CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

PART I

Research in the field of reproductive biology leads to the inescapable conclusion that human reproduction is simultaneously wonderous and imperfect. The almost endless series of delicate steps leading to conception require reproductive tract structures capable of carrying out highly specialised functions, precise timing and a meticulously programmed sequence of events.

The problem of infertility has long since challenged investigators and clinicians. Without contraceptive practices pregnancy can be achieved within 12 months by 75% of couples (Southam, 1960). Therefore, a couple is medically defined as infertile if one year of normal sexual practice without contraception has not resulted in pregnancy. It has been estimated that 10% to 15% of couples have difficulty in establishing a pregnancy. When this estimate is compared with global problems of overpopulation and starvation, the figure may seem insignificant - but not for those couples who wish to conceive and find that they cannot. For such couples, fertility may become an all consuming goal. For one of six couples of child-bearing age, fertility is not a force to be turned on at will. Hence considerable interest has centered on the management of infertile couples.
MALE FACTOR IN INFERTILITY:

While the causes of infertility in women are well understood and rational, treatments are now feasible, the same cannot be said for men with infertility. Infertility in the male is the result of an anomaly at one or more of the steps involved in reproductive processes. Approximately 15% of couples attempting their first pregnancy meet with a failure and in one-third of these cases, the man is infertile and in another 20%, both the partners are responsible for the failure. Thus in roughly 50% of infertile couples, the male factor is at least partly responsible for the failure to conceive (Macleod, 1971; Simmons, 1956). Male factor infertility has a relatively poor prognosis in terms of successful conception (Pampiglione et al., 1993). Therefore, male infertility is a more prevalent problem than is generally appreciated.

Inspite of recent extensive researches on the male reproductive tract, testicular and spermatozoal functions continue to remain an enigma. Current clinical and laboratory diagnoses may well be incapable of detecting numerous molecular and functional abnormalities that could result in human infertility (Collins, 1989). The present research work will therefore consider those areas that we believe have greatest influence on spermatozoal quality, as assessed by fertilizing potential, and suggest some avenues that may be explored to help us improve the definition of a fertile spermatozoon, viz. spermatozoon which is viable and has good fertilizing ability.
Interaction of three pituitary hormones (FSH, LH, PRL) and the androgens (T, DHT) spermatogenesis. ABP - androgen binding protein, PRL - prolactin; T-Testosterone; DHT - dihydrotestosterone; LH - Luteinizing hormone; FSH - follicle stimulating hormone.

Figure 1:
Anterior lobe of Pituitary.

LEYDIG CELLS

ALOOD

SEMENIFEROUS TUBULES

INTERACTION OF THE PITUITARY HORMONES AND THE ANDROGENS

FIGURE 1.
REQUIREMENTS FOR MALE FERTILITY

Endocrine functions sufficient to support spermatogenesis, an intact system of ducts to transport spermatozoa from the testes to arrive eventually in the urethra, normal epididymal function to allow maturation of spermatozoa, functional accessory glands to produce seminal plasma, and an intact nervous system are necessary for normal fertility.

The semen quality, sperm motility and ability to migrate through cervical mucus and into the upper female genital tract and fertilize the ovum also play an important role.

ENDOCRINE REGULATION OF REPRODUCTIVE FUNCTION

Reproductive activity and function in both the sexes is regulated by an elaborate neuroendocrine system, involving the hypothalamus, adenohypophysis and gonads. Internal and external cues are relayed to the hypothalamic neurons which translate these inputs into neurosecretory endocrine outflow that regulate the pituitary production of gonadotropins and prolactin (Moore, 1978; Reichlin, 1981). The pituitary endocrine products in turn, regulate steroidogenesis and gametogenesis in the gonads, which consequently exert a feedback regulation on the hypothalamus and pituitary (Scharrer and Scharrer, 1962) (Fig. 1). A primary neuroendocrine complex operates spontaneously and autonomously between the medial and basal hypothalamus, adenohypophysis and gonad, which drives the functions necessary for reproduction and fertility (Knobil, 1980). The primary mechanism involved in the regulation of Gn-RH-gonadotrophin system depends on the negative feedback
Figure 2: Hormonal control of testicular function. DA - dopamine, PIF - prolactin inhibiting factor; PRF - prolactin releasing factor; GnRH - gonadotropin releasing hormone; PRL - prolactin; FSH - follicle stimulating hormone, LH - Luteinizing hormone.
Hypothalamus

Pituitary

Germinai tissue of testis

Release of sperm into the epididymis

Release of androgen into the blood

Androgen feedback

Hypothalamus, PRF → GnRH

Pituitary

PRL

FSH

LH (ICSH)

Germinai tissue of testis

Interstitial tissue of testis

HORMONAL CONTROL OF TESTICULAR FUNCTION.

FIGURE 2.
exerted by gonadal hormones (androgens and estrogens) and a protein (inhibin). The androgens primarily inhibit LH, while inhibin affects FSH production.

The hormones primarily concerned with reproduction include those from hypothalamo-hypophyseal gonadal axis as well as hormones of thyroid, adrenal, pineal glands also influence reproduction.

Testicular steroid and spermatozoal production is mostly under the control of the hypothalamic-pituitary gonadal axis, with follicle stimulating hormone (FSH) and luteinizing hormone (LH) being episodically released from the anterior pituitary to stimulate the Sertoli cells and Leydig cells respectively (Fig. 2).

A key to understanding the cellular interactions within the testis is the localization of a particular non-hormonal stimuli. The local hyperandrogenic environment created by testosterone release from Leydig cells, induced by LH, in turn acts upon the Sertoli cells. Meanwhile, Sertoli cells locally release an LH releasing hormone (LHRH)-like peptide that stimulates the Leydig cells (Sharpe and Cooper, 1982). Other complex sequences of interactions to regulate the growth and differentiation of the male gonads presumably exist and are slowly being discovered (Cheng et al., 1987; Bellve and Zhing, 1989; Cheng et al., 1989), ultimately to describe a fabric of molecular communication that is at once both subtle and vulnerable to minor environmental changes. The importance of a carefully balanced microenvironment is highlighted by the need for an intact blood-testis barrier for spermatogenesis to proceed (Setchell, 1977).

Complete failure of spermatogenesis leads to sterility, while impaired spermatogenesis, although allowing production of some spermatozoa, will
### TABLE I

NORMAL VALUES OF SEMEN VARIABLES

<table>
<thead>
<tr>
<th>Standard Tests.</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>2.0 ml or more</td>
</tr>
<tr>
<td>pH</td>
<td>7.2 - 8.0</td>
</tr>
<tr>
<td>Sperm Concentration</td>
<td>$60 \times 10^4$ spermatozoa/ml or more</td>
</tr>
<tr>
<td>Total sperm count</td>
<td>$40 \times 10^4$ spermatozoa per ejaculate or more.</td>
</tr>
<tr>
<td>Motility</td>
<td>50% or more with forward progression (grade 3 and 4) or 25% or more with rapid progression (grade 4) within 60 min. of ejaculation.</td>
</tr>
<tr>
<td>Morphology</td>
<td>30% or more with normal forms.</td>
</tr>
<tr>
<td>Vitality</td>
<td>75% or more live i.e. excluding dye.</td>
</tr>
</tbody>
</table>

Reference:

