CHAPTER - 2

Review of Literature
2.0 REVIEW OF LITERATURE

The prostate is the organ of the body most frequently affected in males with advancing age, and by far the single most common pathological process experienced is BPH (benign prostatic hyperplasia / hypertrophy). This common pathology which is generally very slowly progressive is characterized by a gradual increase in both glandular and fibromuscular tissue in the periurethral and transition zones of the prostate with resultant urethral compression. The precise causes of BPH remain enigmatic, there is an interplay of a number of factors which have been identified; ageing is a prerequisite factor as is the 5α dihydrotestosterone (DHT). DHT levels are not supranormal in BPH but 5α reductase activity and the density of androgen receptors may be significantly increased. Increasing estrogen levels in later life may also play a role, either by inducing androgen receptors or decreasing the rate of either epithelial or stromal cell death. Autocrine or paracrine growth factors such as epidermal growth factor (EGF), transforming growth factor (TGF-β) and fibroblast growth factor (FGF), may provide the signaling mechanisms for the stromal –epithelial interactions that result in BPH nodule formation and eventually bladder outlet obstruction.

At least 70% of men who reach 70 years of age develop histological BPH and 40% or more of them have some symptoms of bladder outflow obstruction; with further ageing the disorder becomes almost universal. However, because the onset is so gradual most patients fail to appreciate that anything is amiss with their bladder or prostate. Therefore many battle on stoically accepting the
progressive reduction in their quality of life as simply a part of ageing until UTI, urinary retention or other complications eventually supervene. The socio-economic implications of this disease are considerable and as the world population continues to age, this burden seems bound to increase in the future (Drummond et al, 1993). In the last few years there has been a flurry of activity directed towards the development of a variety of new treatments other than TURP (transurethral resections of the prostate), to relieve bladder outflow obstruction due to BPH. The driving force behind this research and development include the wish to spare the frail and elderly male the need for surgery; and the perception that with the demographic trends towards increasing longevity, a huge potential market exists for any device or pharmacological product that can be both safe and effective.

2.1 Anatomy of the Prostate
The prostate is the largest gland in the male accessory reproductive system. Anatomically, it is the shape of an inverted pyramid, where the base (the vesticular surface) is the superior surface adjacent to the bladder, while the apex is inferior (Fig.1). The normal prostate weighs about 20 g. The prostate measures between 3 and 4 cm at its widest portion, it is 4-6 cm long, and 2-3 cm thick. The prostate is partly glandular (50-70%) and partly fibromuscular (30-50%), lying in the true pelvis below the inferior border of the symphysis pubis in front of the ampulla of the rectum. The upper end of the prostate is continuous with the neck of the bladder, and its apex rests on the superior
Fig. 1 (a) Schematic diagram of a midline section through the male lower urinary tract. The lumen of the bladder and of the urethra is dilated and the right half of the trigone (T) is shown as a surface feature. The detrusor muscle (D) is in direct continuity with the deep trigone (DT). The superficial trigone (ST) extends inferiorly as far as the verumontanum. IS, internal sphincter; the external striated urethral sphincter (ES) surrounds the membranous urethra. (b) Viewed from in front, the trigone (T) is represented as a surface feature on the luminal aspect of the trigonal detrusor thickening. ES, distal or external striated urethral sphincter; IS, internal sphincter; PS, periurethral striated muscle.
fascia of the urogenital diaphragm, the medial margins of the levator ani muscles, and the sphincter urethra muscle. The prostate is the size of a walnut and surrounds the prostatic urethra, which runs through the prostate from base to apex, making an anterior 35° angulation at the proximal part of the verumontanum (colliculus seminalis). This angulation divides the urethra into proximal and distal portions, each approximately 15 mm long.

2.1.1 Zonal Anatomy of the Prostate
Lowsley (1912) first presented a detailed description of the prostate, based on embryonic and fetal studies he proposed that the prostate was an outgrowth of five lobes, namely two lateral, medial, anterior and posterior. Franks (1954) challenged this theory and revised the zonal anatomy of the prostate. This map of the prostate was modified many times by numerous others (McNeal, 1978; McNeal, 1980; Tissell and Salander, 1975; Tissell and Salander, 1984). In 1972, McNeal proposed a concept of zonal anatomy based on histology and anatomy that is currently used as the basis for describing the location and perhaps the origin of neoplastic processes within the prostate (McNeal, 1972; McNeal, 1978; McNeal, 1980). McNeal defined four distinct regions of the prostate, each of which develops from a different segment of the prostatic urethra (Fig. 2). According to this concept, there is fibromuscular, non glandular region (comprising 1/3 of the total gland and glandular portion of the prostate (comprising 2/3 of the gland); the latter region is composed of large peripheral zone (70-80%) of the total glandular area) and a small central
Fig. 2 Diagram of a sagittal section of prostate to show anatomical subdivisions: CZ, central zone; PZ, peripheral zone; TZ, transitional zone; V, verumontanum; FS, fibromuscular stroma; D, detrusor; P, preprostatic sphincter; ES, external sphincter; ST, superficial trigone; BL, bladder lumen; U, urethral lumen.
zone, which together constitute about 95% of the glandular part of the gland. The other 5-10% of the glandular region is formed by the transition zone, located adjacent to the urethra at the verumontanum, and is composed of the periurethral glands. The fourth region, described by McNeal as the largest, is the anterior fibromuscular (non-glandular) cap, which consists of stromal and muscle tissue, partially covering the urethra and bladder neck anteriorly; this region has no glandular epithelial cells. The central and peripheral zones are not demarcated easily as separate regions, whereas the transition zone is well demarcated from the other two zones.

2.1.2 Histology of the Zones

The prostate gland is enveloped in a thin, dense, fibrous capsule (called the true capsule), which is enclosed within a loose sheath, call the prostatic sheath or the false capsule. The glandular zones of the prostate contain duct-acini systems lined by columnal secretory cells. The ducts originate near the urethra and terminate near the capsule in acini that are saccular structures with undulating borders. The ducts of the prostate arise every 2mm from the distal prostatic urethra. The ducts branch giving rise to acini. Acini appear uniformly except close to the urethra. The central zone of the prostate consists of large glands with papillary epithelium surrounded by a dense stroma. The ducts of these glands are wide and terminate into large irregularly contoured acini and the stroma is long and compact, closely associated with acini. The epithelial cells have granular darker cytoplasm and
enlarged nuclei located at various levels from basement membrane. The glands in posterior zone are small and spherical. The ducts of the glands are narrow and straight and branch into small, regular acini, these ducts eventually drain into distal urethra. The stroma is loose and randomly associated. The epithelial cells have clear cytoplasm and small, dark nuclei located uniformly along the basal aspect of basement membrane. The transition zone glands are normally the smallest glandular part and are identical to peripheral zone glands, but they are less numerous and are surrounded by dense and compact stroma.

It has been shown through numerous studies by Franks 1954; Tissell and Salander 1975; McNeal 1980 that the transition zone is involved with BPH, whereas 70-80% of prostatic cancer occurs in the peripheral zone, 10-15% in the transition zone, and 5-10% in the central zone. In normal prostates the border between the central gland and the peripheral gland is indistinct. As the transition zone (which can grow up to >90% of the gland volume) enlarges, a distinct demarcation between these two regions can readily be appreciated. With further enlargement, the transition zone can compress the central zone as well as the peripheral zone.

