INTRODUCTION

THE PROSTATE GLAND

The prostate gland is a firm, partly glandular and partly muscular body surrounding the commencement of the urethra in the male. It originates from the urogenital sinus of the embryo (1). The prostate gland of the sexually mature rat is formed by a complex arrangement of lobes, which empty their serous secretions into the prostatic urethra through a number of ducts opening near the prostatic utricle. It is considered to be formed by discrete, i.e., it forms a definite body outside the urethral muscle, paired, ventral lobes at the neck of the bladder, a dorsal and lateral group also at the bladder neck and a cranial or anterior lobe (coagulating gland) which lies in the curvature of the seminal vesicles.

Structure - The prostate gland is enveloped by a thin but firm capsule. The glandular substance is composed of numerous follicles, the lining of which frequently shows papillary elevations. The follicles open into elongated canals many of which join to form excretory ducts. The epithelium which lines the follicles may be cuboidal but in most places it is simple or pseudostratified columnar. There is no distinct basement membrane and the glandular epithelium rests upon a layer of connective tissue with dense elastic network and numerous blood capillaries.
The normal adult columnar epithelium has basal nuclei with conspicuous nucleoli and chromatin particles. Mitochondria are distributed in all parts of the cell. Electronmicroscopy reveals rough endoplasmic reticulum, Golgi complexes and microvilli at the luminal surfaces of the cells. The apposed borders of the cells are mainly straight and the plasma membranes are united by desmosomes.

The muscular tissue constitutes the proper stroma of the prostate gland, the connective tissue being very scanty forming thin trabeculae in which the vessels and nerves of the gland ramify.

**VASCULATURE OF THE PROSTATE GLAND**

*Arterial supply* - According to Jesik *et al.* (2), the arterial supply to the prostate gland can be traced from the aorta to the common iliac arteries, which divide to form the internal and external iliac arteries. The former, otherwise known as hypogastric artery supplies the prostate, bladder and other accessory sex organs (ASO). The superior vesical artery leaves from the ventral surface of the internal iliac and supplies to the dorsal, lateral and ventral prostatic lobes. The inferior vesical artery leaves the superior vesical artery at the point where it meets the urinary bladder and supplies the dorsal surface of the prostate anastomosing with the superior vesical artery.
Venous drainage - The venous drainage of rat accessory glands has been studied in detail by Lewis and Moffat (3). They have shown that deferential vein draining the cauda epididymis and ductus deferens empties into the hypogastric vein, as does the left spermatic vein from the testis and veins from the seminal vesicle and coagulating gland. The hypogastric vein drains at one end into the external iliac vein, but at its other end it joins a single large circular anastomosis formed by dorsal and ventral veins surrounding the base of the bladder. The ventral prostatic lobes drain by straight veins directly into this venous circle around the bladder and the term "prostatic veins" has been suggested for them. These authors believe that veins from the dorso lateral prostate also drain into the venous circle, but there are observations contrary to this belief.

The particular anatomy of the venous system may allow intermittent reversed flow from the hypogastric vein, thus carrying the drainage from the cauda epididymis and ductus deferens into the prostatic complex. It is assumed that androgens from the testes exerts a local control of the accessory sex glands.
INNERVATION OF THE PROSTATE GLAND

The prostate gland is devoid of somatic innervation, but receive dual innervation from branches of the autonomic nervous systems and possess, in addition, a sensory afferent system.

Nerves supplying the ASO's arise on each side from the pelvic plexus. Peripheral ganglia are located along the neural pathway to the accessory organs distal to the pelvic plexus and at a short distance from, adjacent to, or within the organs they innervate.

The existence of short postganglionic fibres with their cell bodies located in ganglia close to the innervated organ is a feature of the male accessory organs. Collectively these nerves have been termed the "urinogenital short neurone system" (4). Nerve bundles, containing both adrenergic and cholinergic fibres, travel in the adventitia or capsule of the gland in association with blood vessels and their perivascular neuroplexuses. These adventitial nerves branch and penetrate the gland, where they ramify in the fibromuscular stroma to form adrenergic and cholinergic perivascular plexuses around arteries and veins. Some stromal nerves do not associate with blood vessels but innervate smooth muscle of the glandular and ductal components. In addition, prominent neuroplexuses may also form
below the epithelium lining the ducts and acini which are predominantly cholinergic while the innervation to the musculature is mainly adrenergic.

