REVIEW OF LITERATURE

Although information about the environment is largely processed at the level of cerebral cortex, subcortical areas of the brain contain important structures through which information processing and arousal are mediated. The cerebral cortex handles memory, symbolic representation, thinking and reasoning. Several subcortical structures direct sensation to the cortex and are source structures for generalized arousal, certain specific emotions and visceral regulations for somatic survival. These structures receive feedback through chemical signals from the pituitary gland and autonomic nervous system via the hypothalamus, and some information from the internal and external environment directly (Mechanic, 1977).

The term stress denotes only those stimuli that have been proved to increase Adrenocorticotropic Hormone (ACTH) secretion in normal animals and humans. The stressors are those noxious stimuli that increase ACTH secretion. Most of the stressful stimuli that increase ACTH secretion also activate the sympathetic nervous system. Precisely all that can be said is the stress causes increase in the plasma glucocorticoids, to high “pharmacologic” levels that in short run are life-saving but in the long run are definitely harmful and disruptive. ACTH not only produces prompt increase in glucocorticoid secretion but also increase the sensitivity of the adrenal to subsequent doses of ACTH. Decreased responsiveness to ACTH is produced by doses of glucocorticoids that inhibit ACTH secretion (Ganong, 1999).
Both basal secretion of glucocorticoids and increased secretion provoked by stress are dependent upon ACTH from anterior pituitary. During severe stress the amount of ACTH secreted exceeds the amount necessary to produce maximal glucocorticoid output. The biological 'stresses' lead to an increase in plasma glucocorticoid concentration resulting in release of corticotrophin-releasing hormone (CRH). It is stored and possibly synthesized in the medial basal hypothalamus from which it is released into the hypothalamo-hypophyseal portal vessels and reach the adenohypophysis to stimulate ACTH (Keele et al., 1982).

The adrenal cortex is formed from the cells of the coelomic mesoderm between the root of the mesentery and the genital ridge. Thus the adrenal cortex is derived from the cells in close relation to those which eventually form the ovary and testis. All these cells synthesize steroids, which may be precursors of hormones or actual hormones, which enter the circulation and produce the characteristic effect of ovarian, testicular, or adrenocortical endocrine function.

During fetal life the adrenal cortex is relatively large organ and its cells depend on ACTH stimulation. The secretion of ACTH from the adenohypophysis is in turn stimulated by corticotrophin-releasing hormone (CRH) from the hypothalamus (Keele et al., 1982).

Secretion of ACTH and cortisol exhibits a circadian rhythm, with the highest rate occurring around the time of the morning awakening. Both ACTH and cortisol
are secreted in episodic bursts. With integrated sum of these bursts giving the characteristic circadian rhythm where mean morning plasma ACTH and cortisol concentration exceed evening. This circadian rhythm appears importantly tied to sleep wake cycle and to food intake (Gill, 1985). In rats, which are nocturnal feeders, peak concentrations of ACTH and corticosterone occurs prior to initiation of feeding period (Miyabo et al, 1980). The circadian rhythm thus favours higher cortisol concentrations during the major period of fasting each 24 hours, whether that occurs during the day or night.

Cortisol production remains constant throughout life as measured under both basal and ACTH stimulated conditions (Pintor et al, 1980). The adrenal secretion increases progressively and reaches maximum around the onset of puberty. This coincides with progressive development of inner reticular zone (Dhom, 1973). After puberty there is progressive decline in adrenal androgen secretion.

ADRENALS - INVOLVEMENT IN STRESS:

Selye (1936, 1937) has reported diminution in fat content of adrenals and its hypertrophy under various conditions of 'stress'.

Tepperman et al (1943a) have observed that removal of one adrenal results in hypertrophy of the remaining one or following enucleation of both adrenals, the cortical remnants quickly assume a mass which is roughly comparable to that present in the normal animal. It is universally accepted that hypertrophy does not
occur and that homotransplants do not 'take' in absence of hypophysis. It has also been demonstrated that measure which is known to cause bilateral hypertrophy of the cortex in intact animals accentuates compensatory hypertrophy in the unilaterally adrenalectomized animals. Thus it is clear that in all species hypophysectomy is followed by adrenal cortical atrophy.

A consistent weight difference has been found between the adrenal of males and females, this is heavier in the latter. Rats with body weights above 100 gm, there is a linear relationship between body weight and adrenal size and that the gland of females were consistently heavier than those of males. This sex difference was confirmed in mice and cats. Cole and Hamed (1942) reported a similar sex difference in the pituitary weight: body weight ratio. Pituitaries of female rats, guinea pig and cattle are richer in adrenotrophic hormone. In male animals all reports are in agreement that castration is followed by adrenal and pituitary hypertrophy. The adrenal hypertrophy was found to be due to an increase in the size of Zona fasciculata and reticularis.

Adrenalectomized or hypophysectomised animals are unable to resist cold environment as well as intact animals do. Selye (1936, 1937) reported adrenal hypertrophy following exposure to cold. There is an increase in voluntary food intake in rats exposed to cold, but inspite of this the animals gain weight at an abnormally slow rate. A possible clue to one of the factors underline the adrenal hypertrophy is the fact that the thyroid gland undergoes marked hypertrophy during exposure to cold. Rats bred for 8 generations under tropical conditions of
temperature and humidity showed a relative increase in adrenal size. In the rat, exposure to heat result in a diminution in spontaneous food intake. Intact animals forced to exercise show a considerable hypertrophy of adrenals within a short time as 12 hours after beginning work. Adrenalectomized animal does not tolerate exercise well.

The adrenal cortex enlarges in response to a great variety of shock-producing agents. Selye et al (1940) has stated that the adrenal hypertrophy seen as a result of shock characteristically occurs during the second stage of shock (Counter shock). During the first phase one can readily imagine that, in addition to increased protein destruction that is often caused by the shock-precipitating episode, the peripheral circulatory failure might result in very extensive tissue breakdown. Teppeman et al (1943b) suggested that events occurring during the catabolism of protein may serve as a stimulus for the hypertrophy of the adrenal cortex in a variety of circumstances.

Deane and Greep (1947) have shown in 2 strains of rats, thyroidectomy results in shrinkage of adrenal from normal 19 to 13 mg% and thymus gland from 160 to 70 mg%. Histologically the adrenal atrophy was noticed in the zona fasciculata. The changes in the zona fasciculata were attributed to a lowered output of adrenotropin in hypothyroidism and an increased output in hyperthyroidism. The increased activity of the zona glomerulosa in the several conditions was tentatively attributed to alterations in salt balance resulting from the different treatment.
Ludewig and Chanutin (1947) have shown that sodium pentobarbital anaesthesia in control rats cause a significant hypertrophy of the adrenals within a few hours. Adrenal ascorbic acid content is depressed within a few hours after anaesthesia and increases above normal within 24 hours. Severe thermal injury causes marked decrease in the adrenal ester cholesterol content during the first 24 hours. Moderate or mild burns caused significant increase in the cholesterol and ascorbic acid content. Cutaneous application of sulfur mustered was shown to be responsible for marked adrenal hypertrophy. The delayed increase in adrenal cholesterol and ascorbic acid contents appear to be the manifestations of adaptation syndrome following injury.

Many kinds of acute stress may not activate the pituitary-adrenal cortical system in non-domesticated animals (Woods, 1954). The author pointed that some rather severe stresses do not result in metabolic depletion of the adrenal cortex in wild rats. Furthermore, Woods in 1957 stated that undoubtedly adrenocortical hypertrophy results due to continued exposure to stress in the domesticated animals. Non-domesticated species may not respond to stresses by producing large amounts of ACTH. The amount of ascorbic acid in adrenals increase as the gland increases in weight.