2.2 Histopathology of Benign Prostatic Hyperplasia.

The characteristic changes in BPH are not simply an increase in stromal and epithelial cell population, but changes in the architecture of the ducts and acini. Nodular hyperplasia which is the characteristic histological feature of
BPH, arises in two zones; the transition zone and the periurethral zone. This process of hyperplasia results in gradual and progressive encroachment on the prostatic urethra. In most cases there are both diffuse and nodular components to transition zone enlargement. The diffuse enlargement appears to be an almost universal feature of the ageing process and increases gradually from 40 year onwards. The development of nodular hyperplasia is more variable and concentrated within the transition and periurethral zones near the distal end of the bladder neck smooth muscle.

Nodular hyperplasia arising within the periurethral tissue may sometimes lead to a mass of tissue dorsally at the bladder neck (middle lobe enlargement), but this is not usually the major component causing bladder flow obstruction in BPH. The majority of periurethral nodules consists mainly of stroma with characteristic abundant pale ground substance interspersed with collagen fibres, but little or no glandular tissue. This is often associated with severe bladder flow obstruction. Unlike nodular hyperplasia in periurethral tissue nodules, transition zone hyperplasia consists of large amounts of glandular tissue which arise by branching from per-existing ducts. Although the precise etiology of BPH remains obscure, its histopathological features are consistent.

2.3.1 Prostatic Neuroendocrine Cells
Endocrine-paracrine or neuroendocrine (NE) cells are intraepithelial regulatory cells dispersed in the urethroprostatic region. They have both
neuroendocrine and epithelial characteristics. Mature nodules characteristic of BPH have substantially less NE cells and secretory products than normal prostate. Regulation of the autocrine-paracrine function of NE cells to prevent uncontrolled growth of either BPH or prostate cancer tumors requires further investigation (Di Sant' Agnese, 1992).

2.3.2 Seminal Plasma Components
Secretions of the sex accessory glands comprise about 99% of the ejaculate, with 1% of the total being spermatozoa. The majority of the ejaculatory fluid is contributed by the seminal vesicles, the prostate and Cowper's glands, in order of decreasing volume. The contents of these glands are released sequentially during ejaculation. Prostatic secretions and sperm are released in the early fraction, while seminal vesicle secretions, which consist mostly of fructose, from the later fraction (Mann and Mann, 1981). Citrate is one of the major anions in human seminal fluid (~60mEq-1) and its major source is the prostate. By secreting, instead of oxidizing citrate (due to low aconitase activity), the prostatic epithelium sacrifice about 60% of the energy potentially available from glucose (Costello and Franklin, 1991). Zinc present in high amounts in prostate cell (140μg/ml) plays a role in aconitase inhibition (Costello and Franklin, 1998). During malignant transformation, intracellular zinc concentration in prostate is decreased, permitting citrate oxidation to proceed like in non prostatic cells. Prostate cancer cells have dramatically lower levels of citrate than normal or BPH cells (Costello, 1998). Prostatic
fluid is the richest source of spermine (polyamine) in the body, resulting in seminal fluid concentrations of 50-350 mg/100ml. Changes in polyamine concentrations with malignant transformations have been identified (Cipolla et al, 1996). Changes in cellular membrane lipid characteristics as a result of BPH effects DHT production by affecting membrane fluidity, lipid-to protein ratio. Recent evidence confirms that androgens co-ordinately enhance the expression of lipogenic enzymes that stimulate fatty acid and cholesterol synthesis (Swinnen et al, 1997a). Swinnen et al, 1997a have shown that androgens increase the expression and activation of transcription factors that affect the sterol regulatory element binding proteins (SREBPs). The hormone sensitive prostate cancer cell-line, LNCaP, responds to androgen treatment by increased expression and activity of fatty acid synthetase (FAS) resulting in intra cellular lipid (Swinnen et al, 1997b). Cancer cells that lack androgen receptors (PC-3 and DU-145) do not show this response.

2.3.3 Other Secretory Proteins

In 1971 Hara et al identified what they considered a protein unique to semen. In 1973, Li and Beling isolated and purified this protein and Wang et al in 1978 characterized it as a semen –specific protein and referred to it as PSA (prostate specific antigen). In 1987, Stamey and associates at Stanford University published the first definitive clinical study investigating the utility of PSA in prostate cancer. Before PSA was available and understood, the only methods to detect this disease was the digital rectal examination (DRE) and
the serum marker prostate acid phosphatase. With these diagnostic tools, nearly 75% of men diagnosed with prostate cancer already had clinically metastatic disease. Currently the men diagnosed with metastatic disease is less than 5%. The reason for this dramatic change is the PSA test and the use of ultrasound-guided prostate biopsies. Because of PSA testing, 30-50% of patients with clinically significant prostate cancer can be diagnosed before the disease can be palpated in the prostate; in more than 90% of patients, prostate cancer can be diagnosed before symptoms occur. Since the test was introduced into clinical practice in 1986, the early diagnosis and management of prostate cancer has been revolutionized. PSA testing not only helps in the early diagnosis but also assists in assessing the response to therapy, determining tumour progression, and, in its most controversial role, screening for prostate cancer.

PSA is a 33-kD protein consisting of a single chain glycoprotein of 237 amino acid residues, 4 carbohydrate side chains, and multiple disulfide bonds. PSA is homologous with the proteases of the kallikrein family. PSA is a neutral serine protease with biochemical attributes that are similar to the proteases involved in blood clotting. The ejaculate primarily contains free prostate-specific (fPSA) in a concentration of 1 million ng/mL. When serum PSA is bound to alpha1-anti-chymotrypsin (ACT), 2 epitopes are left unmasked and can be detected by immunoassays. By measuring PSA before and after transurethral resection of the prostate, Stamey et al (1987) were able to calculate the amount of PSA per gram of benign prostatic tissue. Comparing
the weights of resected tissue and the change in serum PSA, the PSA in ng/mL/g of hyperplastic tissue was 0.31 plus or minus 0.25. The interpretation of PSA may vary according to the amount of BPH tissue and the epithelial–stromal ratio. Most PSA is produced in the hyperplastic TZ of the prostate. A relatively small amount of PSA is produced in PZ where 80% of prostate cancers originate. The PSA produced by cancer cells may vary according to the grade of the cancer. A Gleason grade 5 prostate cancer produces less PSA than a grade 3 cancer. Some patients with advanced prostate cancer may have low or undetectable PSA levels.

Sutkowski et al (1999) suggested that PSA may regulate the volume of stromal tissue in men with BPH. PSA represents a major indicator for the diagnosis and management of prostate cancer. However, within the range of 4-10 ng/mL, in which 75% of men do not have cancer, the PSA lacks specificity. After the development of an immunoassay, investigators demonstrated that the ratio of free to total PSA (fPSA/tPSA) was lower in men with prostate cancer. In the PSA range of 4-10, tPSA segregates adequately between men with or without cancer.