CHEMICAL COMPOSITION OF THE PROSTATE GLAND SECRETION

i) Electrolytes: Water is the main constituent of prostatic secretions which is iso-osmotic with respect to blood serum. In general, sodium is the main cation and chloride tends to be the main anion. The human secretion has a much greater citrate and calcium content and a much smaller chloride level.

ii) Zinc: Large amounts of this element is found in human semen. In rats, the zinc content of the dorsolateral prostate is especially high (5) and the lateral portion is known to concentrate Zn (6,7), both of which are under hormonal control (7,8). Some investigators have shown a direct role of zinc as a prostatic antibacterial factor (9).

iii) Fructose: This monosaccharide is secreted by the prostate gland (10) in comparatively small amounts compared to other ASO’s.

iv) Polyamines: Large amounts of spermine and spermidine are present in the prostate gland of many species (11).

v) Citric acid: Citric acid is secreted by the prostate gland and have often been used as chemical indicators of prostatic function (12).
vi) **Cholesterol and Lipids**

The prostate gland is a partial source of cholesterol in the seminal plasma. It has been reported that the human prostate gland can synthesise cholesterol (13).

vii) **Enzymes**

(a) **Phosphatase**

- Phosphatase enzymes hydrolyze many types of organic monophosphate esters to yield inorganic phosphate ions and alcohol. According to the pH in which they exert maximum activity, they have been classified as acid and alkaline phosphatase. The former is secreted by the prostate gland in copious amounts. Many isozyme patterns have been reported by several investigators (14). In rats, alkaline phosphatase has been detected in prostatic tissue. Both the enzyme activities are controlled by androgenic hormones (15).

(b) **Leucine Aminopeptidase**

- Leucine aminopeptidase is a product of the epithelial cells of the prostate gland. Human prostate is a rich source of arylamidase type of leucine aminopeptidase, because their appropriate substrate is L-leucyl β-naphthylamine (16).

viii) **Prostatic proteins**

(a) **Prostate-Specific Antigen**

- This is an androgen dependent protein. It is an enzyme, specifically an arginine esterprotease. The protein is an important marker of prostatic function (17). This is a 33,000-dalton glycoprotein containing 7% carbohydrate which can be immunologically detected only in the epithelial cells (18).
(b) Prostatein: This is composed of several chains - C-1, C-2, & C-3. C-1 is a 6000-dalton subunit, C-2 is a 10,000-dalton subunit, and C-3 are two 14,000-dalton subunits (19).

(c) Thyrotropin releasing-hormone: TRH occurs in high concentrations in the rat prostate gland. This is synthesized in prostate gland (20).

(d) Endorphin: This peptide has been located in various sites of the male reproductive tract of rat including the prostate gland (21).

(e) Adrenocorticotrophic hormone: Prostate gland has been demonstrated to be a source of ACTH (22).

(f) Inhibit: Biosynthesis of inhibit-like material has been reported in human prostate gland (23). This compound is biologically and immunologically active.

(g) Prostatic EGF-related mitogen (PEM): PEM has been isolated, purified, and characterized by several workers. This is capable of stimulating proliferation of rat prostate epithelial cells in vitro (24).

(h) Insulin-like peptide: The prostate gland has been shown to be positive immunocytochemically to insulin. There is a strong evidence to show that it is synthesized inside the gland (25).
The physiology of the prostate gland greatly depends on the supply of a number of steroids and peptide hormones. Among these hormones, the testicular androgens have been shown to play a prominent role in the differentiation and growth of this gland from the time of embryogenesis to puberty and throughout adulthood.

Role of androgens - John Hunter in 1792 (26), first noted the atrophy of the prostate gland following castration. This clearly demonstrated the prostatic dependence on testicular function. Later, with the isolation and characterization of the steroid hormones, the details of this dependence were elucidated. Following castration, the decrease in the secretory activity precedes alterations in epithelial morphology. Citrate secretion decreases markedly. There is regression of the epithelial ergastoplasm, progressive degranulation of the rough endoplasmic reticulum. Increase in lysosomal activity is noted. Orchiectomy prior to puberty causes differentiation of the prostate gland only up to the age of 30-40 days following which regression starts (27). These group of workers (27) have also shown the reversal of castration-induced atrophy of the prostate gland following administration of androgenic substances. This emphasizes the critical role of testicular androgens. The important
androgenic steroids secreted by the testes include testosterone, androstenedione, dehydroepiandrosterone, dihydrotestosterone and androstenediol.

Recently, Lee and his associates (28-30) have shown that the castration induced atrophy is an active process. These group of workers have shown that if protein or RNA synthesis is blocked by actinomycin D following castration, the ability of the prostate gland to involute was markedly reduced. This suggests that androgen dependency is regulated both by inducible and repressible mechanisms.

The adrenal cortex is also a source of androgen production which has been shown to influence prostatic growth. In 1936, Davidson and Moon (31) observed a stimulation of ventral prostate growth following the treatment of young castrated rats with ACTH. Price and Ingle, in 1957 (32) showed that adrenals autotransplanted into seminal vesicles and ventral prostates of adult castrates caused a marked local stimulating effect on prostate weight and histological structure. All these observations demonstrate the adrenal-dependent growth of the prostate gland. The main androgens secreted from the adrenal glands are dehydroepiandrosterone and androstenedione.