Mikhail (1961) studied hypertrophy of the adrenal cortex of the rat following prolonged administration of ACTH. Unilateral adrenalectomy, castration and pregnancy are associated with narrowing of the glomerulosa and outward
displacement and increased cellularity of the subglomerulosa. Only late in pregnancy did the glomerulosa become wider than usual. The main enlargement occurred in the fasciculata and reticularis. Alterations in the inner zones of the gland differed in each case and they might have been produced by the specific effect of either ACTH or gonadotrophins.

Mikhail and Mahran (1965) reported their study on adrenals of rats from newborn to 9 weeks. 15 μ sections were stained with haematoxylin and eosin, Holmes, Bodian and Bielschowsky method to show the nerve fiber and cells in the adrenal gland. In the newborn rat adrenals, nerve fibers extend from capsule and traversed the cortex and in the central part of the gland. Collection of autonomic nerve cells were already present in the central part of the gland. By the end of the first week postnatal and in subsequent ages the definite pattern of innervation became established. With the appearance of the zona reticularis in the third week the intrinsic adrenal plexus was found to lie in the inner fasciculata and reticularis. After complete growth of zona reticularis the nerve plexus was largely in the zona reticularis. The presence of the intrinsic adrenal plexus in the inner fasciculata in immature rats and in the zona reticularis in adult emphasize the functional significance of the inner zones of the adrenal cortex.

Mikhail and Amin (1969) studied the adrenal gland of human newborn, 1, 3, 5 year old children and adult. 12 to 15 μ thick sections of the adrenal gland were stained by H & E, Mallory Azan, Holmes and Bodian method. In findings, sympathetic ganglions were found embedded beneath the capsule. Similar but
smaller ganglia were found in the three zones of the adrenal cortex as well as in the medulla. Other autonomic nerve cells, parasympathetic ganglion-like cells were seen in all layers of the cortex and medulla. At birth, fetal cortex is occupied by a thick layer of nerve cells. At 3 to 5 years the intricate plexus of the zona reticularis appeared. The significance of the intrinsic nerve supply lie in its many regulatory function in the secretion of various steroids and catecholamines.

In rat, Mikhail (1973a and b) found that in the first week PN the zona reticularis appeared. Before its development completed, in several parts of the gland, the columns of fasciculata extended to the medulla of the gland. It consisted of short anastomosing cords of small cells separated by sinusoids. In second and third week PN, the reticularis, though apparent, constituted of an outer zona glomerulosa and a much wider inner fasciculata. From fifth to seventh week, the cells of reticularis were small and were arranged in anastomosing cords of cells separated from each other by wide sinusoids. Nuclei of the cells were large, cytoplasm contained vacuoles. At tenth week zona reticularis was quite apparent but it was smaller than the fasciculata. At 16th week the zona reticularis appeared similar to the previous age groups. Signs of degeneration did not appear at any stage of the study.

Sakellaries and Vernikos-Danellis (1975) recorded a rapid increase of glucocorticoids within short period of 10 to 15 minutes. With prolonged stress, this initial rise in the plasma corticosterone was followed by a decline to prestressed level; with further stress, eventually the plasma corticosterone level again rose to a
high level. The authors cited several investigators who have proposed that under some circumstances pituitary-adrenal functions lacks precise negative feedback control since large doses of corticosteroids or elevation of endogenous steroids fail to block the release of ACTH in response to acute stress.

Kuhn et al (1978) stated that maternal deprivation or in other words interruption of mother-infant interaction is a "stressful" experience that has adverse biochemical, physiological and behavioural consequences for the offspring. They also reported that ornithine decarboxylase (ODC) activity, a sensitive index of organ growth and differentiation decrease in brain and heart of rat pups after just 1 hour of maternal deprivation and increase rapidly when the pups were returned to the mother. The decline in ODC appears to be mediated by a metabolic or endocrine signal rather than from a direct neural stimulus. The decline in ODC is independent of adrenal steroid but several suggestions points the involvement of other hormones responsible for decline in ODC in maternally deprived pups.

Jean-Faucher et al (1981) have shown that increased population size induced decreased body and seminal vesicle weight; testicular, pituitary and adrenal weights were little affected. Plasma testosterone levels were lowered by increasing population size over a period from weaning to 50 days but they were increased at 60 and 90 days. The age at which first fertile mating occurred was not affected. These results indicated that the endocrine function of the testis as determined in the experiment was specifically affected by the differential housing.
Mazzocchi et al (1982) tested effect of Nafenopin, a hypolipidemic drug on zona fasciculata of the rat adrenal cortex. Results showed chronic nafenopin treatment (5 days) significantly decreased serum cholesterol level, while it did not alter blood corticosterone concentration. Stereologically observed, a significantly increased volume of zona fasciculata was noted. One day of Nafenopin administration provoked a slight but significant lipid depletion in adrenocortical cells, while three days of continuous drug treatment induced an extreme lipid depletion. It was inferred that Nafenopin elicits compensatory response enabling adrenocortical cells to maintain an adequate level of hormonal output.

Armario et al (1984) observed that crowded rats (10 per cage) showed lowered body weight than control rats (3 per cage). Crowding decreased food intake and increased water intake. Crowding did not modify the weight of endocrine glands. Only relative testicular weight was significantly increased by crowding. Chronic noise did not modify basal levels where as acute noise decreased serum glucose and insulin levels, although hypoglycemia was transient.

Forced swimming decreased insulin and increased glucose levels. In summary (1) the serum insulin levels were sensitive to both physiological and psychological stresses, (2) forced swimming cause more marked glucose and insulin responses than noise exposure, (3) chronic intermittent noise did not alter pancreatic function and (4) no sign of adaptation was apparent after repeated exposure to noise (Armario et al, 1985a); further chronically stressed rats showed a slight decrease in body weight gain and an increase in relative adrenal weight.
Chronic stress did not modify defaecation rate but reduced exploratory activity in the holeboard. Neither basal nor acute-stress induced levels of adrenocorticotropic (ACTH) were modified by previous chronic stress. Corticosterone response to acute restraint stress was higher in chronically stressed than the control rats (Armario et al., 1985b).

Gallo-Payet and Escher (1985) have shown that glomerulosa cells possess a higher concentration of ACTH receptors than fasciculata cells. However, if it is considered that the tissue volume of zona fasciculata is about 7-fold that of the zona glomerulosa, the total number of receptors should be higher in zona fasciculata. This should be in accordance with the functional activity of the two zones, since the zona fasciculata is responsible for the major part of the glomerulosa production.

By a stereological study of the adrenal cortex of unilaterally adrenalectomized hamsters, Kasprzak and Malendowicz (1985) observed two noteworthy points. Firstly, unilateral adrenalectomy did not change the weight of the remaining adrenal gland. Secondly, due to unilateral adrenalectomy, the males and females showed clear differences in the compensatory hypertrophy of the adrenal cortex. Adrenalectomy evoked an increase in the average volume of zona fasciculata cells in males and zona reticularis cells in female hamsters.

In 1986, Miskowiak et al. reported that both CRF and ACTH treatment resulted in an increase in weight of the supra renal gland. Both treatments have
shown increased volume of adrenocortical cells as well as enhanced total number of parenchymal cells in the paired glands. However, no significant difference could be seen in the functional parameters of adrenocortical cells of CRF and ACTH treated rats. Thus in conclusion, long term treatment of adult male rats with CRF evoked adrenocortical changes similar to those observed after ACTH administration.