### TABLE II

**NOMENCLATURE FOR SOME SEMEN VARIABLES**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normozoospermia</td>
<td>Normal ejaculate as defined in Table 1.</td>
</tr>
<tr>
<td>Oligozoospermia</td>
<td>Sperm concentration fewer than $40 \times 10^6$/ml.</td>
</tr>
<tr>
<td>Asthenozoospermia</td>
<td>Fewer than 50% spermatozoa with forward progression.</td>
</tr>
<tr>
<td>Teratozoospermia</td>
<td>Fewer than 30% spermatozoon with normal morphology.</td>
</tr>
<tr>
<td>Oligoasthenoteratozoospermia</td>
<td>Signifies disturbance of all three variables (combination of only two prefixes may also be used).</td>
</tr>
<tr>
<td>Azoospermia</td>
<td>No spermatozoa in the ejaculate.</td>
</tr>
<tr>
<td>Aspermia</td>
<td>No ejaculate.</td>
</tr>
</tbody>
</table>

**Reference:**

commonly mean reduced numbers of spermatozoa of increasingly abnormal morphology, reduced motility and functional ability. This can culminate in male factor infertility (Tucker and Chan, 1993). Unfortunately, primary endocrinological defects giving rise to failure or impairment of spermatogenesis is rare, constituting only 2 - 3% of all cases of male infertility (Ressler, 1989). Consequently, hormonal replacement therapy in male infertility has only a small role to play. It has been suggested (Turner et al., 1990) that while systemic serum or plasma levels of reproductive hormones may be in the normal ranges, lowered intratesticular concentrations may be causing otherwise unexplained male subfertility, possibly correctable by local intratesticular hormone administration.

SEmen ANALYSIS IN MALE FERTILITY EVALUATION

The semen analysis is a critical test in the evaluation of male infertility. It is the most reliable method for prediction of fertilizing potential of spermatozoa. The bulk volume of the semen is contributed by the accessory glands. Low volume or an excess volume is reported to be a cause of infertility (Dubin and Amelar, 1971). High and low semen values are associated with higher incidence of spermatozoa exhibiting subfertile characteristics, such as increased percentage of sperms with abnormal morphology, low motility and low sperm density (Table 1) (Gopalkrishnan et al., 1992). In the present investigation, some recent techniques were used to assess the nuclear membrane and acrosomal integrity of spermatozoa (Highland et al., 1991). A modified method for the differential staining of spermatozoa using alcoholic acidic silver nitrate (Chinoy et al., 1992) was used for
the assessment of sperm morphological anomalies and membrane and acrosomal integrity. Since the gametes of the male, unlike those of the female, are readily available for observation, considerable information could be obtained from a properly performed analysis. What follows is a discussion of various aspects of the semen analysis and a description of some techniques which have been used for evaluation of male infertility.

CONVENTIONAL SEMEN ANALYSIS

Although spermatozoa were first observed more than 300 years ago, the concept of semen analysis is relatively recent. Macomber and Sanders (1929) were the first to perform sperm counts in a large number of men. They were also the first to compare fertile and infertile populations in an attempt to establish a normal range for sperm number. According to them 60 million/ml was the lower limit for male fertility, despite subsequent reports that 15% to 25% of fertile men had sperm counts below 60 million/ml (Hotchkiss et al., 1938; Falk and Kaufman, 1950).

In the 1950s John Macleod published several papers which set standards for semen evaluation that have persisted to the present (Macleod, 1950, 1951; Macleod and Gold, 1951 a, b, c, 1953). Macleod's work serves as the foundation for modern semen analysis using five measures of semen quality (semen volume, sperm concentration, proportion of motile sperm, quality of motility and proportion of sperm with normal morphology) in 1,000 fertile and 1,000 infertile men. The data clearly showed that 60 million/ml limit of sperm count was too high. Thirty per cent of Macleod's fertile population had counts below 60 million/ml, but only
5% had counts below 20 million/ml. This number was the value subsequently adopted as the new minimum for male fertility (Macleod and Gold, 1951).

While some argued that Macleod's criteria were overly strict (Nelson and Bunge, 1974; Smith and Steinberger, 1977), others refuted the claim (Macleod and Wang, 1979). Today, the standards promulgated for conventional semen parameters are essentially those proposed almost 40 years ago. The critical question is: are the traditional measures of semen quality and the standards defined for normality clinically useful predictors of fertility? In answering this question, it is important to keep in mind the goals of semen analysis and the pitfalls that can hinder the attainment of these goals. The goals are: (1) to identify measures of human spermatozoa that will reliably distinguish individuals who are infertile from fertile males; (2) to use those measures to detect the earliest signs of altered reproductive potential, before the onset of clinical symptoms; and (3) to relate those measures to the physiology of sperm in vivo in order to understand the biological meaning of such measures. The ability of any test to distinguish normal from abnormal depends on (1) the reliability of the measuring tool, i.e. its precision and accuracy, (2) the variability of measured values in the disease-free population, and (3) the variability of measured values in the diseased population (Reigeleman, 1981). Traditional semen tests have been unreliable because of shortcomings in all three categories.
PROBLEMS WITH CONVENTIONAL METHOD

Among the semen variables recorded in conventional analysis (volume, sperm concentration, percentage motile, grade of motility and percentage normal morphology), only the volume and concentration have been measured objectively. Unfortunately, they are the ones least correlated with fertility.

Sperm concentration involves manual counting of sperm cells in a haemocytometer (WHO, 1987) or Makler chamber (Makler, 1978; 1980). The former method requires sample dilution, a potential source of error, and some studies have shown poor reproducibility in this method. Modern micropipettes have improved accuracy and precision, and the haemocytometer method is still widely used. The Makler chamber requires no dilution, but variations in chamber volume and spatial distribution of sperm are potential sources of error. Electronic, automated cell counters have also been adopted for sperm counting. Since the electronic methods count particles and not sperm per se, cellular and crystalline debris result in erroneous counting, especially at low sperm concentrations (Gordon et al., 1967).

Traditional motility assessment poses an even larger problem. Quantitative motility (percentage of motile sperm) is determined by counting both motile and immotile sperm in at least ten randomly selected, nonoverlapping microscopic fields. The percentage of motile sperm is calculated after counting a minimum of 100 sperm cells. This method may overestimate the number of motile cells for several reasons:
1) moving cells attract more attention than non-moving cells.

2) moving cells may be counted more than once as they swim into and out of the microscopic field, and

3) swimming cells may induce motion in intrinsically non-motile sperm.

The greater problem, however is the assessment of the quality of sperm motion. Macleod (1951) originally described a grading system scaled from 1 (extremely sluggish, barely progressive movement) through 4 (rapid, undeviating progression of cells). Intermediate scores were described as 1+, 2-, 2, 2+, 3-, 3 and 3+. Many grading systems have subsequently been described eg. Rochester motility scoring system (Jenks et al., 1982; Mahadevan and Baker, 1984; Talbert et al., 1987). The subjective nature of such systems is obvious and, while a trained observer may produce internally consistent results, the between observer variability prevents valid comparisons among investigators.

The inadequacies of the conventional methods led to the automated analysis of semen by Computer Assisted Sperm Analysis (CASA).