PSMA (Prostate-Specific Membrane Antigen). Although it is not a secretory protein, prostate specific membrane antigen is a very important membrane bound protein of the prostatic epithelial cell. This trans membrane glycoprotein of 100,000Mw is found on the surface of prostatic epithelial cells (Troyer et al, 1995). PSMA is a protein that can be distinguished from PSA. It is a selective marker for prostate epithelial cells and is expressed to a greater
extent than PSA in higher grade cancers. Bostwick et al (1998) found that
70% of benign epithelium, 78% of prostate intraepithelial neoplastic cells and
80% of invasive cancer cells express this marker.

The development of immunoassays for PSMA has permitted an increasing
number of studies. PSMA levels seem to correlate with stage and tumour
volume. Following radical prostatectomy, PSMA levels become undetectable
and rise when the tumour recurs. PSMA primers used in reverse transcriptase
polymerase chain reaction (RT-PCR) have been used to detect circulating
prostate cancer cells. This method detects as few as 1 tumour cell in 10
million lymphocytes. The use of this method for clinical decision making is
limited. With this technology cancer cells can be identified in the circulation
and in the bone marrow of patients with all stages of prostate cancer. This
indicates that cancer cells begin leaving the prostate early in the
development of the disease, but most of these cells do not survive, and their
identification does not correlate with patient prognosis and survival. PSMA
serves as the basis for the ProstaScint scan. This is an imaging study used to
detect metastatic cancer. Its primary use has been to identify prostate cancer
cells in lymph nodes and in the prostate base. A monoclonal murine IgG1
antibody (7E11-Cs) prepared against purified PSMA recognizes the antigen
on prostatic epithelial cells (Horoszewicz et al, 1997). 7E-11-Cs
immunoreacts weakly with normal and BPH prostatic epithelium and strongly
with malignant epithelium from the prostate, and does not react with other
tumours and normal tissue, this antibody linked to indium (in-CYT-356) is
investigated as an imaging tool to localize prostate cancer metastases and disease recurrence following radical surgery.

- HPAP (prostatic acid phosphatase) is a secretory enzyme in the human prostate with high enzymatic activity. It is a glycoprotein dimer of 102,000 M\textsubscript{W} and contains about 7\% by weight of carbohydrates (Chu et al, 1997).

- Leucine aminopeptidase is another product of prostatic epithelial cells and is secreted into the lumen of acini.

1.4 Hormonal Control of Prostate Growth and Function

1.4.1 Androgen effects on Prostate Cells

Studies from the early 1970s have show that hypothalamus, anterior pituitary and the gonads (testes or ovaries) comprise an endocrine "loop", which is responsible for maintaining the appropriate levels of steroid hormones in the serum and target tissues. The prostate requires the presence of adequate levels of circulating testosterone in order to develop and grow (Isaacs and Coffey, 1981). This effect is depicted schematically in Fig. 3. The decapeptide Luteinizing Hormone Releasing Hormone (LHRH) is released in a pulsatile fashion from the hypothalamus and stimulates the pituitary to secrete Luteinizing Hormone (LH). LH then in turn acts directly on the Leydig cells within the testis stimulating them to secret 95\% of the 6-7 mg of testosterone produced in the body each day. The remaining 5\% of daily testosterone production is either directly synthesized by the adrenal gland or produced by peripheral metabolism.
Fig. 3 ‘Endocrine loop’ showing the hormonal control of Prostate growth and function.
In the bloodstream, 98% of circulating testosterone is bound to several plasma proteins. The most important are human serum albumin (HSA) and sex hormone-binding globulin (SHBG). As a consequence only the remaining 2% or so of the free testosterone is available to enter prostatic cells, doing so by simple diffusion. Two forms of the enzyme 5α-DHT reductase, present on the nuclear membrane of cells of many tissues, convert testosterone to 5α-DHT (dihydrotestosterone). The Type 1 isozyme is present in liver, skin and other tissues and contributes to circulating levels of DHT. The Type 2 isozyme predominates in urogenital tissue. Individuals with inherited deficiencies in the Type 2 isozyme do not develop normal external genitalia or prostates (Silver et al, 1994). In the prostate, most of the DHT is produced in the epithelium, while the majority of the androgen receptors (ARs) reside in the stromal compartment.

The metabolite DHT is a considerably more potent androgen within the prostate than testosterone by virtue of its greater affinity for the androgen receptors within the nucleus. Binding of DHT to these receptors produces a conformational change in the chromatin, which facilitates transcription of specific sequences of DNA into mRNA. This sets off a complex orderly series of events, including signal transducing protein synthesis, rRNA production and finally cell replication (Coffey, 1986). Administration of DHT to 24 male pseudohermaphrodites with vestigeal prostate and normal serum testosterone levels (Imperato-McGinley et al, 1974) resulted in prostatic growth. This data
lead to the conclusion that DHT is the key androgen, modulating prostatic growth with testosterone simply acting as a prohormone.

2.4.2 Estrogen effects on Prostate Cells

The effects of estrogen and testosterone on the sex accessory glands are antagonistic. Estrogens can act directly on the prostate, with similar effects as seen with castration, or can act indirectly via the hypothalamus or pituitary suggesting that it plays an important role in the development of BPH (Kumar and Majumdar, 1995; Suzuki et al, 1995). Administration of estrogen to normal male rat results in a decrease in size and function of the sex accessory glands, epithelial cells, probably by a reduction in testosterone synthesis via a negative feedback to reduce LH release (Suzuki et al, 1995).

2.4.3 Prolactin effects on Prostate Cells

Prolactin receptors have been detected in prostatic epithelial cells. Prolactin has a synergistic action on androgen induced weight gain and citrate secretion by the lateral prostate. It also stimulates accumulation of testosterone and DHT by the epithelial cells (Kumar and Majumdar, 1995). Transgenic mice overexpressing the prolactin gene were found to have dramatic enlargements of the prostate, increased DNA content, increased secretory fluid and increased levels of testosterone (Wennbo et al, 1997). This suggests that prolactin may play a role in prostatic hyperplasia through direct actions on the prostate or via stimulating increased circulating testosterone.
2.5 Stromal – Epithelial Interactions

BPH contains a considerable amount of fibromuscular stroma, and Franks and Barton (1960) were the first to suggest that the growth of epithelial cells might be stimulated in some way by prostatic stroma. This has been termed 'epithelial reawakening'. Subsequently Cunha (1972), in an impressive series of experiments recombined isolated mouse urogenital sinus mesenchyme (embryonic prostatic stroma) with adult mouse bladder epithelium and transplanted these combined tissues beneath the capsule of the kidney of the nude mouse. With the testis intact the epithelial cells differentiated and developed into mouse prostatic epithelium, an effect not seen when the nude mice were castrated. When similar recombination's were made using the same urogenital sinus mesenchyme, but epithelium from mice with testicular feminization syndrome, the epithelial tissue still differentiated and grew in intact nude mice. No development of prostatic epithelium occurred, however if embryonic stroma from mice with testicular feminization syndrome was combined with normal bladder epithelium. These experiments clearly demonstrate that differentiation and development of prostatic epithelium are indirectly controlled by androgens through androgen-dependent mediators of stromal origin.