Gamello et al (1986a) have shown that in rats lack of adaptation to moderate and intensive stress leads to decrease of activity variables, and increase of defaecation rate and high sensitivity to restraint ulcer. The effect of crowding conditions show higher values for the basal secretion of corticosterone and atrophy of the thymus gland besides which crowd reared animals showed adrenal hypertrophy. Further, Gamello et al (1986b) showed that crowd reared rats (10 rats per cage) have significantly lower body weight at the end of the crowding period compared to the control rats (5 rats per cage). After 200 days of being reared in normal condition the body weight of crowd reared rats were still significantly lower than those of the control rats. Crowd reared rats also had significantly lower thymus weight and higher adrenal gland and testis weight compared to controls. Food intake was similar in both groups.

Kant et al (1987) observed that plasma corticosterone levels were elevated during the first 7 days in the stressful environment but returned to control values by day 14. Levels of stress induced changes in glucorticoids but not in ACTH or
Prolactin might mediate some of the physiological changes that occur as a result of chronic stress.

In male Wistar rats, Alario et al (1987) observed that noise stress reduced body weight gain and food intake. No adrenal hypertrophy but an increase in relative DNA content by stress was found. ACTH and dexamethasone-treated rats showed a body weight and food intake decrease. Adrenal hypertrophy and hyperplasia were found in ACTH treated rats, whereas dexamethasone provoked adrenal atrophy with a decrease in DNA content.

Observations of Cure (1989) have shown reduction in the body weight of male rats during chronic exposure to heat (34°C) continuously for 25 days. There were major alteration of behaviour specially during nocturnal period of light: dark (L-D) cycle. Heat altered circadian plasma corticosterone rhythm. This could be linked to changes in feeding behaviour and reduced metabolic activity. Further, chronic heat exposure induced poor adaptation than intermittent heat exposure.

The development of fetal sheep adrenal cortex between 53 days of gestation and 2 days PN occurs in 3 phases. The establishment of the functional zone occurs from 53 days to 100 days, 100 days to 130 days is a period of quiescence and reactivation takes place between 130 days to 2 days PN which is the period of structural and functional maturation. The development of cortical and medullary compartments of the adrenal gland are closely linked, for both showed rapid mid-gestational growth which slowed with attainment of definitive tissue.
organization. The second phase of growth associated with increased and controlled catecholamine secretion in the medulla and cortisol in the cortex in late gestation (Boshier et al, 1989a and b).

Hennessy et al (1989) have shown that pre-weaning guinea pigs subjected to maternal deprivation exhibited higher plasma levels of ACTH. The results of this study provide evidence that brief maternal separation can serve as a potent stimulus for activation of the hypothalamo-pituitary-adrenal system in guinea pigs. The combined influence of separation and other aspects of manipulation (e.g. exposure to novelty) evoked sympathetic responses. The number of vocalisations emitted by pups during the first 20 min of isolation in the test cage was positively correlated with concentrations of ACTH, epinephrine, and norepinephrine following 24 hr of maternal separation, but not following 90 min or at baseline. Thus initial behavioural response appears to be predictive of the levels of both hypothalamo-pituitary-adrenal and sympathetic activity 24 hr after separation is initiated.

In the pre-weanling rats, Rosenfeld et al (1991) investigated the effect of 24 hr maternal deprivation on the infant's hypothalamic-pituitary-adrenal system. The study has shown that at least 2 different aspects of the infants HPA axis are strongly modified by maternal cues. First, prolonged-maternal deprivation leads to a marked potentiation of responsiveness of the HPA axis. Second, the stress response can itself be suppressed by a lactating dam. Contact per se may in this case be the critical maternal variable. Based on these findings it was proposed that during development the mother should be considered as an integral part of the
system regulating the infant's HPA axis. Thus the data indicated that the infant rat shows a marked increase in adreno-cortical responsiveness to stress immediately following prolonged maternal deprivation.

In the ensuing year, Rosenfeld et al (1992) stated that short period of deprivation did not have persistent effect. The authors further stated that these effects do not appear to be additive, so far as repeated maternal deprivation did not have a cumulative effect. If the period of deprivation is sufficiently long (24 h), the induced hyper responsivity to stress will persist for at least 4 hrs following maternal reunion. In summary: (1) Short periods of deprivation did not have cumulative effect and (2) there is a critical length of deprivation beyond which persistent changes in adrenocortical responsivity ensue. It was suggested that basal and stress-induced corticosterone secretion in the infant rat are sensitive to different aspects of maternal deprivation.

Kant et al (1992) sacrificed rats following 3 or 14 days of stress and found that after 3 days of stress, plasma corticosterone and prolactin levels were elevated in both stress groups compared to controls. ACTH levels were similar in stressed and control rats. After 14 days of stress, ACTH and corticosterone levels in both stress groups were similar to control levels. The results suggest that control over stressors attenuate the effect of stress on physiology and demonstrate that two hormones with diverse biological effects are elevated by chronic stress.
Kant et al (1997) demonstrated that stress may impair both the rate and patterning of behaviour and suggest that this rodent paradigm may usefully model some aspects of the effects of stress in human.

Two types of stresses, forced swimming and supine restraint for a period of 15 days have resulted in significant increase in the weight of cerebrum, cerebellum, pituitary, thyroid and adrenals. However, testis and seminal vesicles did not show significant change in weight (Desai et al, 1993).

Loesser et al (1994) have studied steroidogenesis in adrenal cortex by immunoelectron microscopy in rat and noted quantitative evidence that isolated unstimulated zona glomerulosa and zona fasciculata cells have different peripheral cytoplasmic properties. ACTH stimulation affect these properties of actin concentration and aggregate microvillar length differently according to cellular zone of origin. Their results suggest that ACTH-induced hormone release from zona fasciculata cells is mediated by increase in peripheral cytoplasmic actin aggregate microvillar length; in zona glomerulosa cells such changes are small or absent.

Prolonged maternal deprivation during infancy increases basal and stressor induced corticosterone levels. Avishaiephner et al (1995) have shown that in rats cold stress induced a robust elevation of plasma corticosterone throughout first 2 postnatal weeks. Maternal deprivation increases cold induced corticosterone levels indicating the basal and stress induced corticosterone after 24 hours of maternal deprivation.
Baez et al (1996) have shown that corticosterone is critically involved in both behavioural strategies selected by the animals when they are forced to confront and inescapable and stressful situation. In addition, under these conditions the functional importance of the hormone release is expressed when the subject further faces a stressful novel situation either with or without escape chance.

TESTIS AND STRESS:

Elftman (1950) reported that the cytoplasmic processes of the Sertoli cells go through a cycle of changes during the maturation of each successive generation of spermatids. As one generation of spermatozoa leave the Sertoli cell; its branches enclose the next generation of spermatids in a cytoplasmic network. This is correlated with a marked acceleration in the rate of maturation of the nucleus and acrosome of the spermatid and its protoplasmic lobe, resulting in a cluster of immature spermatozoa attached to stout Sertoli trunks. A migration of the heads of the immature spermatozoa towards the basal membrane is followed by their reversed migration towards the lumen. This results slender Sertoli trunks, still attached to the periphery of the tubule with their nuclei temporarily displaced a short distance towards the lumen. With the return of the nucleus to its basal position and the liberation of the spermatozoa, the Sertoli cell envelopes the next generation of spermatids, beginning another cycle.

The acceleration of the spermatids when the Sertoli cytoplasm surrounds them emphasizes the critical importance of this phase in the history of both the
Sertoli cell and the spermatid. Full visualization of the Sertoli cell re-emphasizes its strategic location for the transfer of materials with or without chemical change, from the vascular supply at the periphery of the tubule to the spermatids.

Leblond and Clermont (1952) vividly described the differentiation and the stages of the cycle of the seminiferous epithelium in rats. In the rat, development of any one generation of spermatogonia, spermatocytes or spermatids is closely integrated with that of other generations present in the same area of the tubule. Thus, the cells are not arranged at random but organised into well-defined cellular association. The preparations stained with haematoxylin cannot be characterized with precision, therefore, cellular associations were defined by stages of development of the spermatids by their staining reaction to the "Periodic Acid-Schiff" (PAS) reagent.