Several workers (Smith and Stienberger, 1977; Zuckermann et al., 1977; Smith et al., 1977) have established semen fertility standards on the basis of sperm concentration and density. Reports by Macleod and Wang (1982) have stressed however, that sperm counts have little meaning, unless qualitative motility and other qualitative aspects of the semen are considered. Moreover, it has been shown (Bustos - Obregon et al., 1981) that in certain cases of male infertility, individuals with comparatively high sperm counts and good progressive motility have poor in vitro sperm penetration and fertilizability. In a separate study, Raboch (1988) has
demonstrated fertility in males, though severely oligospermic, through reports of successful pregnancies in such couples. Hence, sperm density by itself is an insufficient criteria to establish semen standards.

Biochemical analysis also constitutes an important part of semen examination. The human sperm has been shown to be sensitive to the effect of a variety of reactive oxygen species (H₂O₂, O₂⁻, OH⁻) which originate from the sperm themselves (Aitken and Clarkson, 1987; Alvarez et al., 1987). The major defence system of the spermatozoon against oxygen free radicals appears to be superoxide dismutase (SOD), the activity of which has been well correlated with the ability to inhibit lipid peroxidation and sustain their in-vitro motility. Pradeep Kumar et al. (1989) described the changes in the levels of the superoxide radical and SOD in maturing mammalian spermatozoa and their involvement in sperm motility. In our laboratory, the important protective role of superoxide dismutase in maintaining the nuclear and acrosomal integrity of spermatozoa was described and therefore its absence may be related to poor sperm fertilizability (Highland et al., Unpublished Observations). Besides they have also shown lower activity of sperm acrosomal enzymes viz., acrosin and hyaluronidase in infertile cases reflecting poor penetrating and fertilizing capacity of the sperm. However, biochemical analysis of the different seminal components have, on the whole, not provided any decisive data in differentiating fertility and sub-fertility (Paz et al., 1977; Guerin et al., 1979). Moreover, variations in the biochemical components are related to physical state, frequency of ejaculation, glandular function (Mann and Lutwak Mann, 1981) as well as different fractions of the ejaculate of the same individual (Eliasson,
The reliability of these analyses is therefore low and conventional semen characteristics, viz. sperm number, motility and biochemical parameters are by themselves, poor predictive features of sperm function or semen quality (Hermanns and Hafez, 1981). Keeping these facts in mind, some relatively simple, reliable and recent techniques have been used to assess the structural and functional properties of the sperm with emphasis on determining sperm viability, membrane function, acrosome integrity, the nature of the nuclear DNA content and sperm morphological abnormalities.

Detailed evaluation of male fertility, therefore, involves careful semen analysis from both quantitative and qualitative aspects. Earlier work in our laboratory also showed that in cases of oligozoospermia and primary sterility, the sperm count and motility was reduced and there was alteration in the sperm metabolites studied (Chinoy et al., 1984). With the advent of Computer Assisted Semen Analyser (CASA), another cause of subfertility in the males viz., impaired forward progressive motility of the sperms was found to be a causative factor (Padman et al. Unpublished Observations). The earlier studies have used conventional methods for semen analysis (Chinoy et al., 1978, Rao, 1981) and so the present investigation holds special significance in providing automated analysis which gives details of the hitherto unknown sperm motion characteristics. The CASA system greatly aids in this direction.

AUTOMATED SEMEN ANALYSIS

The analysis of human spermatozoa in semen, promises to provide vital
information about male reproductive health and fertility. In the last decade, there have been major advances in techniques for human semen analysis. Subjective measures from single unrecorded observations are being replaced by objective measurements from stored, digitized sperm images (Boyles et al., 1989). Manual methods are being replaced by computer-assisted systems, which couple video technology and sophisticated micro-computers to perform automated image digitization and processing. Computer assisted sperm analysis (CASA) promises both technical and practical advantages over previous methods. The CASA system enables the objective assessment of large numbers of sperm samples in a variety of research and clinical settings with a speed and economy hitherto not possible. Automated semen analysis for studying motion characteristics have been well established (Vantman et al., 1989; Mack et al., 1988; Boyers et al., 1989).

While their potential as clinical and research tools is undeniable, CASA systems today provide information, the biological and clinical value of which is still unclear. In the light of this, the rapid proliferation of CASA systems is a triumph as much of marketing as of science, and reflects an uncritical acceptance of new technology.

The present work deals with sperm analysis, using CASA to study sperm motion and morphology, and about the clinical and biologic relevance of the measures that these tools provide.
The cases investigated were grouped in 4 major categories as follows:

GROUP 1 : Normal healthy males in the age group of 20-35 years with 2-5 years of married life and proven fertility were selected as controls.

GROUP 2 : Males diagnosed as having primary sterility, absence of spermatozoa (azoospermia) with certain chromosomal anomalies like Klinefelter syndrome were classified in Group 2.

GROUP 3 : Males diagnosed as having oligozoospermia, gynaecomastia, hypogonadism.

GROUP 4 : Miscellaneous group - Ambiguous genitalia, hypospadias, precocious puberty, intersex, etc.

PRIMARY STERILITY

While the causes for female infertility are still better understood, the actual difficulty arises in investigating male infertility, which contributes to 40% of infertility of couples. The real question involves differentiating the sub-fertile and infertile males. Moreover, in a stringent social set-up such as ours, particularly in couples from rural backgrounds, the male partner is extremely reluctant to face investigation. It is therefore all too common, to subject the female to a series of investigations with little attempt to evaluate the male. The present study however,
concentrated on the evaluation of the male, before any conclusion could be made.

Papadimas and Mantalenakis (1983) have shown that hormonal evaluation is an important diagnostic tool in assessing male infertility. Much attention has also been given to assessing some seminal parameters such as sperm density and motility in relation to hormone profiles (Rodrigues - Rigau et al., 1978; Nieschlag et al., 1979; Pierrepoint et al., 1962; Aitken et al., 1982; Bruno et al., 1986). However, there have been conflicting opinions regarding these parameters and male factors in infertility.

Lipshultz and Howards (1983) have suggested a holistic approach to the evaluation of the infertile male which utilizes a combination of careful clinical history, physical examination, detailed semen analysis and specific hormone assays to assess the patient's problem. Semen analysis is the most important test to evaluate seminiferous tubular function (Fredricsson, 1984). Earlier work carried out in our laboratory (Highland, 1989) showed that there was significant variation in the seminal characteristics rather than endocrine variation in the primary sterility cases as compared to control.

AZOOSPERMIA

Since the testis is under the control of the pituitary, which in turn is modulated by hypothalamic activity, it is obvious that several different links in the chain of control, however remote they may be from the testis itself, might be broken or absent and may interfere with normal semen production. In other words, lesions in the presiding neuroendocrine system that occur outside the testis itself
may give rise to abnormal testicular and accessory gland functions. A lesion in the hypothalamic or abnormal signals from other parts of the brain resulting in defective production of FSH and/or LH, could affect sperm output and other aspects of testicular function. A failure of normal testosterone feedback or deficient synthesis or release of gonadotropins could jeopardise normal function of the testis. The testis itself may also be a source of trouble in many cases. If receptors for the protein hormones on Leydig cells or Sertoli cells are absent, desensitized or down regulated, it will not be possible for those parts of the testis to respond to circulating hormones. Under these circumstances, levels of circulating LH or FSH may be correspondingly elevated as a concomitant to aberrant or absent spermatogenesis. In such cases, depending upon whether FSH or LH receptors are affected, spermatogenic activity may be absent or may be arrested at the spermatocyte stage. Azoospermia would result in either of these cases. The other causes resulting in azoospermic conditions are Klinefelters syndrome or other genetic disorders, Sertoli-cell-only syndrome, Seminiferous tubule or Leydig cell failure, ductal obstruction, including Young's Syndrome Varicocoele and certain exogenous factors.