Stromal mediators (i.e., growth factors) such as Epidermal growth factors (EGF) (Davies & Eaton, 1989), insulin – like growth factor (IGF) and fibroblast growth factor (FGF) (Mori et al, 1990) are characteristic of tyrosine kinases and all show increased gene expression in BPH tissue and ‘they’ not
androgens exert a marked mitogenic effect on prostatic epithelial cells in vitro (McKeehan et al, 1998). TGF β (transforming growth factor) induces fibroblast proliferation, while inhibiting that of epithelium, and it can specifically abolish EGF activity, however βFGF can prevent inhibitory effects of βTGF. TGFα and EGF have 35% sequence homology and have been found to promote proliferation and development of prostate epithelium (Steiner, 1993; Massague, 1990). Castration results in a decrease in EGF and FGF expression, while EGF receptor is upregulated in an attempt to maintain growth. At the same time, TGF β1 and its receptor levels are increased, thereby preventing epithelial cell proliferation and possibly initiating apoptosis, the net effect of androgen deprivation. In BPH, β FGF and TGF β2 expression is elevated, while EGF, the EGF receptor, and TGF-β1 remain constant. β FGF is thought to be the major factor in BPH development, causing both the epithelium and mesenchyme to proliferate. Since TGF-β2 can stimulate the mesenchyme, induction of both these GF may result in glandular module. The overall ratio of TGF-β2 and β FGF may determine whether BPH is predominantly glandular or stromal.

2.6 Regulation of Cell Growth

Cell growth in the normal prostate is regulated by delicate balance between cell death and cell proliferation (i.e., apoptotic vs. proliferative activity). Disruption of molecular mechanisms that regulate these two processes may underline the abnormal growth of the gland leading to BPH. BPH could be a
stem cell disease and not due to an increase in cell replication but rather be caused by a decrease in cell death (Kyprianou, 1996).

2.7 Medical Treatment Options:-

The idea of 'pill' for the prostate has long been an alluring one for patients, but the concept has been viewed with scepticism by surgically oriented urologists for many years. Data are however accumulating to suggest that a number of pharmacological agents may have reasonable safety and efficacy in the long term treatment of obstructive benign prostatic hyperplasia.

2.7.1 \(\alpha\)-Adrenoreceptor Blockers

\(\alpha\)-1A, a subtype of the \(\alpha\)-1 receptor is predominant in the prostatic smooth muscles and forms 70\% of these receptors (Caine et al, 1975b). The scientific rationale for using \(\alpha\) blocker therapy is based on the fact that a) prostatic smooth muscle contracts with \(\alpha\) receptor sympathetic stimulation b) contraction of smooth muscles in the prostate capsule and bladder neck results in decreased outflow \(\alpha\)-adrenergic blocking drugs such as short acting phenoxybenzamine, prazosin and long acting terazosin, doxazosin, alfuzosin and are used for the management of BPH (Kirby, 1995).

The downside of these agents is that they are also approved for use in the treatment of hypertension, and therefore there is an incidence of postural hypotension with flu-like syndrome. They also have additional disadvantages in that they need to be titrated up to the optimal dose over a period of 3-5 weeks. While most \(\alpha\) blockers do not lower the blood pressure 4further in
patients who are normotensive, there is a fear of this event occurring and therefore titration of other antihypertensives needs to be watched while patients are on \(\alpha\)-blockers.

\(\alpha_1\) adrenoreceptor antagonist Tamulosin (Flomax\textsuperscript{R}, Boehringer Iganelheim, Pharmaceuticals) having 10-12 times higher selectivity for prostatic receptor subtypes devoid of such side effects has been introduced (Kawabe, 1995). However the side effect of Tamulosin are abnormal ejaculation and dizziness.

2.7.2 5 Alpha Reductase Inhibitors: 
Since DHT rather than testosterone is the main intracellular androgen modulating prostate growth, a drug that could effectively inhibit 5\(\alpha\)-reductase would reduce intraprostatic DHT levels and prevent development of BPH. Finasteride a neutral – azasteroid synthesized by Merck & Co acts as a pure 5\(\alpha\) reductase type inhibitor without detectable androgen receptor blocking properties (Rasmusson et al, 1984).

2.7.2.1 Other 5 \(\alpha\) Reductase Inhibitors.
The compound permixon, which is the hexane extract of the fruit of the American dwarf palm tree (\textit{Serenoa repens} \textit{B}) has been reported to produce 5\(\alpha\) reductase inhibition (Stenger et al, 1982). However Champault et al (1984) were unable to demonstrate any significant difference in effect from placebo.
2.7.3 Endocrine Therapy for Benign Prostatic Hyperplasia

John Hunter, regarded by many as the father of modern scientific surgery, observed in the eighteenth century that prostate atrophies after castration. Stimulated by this observation Cabot (1896) performed bilateral orchiectomy for the treatment of bladder outflow obstruction due to BPH in 79 patients and reported an improvement in 80%. Huggins and Stevens (1940) reported three cases of BPH treated by castration. In case one, the prostate was removed 29 days after castration, when there was no change. In the other two patients, the remaining gland was removed 86 and 91 days respectively after castration; both microscopic and macroscopic evidence of glandular atrophy was evident. Following this, Peirson (1946) reported shrinkage of the prostate with stilboestrol therapy in 10 of 13 patients, and noted symptomatic improvement in 70% of patients. Subsequently, Kaufman and Goodwin (1959) tried a combination of testosterone propionate and diethylstilboestrol (DES) in 42 patients with BPH, they noted an improvement in symptoms and uroflow, as well as histological evidence of prostatic epithelial atrophy.

2.7.3.1 Androgen Receptor Inhibitors

Antiandrogen drugs bind to androgen receptors on target tissues, thus preventing the stimulating effects of exogenous or endogenous androgens. At the same time, they exert no such action of their own. In the treatment of prostate cancer, antiandrogens block the stimulating effect of testosterone on cell growth.
The first antiandrogen drug was cyproterone acetate, a synthetic steroidal antiandrogen discovered in the early 1960s. Scott and Wade (1969) described the use of cyproterone acetate in 13 patients with BPH and noted improvement in more than half of the patients who received the drug. Cyproterone also possesses progestational activity that provides negative feedback to the pituitary, causing a decrease in LH release. Although claimed to be as effective as orchiectomy or estrogen, cyproterone offered no survival advantage and its steroidal side effects made it less desirable.

To circumvent steroidal side effects, researchers began developing non-steroidal antiandrogens such as Flutamide, in the 1970s. Caine et al (1975a) reported improvement in uroflow with Flutamide. Without steroidal side effects, flutamide blocks the binding of testosterone to the cytosolic androgen receptor and/or inhibits the nuclear binding of androgens in target tissues. For this reason, it was termed a "pure" antiandrogen. As a single agent, this drug produces significant benefits in some patients including relief of metastatic bone pain, reduced primary tumour size and improved urine flow. However because of its short half life (a 250 mg dose is completely metabolized in 8 hours) relatively frequent dosing (250 mg every 8 hours) is required. Flutamide treatment for BPH has shown some promise in some clinical trials (Stone et al, 1989). However, the high incidence of adverse effects has caused many participants in these studies to drop out. These adverse effects, such as diarrhoea, breast tenderness and gynecomastia
were the limiting factors in this study, until these problems are overcome, flutamide will remain an investigational drug (Narayan et al, 1996).