Spermatogenesis can be divided into 19 stages (1 to 19). During first 8 stages of the development of young spermatids, the seminiferous epithelium also contains a generation of older spermatids, which are released when the younger generation completes Stage 8. During the next few stages of development, the remaining spermatids are the only one present, but when they reach Stage 15, a new generation again arise in the same area of the tubule. The cellular associations present at that time include Stage 1 and 15. Again, the young spermatids go through their development, as different cell associations succeed one another in the tubule. When this generation reaches Stage 15, another group of young ones arises in the same area of the tubule and again there is a cellular
changes occurring between two successive appearances of this cellular association makes up a cycle of the seminiferous epithelium. Since, during a cycle of seminiferous epithelium, the new generation of spermatids go through 14 well-characterized stages, the cycle was subdivided into 14 corresponding periods.

Further, it was decided to designate the stages of spermatogenesis by Arabic numerals. The corresponding periods of the cycle of the seminiferous epithelium with their definite cell associations are also referred to as "Stages" or "Stages of the cycle" but are designated by Roman numerals. Accordingly, Stage I of the cycle will include amongst other cells, spermatids at Stage 1 of spermatogenesis; Stage II of the cycle, spermatids at Stage 2, and so on, up to Stage XIV. The characteristic features of the stages are as under.

Stage 1-3 (Golgi phase) is characterized by the appearance of acrosomic granules in the idiosome of the spermatid.

Stage 4-7 (Cap phase) is characterized by the acrosomic granules and covers one pole of the nucleus.

Stage 8-14 (Acrosome phase) is characterized by the nucleus that undergoes a series of changes leading to the formation of sperm like nucleus.

Stage 15-18 (Maturation phase). In this phase, the immature sperm completes with little change of its morphological evolution into a "free spermatozoan".

The occurrence of the various stages in the evolution of spermatocytes and spermatogonia may be related to the first 14 stages of the spermiogenesis within which the 5 mitotic peaks are found in the stages IX, XII, I, IV and VI of the cycle.
and each one of them appears to double the number of spermatogonia present in the tubule except at Stage VI, when the cells resulting from mitosis are young spermatocytes.

Some of the nuclei of the sertoli elements transform from the "parallel" type (with long axis, "parallel" to the basement membrane) to the "perpendicular" type (with long axis perpendicular to the basement membrane). This evolution takes place from Stage I to Stage VIII of the cycle, when the number of "perpendicular" type nuclei reach a peak. At Stage IX, these nuclei flattened on the basement membrane and remain so for the rest of the cycle.

Roosen-Runge (1952) stated that in rat the spermatogenesis has been the prototype of sperm development in mammals. The story begins with a large cell which may be called a stem cell; which in adult, has been termed as spermatogonium of Type A. Usually, it divides twice and become irreversibly into spermatogonia of Type B. If for reasons unknown, this division does not occur, or does not occur in time, the stem cell will finally revert to its initial size and at a definite time, begin again to divide, thus starting another generation. But only two or three cells in a hundred revert like this. The majority metamorphose into the smaller Type B spermatogonia with a "crusty" nuclei, and generally undergo two more synchronous division after which they can be regarded as early primary spermatocytes. The duration of this phase of development, from stem cell to spermatocyte is one-fourth the duration of spermatogenesis. Some authors have called the cells resulting from the last division of spermatogonia "spermatocyte"
when the nuclei, however, are restored after this division, the cells do not immediately appear like spermatocytes. They are small, but resemble the spermatogonia in cell shape and nuclear structure. After a resting stage of many hours, they transform rather suddenly into round cells with an early leptotene nucleus. Further, they develop slowly through leptotene, zygotene and pachytene stages. Then they undergo reduction division into secondary spermatocytes and these divided after a very brief resting stage into spermatids. The spermatids do not usually divide again in the rat but undergo spermiogenesis. The process involves the formation of acrosome system, the head cap, finally, the head and the tail of the spermatozoon and the loss of most of the cytoplasm of the spermatid. Thus, the highly individualistic motile spermatozoon emerges.

Since the main feature of this process is the reappearance at each cycle of a new dormant cell which acts as the stem cell of a subsequent generation of spermatocytes, it is described as the "Stem Cell Renewal Theory". This process accounts for the cyclic formation of the spermatocytes as well as for the maintenance of spermatogonia in rats and other species.

By applying histometric techniques, Clermont and Morgentaler (1955) studied the spermatogenesis in hypophysectomised rats. They observed the first cell to disintegrate and disappear from the seminiferous tubule after hypophysectomy were step 9 to step 19 spermatids. Upto 25 days after hypophysectomy, the frequency of stages of cycle was found to be closely similar to that observed under normal conditions. A partial degeneration of Type A
spermatogonia takes place during their proliferation. Type B spermatogonia divide in to resting spermatocytes without loss. Marked degeneration of spermatocytes occur before and during meiosis in such a way that at 44 and 61 days after hypophysectomy, only 4% of spermatocytes appearing in the testis have produced spermatids. These spermatids evolve up to step 7 of spermiogenesis and then degenerate. In conclusion, spermatogenesis in rats may proceed independently of hypophyseal hormone stimulation up to step 7 of spermatogenesis but only a very small number of cells reach this stage.

Roosen-Runge (1955) reported the finding of his histological study of the seminiferous tubules of the rat in 8 different stages of the spermatogenic cycle. The report states that the growth of the primary spermatocyte is approximately exponential. This cell appears to have the deceleration growth phase before it enters the first mitotic division. The total increase in volume from late spermatogonium to spermatocyte just before the first maturation division is at least 7 fold. The sertoli cell undergoes cyclic volume changes, the most pronounced of which is the loss of about 40% of volume immediately after or during the release of the spermatozoa from the tubule. The total volume of Leydig cells was found to be 1.7% of the total volume of the testis.

Clermont and Perey (1957), by means of histological study reported that the mean number of resting and dividing supporting cells and gonocytes (and their progeny) per cross section of cord or tubule revealed that the supporting cells proliferate actively at and soon after birth but stop dividing in 15-day rats. By 45
days supporting cells, which never give rise to spermatogonia become typical Sertoli cells.

The gonocytes start proliferating in 4-day rats and give rise to type A spermatogonia (However, many gonocytes fail to divide and degenerate). In 6 day rats the type A cells proliferate and give rise to new type A cells. Stem cells which at later time intervals provide for the formation of new generation of spermatogonia. By the 15th day, the yield of spermatocytes per type A stem cell is identical to that found in the normal adult. This indicates that a quantitatively normal spermatogenesis is initiated as the type A spermatogonia appear, that is, as early as 4 days after birth. On the 15th day, it becomes evident that cyclic changes of the seminiferous epithelium similar to those observed in the adult are already established. Presumably the cycle begins as type A spermatogonia start proliferating and from the start the duration of the cycle approximates 10 days as in the adult.

Steinberger and Dixon (1959) experimented on the adulthooded rats. A 15 minute exposure to a temperature of 45°C produced progressive destruction of the entire germinal epithelium. The earliest cytological changes were observed in spermatids. A 15 minute exposure to temperature below 43°C produced inconsistent tubular damage in some animals. A 15 minute exposure to 43°C produced testicular damage in the majority of animals. The damage was characterized by a selective destruction of the spermatocytes. In conclusion, the
authors stated that heat produces a specific type of germinal epithelium damage which is masked when an excessive amount of heat is applied.

