES

1) All the cases referred showed insignificant alterations in the haemoglobin, blood glucose and serum cholesterol levels suggesting that metabolic factors did not play a significant role in inducing fertility in the cases investigated.

SECTION II

SEMEN ANALYSIS

1) Significant alteration in the semen volume of azoospermic males suggests reduced accessory gland function. The secretory function
of accessory gland is androgen related which explains the reduced T levels in these cases.

2) Significant decline in sperm motility by both manual and automated methods was seen in all the groups studied.

3) In the primary sterility cases, the patients were further classified into 2 sub-groups based on their sperm count. Sub-group A had reduced counts while sub-group B had counts comparable to normal.

4) The computer automated semen analysis (CASA) revealed that despite a normal sperm count a significant reduction obtained in forward progressive motility sub-group B patients resulted in impaired movement of sperms and therefore reduced fertility.

5) The CASA system, therefore greatly aids in diagnosis and often pinpoints the root problem that leads to infertility.

6) Thus both endocrine and seminal factors related to infertility should be investigated in detail when evaluating an infertile male.

**FUTURE LINES OF WORK**

1) Follow up studies of patients which would include monitoring of hormonal levels periodically after therapy, would be beneficial in determining the endocrine changes or response of the patient to the hormonal therapy.

2) The action of hormones is often impaired at the receptor site of the
target organ. Receptor assay, if carried out, would be of utmost importance in determining end-organ response and insensitivity of the target organ to hormone action.

3) Further detailed studies using CASA which includes sperm linear velocity and lateral head displacement and tail movements would be beneficial.

Thus male infertility is a very complicated medical problem and therefore detailed investigations of these patients is of significance in this state (Gujarat) and its neighbouring states as it could aid in appropriate counselling and therapy and alleviate the emotional stress experienced by the patients.
PART II

Oral administration of benzene and alcoholic extracts of *Carica papaya* seed for a period of 30 days brought about a significant decrease in the fertility rate of male rats (*Rattus norvegicus*). The decrease in fertility rate could be attributed to the alterations caused by the treatment in sperm motility, forward progressive motility, viability and also the significant alterations in the sperm acrosomal enzymes, viz. acrosin and hyaluronidase. The treatment did not affect the testicular steroidogenesis as is evident from the unaltered levels of $3\beta$HSD, $17\beta$HSD and cholesterol and also the circulating levels of serum testosterone. The unaltered sperm count is suggestive of normal spermatogenesis and therefore the extracts acted at the post testicular level probably by altering the epididymal milieu which plays a very important role in sperm maturation.

The study was carried out following the WHO protocol. The extract treatment did not reveal any estrogenic effects since, estrogen dependent changes in ovariectomised immature female rats were not observed in treated rats when compared to the estradiol treated standard group of animals.

The study also elucidated that both the extract treatments did not manifest any toxic side effects since the body weights were not altered and the osmolality of the blood was maintained as is evident from the balanced $\text{Na}^+$, $\text{K}^+$ and $\text{Ca}^{++}$ levels in the serum.

In order to ascertain the reversibility of the induced effects, the treatment was withdrawn for a period of 1 month after 30 days treatment and the data revealed significant recovery in all the induced effects. The sperm motility and
fertility rate recovered to control levels suggesting that the effects of the extract treatment were transient and reversible after withdrawal of treatment.

The mechanism of action of the papaya seed extract seems to be via selective action on epididymis by probably altering the hormone receptor interaction and/or target organ response to androgen by a likely interference with the conversion of testosterone to its potent metabolites. The extracts were also believed to interfere with energy metabolism of the sperm cell thereby causing significant reduction in progressive motility of the spermatozoa and rendering them incapable of fertilizing an ovum.

Thus functional sterility could be induced in male rats by the benzene and alcoholic extracts of *Carica papaya* and based on the present findings which supports earlier work carried out in our laboratory, it can be concluded that both the benzene and alcoholic extracts were effective, reversible, non-estrogenic, safe oral male contraceptive agent in rodents.

From the work presented in this part of the thesis, the following conclusions can be drawn:

1) The benzene and alcoholic extracts of papaya seeds on oral administration were effective in bringing about a contraceptive effect.

2) The two extracts revealed no effect on the body weights of the treated animals.

3) The extracts caused a significant decline in the fertility rate which could be correlated with the reduction in sperm motility, forward
progressiveness as well as sperm viability.

4) The extracts did not hamper testicular steroidogenesis as is evident from the unaltered 3βHSD, 17βHSD and cholesterol levels.

5) The extracts manifested a non-estrogenic effect, since estrogen dependent changes were not observed in the extract treated immature ovariectomised female rats in comparison to the estradiol treated standard group.

6) The two extract treatments maintained the electrolyte balance as evident from the unaltered levels of Na⁺, K⁺ and Ca²⁺ in serum.

7) The extract treatment significantly altered sperm acrosomal enzymes which are a prerequisite prior to fertilization and therefore a decline in these enzymes could result in the poor penetrating and fertilizing ability of the spermatozoa leading to reduced fertility.

8) The sperm mitochondrial activity index and sperm nuclear integrity were however maintained following both the extract treatments.

9) The extracts acted at the post testicular level since the sperm count was unaffected following treatment.

10) Withdrawal of treatment for 30 days revealed that all the induced effects were transient and reversible.

11) Functional sterility could be thus induced by both benzene and alcoholic extracts of papaya seeds. It has thus proved to be an effective, reversible, safe, post testicular male contraceptive agent in rodents.
FUTURE LINES OF WORK

* Ultrastructural studies of testis, caput and cauda epididymis of treated animals is called for.

* Receptor studies to detect the level of androgen binding proteins in the target organs.

* Isolation, characterization and identifications of the active component(s) in the papaya seed extract and testing of their biological activity.

* Since the contraceptive efficacy of the papaya seed has been established and tested in different rodents, phase I trials should be initiated to test their effect in non-human primates which is presently underway.