Nilutamide is another pure antiandrogen that, like flutamide, has no androgen agonist properties. The long half life (approx. 46 hours) of this drug allows once a day dosing (Tremblay, 1987). Nilutamide has been tested extensively in combination with other treatments that cause either medical or surgical castration. Clinically significant adverse effects associated with therapy include pulmonary fibrosis and light-dark adaptation (Ojesoo, 1987).

One extremely potent pure antiandrogen discovered in the 1980s is ICI 176,334 (Casodex, ICI). In rats, the binding of ICI 176,334 to prostate receptors is 50-fold less than that of 5-α DHT but fourfold greater than hydroxyflutamide. It is 5-10-fold more potent than flutamide in inhibiting the action of exogenous testosterone in castrated rats and endogenous testosterone in intact rats (Furr, 1988). In multicentre dose sighting study ICI 176,334 was generally well tolerated; the most common adverse events were nausea and/ or vomiting, breast tenderness, gynecomastia and hot flushes (Denis, 1989).

2.7.3.2 Exogenous Estrogens
A highly effective nonsurgical alternative for suppressing plasma testosterone levels has been the administration of exogenous estrogens. Estrogens block
the release of LH from the pituitary, thus breaking the hormonal chain that leads to the production of testosterone by the Leydig cells in the testis.

- One of the most commonly used estrogens has been diethylstilbestrol (DES). At a oral dose of 3mg or more, DES produces castration levels of testosterone within 7-21 days (Shearer et al, 1973). In general DES has a palliative effect equivalent to that of orchiectomy. The side effects associated with DES are potentially serious including thromboembolism and cardiovascular complications (The Veterans Administration Cooperative Urological Research Group, 1967), virtually nullifying any survival benefit of estrogen treatment.

- Several other hormonal interventions have also been tried with varying degree of success. Estramustine, an estrogen complexed to nitrogen mustard moiety is thought to have a direct cytotoxic action on tumor cells and also inhibits prostatic cell growth by binding to protein receptors in cytoplasm (Trachtenberg, 1987). Early studies reported a high response rate to estramustine as first line therapy (Pavone-Macaluso, 1986) but overall it is no better than DES with similar side effects (Trachtenberg, 1987). Although drugs other than DES were used extensively for the treatment of advance prostatic cancer, none can be said to have gained universal acceptance. Such compounds included other estrogens such as long acting polyestradiol phosphate (Estradurin® Wyeth-Ayerst), ethinylestradiol, and Premarin® (Wyeth-Ayerst) a mixture of conjugate equine estrogens. Various progestational steroids, such as medroxyprogesterone caproate (Provera®,
Upjohn), hydroxyprogesterone caproate (Delautin®, Squibb), chlormadinone acetate and cyproterone acetate (Androcur®, Schering) also have been used for the treatment of advanced disease; however none can be said to have an established role in the management of prostate cancer.

Megestrol acetate (Megase®), an antiandrogen approved for the treatment of breast cancer, has been studied as a treatment for prostate cancer because it competitively bind to the DHT receptor, blocks 5α reductase and also inhibits LH release (Geller, 1976). Its favourable effects on prostate cancer (≥ 70% overall response) and its tolerability by patients has been shown in several small studies when megestrol doses of 120-160 mg per day were given as initial hormone therapy (Bonomi et al, 1985). A limitation of megestrol therapy is the gradual increase in testosterone levels after 6 months of treatment (Geller et al, 1978).

2.7.3.3 Aromatase Inhibitors

Aromatase is a enzyme complex responsible for converting androgens to estrogens. The best known and most widely used aromatose inhibitors are aminogluthethimide and ketoconazole (antifungal agent). Ketoconazole is used to avoid the increase in plasma testosterone levels that sometimes occurs early during treatment with LHRH agonists (Wenderolt and Jacobi, 1983) but again side effects predominate like hepatitis, which is reversible on discontinuation of the therapy but occasionally may be progressive and fatal.
2.7.3.4 Combination Therapy

The two proven safe and effective strategies for the pharmacological treatment of BPH involve decreasing prostatic-tone with $\alpha$-adrenoreceptor blockers or reducing prostatic volume with antiandrogen therapy. Lepor and Machi (1992) compared terazosin alone versus flutamide and found that flutamide added little in terms of efficacy but caused considerable toxicity in the form of diarrhoea and gynaecomastia. A more logical and less toxic combination would be an $\alpha$ 1-selective blocker plus 5$\alpha$ reductase inhibitor Finasteride. Lepor et al (1996) completed a study of terazosin and finasteride combination. Linasteride did not significantly improve symptoms, despite reducing the size of the gland.

2.7.4 LHRH Analogues

Surgical castration ‘orchiectomy’ remains the ‘gold standard’ against which other alternative forms of therapy must be measured. Surgical castration, however has serious psychologic overtones for many men. The past decade has been dominated, however, by the introduction of a new form of medical castration, namely the use of innovative LHRH analogues as primary endocrine therapy. Physiologic doses of the analogues were show to mimic the action of LHRH, whereas long term administration produced antigonadal effects. The result is persistent suppression of Leydig cell function and a subsequent fall in serum testosterone and 5$\alpha$ DHT (Schally and Comaru Schally, 1987; Dutta and Furr, 1985).
LHRH analogues are absorbed poorly and are weakly active when administered orally. Therefore, a convenient effective route of administration that would promote compliance has been a primary goal of researchers. Among the routes of administration that have been tried are daily subcutaneous and i.m injections, nasal sprays, percutaneous and i.m depots. Buserelin was the first LHRH agonist formulated as a nasal spray and studied clinically (Sandow, 1983) followed by nafarelin (Anik et al, 1985). However its use leads to non compliance in elderly patients.

One of the earliest LHRH agonist administered by s.c injection and tested clinically was leuprolide (Smith et al, 1985). In a randomised study of 199 patients with metastatic prostatic cancer, the response to treatment with leuprolide (1mg daily) was equivalent to that of DES (3mg daily) but with less incidence of side effects (The Leuprolide Study Group, 1984). To be the most effective drugs in this class require some type of long acting formulation. Currently in clinical use is a 7.5 mg depot form of leuprolide injected i.m once a month. In an uncontrolled trial, the Leuprolide Study Group compared 1-mg and 10-mg daily subcutaneous doses of leuprolide and found no dose-response effect. During the first 6 months of the study, 86% of patients had at least partial response to treatment (Sharifi and Soloway, 1990). Despite much Phase II data on this dosage form of leuprolide there are no published controlled Phase III trial comparing it with standard therapy.