Clermont (1962) stated that the ratio of average number of type A stem cells to the average number of other germ cells calculated from corrected counts by Abercrombie's (1946) formula. The counts were similar to the ratios calculated from uncorrected counts. The report states that a pair of type A stem cells starts proliferating at stage IX of the cycle, thereafter the daughter cells divide successively at stage XII and XIV of the cycle, their number increasing in geometrical progression. Following the third spermatogonial mitosis, a pair of cells become dormant and form new type A stem cells which will later provide for the new generations of spermatogonia. The other spermatogonial elements arising from the third spermatogonial divisions differentiate into intermediate type spermatogonia which give rise to type B spermatogonia during stage at IV of the cycle. The type B cells divide at stage VI to produce a generation of primary spermatocytes. This revised "Stem Cell Renewal" model, still considered as statistical, gives an accurate but theoretical representation of the maintenance of the stock of spermatogonial stem cells and of the periodic formation of generations of spermatocytes produced per stem cell is in actual fact lower than the theoretical number proposed by the model due to the degeneration of some (10.6%) type A spermatogonia at stage XII of the cycle. Finally, the number of spermatid produced by the spermatocytes is only 72.6% of the expected yield due to degeneration taking place during the first and second maturation division of spermatocytes.
Chowdhuri and Steinberger (1964) in progression to their previous work exposed adulthooded rats to 43°C for 15 minutes and studied testis at intervals of 2 to 26 days. The authors found that the frequency distribution of various stages of spermatogenesis, the resting spermatocytes, spermatogonia and Sertoli cells were not affected by exposure to heat. On the other hand, primary spermatocytes in stage IX (leptotene), and including dividing spermatocytes in stage XIV, were injured, excepting for pachytene spermatocytes in stage V and VI of spermatogenesis. The spermatids were affected by heat only in step 1 and the early part of step 2 of spermatogenesis. Those beyond step 2 continued to mature and form adult spermatozoa. The study indicated that heat produces selective damage to the germinal epithelium affecting only specific types of germinal epithelial cells.

Clegg (1966) using histometric methods studied Wistar rat testis and accessory reproductive organs. The findings revealed that the growth increments in the Leydig cells decline exponentially with decrements after the age of 51 days, the accessory organs show a pubertal growth spurt. The findings suggest that at puberty there is both an increased output of testicular androgens and increased sensitivity of the accessory reproductive organs to these substances.

Clermont and Bustos-Obregon (1968) reported that there were five distinct classes of type A spermatogonia. The type A1 found in stages II-VIII of the cycle of the seminiferous epithelium had round, pale-stained nuclei, typically arranged in linear clusters of four or eight along the tubular wall. They all divide at stage IX to
produce type A₂ cells. These in turn divide at stage XII to produce type A₃ spermatogonia. The type A₂ and A₃ cells have large ovoid nuclei containing globular masses of deeply stained chromatin and were randomly distributed in the space between Sertoli nuclei. The type A₃ spermatogonia divide at stage XIV to produce A₄ cells. These have similar nuclei sometime lobulated containing more deeply stained chromatin granulation. They divide in stage I of the cycle to yield two classes of spermatogonia: intermediate type and new type A₁. Hence, type A₁ - type A₄ spermatogonia were considered as "renewing stem cells". The fifth class of type A spermatogonia (A₀) was found at all stages of the cycle. Rare, isolated or in pairs, they did not appear to be actively involved in cell renewal and were tentatively considered as "reserve stem cells".

Dym and Clermont (1970) stated that in normal adult rat testis, type A₀ spermatogonia do not appear to participate to a significant extent in the production of spermatocytes, while type A₁ spermatogonia periodically initiate a series of divisions resulting in the production of spermatocytes and new type A₁ spermatogonia. The behaviour of type A₀ and A₁ spermatogonia was investigated following administration of a single dose of X-rays to testis. At 8 and 13 days after irradiation, type A₁ spermatogonia reached the lowest value, i.e. 6% and 13% of non-irradiated control, while type A₀ reached the lowest value, i.e. 62% of control at eight days. Therefore, the numbers of type A₀ and A₁ progressively increased to return to normal at 39 days. It was thus concluded that A₀ were comparatively more resistant to X-irradiation than type A₁ spermatogonia.
Huckins (1971) identified and reported that according to topographical arrangement, three groups of undifferentiated A spermatogonia can be classified, paired, isolated and aligned spermatogonia. It was proposed that the isolated (as well as a few paired) spermatogonia, which were always present throughout the seminiferous epithelium, are the functional stem cells and should therefore be designated as $A_0$. Through sporadic divisions, the $A_s$ spermatogonia both maintain their own numbers and give rise to pairs of cells which are designated to eventually differentiated. The latter undergo several synchronous divisions in succession, thereby forming increasingly longer chains of aligned spermatogonia. The proliferation of these chains, primarily in stages I-V, leads to a gradual expansion in the size of the undifferentiated type A population. When the population attains its maximal size in stage V, mitotic activity among the aligned cells ceases, and all of these cells morphologically transform without further division into typical $A_1$ spermatogonia. Subsequently, the cohort of $A_1$ cells synchronously divides in stage IX to begin the long process of spermatogonial maturation. The isolated (and a few paired) cells, which do not undergo this transformation and remain quiescent during the stage IX peak of mitosis, form a residual stock of stem cells, that during the course of another cycle, rebuild the population of aligned A spermatogonia which will cyclically differentiate is insured.

Hayashi and Harrison (1971) histologically studied the development of the interstitial tissue of prepubertal, cryptorchid and normal human testes and reported that the absolute number of undifferentiated cells within a unit volume of 1 cubm of interstitial tissue is higher in undescended testes than in normal scrotal testes from
individuals of the same age. Leydig cells are absent from the interstitial tissue of prepubertal undescended testes, but are present during the 1st year of postnatal life in normal testes; they appear first in undescended testes near puberty, by differentiation from mesenchymal cells.

Both LH and FSH are required for spermatogenesis. It is currently held that all the effects of LH are mediated by way of testosterone from the Leydig cells. As a consequence, testosterone and FSH are the hormones which acts directly on the seminiferous tubular epithelium. In immature animals, initiation of spermatogenesis requires both testosterone (or LH) and FSH. Once the normal germinal epithelium is established, testosterone alone can maintain sperm production in the hypophysectomized animals, provided treatment is begun immediately after the removal of pituitary. However, if the seminiferous epithelium is allowed to regress, then both testosterone and FSH are again required to reinitiate spermatogenesis (Steinberger, 1971).

Clermont (1972) reviewed the past literature on testis more in particular, about the seminiferous tubular epithelium. Accordingly, he described spermatogenesis produced as a result of spermatogonial mitotic division; a second phase, during which spermatids are produced as a consequence of the meiotic divisions of spermatocytes; and a third phase, during which spermatozoa are formed as a result of the metamorphosis of spermatids.
There are certain features of spermatogenic process. One is that, for a given species, each step of spermatogenesis has a constant duration; thus, germ cell differentiation unfolds as if regulated by a rigid time-scaled program. Another feature is that many spermatogonial stem cells enter spermatogenesis simultaneously and almost synchronously. Consequently, large groups of germ cells, called “generations” evolve synchronously throughout the spermatogenic process. The seminiferous epithelium is composed of five or six such generations of germ cells that are not randomly arranged but form cellular association of fixed composition. Due to the precise and regular timing of the steps of spermatogenesis, spermatids at a given step of spermiogenesis are always associated with spermatocytes and spermatogonia at given steps of their development. The cellular groupings, which reappear at regular intervals, were taken to represent stages of a cycle of the seminiferous epithelium. The number of stages composing the cycle vary with the species. The duration of the cycle of the seminiferous epithelium varies among several mammals; it ranges from 8.6 days in mouse to 16 days in man. There are marked differences in the modalities of spermatogonial renewal from one species to another; thus for example, the number of successive spermatogonial mitoses leading to the production of spermatocytes varies from 3 in man to 6 in rat. The spermatogonial stem cells that belong to the type A category may be classified as “reserve” and renewing stem cells. The cells, which include some type A spermatogonia, intermediate type (when present), and type B spermatogonia, are destined to produce spermatocytes.
Kothari et al (1972) stated that the Leydig cells in dog testis constitute about 15% (14.2 to 17.1%) of the testicular volume. In absolute terms it comes out to be $1.56 \pm 0.45$ ml of Leydig cells per testis. Larger testes contain proportionately greater amount of Leydig cell tissue, the relationship being almost linear.