**KLINEFELTER SYNDROME (47, XXY)**

This chromosome abnormality is one of the most commonly encountered among infertile men (Chandley, 1979). The patient exhibits an XXY chromosomal pattern. The testis in classical Klinefelter's syndrome shows a progressive failure of spermatogenesis, accompanied by tubular sclerosis and a marked increase in the
number of Leydig cells. The earliest derangement is a reduction in spermatogenic activity, leading to gradual disappearance of the germ cells. This is followed by atrophy of the Sertoli cells and thickening of the tunica propria. Finally, both the germ cells and the Sertoli cells are absent, and the lumen of the tubules are obliterated by thickened, hyalinized, and contracted tunica propria. The clinical signs of Klinefelter's syndrome generally appear some time after puberty, when the intrinsically abnormal testis, unable to respond to pituitary gonadotropin stimulation, begin to undergo the typical pathological changes (Becker, 1972).

Earlier work carried out in our laboratory has described cytogenetic findings in cases referred from major hospitals of Ahmedabad and its vicinity, for suspected chromosomal abnormalities because of clinical features of Klinefelter's syndrome, Down's syndrome, Turner's syndrome, ambiguous sex etc. (Shah et al., 1989).

OLIGOZOOSPERMIA

Oligozoospermia is the term used to represent those patients whose sperm count is less than 20 million sperm per milliliter. The usual quantity of ejaculate has approximately 120 million sperms in each million of semen, though in normal persons this can vary from 60 to 200 million. When the number of sperm in each milliliter falls below 40 million approximately, the person is likely to be infertile (WHO Manual, 1990). Such infertile subjects are said to be oligospermic. This condition arises as a result of genetic disorders, endocrinopathies, including androgen receptor defects, Varicocele and other anatomic disorders, maturation arrest, hypospermatogenesis or due to some exogenous factors. The acute cases of
oligozoospermia, in which the count is below 1 million of sperm cells is found to be associated with testicular malfunctions, hypothalamic dysfunction, hypogonadism zinc deficiency or malnutrition etc. (Table II).

According to Kumar et al. (1989, 1991), oligospermic sperms revealed defective SOD with poor radicle generation capacity. In contrast to the normal human spermatozoa which have a low SOD activity with the high SOD anion radicle generation capacity. Hence inherent biochemical and physiological defects in the membrane architecture of the sperm is responsible for infertility. These authors have further reported that human oligozoospermia is associated with erroneous dynamic behaviour of sperm cell membranes including round the axis lipid rotation, lipid packing and transmiceller protein diffusion. These abnormalities could be a cause of the inherently damaged O$_2$/SOD dependent membrane modulator operating in these cells. An alteration in thiol topography and spermatozoa in connection with human oligospermia has also been reported (Sumita Sinha, 1994).

GYNAECOMASTIA

Gynaecomastia is defined as the presence of a discrete disc of sub-areolar tissue greater than 2 cms in diameter in the males, is another common problem found in adolescents who are virilizing rapidly. Gynaecomastia results from a disturbed hormonal balance and hence warrants analysis to identify the possible endocrine disturbances. Nuttal (1979) noted an incidence as high as 30% of glandular enlargement in 306 healthy males from 17-50 years and hence the
pathological presence of gynaecomastia due to endocrine imbalance, must be separated from the temporary development of gynaecomastia.

HYPOGONADOTROPISM

Paramount among the pretesticular causes of infertility is hypogonadotropism. There are two types of cases, (1) prepubertal, (2) postpubertal. The end result is testicular failure regardless of the causes of the gonadotropin insufficiency. In prepubertal cases patients have small, immature seminiferous tubules. Patients with genetic defects in gonadotropin secretion are usually tall and eunuchoid, and exhibit no signs of deficiency of growth hormone. Hence clinical syndrome embodied by such patients is referred to as hypogonadotropic eunuchoidism (Paulsen, 1974; Santen and Paulsen, 1973). In postpubertal period, the seminiferous tubules, once having attained full development, do not revert to prepubertal state. Instead they show in succession, maturation arrest, loss of germ cells, reduction in diameter and progressive thickening and hyalinization of the tunica propria. Leydig cells are shrivelled and inconspicuous.

GROUP 4

This group included patients with a variety of sex anomalies as described below. Some of the patients in this group were of pre-pubertal ages.
**AMBIGUOUS EXTERNAL GENITALIA**

Sexual ambiguity at birth reflects abnormal foetal sexual differentiation. The presence of ambiguous external genitalia is not only a source of social embarrassment and psychologic problem for parents but one that necessitates detailed investigation and examination of the patient. The adrenals secrete an abnormally large amount of virilising steroid hormone, even during embryonic life resulting in the formation of ambiguous genitalia (Jones and Klingensmith, 1985).

In our laboratory, the clinical and histopathological studies of a case with 45,X/46,X inv (Y) mosaicism was studied (Radhakrishna et al., 1989). Besides cytogenetic studies were also carried out in 292 cases with abnormal sex phenotypes (Shah et al., 1990). A case of a 21 year old male with absent gonads who had female type genitalia was also reported (Sheth et al., 1989).

**HYPOSPADIAS**

Hypospadias, without any other form of abnormality of sexual differentiation, is the mildest form of male pseudohermaphroditism (Jost, 1971; Devine et al., 1980). Murphy and Zacur (1987) have reported the occurrence of hypospadias as 0.8 to 7.6 per 1000 live births and its endocrine causes. Earlier work carried out in our laboratory on various age groups having sexual disorders, e.g. ambiguous genitalia, hypospadias, hypogonadism, Klinefelter's syndrome etc., was undertaken to investigate the possible involvement of 17 ketosteroids in these anomalies (Shah et al., 1984).
PRECOCIOUS PUBERTY

True sexual precocity is the inappropriate appearance of secondary sexual characters for chronological age. The diagnosis of precocious puberty is a clinical one supported by characteristic growth charts and radiological evidence of advanced bone age. Confirmation and determination of the source of the cause of precocious puberty however, requires assessment of the hormonal status of the child who has achieved premature pubertal development. Rudd (1988) has drawn attention to the limitation of measuring basal or stimulated levels of FSH, LH and the sex steroids for the diagnosis of these conditions. Precocious puberty however, is extremely rare in occurrence and hence generates special interest in the investigation of this disorder.

INTERSEX

The diagnosis of true hermaphroditism is made when testicular tissue as well as ovarian tissue ie. both primordial follicles and seminiferous tubules are present in the gonads (Shearman, 1985). External genital sex and internal sex vary widely, the former, as a rule being predominantly male. This chromosomal pattern vary from 46, XX female karyotypes, mosaicisms (46, XX/46, XY) to rarely 46, XY male karyotypes. The management of these cases require investigation to determine the development of internal and external genitalia, the hormonal imbalance and chromosomal constitution in relation to the sex of rearing. In our laboratory, the clinical, cytogenetic and endocrine studies were carried out in a 46, XX male (Multani et al., 1989). Such males with XX karyotype and masculine
development associated with infertility are relatively rare as compared to XXY individuals.