Androgen metabolism in the prostate gland

Testosterone enters the prostatic cells and is rapidly metabolized irreversibly to dihydrotestosterone (DHT) in presence of the enzyme 5α-reductase. DHT is further converted to
3α- or 3β - diol by a reversible reaction catalyzed by 3α- or 3β- hydroxy steroid oxidoreductases. The steroids including DHT bind to cytoplasmic androgen receptors which help in its entry into the nucleus where it binds to the nuclear receptor. Finally, the androgen-receptor complex binds to the specific sequence of DNA and transcription and expression of genes are initiated. At last, proteins are formed as a result of these complicated processes.

Role of prolactin (PRL) - The atrophy of the prostate gland was found to be more profound in castrated dogs in absence of the pituitary gland (33). In 1955, Grayhack et al. (34) reported that in castrated and hypophysectomized rats, administration of testosterone alone was not capable of restoring normal prostatic weight unless it was given with a supplement of luteotrophins. A fall in prostatic uptake of labelled testosterone following hypophysectomy was also reported (35). Asano (36) has shown that the pituitary content and release of PRL increased following prostatectomy. Specific binding sites of PRL have been detected in membrane preparations of the prostate gland (37). Investigations on the properties of PRL receptor in rat prostate gland suggest that low levels of PRL are associated with increase in binding (up-regulation), while high doses of the hormone cause a dose-dependent decrease in binding (down-regulation) (38). These studies have provided some experimental evidence for a direct effect of PRL on the prostate gland.
The studies made by Ghosh *et al.* (39) reveal that the role of PRL on the prostate gland is synergistic with the testosterone as far as the regulation of prostatic acid phosphatase is concerned. Farnsworth (40) has suggested that PRL accentuates the effect of androgens on the prostate gland. In addition, PRL increases zinc uptake in the tissue (41), alters androgen uptake and metabolism (42) and regulates citric acid and fructose level. PRL has a permissive effect on the growth of the lateral prostate gland of rat and this may be associated with increased nuclear androgen levels (43–45). Even a direct action of PRL on prostate gland has been suggested by several workers (44–47). From the above observations it may be surmised that PRL contributes significantly for the maintenance of prostatic physiology.

**Role of gonadotrophins** - The effect of gonadotrophins (LH & FSH) on the prostate gland is indirect, acting through the regulation of testicular androgen secretion. The hypothalamic-pituitary-gonadal axis which is involved in a feedback control mechanism plays an extremely important role in regulating the supply of androgens and thus influencing the growth and functional activity of the prostate gland.

**FUNCTION OF THE PROSTATE GLAND**

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The prostate gland is not essential for life. Its
secretion functions as a diluent and may increase the motility of the small volume of sperm delivered by the vas deferens. It is therefore essential for the fertilization by the natural methods (48). The observations of Queen et al. (49) suggest that the dorsolateral lobes of the gland are essential for fertility in rats. These results are consistent with the findings of Pang et al. (50) whose experimental design was somewhat different from Queen et al. (49), nevertheless, the latter group has demonstrated the role of dorsal prostate in fertility of mice. Biochemical studies have demonstrated that, apart from furnishing an osmotically balanced milieu for the spermatozoa, the seminal plasma contains substances, the role of some of which have been at least partially evaluated. The rest is still speculative. In this connection it is worth noting, that in rats and guinea-pigs, the secretions of the prostate gland has been reported to stimulate uterine motility (51). Thus, it may be concluded that the secretions of the gland though not essential for fertilization, yet it may optimize conditions for sperm motility, survival and transport in both male and female reproductive tracts.
One of the important functions of testis is to produce androgens mainly testosterone and androstenedione. The Leydig cells situated in the interstitial space are the chief site for the synthesis of androgens, while seminiferous tubules may contribute certain amount of testicular androgens. However, controversy still remains over androgen production and the enzymes involved in androgen metabolism in specific compartments.

The biosynthesis of androgens take place in different steps which are well established. Oxido-reduction and hydroxylation processes are the most important and rate-limiting reactions in androgenesis (52-54). The oxido-reduction reactions are catalyzed by dehydrogenases such as $\Delta^5 - 3\beta$-hydroxysteroid dehydrogenase enzyme (52-54) and $17\beta$-hydroxysteroid dehydrogenase enzyme (55,56), while introduction of hydroxyl group into the steroid moiety is catalyzed by NADPH hydroxylase enzyme.