Erickson and Martin (1973) found no significant difference in the number type A spermatogonia in testes of non-irradiated rats between the ages of 2 and 16 months. Irradiation reduced spermatogonial numbers to 21 to 25% of control in both age groups. The results of the study revealed that irradiation produces a permanent decrement in the stem spermatogonial population with the magnitude of the decrement being age dependent.

Clermont and Mauger (1974) mentioned that as stated previously (Dym and Clermont, 1970) postulated an interaction between the two main classes of type A spermatogonial stem cells of adult rats, i.e. the renewing type $A_1$-$A_4$ cells were possibly inhibiting the reserve type $A_0$ spermatogonia from entering mitosis. The present paper supported this theory and suggested that the former cells may be involved in the production of a spermatogonial chalone. The existence of ‘chalones’ defined as ‘an internal secretion produced by a tissue for the purpose of controlling, by inhibition, the mitotic activity of that same tissue (Bullough, 1967).

Clermont and Mauger (1974) stated that chalones are factors inhibiting mitosis under normal conditions and thus keep growth under control. Since after irradiation of the testis there is a recovery requiring mitosis of type A spermatogonia
(presumably of the reserve category) it was thought that an extract of normal adult
testes may contain a chalone controlling its growth and that the chalone might
inhibit the recovery taking place after irradiation. The results of this paper indicate
that a saline extract of normal adult testes contains a substance that can inhibit
specifically the proliferation of type A spermatogonia during the repair phase of the
spermatogonial population following irradiation. The substance was tentatively
considered as a spermatogonial chalone. Further, Clermont and Mauger (1976) by
similar experiments found that the saline extract of adult testes contained a
substance, a spermatogonial chalone, inhibiting specifically the proliferation of
some type A spermatogonia. This supports the concept that a spermatogonial
chalone may intervene, through its action on the spermatogonial stem cell
population, to arrest the growth of the seminiferous tubules as the animal reaches
maturity.

Clermont and Hermo (1975) stated that spermatogonia perpetuate
themselves while producing generations after generation of spermatocytes. In the
rat, resting and dividing spermatogonia of the three types present (A, B and a
transitional cell referred to as the intermediate type). The divisions of
spermatogonia occur in five successive peaks during the cycle. Four of these
mitotic peaks (Stages I, IV, IX and XII) are followed by an increase in the number of
spermatogonia, while the 5th (Stage VI) is followed by the appearance of
spermatocytes. At approximately the beginning of each cycle, spermatogonial
mitoses give rise to type A spermatogonia, a small number of which are described
as "dormant" since they do not divide during the first 8 stages of the cycle. At Stage
IX, however, each dormant cell initiates a series of divisions, the first two of which produce 4 type A cells. By the beginning of the next cycle, one of these 4 cells stops dividing to become a new dormant type A cell, while the three others give rise to 8 "intermediate type" spermatogonia, which in turn divide to give rise to 24 young spermatocytes.

Russell and Clemont (1976) stated that near the end of spermatogenesis, the late spermatids remain attached to the superficial layer of the seminiferous epithelium for 3 to 4 days. The sickle-shaped head of the spermatids are embedded in an apical process of sertoli cell cytoplasm which is connected to the rest of the cells by a narrow stalk. In the concavity of the head several long (2-3 μm) and very narrow (50 nm) tubular projections of the spermatids plasma membrane invaginate the Sertoli cell cytoplasm. These tubular processes terminate by a bulbous swelling. Along the process of the plasma membrane of the Sertoli cell is closely opposed to the spermatid's membrane, the intercellular space being only 6-8 nm wide. In the Sertoli cytoplasm immediately over-rounding the tubular portion of the structure there is an accumulation of filamentous material, while next to the bulbous extremity there are at a short distance, smooth surfaced cisternae of endoplasmic reticulum, the whole structure was referred to as a tubular tubulobulbar complex. These complexes, of which there are up to 24 per spermatid, appear as these cells complete their migration towards the apex of the Sertoli cells. Then disappear just before the release of the spermatids in the lumen of the seminiferous tubule as a result of the fragmentation of the spermatids plasma membrane followed by a resorption of the Sertoli plasma membrane. Morphological
evidence suggests that the tubulobulbar complexes serve as an anchoring device that retain the spermatids at the surface of the seminiferous epithelium, while their dissolutions contribute in part to the process of spermiation. Similar tubulobulbar complexes were also formed by the plasma membranes of two adjacent Sertoli cells close to the Sertoli tight junction near the tubular limiting membrane.

Nazian and Piacsek (1977) have shown that ambient temperature has no function on serum FSH levels. Serum LH level was elevated (>45 mg/ml) in cold raised rats. Total serum androgens showed no pattern. Relative testicular weight was maximal between 49 and 60 days in cold exposed animals. Mature sperm count was found decreased in cold exposed rats. The authors conclusively stated that low ambient temperature appeared to delay sexual maturation in the male rats. This delay make the result of a delay in the appearance of an LH "Surge" or by a depression in prolactin or a change in some other hormone.

Based on then existing evidences that the aging human male experience a gradual decline in testosterone production, Kaler and Neaves (1978) highlighted a phenomenon that should be reflected on the Leydig cell population of the testis. The authors reinvestigated this by histometric analysis of testes in men aged between 18 and 87 years. Results showed average single Leydig cell volume (2.43 ± 623 μm³) did not change significantly with increasingly age, suggesting that surviving cells remain active. Total testis weight (43.5 ± 13.9 g) also did not change with age. However, both total Leydig volume and absolute number of Leydig cells per individual decreased significantly as functions of age. Analysis of the
relationship between these two parameters indicate that the total volume of Leydig cell cytoplasm contained within the human testis is determined by the number of cells present. The results of this study has shown that a pair of young adult testes endowed with more than 700 million Leydig cells at 20 years of age may be expected to undergo an attrition rate of approximately 80 million cells per subsequent decade of life. Thus, Leydig cell attrition is an important correlate of declining androgen status in aging men.

By a histologic, histomorphometric and histochemical study of pig testis, Vanstraaten and Wensing (1978) reported that during fetal period and sexual maturity Leydig cells show two developmental and activity phase. One perinatally and the other from 13 weeks postpartum onwards. Before the perinatal phase Leydig cells are scarce and poorly developed, almost excluding any involvement by them in the process of testicular descent. The perinatal development results in a large volume of well differentiated Leydig cells. Near their maximal development two cell types can be distinguished by most of the parameters. The most obvious cell type is the intertubular Leydig cells these cells are the first to be formed, are the best developed type and are located in the spaces between the sex cords. The other type is the pentubular Leydig cell. They appear to be less developed and are surrounded by the coils of the sex cords. After two to three weeks postpartum both types of Leydig cells undergo regression, but distinctions persist.

The development of Leydig cells from 13 weeks postpartum onwards differs from that of perinatal phase. The intertubular cell now remain small, histochemically
inactive and located along the testicular septa. Peritubular cells develop to well differentiated cells, constituting the predominant type.