CAUSES OF MALE INFERTILITY

A number of schemes have been used to classify the etiologic factors in male infertility. The scheme divides the factors into the following main categories: anatomic, endocrine, genetic, inflammatory, idiopathic, immunologic, exogenous, neoplasia and ejaculatory dysfunction.

Anatomic factors include varicocele, cryptorchidism and congenital anomalies which include hypospadias or epispadias, which might interfere with delivery of semen into the vagina, congenital testicular hypoplasia or eplasia, partial or total absence of vas deferens, failure of vas deferens to join the epididymis and epididymal hypoplasia.

Endocrine factors in male infertility include acromegaly, Cushing’s disease, isolated gonadotropin deficiency, pituitary tumor and hypogonadotropic hypogonadism. Testicular failure may be idiopathies or secondary (genetic, post-traumatic or postinflammatory). A case of an enzymatic defect in testosterone synthesis has been reported (Steinberger et al., 1974) and androgen receptor deficiency may occur.

Chromosomal changes and infertility

Alteration in the structure and number of sex chromosomes are frequently responsible for sex abnormalities, in the dysgenesis of the foetal gonads, leading
to endocrine imbalance and phenotype anomalies. In our laboratory, a triple X-female with long arm deletion of one of the X-chromosomes associated with primary amenorrhoea: 47,XX,+del(X)(q27.3) was reported (Radhakrishna et al., 1991). In another case, a female with isodicentric X chromosome idic(Xq) associated with ovarian dysgenesis was reported (Sheth et al., 1994). Among the other genetic factors are sex chromosome abnormalities including Klinefelter’s syndrome (47 XXY). Others associated with infertility include XX/YY, XXX/XY, XX/YY, XX/XXY and XY/XXY patterns as well as XX with male phenotype. Autosomal translocations may also be associated with infertility.

Inflammatory conditions may cause infertility. Orchitis damaging the germinal epithelium may be the result of mumps or pancreatitis. Obstructive epididymis can result from tuberculosis, gonorrhea, Chlamydia trachomatic and other bacterial inflammations. Prostatitis could be caused by a variety of bacteria and other agents.

Idiopathic factors include Sertoli-cell-only syndrome (germinal cell aplasia), maturation arrest of spermatogenesis and hypospermatogenesis. Isolated abnormalities of sperm morphology and ultrastructure (Lalonde et al., 1988; Eliasson et al., 1977) or sperm function could also be included in this category.

Immunologic factors such as autoimmune reaction may result in the formation of sperm antibodies that may agglutinate or immobilize sperms or otherwise interfere with sperm function. Sperm antibodies commonly develop after vasectomy (Bernstein et al., 1979).

A wide variety of exogenous factors may cause infertility. These include
prescription drugs, hormones, recreational drugs, antineoplastic drugs and radiation including X-rays and isotopes (Sherins and Molvihill, 1989). Other toxic substances include metals, dyes and environmental pollutants. In our laboratory the effect of fluoride on reproductive organs in male rodents has been studied extensively and fluoride is known to impair fertility in male mice and rats due to alterations in the structure and metabolism of their testis, epididymis as well as reduction in sperm motility, changes in its morphology, metabolism and fertilizability (Chinoy and Sequiera, 1989a,b, 1992; Narayana and Chinoy, 1994a,b). The human spermatozoa were also rendered immotile by NaF treatment in vitro (Chinoy and Narayana, 1994). The adverse effect of lead on human semen was also reported (Roy Chowdhury et al., 1986, 1987). High doses of lead exposure was known to cause permanent damage to the male reproductive organs which was irreversible (Rao, 1989).

Other miscellaneous factors causing infertility are:

(a) Mechanical damage, such as trauma to or torsion of the testes, injury to vas deferens or spermatic artery during hernia repair etc., result in reproductive dysfunction;

(b) Testicular and Pituitary neoplasms may cause impaired spermatogenesis and infertility;

(c) Ejaculatory dysfunction may result from a variety of conditions. Retrograde ejaculation may occur secondary to diabetic neuropathy, prostatectomy, spinal cord injury, etc.

Other factors include psychological dysfunction that may cause impotenc
or premature ejaculation. Psychological and physical stress (Cockett and Urry, 1977) may have adverse effect on semen quality.

IMPORTANCE OF THE STUDY

The role of hormones, endocrine control and regulation of reproduction, as well as importance of detailed semen analysis in cases of male infertility have been elucidated. Endocrine studies and assay of seminal factors are therefore vital in understanding and determining the causes and factors responsible for infertility in the male, with probable suggestive measures towards appropriate therapy.

In an over-populated world, so preoccupied with fertility control measures owing to the rampant child-boom, in particular in the third world and developing countries, there seems to be something incongruous about focussing the ingenuity of research and investigatory medicine towards enhancing reproduction. But while the pressing need of times today is to curb the ever growing population, one cannot ignore or overlook the population (10 to 15%) which represents one of the most neglected and silent minority groups in our country who wish to conceive but fail to do so. For these couples, fertility becomes a burning, all-consuming goal. The problem of infertility directly affects the individual at a personal level, resulting in much physiological and psychological stress and trauma. The alleviation of infertility is now increasingly accepted as an urgent social and clinical need (Seppala and Edwards, 1985). Research centered around reproductive disorders and its associated causes, is consequently research oriented towards an individual.
In a socially orthodox society such as ours, with traditional emphasis on child-bearing, infertility represents a social stigma. Siebel and Taymor (1982) have reviewed the emotional aspects of infertility and have shown that such couples suffer extreme social pressure, frustration and anxiety. When such cases are neglected in the course of investigation or when inadequately counselled, the consequences are emotional anxiety, marital stress and instability. Such trauma could be alleviated through the use of updated investigations to identify the cause and help the patients understand the condition more comprehensively.

Infertility is a rather difficult subject for most people to discuss and especially so in males, who are often extremely reluctant to face investigation. Thus, although difficult, it becomes increasingly important to carry out detailed investigation in order to be able to elucidate the causes and probable mechanisms related to certain disorders, counsel and explain lucidly to the patient the sources of the problem its implications and if possible, the remedy.

The cases included in the study were referred for investigation from Ahmedabad city, various regions of Gujarat and neighbouring states to our Departmental clinic consisting of a gynaecologist, pediatrician, sonologist, endocrinologist and human cytogeneticist. A special attempt was made in the work carried out in this thesis, to investigate the infertile males for both endocrinological and seminal factors which may contribute to reduced fertility.
PART II

STUDIES ON MALE CONTRACEPTION

In the present day context, where one of the major burning issues of immense global concern is the 'population explosion boom', contraceptive biology has a tremendous role to play. For many years research in the field of contraception, or the application of contraceptive methods, has been almost exclusively limited to the female tract. In the last decade, there has been renewed interest in developing methods for male fertility control. The impetus for this activity has resulted partly from a sincere concern with possible reactions associated with a 'pill-iredness' in women, partly from a definite social trend towards a sharing of responsibility for family planning between partners, and partly from a desire expressed by many men to achieve control over their own fertility.