Cholesterol is the obligatory intermediate for the synthesis of androgens from acetate (57). The cholesterol is then converted to pregnenolone in the mitochondria (58) through the formation of $20\alpha$-22$\epsilon$-dihydroxycholesterol which is then cleaved by lyation to produce pregnenolone. Pregnenolone is then converted to testosterone through five important enzymatic reactions. These are $17\alpha$-hydroxylation, lyation, $3\beta$-hydroxysteroid
dehydrogenation, \( \Delta^5 \)-3-ketosteroid isomerisation and
17\( \beta \)-hydroxysteroid dehydrogenation. There are two pathways of
testosterone synthesis from pregnenolone - \( \Delta^4 \) pathway and \( \Delta^5 \) pathway. The \( \Delta^4 \) pathway proceeds through the formation of
progesterone, 17\( \alpha \)-hydroxy-progesterone and androstenedione (59),
while the \( \Delta^5 \) pathway runs via 17\( \alpha \)-hydroxypregnenolone, dehydroepiandrosterone and androstenediol (60). It appears that
both pathways are operative to a greater or lesser extent in many
species (61). The direction of the metabolic pathway is dependent
on the competitive function between \( \Delta^5 \)-3\( \beta \)-hydroxysteroid
dehydrogenase and 17\( \alpha \)-hydroxylase over a single substrate
pregnenolone. Once the metabolic direction is decided, the
synthesis of testosterone proceeds independently of the other
pathway (62). The biosynthetic pathway with enzyme system are
schematically represented in the following way:
Cholesterol

20α- hydroxycholesterol 22R-hydroxycholesterol

20α - 22R-dihydroxycholesterol

Pregnenolone

4 & 5

Progesterone

17α - hydroxypregnenolone

17α - hydroxyprogesterone

Dehydroepiandrosterone

Androstenedione

Androstenediol

1. Cholesterol 20α-hydroxylase
2. Cholesterol 22R-hydroxylase
3. C20 - C22 lyase
4. Δ-3β-hydroxysteroid dehydrogenase
5. Δ- Δ-isomerase
6. 17α-hydroxylase
7. 17-desmolase
8. 17β-hydroxysteroid dehydrogenase

--- Invitro pathway ---

Invivo pathway

15
The androgenesis by the mammalian testes is under the control of the pituitary gonadotrophic hormones (61). Although the pituitary-testicular axis is the principal pathway in the control of androgen biosynthesis, there are several reports regarding the existence of intra-testicular circuit, i.e. Leydig cell-Sertoli cell interconnection which play a role in the control of androgen production (63, 64).

Effect of LH (ICSH) on androgenesis

The androgenesis in the testes is the result of trophic stimulation of luteinizing hormone. This hormone enhances the synthesis of testosterone, androstenedione, dehydroepiandrosterone both in vivo (65-67) and in vitro (67-69). The acute response of Leydig cell to LH depends largely upon an activation of the mitochondrial enzymes controlling cholesterol side-chain cleavage (70) while chronic effect of LH on testicular steroidogenesis appears to involve the synthesis of new protein with an increase in the activity of several enzymes responsible for the conversion of pregnenolone to testosterone (71). LH also activates cholesterol esterase enzyme which in turn stimulates the release of free cholesterol from esterified cholesterol (72). Moreover, the uptake of cholesterol by the mitochondria, activation of phosphorylase enzyme and NAD-dependent mitochondrial catalytic steps for transformation of
cholesterol to pregnenolone and the exit of newly formed pregnenolone from mitochondria are influenced by LH (72-74).

In immature rats, testicular tissue possesses low activities of both $\Delta^5-3\beta$-HSD and $\Delta^4-\Delta^5$ isomerase which can be increased by exogenous gonadotrophins (75). In immature state pituitary content of LH is very low than adult level (76) indicating that androgenesis at low level in immature state is primarily due to low production and secretion of pituitary gonadotrophins. So, treatment of animals with various gonadotrophins resulted in enhancement of overall synthesis of testosterone from acetate (77-79) by influencing the steroidogenic enzymes (80-83).

The action of LH is mediated through specific membrane receptors of Leydig cells (84,85). The binding of LH with the specific receptor of Leydig cell is essential for the initiation of testosterone secretion (86).

From the characteristic study of gonadotrophin receptors so far carried out, it may be said that they have a definite site and specific binding affinity for specific hormones. The membrane receptors appear to be glycoprotein in nature (87) and they lose their binding properties after removal of phospholipids (88). Stimulation of adenyl cyclase by LH or hCG in rat testicular tissue leads to the formation of cyclic AMP (89). The data so far obtained indicate that cyclic AMP may be a
second messenger of LH action in the testis (90) as cyclic AMP or
dibutyryl cAMP (91) stimulates the testosterone production by intact testis.