There is a considerable animal to animal variability in terms of number of undifferentiated types of spermatogonia (Huckins and Oakberg, 1978). Seminiferous tubule in the mouse reveal a pattern of behaviour which is consistent with that described for the rat. Within the proliferating compartment of the seminiferous tubule, the pair of cells may undergo one or several additional divisions to form chains of aligned spermatogonia (A1). Eventually, however, mitosis ceases, and all the spermatogonia in the proliferating compartment transform without further division into A1 cells. This cohort of A1 spermatogonia synchronously enters the differentiating compartment to initiate maturation, and the whole process begins again. Thus the stem cell compartment maintains a constant size which the proliferating compartment is regularly depleted and rebuilt again. By this mechanism, generations of A1 spermatogonia cyclically begin differentiation.

Further, in 1978 Russel used, lanthanum (an electron-opaque substance) to determine when germ cells of the rat first cross the blood-testis barrier in adult spermatogenesis. Lanthanum was shown to surround all spermatogonia, preleptotene spermatocytes and early leptotene spermatocytes of stage IX in the adult rat testis. Lanthanum was excluded from the spaces around more mature cells by newly formed tight junctions between adjoining Sertoli processes. These processes had previously intervened between the leptotene cells and basal lamina. Referring to his previous work the author stated that leptotene cells are the first
cells of adult spermatogenesis to enter the intermediate compartment and to reside beyond a permeability barrier.

Iron and Clermont (1979) observed that spermatogonia and Sertoli cells occupy a basal position in the seminiferous epithelium, being located next to the limiting membrane when viewed from above in tubules mounted in toto, these cells were seen as the most superficial layer, with the exception of the pale-staining cells of the limiting membrane. Groups of mitotic figures were seen in clusters or chains of four or more in stages IX, XII, XIV and I. These were identified as type A spermatogonia. Intermediate and type B spermatogonia also divide in compact groups in stages IV and VI. These mitotic figures could also be readily identified in all stages of the cycle and their chromosomes are longer and thinner than those of the other dividing type A spermatogonia.

Mendez and Emery (1979) studied the seminiferous tubules of stillborn children between 24 and 40 weeks of gestation and in children dying between the age of birth and six years. There was general diminution in the diameter of seminiferous tubule throughout the last trimester and immediately before birth, followed by a slow and steady increase in the diameter of the tubule throughout childhood. The germinal cells within the seminiferous tubule appears to be under some slightly increased stimulatory influence during the last few weeks prior to birth, and under considerable stimulation in the first 4 or 5 weeks after birth. Following this, these cells appear to diminish in size and become hidden among the Sertoli cells, to be restimulated around the time of puberty.
Johnson et al (1980) stated that based on enumeration of maturation-phase spermatids in testicular homogenates from adult men and rat, daily sperm production per gram (DSP/g) was found to be almost seven times greater in rats. The relative inefficiency of the human testis was uniformly expressed in cranial, equatorial and caudal regions. Histometric analysis revealed that in relative composition between humans and rats include higher proportion of parenchyma, seminiferous epithelium and germinal cells in the rat testis. Humans exceed rats only in the proportions of testis occupied by nongerminal components such as tunic, interstitium, tubule boundary tissue and Sertoli cells. The authors stressed upon by stating that the homogenization method remains a rapid and precise technique for estimation of DSP/g in humans. Histometric analysis of DSP/g support the concept that the human is less efficient in sperm production than the rat, but these methods suggest that the rat is only about twice as efficient per gram of testicular parenchyma as the human.

Tache et al (1980) subjected Charles River CD rats to 6 hr a day for 6 days of restraint stress and observed that (1) plasma testosterone suppression induced by chronic restraint is not initially mediated by a direct action at the testis level, (2) the sustained endogenous release of glucocorticoid associated with chronic restraint exposure does not play an important role in the decrease of LH and testosterone secretion but could participate in the drop of prolactin induced by long term exposure to stressor, (3) the decrease in LH secretion may be partly
Lalli and Clermont in 1981 have shown the structural changes of the head components of the rat spermatid during late spermiogenesis. The study describes the maturation phase, i.e. step 14 to 19 of spermatogenesis. Following partial condensation of chromatin from step 11-14, the nucleus continues to condense during the following steps until the end of spermiogenesis. The redundant nuclear envelope which forms along the apical and ventral aspects of the nucleus and around the implantation fossa regresses during steps 17-19. The acrosomic system splits into two portions early in step 15 to give rise to: a) the main portion with its crest-like acrosome running along the dorsal aspect of the nucleus and head cap extending over the lateral surface of the nucleus; and b) a smaller head-cap segment which is seen in step 15 and 16 along one side of the nucleus at its apical extremity. This separate head-cap segment reaches the apical-ventral aspect of the head during step 17 and condenses in synchrony with the rest of the acrosomic system in step 19 of spermatogenesis. The large crescentric acrosome, which in step 15 forms a large fin at the caudal extremity of the acrosomic apparatus, move anteriorly during step 16 and 17, while the whole acrosomic system extends further apically beyond the tip of the nucleus. The perforatorium and post-acrosomal dense lamina form a rigid capsule (perinuclear theca) that covers tightly the sickle-shaped nucleus and bind the inner acrosomal membrane and the post-acrosomal membranes. The post acrosomal dense lamina, which includes the ventral spur, appears early in step 15 as a dense cytoplasmic layer.
applied to the nuclear envelope at the caudal extremity of the nucleus except over the perifossal zone. The perforation forms during the step 19 of spermatogenesis as a result of the condensation of a wispy cytoplasmic material which has accumulated in the subacrosomal space during steps 14-18. Thus the spermatid's head is deeply modified during the maturation phase and takes its definitive shape only at the last step of spermatogenesis.

Tait and Johnson (1982) stated that in the testis of sexually active gray Squirrels 8 stages of spermatogenesis were recognizable each with a characteristic association of germ cells. The low number of spermatozoa in squirrel testis are the result of fewer spermatogonial divisions compared with those of most other mammals as well as a low efficiency of spermatogenesis, with only 42% of germ cells becoming spermatozoa. A period of testicular regression which may be prolonged follows the breeding season in June/July. In a fully regressed testes no stages of spermatogenesis are recognized, the lumen of the seminiferous tubule was occluded and no stages later than primary spermatocytes could be seen.

Lok et al (1982) have shown that the striking similarities that were found between the ram and Chinese hamster with respect to the course of the numbers of undifferentiated plus A, spermatogonia during the cycle of the seminiferous epithelium indicate that the model of spermatogonial stem cell renewal is not confined to rodents alone. The findings that such widely different animals like rams and rodents are the same in this respect suggests that the model is very general.
Nistal et al (1982) reported that the testis of human preterm, children histometric study revealed a progressive decrease in Sertoli cell number per transverse tubular section as well as per unit area of the testicular parenchyma, mainly from 3 year onwards. However, this decrease seems to be the result of progressive increase in the testicular volume as well as in the length and width of the seminiferous tubules, without change in the total Sertoli cell number per testis. This number may be considered constant, not only for adult testis but also for the postnatal developing testis. Ultrastructure findings revealed that immature Sertoli cells, present from birth to puberty, show round to elliptical nuclei, with regular outline and small nucleoli.

Mori et al (1982) studied in normal mice and reported that in a decapsulated testis, the seminiferous tubules occupy 89.3% and the interstitial tissue makes up 10.7% of the volume of the testis parenchyma. The Leydig cells comprise 3.8% of testicular volume. There are 24.9 million Leydig cells per cm³ (or gm) of tissue. An average Leydig cell has a volume of 1,533 μm³ and a surface area of 1150 μm².