Despite phenomenal advances in fertility control, it is clear that every method falls short of possessing all the ideal characteristics. Different methods fulfill different criteria, and no method is completely devoid of risks, be they serious side effects or failure. As newer methods enjoy varying degrees of popularity, expanded clinical and laboratory data raise newfound concerns. Awareness of a method's limitations and risks, appropriately or not, soon raises apprehension in a medically conscious patient population and limits its acceptability.

The quest for a safe, effective and easily reversible contraceptive method in the male continues. The search for new techniques has followed several
pathways: use of steroids to interfere with the hypothalamic-pituitary-testicular interaction, agents to interfere with sperm maturation and agents that might disrupt spermatogenesis.

A number of major categories of compounds have been evaluated as possible agents for male fertility regulation. These include steroidal compounds, anti neo-plastic compounds, LH-RH and its analogues, plant extracts and agents that interfere with sperm maturation. The maturation process of the spermatozoa in the epididymis depend on several factors (Prasad and Rajalakshmi 1976, 1977; Chinoy 1984). α-chlorohydrin and certain alkalizing substances which are used for medical tumor therapy, interfere with the maturation process of the spermatozoa in the epididymis. Experiments on rats, however have shown that these agents have a number of serious side effects, so that this method cannot be used as a male fertility regulating procedure (Ericsson, 1970).

SYSTEMIC MALE CONTRACEPTIVE AGENTS

THE UNSOLVED PUZZLE

Research in the area with antispermatic compounds has increased in recent years due to the growing interest in the development of systemic male contraceptive agents.

In seeking antifertility compounds, it should be remembered that the process of spermatogenesis comprises several steps - the initial mitotic divisions of undifferentiated stem cells in the basal compartment of the tubules; the stem cells that may participate in the reinitiation of the spermatogenic process; the movement...
of rapidly proliferating cells destined to become spermatozoa into the adluminal compartment of the seminiferous tubules; the completion of meiosis in the adluminal compartment, and the metamorphosis of the prospective gamete (round spermatid) into the spermatozoon. Many of these events occur only if the germinal cells are under the influence of steroids and protein hormones derived from either the nearby Sertoli cells or Leydig cells or from the distant, but equally important hypothalamus and pituitary gland.

Any compound that would destroy the stem cells would result in irreversible infertility, which is not a goal in the development of male contraceptive agents. It is also important to consider that, after a series of spermatogonial divisions, primary spermatocytes are formed which undergo meiosis. During the long prophase of the first meiotic division, reduplication of the genetic material of the germ cells occur and a complex pairing and interchange of genetic material of the germ cells takes place between chromosomes. Therefore, it would be important to avoid drugs that might affect this phase of spermatogenesis because of the possibility of mutation or other alteration of the genetic material that might result in abnormal offspring. The exclusion of spermatogonia and spermatocytes as targets for drug action would leave, as relatively safe candidates for pharmacological interruption, cells in the later stages of spermatogenesis.

There have been many attempts to induce infertility in men by taking advantage of the negative feedback regulation of gonadotrophin release. The capacity of excess testosterone to lower plasma gonadotropin concentrations and bring about suppression of spermatogenesis has been recorded (Lee et al., 1972.
Sherins and Loriaux, 1973). A synthetic derivative of ethinyl testosterone, danazol, appears to act directly on the Leydig cells to suppress their steroidogenesis, resulting in diminished sperm count (Sherins et al., 1971; Skoglund and Paulsen, 1973). Suppression of gonadotrophins can also be achieved by administration of oestrogens and progestogens. These treatments have several drawbacks, such as the possibility of serious toxic side effects resulting from the chronic administration of high doses of testosterone; loss of libido as well as painful gynaecomastia which results from the administration of oestrogens. Still unknown are the mutagenic effects of these compounds.

In the past 40 years, the effects of testosterone esters (either enanthate or cyclopentylpropionate) administered intramuscularly, the progestogens, depo-medroxy progesterone acetate and danazol, and combinations of testosterone esters and progestogen have been extensively studied (Bremmer and de Krester, 1976; Ewing and Rohaire, 1978). Available data (Brenner et al., 1977; Steinberger and Smith, 1977; and Patanelli, 1977) suggests that it may be possible to induce azoospermia or oligozoospermia in 70-100% of normal men without major adverse reactions, provided appropriate drug and dosage selection are made. Recovery of spermatogenesis is also observed. It will probably not be possible to induce azoospermia in over 70% of men using presently available compounds without risking unacceptable toxicity. Thus the problem of oligospermia remains, with the risk of unplanned pregnancies. Furthermore, it will be important to determine whether induced oligospermia in normal men represents dilution of normal sperm or lesioned sperm.
Another class of compounds displaying antispermatic properties also under investigation are the indazole carboxylic acids or 1-substituted-indazole-3-carboxylic acid compounds and their derivatives, particularly lonidamine. These compounds have been intensively investigated in animals in the past years (Coulston et al., 1975; Lobl et al., 1979). There is no available information on the effect of these compounds in the human. The possibility of toxic and mutagenic effects cannot be excluded.

Other approaches in the control of spermatogenesis have been thought to be found in the possible use of 'inhibin'. However, this approach is speculative (Franchimont et al., 1978). Also, the application of potent analogues of LH-RH/FSH-RH which can exert an inhibitory effect on gonadotropins at the pituitary level, as well as an inhibitory effect on gonadotropins at the testicular (Leydig cell) level has been investigated (Wiegelmann et al., 1977; Smith et al., 1979; Bergguist et al., 1979). One major shortcoming of such an antispermatic agent is that the lack of androgen would result in a failure of libido and potency thereby necessitating androgen substitution.

The WHO Task Force on Methods for the Regulation of Male Fertility was established in 1972. Recently, through increased public awareness, statements supporting research on male methods and greater involvement of men in reproductive health have been forthcoming from several quarters. The clinical and scientific basis for the research have been well reviewed in recent years (Wu, 1988; Swerdloff et al., 1989; Nieschlag et al., 1992; Ray et al., 1991 and Waites, 1991).

In pursuit of new and affordable methods of contraception in men, the
following methods are available. The prevalence of vasectomy can be taken as a measure of male participation in family planning programmes. A major concern here is that vasectomy might be associated with increased risk of cardiovascular ill-health (Tang et al., 1988, Petitti, 1986). Two main factors limit the acceptability of vas occlusion, one is the necessity for a skin incision which is unacceptable in some cultures, and the other is difficulty of reversibility should the circumstances require this.

HORMONAL METHODS

The suppression of sperm production by hormonal means is another strategy that has involved, (i) Suppression of the secretion of gonadotrophins, either of both LH and FSH or of FSH alone, (ii) The recovery of circulating androgen to physiological levels without restimulation of spermatogenesis and (iii) assessment of the functional capacity of residual sperms, should the treatment fail to achieve azoospermia in all cases.

FSH inhibition: The concept of dual hormonal regulation of testicular function, in which FSH is considered essential for spermatogenesis, while LH by acting on the Leydig cells regulates androgen biosynthesis and secretion, has been exploited to develop endocrine based approaches to male fertility regulation. Non-human primates have been used to study the effects of selective inhibition of FSH by active (Modugal and Rao, 1985; Srinath et al., 1983) or passive immunization (Murthy et al., 1979, Wickings et al., 1980). The treatment results in a decrease in
sperm counts, but consistent and uniform azoospermia could not be induced.