**Effect of FSH on androgenesis**

Regarding the role of FSH on testicular steroidogenesis Hall & Eik-Nes in 1962 (69) have suggested that FSH augments the testicular steroid production. It has been reported that in hypophysectomized immature rat FSH enhances the effect of LH on testicular androgen production (92). Studies by Chen et al. (93,94) indicated that FSH increases both the LH receptors and LH sensitive testosterone synthesis in the testis. The activities of $\Delta^5 - 3\beta$-HSD and $17\beta$-HSD are increased by FSH in hypophysectomized immature rats (55), but, in mature rats FSH acts synergistically with LH in enhancement of $\Delta^5 - 3\beta$-HSD activity (56) leading to testosterone synthesis (95).

In 1973, Lacy (96) has suggested that testosterone secretion within seminiferous tubule is increased in response to FSH. It has also been reported by several workers that FSH specifically binds to the Sertoli cell of seminiferous tubules and can stimulate the conversion of androstenedione to testosterone in immature rats (97,98). It has also been observed that FSH can stimulate adenyl cyclase activity in rat testis leading to the formation of cyclic AMP (99–101).
Effect of PRL on androgenesis

The role of PRL in the regulation of testicular steroidogenesis is poorly understood. In rat, PRL potentiates the action of LH on testicular steroidogenesis (102). This effect can be explained by a direct action of PRL on Leydig cells as it possess specific PRL receptors (103). Treatment with PRL also increases the concentration of esterified cholesterol in the testes of mice which appears to serve as a precursor in the biosynthesis (104). It has been suggested that PRL affects testicular steroidogenesis by increasing the availability of precursors for the biosynthesis of androgens, perhaps, by regulating the activity of cholesterol ester synthetase in a manner similar to that described in the ovary (105). Studies also reveal that PRL increases the synthesis of testosterone in the testes through increasing the activity of 3β-HSD and 17β-HSD in hereditary dwarf mice (106, 107). It has also been reported that PRL increases the number of LH receptors on Leydig cells by acting synergistically with FSH and growth hormone (108).

A BRIEF REVIEW OF SPERMATOGENESIS IN RAT

Histology of testis - The testis is composed of both exocrine components (seminiferous tubules) and endocrine components (interstitial cells).

Seminiferous tubules - The exocrine component of the testis, seminiferous tubules are primarily responsible for the
formation of male gametes, i.e., spermatozoa, which are continuously produced within the seminiferous tubules through a complicated series of events, i.e., both by cell differentiation and cell division. The spermatogenic epithelium (germinal epithelium) and tall columnar non-germinal cells of irregular shape (Sertoli cells) are present within the seminiferous tubules. Histological study reveals the following types of germinal cells in seminiferous epithelium.

(1) Spermatogonia - These germ cells arise from the gonocytes. Depending on the stage of maturation, spermatogonia are of three major types, i.e., type A spermatogonia, intermediate spermatogonia and type B spermatogonia. There are several differentiated type A spermatogonia viz. A₁, A₂, A₃ & A₄ (109, 110) which occurs during the process of spermatogenesis.

(2) Spermatocyte - (a) Primary spermatocytes: These are the germ cells which are produced after the mitotic division of spermatogonia type B. This stage is called resting or preleptotene spermatocyte which represents the pre-meiotic interphase preceding the prophase of the first meiotic division. The next stage in the evolution of primary spermatocytes initiates the prophase of the first maturation division and the different varieties of primary spermatocytes are found. These are leptotene, zygotene, pachytene, diplotene, and diakinesis.

(b) Secondary spermatocytes: These are germ cells which are obtained after the completion of first meiotic division.
Spermatid - These germ cells contain haploid number of chromosomes which are derived from the second maturation division. These cells are comparatively small in size and spherical in shape.

Spermatozoa - These germ cells are produced through morphological transformation and modification of the components of spermatids.

Sertoli cells - In the intra-tubular region some non-germinal columnar cells (somatic) are present which are known as Sertoli cells.

Interstitial tissue - The interstitial tissue constitutes the skeletal framework of the testis and support for the seminiferous tubules. This tissue consists of different types of cells such as mast cells, plasma cells, reticular cells, lymphocytes, fibroblasts etc. Moreover, another type of epithelial cells are found which are called Leydig cells which perform the major endocrine function of the testis.

Kinetics of spermatogenesis - Spermatogenesis is an orderly and well-defined process by which spermatozoa are produced from spermatogonia. The spermatogenic process can be divided into three distinct phases: (a) The first phase constitute the proliferation of spermatogonia leading to the formation of spermatocytes along with the maintenance of the number of spermatogonia through cell renewal system. (b) The second phase involves the primary and
secondary spermatocytes which go through the process of reduction division leading to the formation of spermatids. (c) The third phase constitutes the metamorphosis of spermatids leading to the production of spermatozoa. This phase is also known as spermiogenesis. Morphologically, the several steps of spermiogenesis can broadly be classified as Golgi phase, Cap phase, Acrosome phase and Maturation phase (111, 112).