One long acting formulation of an LHRH analogue that is effective and for which extensive data are published is Goserelin (ZoladexR, ICI, Wilmington
DE) as 3.6 mg depot formulation (50:50 PLGA) (Perren et al, 1986). Preclinical investigations of depot Goserelin indicated a potency 50-100 fold greater than that of naturally occurring LHRH (Furr, 1989). Preliminary animal pharmacology studies have confirmed that this drug acutely stimulates gonadotropic secretions but it inhibits these secretions when given chronically (Maynard and Nicholson, 1979; Furr and Nicholson 1982). In subsequent investigations the drug inhibited the growth of transplantable prostate tumours in male rats and caused predictable reversible involutional changes in reproductive organs (Furr, 1989). Typically, serum testosterone levels rise during the first week of depot Goserelin therapy; then they gradually decrease to castration levels by day 22. LH tends to reach peak levels within 24 hours after the first injection of depot Goserelin. Within the first week, LH levels drop back to their initial level and maximum suppression of LH occurs after 3 weeks (Denis et al, 1987). Subsequent injections of the drug cause no change in the LH level. A Phase III randomized clinical trial inviting 187 urologic clinics of British Prostate study group indicated that administration of Zoladex\textsuperscript{R} (3.6 mg monthly) was as effective as orchiectomy for the treatment of prostate cancer (Kaisary et al, 1988:89)

2.7.5 Active immunization against LHRH

LHRH controls the synthesis and release of the pituitary gonadotropins (Schally et al, 1973), which in turn regulate gonadal steroidogenesis, sperm production, follicular development and ovulation (Conn et al, 1987).
Immunization against the hormone prevents circulating LHRH from binding to pituitary receptors and hence blocks further interaction and physiological events, by immunoneutralization (Meloen et al, 1995).

Andrew V. Schally in 1971 isolated LHRH hormone from the hypothalamus, after isolating LHRH from 160,000 hypothalami and determined its structure. Schally was rewarded for his contributions when he received the Noble Prize for Physiology and Medicine in 1977. Schally recognized that active immunization against LHRH was a potential means by which the reproductive system of mammals might be shut down for various practical and clinical reasons. The application of LHRH neutralization is far reaching in human and animal therapies and anti-LHRH vaccines are being developed for the treatment of hormone dependant malignancies and non-surgical castration (Talwar et al, 1992; Ferro et al, 1995).

LHRH being a "self" molecule to the immune system is non immunogenic and requires chemical linkage to a 'foreign' carrier molecule to mobilize T helper cell function to activate B cells to produce antibodies. Native LHRH molecule has a pyroglutamic acid (<GLu) at the N-terminal and Gly-NH$_2$ at the C-terminal. Chemical linkage to a foreign carrier, an essential step for making LHRH immunogenic, can only be carried on through hydroxyl group of Tyr$^5$ or Ser$^4$ or through carboxylated derivative of His$^2$ using coupling agents such as ECDI (1-ethyl-3-3 dimethylaminopropyl) carbodimide. Such conjugates are, however ill defined and have inconsistent immunogenicity. Conjugates made
through the N-or C-terminal have been reported to be immunogenic. The
carrier conjugate at N-terminal is the vaccine developed by Ladd et al (1988)
at Population Council of India. The vaccine developed by Talwar et al (1992)
has utilized the amino acid position at position 6, (Gly^6) has been replaced by
D-lysine this was linked to amino caproic acid, a spacer molecule to which the
carrier DT/TT was attached via its E-amino group. This replacement has a
stabilizing effect on the molecule without affecting the receptor recognition
site and also makes it refractory to catabolic cleavage (Gupta et al, 1993).
The vaccine generates antibodies in rats with concomitant decline of
testosterone. The prostate undergoes drastic atrophy, which reverses with
decline of antibodies (Jayshankar et al, 1989). These finding were confirmed
by Rovan et al (1992), who studied also the histology of testis, epididymis,
seminal vesicles and prostate at various stages of the action of the vaccine.
While the size and the function of the testis is fully recovered on decline of
antibodies, the weight of the regenerated prostate stays somewhat lower than
in the controls.
The vaccine induces also hypotrophy of prostate in monkeys (Girl et al,
1991). Acute, subacute and chronic toxicology studies in rodents and
monkeys showed that the vaccine was safe and devoid of side effects. The
vaccine has also been tested on Dunning tumour cells *in vitro* and *in vivo*. It
inhibits very effectively the growth of Dunning R3327-PAP cells *in vitro* and in
rats. (Fuerst et al, 1997). This clone of the Dunning tumour is androgen
dependant. Immunization with the vaccine inhibited partially (but significantly)
the growth of the androgen independent Dunning Cells R3327-AT 2.1. No such inhibition of proliferation of these cells was seen in orchiectomized rats with castration levels of testosterone. With the permission of the Regulatory Authorities, Phase-I/II clinical trials were conducted with this vaccine in 28 patients of advanced stage carcinoma of prostate in two centres in India (All India Institute of Medical Sciences, New Delhi and Post Graduate Institute of Medical Education and Research, Chandigarh) and one centre in Austria (General Hospital, Salzburg). The vaccine given at 200 & 400 μg per dose was well tolerated by all patients and no undesirable reactions attributable to the vaccine were observed. 400 μg dose was more immunogenic than 200 μg. Patients generating antibodies above 400 pg/ml benefited clinically as reflected by decline in PSA (prostate specific antigen) and acid phosphatase levels. Serial ultrascans showed diminution of prostate tissue mass (Talwar et al, 1999).

This section describes in brief the numerous studies, performed since 1970’s to determine the applicability of the technique as an alternative to surgical removal of the testes.

Clarke et al (1978), reported the active immunization of ewes against LHRH, and its effects on ovulation and gonadotrophin, prolactin and ovarian steroid secretion. Three Scottish Blackface ewes were immunized against LHRH conjugated to bovine serum albumin (BSA) and three control ewes were immunized against BSA alone. When the antibody titre to LHRH was raised
the treated animals failed to show oestrus or ovulation; they had significantly lower levels of plasma luteinizing hormone (LH) and higher levels of prolactin than the controls, whereas the levels of follicle-stimulating hormone (FSH) were unaltered. Similar results were obtained by McNeilly et al (1986) who showed that active immunization of 6 Damline ewes against LHRH during seasonal anoestrus resulted in an inhibition of ovarian cyclicity throughout 2 subsequent breeding seasons.

Schanbacher, (1982) reported that active immunization of young ram lambs against testosterone and luteinizing hormone-releasing hormone (LHRH) was shown to block the growth attributes characteristic of intact ram lambs. Testosterone and LHRH-immunized lambs grew at a slower rate and converted feed to live weight gain less efficiently than albumin-immunized controls. LHRH immunoneutralization effectively retarded testicular development and produced a castration effect in young ram lambs. Similar results were reported by Kiyma et al (2000) who studied the effect of active immunization against LHRH on production, carcass, and behavioral traits in ram lambs fed to a uniform slaughter weight. In these studies ram lambs were untreated, castrated, or actively immunized against LHRH using a LHRH -keyhole limpet hemocyanin conjugate (1 mg) emulsified with either Complete Freund's adjuvant (CFA) or another oil-based adjuvant (ISA). Animals were housed individually and slaughtered at 58 kg body weight. Immunoneutralization of LHRH reduced testes weight and the concentration
of testosterone in serum at slaughter. Suppression of testicular size and function was most clearly evident in animals immunized with LHRH-KLH using CFA. Final anti-LHRH titer was also highest in lambs immunized using CFA. Immunization against LHRH decreased testicular weight and reduced feedlot performance and sexual behavior to levels comparable to those of castrated males.