Passive immunization of adult rats, hamsters and marmosets with rabbit anti-seminal inhibin resulted in complete or partial block of fertility (Sheth et al., 1992). The antiserum treatment presumably neutralized endogenous inhibition resulting in an unopposed rise in FSH. This probably led to a refractoriness of the testis to FSH resulting in complete spermatogenic arrest.

Here, the affordability of the drugs for the developing countries is a major consideration.

GnRH analogue - androgen combinations

Clinical studies (Pavlou et al., 1989, 1991) and studies in non-human primates (Weinbauer et al., 1989) have shown that GnRH antagonists are more potent in the suppression of gonadotrophin secretion and of sperm production than are GnRH agonists. Here again the cost of synthesis of peptide hormones such as the GnRH antagonists is likely to remain too high for contraceptive use in developing countries.

NON HORMONAL AGENTS ACTING DIRECTLY ON SPERMATOGENESIS

A large number of chemical agents have been described (Ray et al., 1991) but all tend to lead to total spermatogenic arrest and ultimately to irreversible sterility.

Physical agents such as irradiation, ultrasound and high temperature also
lead to spermatogenic arrest when applied at certain dose levels. Their limitations for contraceptive application lie in the equipment needed and the careful monitoring of the dosage that is required to avoid irreversible damage. One exception is the application of heat (Rock and Robinson, 1965; Kandeal and Swerdloff, 1988). Clinical studies showed that long term, mild elevation (1-2°C) of temperature by the apposition of the testes to the abdominal cavity during working hours can lead to azoospermia or oligozoospermia (Mieusset et al., 1987, 1991 and Shafik, 1992). The concern is about safe reliability and reservations about compliance during introduction into family planning programmes.

Sperm surface Antigens

Sperm surface proteins, crucial for sperm-egg interactions, offer hope as immunogens for the development of a vaccine (Wu, 1988; Primokoff and Myles, 1990). However, such a vaccine would more likely lead to a contraceptive method for the female, given the difficulty of access of antibodies to the male reproductive tract.

The present research work deals with male contraception and it is an attempt to develop a safe and effective oral agent for fertility control in the male from an indigenous source, the papaya seed. The antifertility effects of the aqueous and alcoholic extracts have been tested earlier in our laboratory (Chinoy and Sam George, 1983; Chinoy et al., 1984/1985; Chinoy and Geetha Ranga, 1984). So the present work was carried out to test the potency of benzene and alcoholic extracts of papaya seed as antifertility agents in male rats.
PLANTS AND PLANT PRODUCTS; SOURCE OF FERTILITY REGULATING AGENTS

Since time immemorial, plants and plant products have been used as a source of fertility regulating agent. The Indian folklore medicines include a large number of plants reputed as oral contraceptives and abortifacients. One of the oldest methods dates back to about 4000 years ago where the paste of ground *Acacia* was prescribed as a means of contraception by the Egyptians (Havemann et al., 1967). Aristotle in his writings, mentioned the use of cedar oil as a method of contraception. In ancient India, the kadamba fruit, the seeds of the red lotus, the palasa flower, the samoli flower and the palm leaf all were used as oral contraceptive agents. The plants that have reputation as a folklore or those which have been tested for antifertility activity constituents have been discussed (Farnsworth et al., 1975; Setty et al., 1977; Garg et al., 1978; Guerra and Andrade, 1978; Oswiecimaka et al., 1980; Atal, 1981; Karkhov and Mats, 1981; Kambhoj and Dhawan, 1982; Nagarajan et al., 1982; WHO Annual Report, 1985; Farnsworth and Bingel, 1985).

The Central Drug Research Institute (CDRI) at Lucknow, in India, have screened numerous plants and their products for their possible use as antifertility agents in both males and females. Similarly, Farnsworth et al. (1981) have indicated the various higher plants useful in fertility regulation in human beings. Many plants are known to have significant antifertility effect on female reproductive system with potent antiimplantation and abortifacient agents showing estrogenic activity. Most of the indigenous plants known to have antifertility effect
on female reproductive system are listed by Nagarajan et al. (1982). However, in the present chapter plants showing potential as male antifertility agents alone have been reviewed.

PLANTS AFFECTING MALE FERTILITY

Nagarajan et al. (1982) have tested numerous indigenous plants having potential spermicidal activity in human beings and/or animals.

Santhakumari et al. (1980) studied the effects of plumbagin on spermatogenesis and accessory reproductive organs of rat. Aqueous and alcoholic extracts of Embelia ribes affected male reproductive organs and exhibited antifertility activity (Munshi et al., 1972; Krishnaswamy and Purushothaman, 1980). The crude extract of Abrus precatorius Linn., caused testicular lesion (Baijal et al. 1981). The green flower extract of Malvaviscus conzatti exhibited antifertility effect in male albino mice (Varma et al., 1980). The cattle grazing on an exclusive diet of Leucana leucoephala became infertile (Holmes et al., 1981). Hyperglycemia induced testicular dysfunction in dogs, after chronic administration of Balenites roxburghii fruit pulp extract was observed (Dixit et al., 1981) with significant fall in protein and sialic acid levels after the treatment.

Chronic administration of Sapindus trifoliatus fruit extract at a dosage level of 10 mg/animal (Oral) every alternate day caused testicular lesions and inhibited the process of spermatogenesis at the primary spermatocyte stage in male gerbils (Dixit and Gupta, 1982a). The study indicated a fall in protein, sialic acid and acid phosphatase level in testis and epididymis. Dixit et al. (1983) also showed
anti-spermatogenic activity of *Gloriosa superba* in male gerbils. Chronic administration of *Solanum xanthocarpum* (Solanaceae) berries brought about testicular lesion in male dogs. The treatment also affected spermatogenesis (Dixit and Gupta, 1982b).

The aqueous extract of *Echeveria gibbiflora* has immobilizing and agglutinating effects on human spermatozoa (Huacuja et al., 1985). Sperm agglutination activity of extracts from roots of *Arum maculatum* and *Arum orientale* was studied by Maldenov (1982). The extract is believed to react specifically with receptors situated on the surface of the tails of human spermatozoa.

The antifertility effect of *Ocimum sanctum* L. in male mice by feeding the leaves along with the normal diet was reported by Kasinathan et al. (1972). The treatment slightly affected spermatogenesis and altered some biochemical parameters. Administration of cold benzene extract of *Ocimum sanctum* leaves, orally at a dose of 100 - 200 mg/Kg body weight showed antispermatic activity in adult male albino rat (Seth et al., 1981). Chinoy and Geetha Ranga (1983) have reported that aqueous extract of *Catharanthus roseus* L., leaf manifested 100% antifertility and strong antiandrogenic effects in adult male albino rats. Chinoy and co-workers, have screened several other plants for antifertility effects in male rodents including betel leaf, bamboo shoot extracts, *Terminalia bellirica, Terminalia arjuna, Aloe vera* etc. (Chinoy and Patel, unpublished observations).