The various types of evolving germ cells, i.e., the spermatogonia, spermatocytes and spermatids are not arranged at random but form well-defined cellular associations. These cell association succeed one another in time in any given area of the seminiferous tubule which constitutes the seminiferous epithelial cycle. Thus, the cycle of the seminiferous epithelium is the series of changes in a given area of the seminiferous epithelium between the appearance of the same developmental stage (111).

Leblond & Clermont (112) have been able to divide spermiogenesis in the rat into 19 steps. In the first 8 steps the germinal epithelium has old spermatids which are released when the younger spermatids reach stage 8, hence the new crop of spermatid is alone until the appearance of stage 15 when new generation of spermatid appears. Thus, stage 1 and stage 15 spermatids appear together and the succession of cells associated with the appearance indicates one cycle. They also described the cycle of seminiferous epithelium according to cellular
association into 14 developmental stages, each of which have well-defined cell association. Further studies on the kinetics of seminiferous epithelium have led to accurate timing of the spermatogenic process (113, 114) and have shown a definite number of each cell type is present in each cross-section of the seminiferous tubules. The application of this knowledge permitted precise quantitative evaluation of changes in the seminiferous epithelium following hypophysectomy (115) or damage induced by noxious agents (116, 117). It is suggested that primordial germ cells or the gonocytes present at birth stop proliferating and in a four-day old rat give rise to type A spermatogonia, which then enter the cycle of the spermatogenesis (118). The same conclusion was drawn by Sapsford (119) and he suggested the presence of a transitional cell which he observed between the gonocyte and type A spermatogonia. He referred to this cell as "immature type A spermatogonium" which nowadays is called "primitive type A spermatogonium". In the "stem cell renewal theory", Clermont et al. (113) showed that type A spermatogonia starts proliferating at stage 9 of the seminiferous epithelial cycle and the daughter cells divide successively at stage 12 and stage 1 of the cycle. During this process, one type A spermatogonium gives rise to the formation of two new type A stem cells. One type A spermatogonium is responsible for the renewal of spermatogonial population and the other type A spermatogonium gives rise to the
formation of intermediate type of spermatogonia which produces type B spermatogonia and ultimately produces primary spermatocytes.

Spermatogenesis in relation to advancement of age

At birth, gonocytes are the only germ cells present in the seminiferous tubules. By the fourth or fifth day of age, they differentiate into type A spermatogonia, which gives rise to type B spermatogonia by the 8th day. The spermatogonia enter the meiotic prophase by day 15 and mature into pachytene spermatocytes by day 20. The meiotic division of spermatocytes are complete by about day 25 when the first round of spermatids appear in the seminiferous tubule. All the stages of spermatogenic cycle are clearly visible by about 60 days of age (118, 120, 121).

Hormonal control of spermatogenesis

In 1927, Smith first observed that hypophysectomy in rat leads to the degeneration of seminiferous tubules (122). Several other investigators observed that replacement of gonadotrophic principles in hypophysectomized rats were capable of repairing the regressive changes in the germinal cells, induced by hypophysectomy (123, 124). The results obtained from earlier experiments utilising replacement therapy in hypophysectomized rats were conflicting and ambiguous. The complexity arose from the unavailability of absolutely pure preparation of gonadotrophins, incomplete hypophysectomy and from qualitative histological evaluation of the spermatogenic process (125–127).
It is now generally believed that an appropriate hormonal milieu must exist for spermatogenesis both in its quantitative and qualitative aspects to culminate in the production of spermatozoa (128,129). Steinberger, in 1971, in his review described that the formation of primitive type A spermatogonia are possibly under the control of testosterone (128). The formation of type A & B spermatogonia and the development of primary spermatocytes up to pachytene stages do not require any gonadotrophic or gonadal hormones (128). The conversion of primary spermatocyte (prophase) to secondary spermatocyte (metaphase) is also under the influence of testosterone (130), thus androgenic hormones provide a major stimulus for spermatogenesis. Spermiogenesis from step 1 to step 15 is not dependent on any hormone, but maturation of spermatid from step 15 to step 19 requires the presence of FSH (128).