Lincoln et al. (1982) reported the studies of antler growth in male red deer (Cervus elaphus) after active immunization against LHRH. Four sexually mature male red deer were actively immunized against LHRH and this caused 3 of the animals to cast their antlers prematurely in the autumn instead of the spring. Development of new antlers was initiated after casting, but the effects on the antler cycle were variable and correlated with the antibody titre, only the animal with the highest titre developed antlers that resembled those of a castrate and remained 'in velvet' for more than 6 months. In October, when all the immunized deer had peak circulating levels of LHRH antibodies, the testes were reduced in size compared to the maximum values of the controls. The changes in the testis confirmed that the immunizations were effective in blocking the secretion of the gonadotrophic hormones.

Five female macaque monkeys (Fraser, 1983) with regular menstrual cycles were immunized against LHRH conjugated to tetanus toxoid (TT). The conjugate was given in CFA. Elevated levels of LHRH antibody were
associated with an absence of preovulatory luteinizing hormone (LH) surges in serum and an inability to respond to an injection of oestradiol benzoate which produced an LH surge (positive feedback) in control animals. Effects on LH and FSH caused inhibition of follicular development and ovulation, which resulted in amenorrhea.

Esbenshade & Britt (1985) reported the active immunization against LHRH in sexually mature gilts. Sexually mature gilts were actively immunized against LHRH by conjugating LHRH to BSA, emulsifying the conjugate in CFA, and giving the emulsion as a primary immunization at Week 0 and as booster immunizations at Weeks 10 and 14. Antibody titers were evident by 2 wk after primary immunization and increased markedly in response to booster immunizations. Active immunization against LHRH caused gonadotropins to decline to nondetectable levels, gonadal steroids to decline to basal levels, and the gilts to become acyclic. Circulating concentrations of LH and FSH increased after ovariectomy in the controls, but remained at nondetectable levels in gilts immunized against LHRH. Prolactin concentrations did not change in response to ovariectomy. The authors concluded that cyclic gilts can be actively immunized against LHRH and that this causes cessation of estrous cycles and inhibits secretion of LH, FSH, and gonadal steroids.

Falvo et al (1986) reported the effect of active immunization against LHRH or LH in boars: Forty crossbred boars were equally divided into eight groups at birth. Four groups were immunized (200 μg/boar) at 12 wk of age against
either (LHRH) conjugated to human serum globulin (LHRH-hSG) in CFA, LHRH-hSG in muramyldipeptide adjuvant (PEP), procine (LH) conjugated to hSG (pLH-hSG) in CFA or ovine LH (oLH) in CFA. Equal doses of boosters were given in either PEP or incomplete Freund’s adjuvant (IFA) at 16 and 18 wk of age. By wk 16, LHRH antibody titers began to rise in those boars immunized against LHRH-hSG. LHRH antibody titers on wk 18, 20 and 22 were greater than those at wk 16. By 22 wk of age, LHRH-hSG boars had non-detectable plasma LH and T and reduced weights of testes and accessory sex glands. Boars immunized against oLH did not respond to treatment, whereas pLH-hSG boars showed a reduction in serum T levels and accessory sex gland weights. Immunization had no effect on average daily gain, hot carcass weights or loin eye area.

Safir et al (1987) investigated the hypothesis that the onset of the breeding season in the mare may be due to a daylength-induced seasonal increase in LHRH pulse frequency. 5 mares were immunized against LHRH. The absence or occurrence of ovulation in LHRH-immunized mares appeared to be related to antibody titre, such that the highest antibody titres were observed in those mares that remained anovulatory throughout the experimental period. These results confirmed that observation, in the mare, the onset of the breeding season is associated with an increase in LH pulse frequency. Furthermore, the results suggested that the increase in LH pulse frequency reflected an increase in pulsatile LHRH release from the hypothalamus. Similar results were obtained by Garza et al (1988) in five
lighthorse mares; Dalin et al (2002) who investigated the effect of active immunization against LHRH in mature Standardbred mares (three experimental and one control mare) on antibody titres, ovarian function, hormonal levels and oestrous behaviour.

Active immunization of heifers against ovalbumin (OV) conjugates of LHRH was investigated by Johnson et al (1988). Significant levels of circulating LH or LHRH antibodies were detected in heifers immunized with each of the hormone conjugates. CFA was the most effective for stimulating antibody response. None of the heifers in the LHRH-OV-CFA immunization groups turned pregnant when placed with bulls, whereas 71% of the OV-CFA control heifers were pregnant.

Reproductive function and feedlot performance of beef heifers actively immunized against LHRH was investigated by Adams and Adams (1990). Heifers were not immunized or were immunized with one of three doses of a LHRH-KLH (keyhole limpet hemocyanin) conjugate in CFA. Antibodies against LHRH were not detectable in non-immunized heifers (n = 9). However, antibodies against LHRH were noted in all immunized animals (n = 30) within 8 wk of primary immunization; anti-LHRH antibody concentrations were at a maximum 16 to 20 wk after immunization. This increased anti-LHRH titer was associated with a decreased serum concentration of progesterone. Ovarian and uterine weight and tissue concentrations of LH and LHRH receptor were reduced by immunoneutralization of LHRH.
Similarly, immunization against LHRH reduced weight gain during feedlot confinement. The depression of weight gain that attends development of anti-LHRH titers may be reversed by use of implants that contain anabolic steroids.

Silversides et al (1988) synthesised cysteine substituted analogues of (LHRH) coupled to carrier molecules. Vaccines were formulated and tested in BALB/c mice for titer development against LHRH. Adjuvants, carrier molecules, dosage and peptide to carrier ratio were considered. Dosages of 50 µg conjugate per immunization per mouse, at conjugation ratios of 3-12 peptides per 10\(^5\) Da carrier molecule, were found to produce immune responses. Adjuvants including Havlogen and dimethyldioctadecylammonium bromide (DDA), and carrier molecules including keyhole limpet hemocyanin (KLH), porcine thyroglobulin (TGB) and equine gamma globulin (EGG) were all found to be effective. The immunobiology of LHRH was explored, to provide a conceptual and practical basis for the use of LHRH for immmucastration (Silversides et al, 1988b).