The discovery of Gossypol, a yellow phenolic compound present in the seed
of cotton plant of Gossypium species (family Malvaceae) was a major lead in male contraception. A good deal of work has been carried out on its effect on male reproduction and toxicity (Wang et al., 1979; Zhou and Hei, 1981; Xue, 1981; Qian, 1981; 1984/85; Liu et al., 1981; Prasad and Diczfalusy, 1982; 1983; Kalla, 1985; Kalla et al., 1982; Wichmann et al., 1983; Rastula et al., 1983; Wong et al., 1983; Weinbaur et al., 1983; Prasad, 1984/85, Liu, 1985). However, Gossypol has shown several harmful and highly toxic side effects on treated animals. The most serious effect was hypokalemic paralysis in men (Qian, 1981; Liu et al., 1987; Liu and Lyle, 1987) and with incidence of irreversibility (Meng et al., 1988). Hence its use was discontinued.

The search therefore continues and various plant products are being tried and tested for their contraceptive efficacy.

CARICA PAPAYA SEED: A PROMISING POST TESTICULAR MALE CONTRACEPTIVE

The papaya (Carica papaya L; family; Caricacea) is a large herbaceous perennial plant cultivated throughout the tropical world and in the warmest parts of the sub-tropics. The genus Carica include about 40 species, of which Carica papaya is the most important and widely cultivated in India. Nearly every part of the tree is said to be of medicinal value. Papaya fruit is not only wholesome, but also digestive, carminative and diuretic. The seeds are rich in protein and contain oil which is reported to have certain insecticidal properties. The most important commercial products which could be produced from papaya are papain, a broad
spectrum proteolytic enzyme and pectin, an important food adjunct.

The cold aqueous extract, hot infusion and the resin fraction of the seeds stimulate rat intestine and depressed frog's heart. All the fractions paralyse the earthworm and rat tape worm in vitro. The resin was the most potent among all the fractions (Bose et al., 1961).

The major alkaloid present in Carica papaya seeds and leaves is carpaine and the only nitrogenous base reported so far from the plant is pseudo-carpaine, a stereoisomer of carpaine found in very small quantity (Govindachari et al., 1954). The alkaloid carpaine in the leaves of papaya was discovered by Greshoff. Carpaine is soluble in ethanol, chloroform, and benzene and insoluble in water.

The alkaloid, pseudo-carpaine has the same gross structure and seems to differ from carpaine only in the configuration of the carbon having the hydroxyl on dehydrogenation. It yields deoxycarpysinic acid (Manske, 1965). Pseudo carpaine on acid hydrolysis yields carpamic acid and pseudo carpamic acids (Govindchari et al., 1965).

The leaf contains a glucoside called carposide and many other glucosinolates (Hanley et al., 1983). The seeds of papaya fruit contain glucoside, caricin (which resembles sinigrin) and also the enzyme myrosin. The seeds also contain a substance named carpasemine with a melting point of 165°C. The chemical properties of carpasemine and its degradation products have been studied and some new derivatives have been produced from it (Panse and Paranjpe, 1943).

Nineteen different carotenoids were identified in the fruits, the major being Cryptoxanthine (48%). Oxycarotenoids were higher in proportion as compared to
carotene hydrocarbons. The percentage of cryptoflavin and B-carotene were 13 and 29.5 respectively. Oxygenated carotenoids present were either hydroxy or epoxy carotenoids of B-carotene (Subharayan and Cama, 1964). The fresh fruit yields 0.001% carica xanthum \((C_\text{4}O\text{H}_{\text{8}}\text{O}_4)\) a colourless substance with 16°C melting point and 0.00004% of violaxanthine \((C_\text{4}O\text{H}_{\text{8}}\text{O}_4)\) as yellowish substance with melting point 184°C.

The alcoholic and aqueous extracts of the seeds were found to manifest antiimplantation and abortifacient effects in female rats (Chinoy and Trivedi, 1983; Chinoy et al., 1995b). The induced effects were reversible by withdrawal of the treatment. The seed is a powerful emmenogogue and will produce abortion if eaten by pregnant woman.

Das (1980) reported that oral administration of aqueous papaya seed powder at a dose of 20 mg/day/8 weeks to male rats inhibited their fertility to about 40%. The histology of the testis and other reproductive organs as well as the weights of adrenals and pattern of sperm motility remained unchanged, whereas, the petroleum ether extract of the pulp of Carica papaya exerted significant antifertility activity in male albino rats (Garg and Garg, 1971).

In our laboratory, 4 different varieties of papaya have already been tested viz. Honey Dew, Ceylon, Ranchi Dwarf and Washington for their antifertility efficacy in the male rodents. The Honey Dew variety was found to be the most potent (D’Souza, 1989) of the four varieties and was therefore used in all future work.

The effect of papaya seeds have been tested on male rats, mice and guinea
pigs and doses ranging from 0.1 mg/Kg body weight/day/animal to 20 mg/Kg body weight/day/animal were tried in our laboratory using im, sc, oral routes. The dose was based on the LD$_{50}$ values which was 15 gm/kg/body weight. According to WHO protocol (1983) a LD$_{50}$ of 5 gm/kg/body weight is considered nontoxic. 0.5 mg and 5 mg/Kg body weight were effective when given intramuscularly. However, higher doses of 10 and 20 mg/Kg body weight were required to bring about the desired effects when administered orally. Studies by Chinoy et al. (1984/85; 1985; Chinoy and Geetha Ranga (1980, 1983, 1984), Chinoy and Sam George (1983) and Chinoy et al. (Unpublished Observations; 1994, 1995a,b) have revealed that aqueous extract of papaya seed at a dose of 5 mg/Kg body weight for a period of 60 days by intramuscular and oral routes brought about significant antifertility effects on male rats, mice and guinea pigs. The loss of fertility was attributed to decline in sperm motility, alterations in sperm morphology, decrease in sperm density, change in the physiology of Vas deferens and alterations in the oxidative metabolism of spermatozoa. On the other hand, the treatment did not alter spermatogenesis and histology of the reproductive organs. The activity of 3B and 17B-HSD, levels of testicular cholesterol, serum testosterone and FSH and LH remained unaltered by the treatment (Chinoy et al., 1984/1985; unpublished observations). Hence the effects were post-testicular and did not seem to affect the hypothalamo-hypophysial gonadal axis. The extracts were also non-estrogenic. All the induced effects were transient and reversible by 2 months of discontinuation of the treatment. The non toxic nature as well as the contraceptive efficacy of aqueous extract of *Carica papaya* at a dose of 5 mg/Kg body weight/animal/day
intramuscular and 20 mg/Kg/body weight/animal/day orally for 60 days has been established (Chinoy et al., 1994, 1995a).

Similarly, Lohiya et al. (1994) and Roy Choudhury (personal communication) also tested our aqueous extracts and found that reversible sterility could be induced in male rats by papaya seed aqueous extract treatment without any effects on libido and toxicological profile. The induction of reversible antifertility effects using crude ethanol and crude chloroform extract of papaya seed in male albino rats were also reported by Lohiya et al. (1994) and Lohiya and Goyal (1992).

The fractionation of the aqueous and alcoholic extracts has also been attempted (Chinoy, unpublished observations) as well as the Phase I trials have been initiated in collaboration with other laboratories.

The present research work takes into consideration the vast knowledge available on the subject of sperm maturation to test the contraceptive efficacy of the benzene and alcoholic extracts of papaya seed as a possible male oral contraceptive and the reversibility of the effects so that functional sterility could be induced.