Role of FSH on spermatogenesis

It is now well established that FSH exerts an important effect on the initiation of the first wave of spermatogenesis. It has been observed that both testicular weight and seminiferous tubular diameter are increased following administration of FSH in rat (131). Administration of anti-FSH in immature rats led to the arrest of spermatogenesis, indicating that FSH is essential for the maintenance of different cells in
the seminiferous epithelium during completion of the first wave of spermatogenesis (132). FSH also affects the multiplication and differentiation of spermatogonia type A₀ & A₁ and increases the number of pachytene spermatocytes along with testosterone (133). Means (134) also reported that FSH increases the mitotic rate and reduces the degeneration of spermatogonia in immature rats. In 1981, Collinset al. (135) reported that FSH may also be essential for spermatid maturation in immature hypophysectomized rats. Furthermore, FSH also plays an important role in the maintenance of spermatogenesis. Hypophysectomy in adult rats suppresses the proliferation and differentiation of the various generations of spermatogonia resulting in a significant reduction in the total yield of type B spermatogonia. Injection of FSH in hypophysectomized male rats maintains the reserve of spermatogonia and restores their divisions (136).

In a review, Steinberger (137) summarised the recent concepts about the molecular mechanism of FSH action on spermatogenesis. He described that FSH is first bound to a specific membrane receptor of the Sertoli cells where it activates adenyl cyclase resulting in the formation of cAMP which in turn promotes DNA-dependent RNA synthesis. This RNA helps in the formation of protein including a specific androgen-binding protein (ABP). ABP is then transported into the intercellular spaces where it binds with androgen which comes from interstitial
area by diffusion. The ABP-androgen complex comes in contact with germ cell membrane and is transferred to a cytoplasmic androgen receptor. The receptor-androgen complex is then transported to germ cell nucleus. Testosterone then serves its function on gametogenesis. But, the mechanism of action of testosterone on germ cells after this step is unknown. The ABP after delivery of androgen can bind with more androgen and the above process may be repeated.

Role of LH on spermatogenesis

The role of LH on the spermatogenic process is an indirect one. Its action is mediated through the production and secretion of testosterone by the Leydig cells.

Role of PRL on spermatogenesis

The direct action of PRL on the seminiferous epithelium has not been demonstrated clearly. Administration of PRL to hypophysectomized rats and mice potentiates the effect of LH on the restoration of spermatogenesis (126, 138). Existence of specific prolactin receptors on the Leydig cells have been demonstrated (103). PRL has also been shown to increase LH receptors on Leydig cells (108, 139) and stimulates testosterone synthesis in them (140).

Role of testosterone on spermatogenesis

It has been observed by several investigators that testosterone plays a major role in the initiation and maintenance of spermatogenesis (128, 137). Administration of low
doses of testosterone in rats produces atrophy of seminiferous epithelium, while high doses of testosterone is unable to exert any adverse effects on seminiferous tubule, indicating that the action of testosterone is dose-dependent. The atrophy of seminiferous epithelium following low doses of testosterone administration was the effect of suppression of pituitary gonadotrophins, whereas, the higher doses of testosterone were capable of maintaining spermatogenesis in spite of markedly diminished gonadotrophin levels (141). In 1975, Desjardines (142) have shown that chronic treatment with testosterone can maintain sperm production and fertility at normal levels in spite of marked reduction in endogenous testosterone production. It has also been reported that testosterone was unable to support fully the initial wave of spermatogenesis and late steps of spermatid maturation (143). In 1976, Lostroh (130) revealed that testosterone is required for the conversion of primary spermatocyte prophase to metaphase and for maturation of spermatids. In 1971, Steinberger (128) has proposed that the formation of primitive type A spermatogonia from gonocyte and the reduction division of primary spermatocyte possibly require testosterone. It has been further shown that neither FSH nor testosterone alone was capable of completing spermatogenesis in hypophysectomized immature male rats. Simultaneous treatment of both hormones could maintain spermatogenesis qualitatively up to step 19 of spermiogenesis (144).
ADRENAL CORTICOSTEROIDOGENESIS

There is a good reason to believe that cholesterol is a significant precursor for the adrenocortical steroids (145, 146). This sterol may be synthesized within the adrenal gland or it may come from the circulating cholesterol. For corticosteroidogenesis, cholesterol is first converted to pregnenolone (147) through the formation of 20α-22-dihydroxycholesterol (148, 149) within the mitochondria (150) which is thought to be a rate-limiting action in steroidogenesis. Once pregnenolone is formed, the subsequent steps in the biosynthetic pathway occur at a rapid rate. In human and dog both cortisol and corticosterone are produced by adrenal glands while in rat only corticosterone is produced (151). Pregnenolone enters into steroidogenic process through two pathways: (1) through the formation of 17α-hydroxypregnenolone and (2) through progesterone. In rodents, major pathway of corticosteroid biogenesis leads through the formation of progesterone while in human and several other species 17α-hydroxypregnenolone is the product of pregnenolone (152–154). Corticosteroid formation from pregnenolone or 17α-hydroxypregnenolone needs the following enzymatic reactions: \( \Delta^5 \-3\beta\)-hydroxysteroid dehydrogenation and isomerisation (155, 156), 21-hydroxylation and 11β-hydroxylation (157). The pathway of corticosteroid synthesis with different enzyme systems can be summarised as follows.
Cholesterol