Studies by Population Council (Ladd et al,1988) with the carrier conjugate at N Terminal of the vaccine developed by Talwar et al showed that active immunization against LHRH combined with androgen supplementation is a promising antifertility vaccine for males. Immunization against LHRH10-TT effectively suppressed fertility (spermatogenesis) in rats and rabbits. The possibility of immunological suppression of spermatogenesis while normal
libido is maintained by exogenous androgen supplementation was tested in male rats (Ladd et al, 1989). Male rats and rabbits were immunized against (LHRH) conjugated to tetanus toxoid (LHRH10-TT) using only materials approved for humans. Testosterone (T)-releasing implants or the long-lasting T ester testosterone-17-trans-4-n-butyl-cyclohexane carboxylate (TE) was used as supplemental androgen for maintaining libido. Active immunization against LHRH administered simultaneously with exogenous androgen supplement caused infertility in 100% of the tested animals, all of which displayed normal sexual behavior. The atrophy of the testes and accessory sex organs was reversible. Active immunization against LHRH could be a convenient and cost-effective method of fertility control in males. The authors found that it took considerable time (up to 5 months) to obtain antibody titers that were sufficiently high for complete suppression of spermatogenesis. The possibility of accelerating the immune response to LHRH by increasing the dose of immunogen was investigated in the male rats Ladd et al (1990). Six doses of LHRH conjugated to tetanus toxoid (TT) in the 10 position (LHRH10-TT), ranging from 2.5 to 612 μg, and three doses of LHRH1-TT (50 to 612 μg) were tested. The magnitude of the immune response did not depend on the dose of the antigen, provided a threshold dose had been surpassed. Antigenicity of LHRH conjugated to TT at either the 1 or 10-position was compared in rats and rabbits. In both species LHRH1-TT induced sufficient antibody concentrations to suppress pituitary gonadotropins (LH and FSH) and, subsequently, serum testosterone (T) levels faster than 10-conjugates.
The authors concluded that active immunization against LHRH conjugated to TT at the 1-position has potential as a fast, convenient method of male contraception.

Awoniyi et al, (1989 & 1993) carried out studies to determine the extent to which increasing doses of exogenous testosterone (T) administered via Silastic implants can restore spermatogenesis and fertility to rats made azoospermic by active immunization against LHRH. Male rats were made azoospermic by active immunization against LHRH. Increasing doses of exogenously administered T (via Silastic implants) were administered for 8 weeks, and testicular sperm concentration and ability to impregnate female rats were evaluated. Testosterone was capable of restoring quantitatively complete spermatogenesis and fertility in LHRH-immunized azoospermic rats. This relationship was dose-dependent, as evidenced by the partial restoration of spermatogenesis and fertility observed in animals replaced with smaller T Silastic implants.

Juorio et al (1991) investigated the effects of active immunization against LHRH conjugated to keyhole limpet hemocyanin on brain and male sexual organ concentration of catecholamines and 5-hydroxytryptamine. The treatment induces antibodies against LHRH with consequent interference with reproductive function as indicated by the decrease in serum FSH, LH and testosterone. Rat striatal and mesolimbic dopamine levels were moderately increased by this treatment but no changes were observed in the
hypothalamus. LHRH immunization also increased brain 5-hydroxytryptamine concentrations as observed in the hypothalamus, olfactory tubercles and striatum. In the male accessory sexual organs, immunity against LHRH alters the density of noradrenergic concentration. It was concluded that LHRH immunization with the consequent reductions in circulating FSH, LH and testosterone are associated with changes in neurotransmitter concentration both in the brain and in some of the accessory sexual organs in the male rat.

Ovariectomized beef cows were actively immunized against LHRH (Stumpf et al., 1992) and the results indicated that decreased stimulation of gonadotropes alters the distribution of LH isoforms, amounts of mRNA for subunits of LH and distribution of intrapituitary LH isoforms without changing the concentrations of LH in the anterior pituitary. Similar results of LHRH immunization on LH levels in sheep were demonstrated by (Zalesky et al., 1993).

Testis function, feedlot performance, and carcass traits were evaluated in bulls actively immunized against LHRH at different ages (Adams et al. 1996). Final scrotal circumference and testis weight in bulls immunized at 4, 7, or 12 month of age were significantly reduced relative to unimmunized bulls. LHRH reduced the masculinity of carcasses from bulls, but did not affect feedlot performance, longissimus muscle area, marbling score, or backfat thickness. These results suggested that single immunization with the LHRH-KLH
conjugate may have practical utility as a noninvasive alternative to surgical castration in management of beef cattle.

The contraceptive effect of active immunization against LHRH was evaluated in beef heifers (Bell et al, 1997). The result showed that immunoneutralization of LHRH may be an effective management tool that will reduce the incidence of unintended pregnancy in heifers destined for feedlots.

Huxsoll et al (1998) assessed testis function, aggressive behavior, and carcass traits in beef bulls actively immunized against LHRH at 1, 4, or 6 month of age. In addition, they examined the effect of combining immunization with insertion of estrogen-containing implants (Synovex C) at 1 month of age. Unimmunized bulls and steers were included as control animals. All immunized calves received a secondary immunization at 12 month of age. Anti-LHRH titer was evident at slaughter in all immunized animals. Neither age at primary immunization nor implant status affected (P > .05) anti-LHRH titer at slaughter. The data suggested that active immunization against LHRH is a practical, noninvasive alternative to physical castration in the management of bull calves.

Because of the very early onset of puberty, long fattening period and relatively harsh circumstances in Chinese pig production, the endocrine response of Chinese breeds to this type of vaccination has generated interest. Zeng et al (2001) investigated, under the normal conditions of local
Chinese pig farming, castration of young male pigs by vaccination with a vaccine against (LHRH). The first group was immunized at 13 weeks of age with a LHRH tandem dimer OVA-conjugate in Specol and received a booster immunization 8 weeks later. The second group was injected with Specol alone and served as untreated controls. The remaining group was surgically castrated at the time of weaning (at 6 weeks of age). Pigs were fed ad libitum from weaning onwards. All animals were slaughtered at 31 weeks of age. Immunized boars had undetectable or low serum testosterone, low fat androstenone levels and very low testes weights. Zeng et al (2002) also reported active immunization of Chinese cross bred female pigs. The data demonstrated for the first time that the anti-LHRH vaccine works very well under practical Chinese pig farming conditions, and can be an attractive alternative to surgical castration.

Dalin et al (2002) investigated the effect of active immunization against LHRH in mature Standardbred mares (three experimental and one control mare) on antibody titres, ovarian function, hormonal levels and oestrous behaviour. All immunized mares produced antibodies against LHRH but the maximum titres varied between the mares as well as the duration of a greater than 10% binding capacity (1:1600 to 1:50 000; 5 to 12 months, respectively). The result showed that the effect of immunization against LHRH in mature mares was not same concerning antibody titre response and the suppression of ovarian activity and hormone levels.
Price et al (2003) reported the reduced incidence of aggressive behaviour's in beef bulls actively immunized against LH relative to contemporary non-immunized control bulls and surgically castrated steers.

To assess the potential efficacy and welfare benefits of non-surgical castration, the effect of active immunization against LHRH on sexual development and growth rate on Tanyang (important sheep bread in northwest China) ram lambs was evaluated by Cui et al (2003) and compared with surgically castrated and entire lambs. The results showed that LHRH immunization can be used alternative to surgical castration and provides animal welfare benefits.

Reasons for such immunocastration include improvement of meat and carcass characteristics for cattle, sheep, goats, and swine; improvement in feed efficiency relative to castrates in these same species; reduction in male aggressive behavior; reduction in male-associated odors in goats and swine; and fertility regulation in pet animals. Although application as a fertility control agent in men is unlikely, there is renewed interest in active immunization against LHRH as a means of treating prostate cancers and related steroid-dependent pathologies. LHRH is essentially a conserved molecule; the sequence of the decapeptide is similar if not identical in all mammals. This is of advantage as the vaccine eventually to be used in humans can be tested for efficacy and safety in rodents and primates.