1. Cholesterol 20α-hydroxylase
2. Cholesterol 22R-hydroxylase
3. 20,22-Desmolase Complex
4. Δ^5 -3β-hydroxysteroid dehydrogenase
5. Δ^4 -Δ^5 -isomerase
6. 17α-hydroxylase
7. 21-hydroxylase
8. 11β-hydroxylase
ACTH and adrenal corticosteroid synthesis

Adrenal corticosteroidogenesis and its secretion is under the control of anterior pituitary hormone. Secretion of corticosteroids are reduced after hypophysectomy (158) but supplementation of ACTH restores the secretion, indicating that corticosteroid synthesis in the adrenal gland is the result of trophic stimulation of ACTH (159-161). NADPH is required in the hydroxylation reactions (162,163) whose production is stimulated by ACTH through glucose-6-phosphate dehydrogenase activity (164). Thus, ACTH influences corticosteroidogenesis by stimulating hydroxylation reactions other than those associated with side-chain cleavage (165-169). It has also been reported that ACTH can increase the activity of 21-hydroxylase (170) and 11β-hydroxylase (171). ACTH induced acceleration of corticosteroid biogenesis in adrenal gland is mediated either by increasing the entry of cholesterol into the mitochondria (172) for serving as steroid precursor or by preventing the product inhibition by increasing the departure of pregnenolone from mitochondria (173). Binding studies of labelled ACTH with rat adrenocortical cells indicate the existence of specific ACTH receptors on adrenocortical cells (174). Thus, at the cell membrane, ACTH activates the formation of cAMP (175-178) which might serve as an intracellular regulator of steroidogenesis (179).
Besides steroidogenic activity, ACTH can maintain the adrenocortical structures. Adrenocortical atrophy in hypophysectomized animal is restored by treatment with ACTH (180).

**PRL and adrenocortical activity**

The literature so far available regarding the effect of PRL on adrenal corticosteroidogenesis is very limited. It has been observed that hyperprolactinemia in rats and mice causes an increase in adrenal weight and in the concentration of corticosterone in peripheral plasma (181). This increased output is due to inhibition in the formation of reduced steroid metabolites in response to inhibition of adrenal 5α-reductase enzyme (182). It has been suggested that PRL interacts with specific adrenal receptors and thereby exerts its action (183).

**ADRENAL–GONADAL INTERRELATIONSHIP**

It is generally agreed that orchiectomy is followed by a rise in pituitary ACTH content and adrenal hyperplasia which can be reversed by administration of testosterone (184, 185) and thus, mediate an existence of relationship between adrenal and testicular activity. In 1963, Kitay (186) reported that testosterone administration to
normal rats resulted in adrenal hyperplasia and decreased ACTH secretion. The action of testosterone on adrenal corticosterone production is dose-dependent. In large doses, testosterone has been reported to reduce adrenal size (187) and corticosterone production (188) while in low doses it can enhance the corticosterone production by adrenal gland (188, 189). Considerably, less information is available regarding the action of different doses of testosterone on corticosterone production. Studies by Sharma & co-workers (190, 191) suggested that this inhibition may be due to impairment of one or more of the hydroxylation steps in steroid biosynthesis. Kitay, in 1968 (189), reviewed that the stimulation of corticosterone formation by low doses of testosterone in rats is due to inhibition of intraadrenal reduction of corticosterone to inactive metabolites.

The effect of adrenalectomy on the testicular function has also been demonstrated by different workers (192, 193). In 1931, Freed, Brownfield and Evans (192) reported that adrenalectomy leads to the degeneration of testes in rats. It has been reported by Herrick and Finerty (194) that adrenalectomy resulted in decreased testicular size in salt-maintained roosters. Compensatory ovarian hypertrophy after unilateral ovariectomy in rats can be prevented by adrenalectomy (195), indicating that adrenalectomy decreases the ability of gonadotrophin output by the hypothalamo-hypophyseal system. There
are many reports regarding the role of exogenous steroids on testicular activity. Heskins & Hoskins (196) in 1916, first demonstrated that feeding of adrenal tissue extract to rats stimulated the growth of gonads in both male and female. Subsequent studies by different investigators indicated that cortisone stimulates the growth of the testes of young rats (197, 198) and can maintain spermatogenesis in hypophysectomized rats (199, 200). Cortisone was also effective in preventing the testicular degeneration caused by alloxan diabetes and chlorpromazine administration (201, 202), indicating that cortisone plays some role in testicular activity.