The smooth endoplasmic reticulum (SER) is the most prominent organelle in the Leydig cells, and has a membrane surface area of 2,428 cm² per cm³ of fresh testis tissue which is 8.5 times the surface area of the plasma membrane and constitutes 56.9% of the total membrane in Leydig cells. Mitochondria occupy 10.1% of the Leydig cell volume or 11.4% of cytoplasmic volume. The inner mitochondrial membrane (including tubular or vesicular cristone) provides a surface area of about 2855 μm²/cell and is 2.26 times that of the outer membrane. There
are approximately 712 cm$^2$ of inner membranes per cm$^3$ tissue. Mouse Leydig cells have numerous lipid droplets, which average 147 per cell and occupy 5.1% of the cell volume.

Altered photoperiod affects the reproductive activity in many animals. Short day light delays reproductive activity in prepubertal deer mice (Whittsett and Lawton, 1982; Whittsett and Miller, 1982).

Hadley and Dym (1983) studied effect of vasectomy on monkeys and found that long after (10 and 18 month), number of spermatogonia, preleptotene spermatocytes, pachytene spermatocytes and step 7 spermatids relative to Sertoli cell nucleoli, were not altered by vasectomy. The study also demonstrated quantitatively that spermatogenesis in the monkey is not inhibited upto 18 months following vasectomy.

Singwi and Lall (1983) reported that spermatogenesis in the non-Scrotal Bat comprises (i) eleven cell associations and (ii) evolutive steps in spermatogenesis. In the seminiferous epithelium, spermatogonia of the A, intermediate, and B type are characteristically present. Leptotene, pachytene and diakinesis stages were easily discerned in dividing spermatocytes. During the 16 step spermiogenesis, the acrosomic system develops through the Golgi, cap, acrosome and maturation phases. The relative frequency of various cell associations differs markedly and is significantly at variance with other mammalian species. Fully formed spermatozoa are diamorphic with conical or blunt heads.
Tingari et al (1984) noted that in one-humped camel, testicular weight is minimal during summer, while the maximum weight was attained during the coldest months. Diameters of the seminiferous tubules were multiphasic with a tendency to be wider during summer. The ratio of the area occupied by seminiferous tubules to that occupied by interstitial tissue revealed that the latter occupied a larger area than that of the seminiferous tubules during winter, while a reversed relationship occurred in summer. There was a difference in the quality and quantity of spermatogenic cells between the monthly groups. Winter (November to January) were the months in which spermatogenic activity was most striking. The cyclic structural changes in the testis appeared to be in parallel with the rutting activity of the camel. It was suggested that the camel is not a typical seasonal breeder as it does not go through sexual quiescence. The reproductive activity of the camel builds up during September and October, and the animal is in actual rut during November to February with a drop in March and thereafter.

Johnson et al (1984) studied effect of microwave-irradiation on rat testis and found no degeneration in spermatogenesis when potential sperm production rates were determined either from type B spermatogonia to spermatids or from type B spermatogonia to a post-testicular approximation of sperm production rate. The authors concluded that regulation of sperm production rates must take place during spermatogonial mitoses, since once the number of type B spermatogonia is determined there is essentially no subsequent alteration in sperm production in normal or irradiated adult rats.
Schultz et al (1984) stated "round body" a spherical structure typically associated with a nucleolus in small germ cells of the rat. Cytochemical analysis indicate that the round body composed of nonhistone protein which appear in the form of 1.6 nm wide fibrils. Development begins in late leptotene when a single round body appears in each spermatocyte as an irregular spheroid located along the inner surface of the nuclear envelope. During subsequent stages of the meiotic prophase, the round body leaves the nuclear envelope, becomes a regular sphere, and gradually enlarges from a diameter of 0.4 μm in leptotene to 1.6 μm in diplotene. At each maturation division, the amount of round body material is decreased by about half, presumably because the constituent proteins are dissociated at metaphase, distributed between two daughter cells at telophase, and reconstituted into half-sized round bodies. As spermatogenesis proceeds, the round bodies shrinks gradually and disappears at step 8. It is possible, therefore, that the round body exerts some control on nuclear activity in meiotic cells.

Paniagua and Nistal (1984) reported on the human testis aged between newborn to 13 years of age. The number of spermatogonia per 10 cross sectioned tubules decreased slightly from birth (15.3 ± 1.2) to 3 years of age (12.1 ± 1.0), and increased afterwards until 8 years of age (28.2 ± 2.6). After a brief decrease between the age of 8 and 9, it increases markedly until 12-13 years of age (49.7 ± 4.6). The number of fetal and transitional spermatogonia per 10 cross sectioned tubules is 5.3 ± 0.5 at birth, and progressively decreased until they disappear at 6
years of age. The number of type Ap and Ad spermatogonia per 10 cross sectioned tubules are similar to one another from birth (about 5.2) to 12-13 years of age (about 21.5). Except during the period between 4 and 10 years of age, when the number of Ad spermatogonia slightly decreases with respect to that of type Ap. This period coincides with the appearance of type B spermatogonia, of which the number per 10 cross sectioned tubules progressively increases from 4 (0.2 ± 0.02) to 12-13 years of age (6.7 ± 0.5). All spermatogonial types are found either resting on the basal lamina or lying towards the lumen. Some of the spermatogonia, but mainly those occupying a more adluminal position, appear hypertrophic, bi- or tri-nucleated, or degenerated. These anomalous spermatogonia are more abundant at 3 to 8 years of age, prior to periods of spermatogonial proliferation. At these times spermatocytes and occasional spermatids are seen in some seminiferous tubules of some children.

Blank and Desjardins (1985) have shown the inhibitory role of limited food intake on the reproductive system.

Breckon and Cawood (1985) have shown how short photoperiod (6L:18D) in hamsters caused a regression in testicular activity within 12 weeks. The spermatogenesis and sperm count regresses within 10 weeks. The most affected cells were Pachytene spermatocytes which did not progress to meiosis I, while in the same period the controls reached meiosis II stage. In addition, short photoperiod exposed hamsters showed fall in the testicular weight and reduction in the mean diameter of the seminiferous tubules.
By a morphometric study of rat testis aged between 5 to 360 days, Gayton et al (1986) reported that the testicular volume increased from $10.28 \pm 0.35 \text{ mm}^3$ (D 5 of age) to $1819.43 \pm 52.76 \text{ mm}^3$ (360 D of age), showing the most rapid increase between 20 and 70 days of age (22.6 times). The mean tubular diameter increased from $62.25 \pm 1.50 \mu \text{m}$ (5 day of age) to $280.81 \pm 9.77 \mu \text{m}$ (360 day of age). The increase in the tubular length was upto D15 of age. The tubular growth was similar in both length and diameter. Tubular development nearly finished at 70 days of age.

Leydig cell number per unit volume of testis was reported to be very high in fetal rat, it fell significantly in testes of day 2 to 3 and subsequently rose significantly. When Leydig cell number was expressed per testis rather than per unit volume, the testes of fetal rats and rats of day 2 to 3 contained the same number of Leydig cells. After the neonatal period, significant increase in the Leydig cell number per testis and increase in testis weight were reported (Zirkin and Ewing, 1987).

In horse, Anighi et al (1987) observed that the interstitial spaces and the number of interstitial cells of Leydig seem to increase while spermatogenesis appear to be arrested in retained testis. Early spermatocytes were the most matured stages of spermatogenic cells in the retained testis. In addition, an extensive vacuolation of spermatogenic cells were found in retained testis.
Abnormal temperature did not hinder the ability of interstitial cells to synthesize hormones. The cells were apparently healthy. However, the apparent changes in the retained testis may result due to high temperatures of the abdominal environment in concert with the altered production of androgens.

By exposing rat testis to 43°C heat for 15 or 30 minutes Bartlett and Sharpe in 1987 reported that moderate seminiferous tubule damage induced by 15 min heat exposure caused a small decrease (20%) in testicular weight. In contrast, after exposure of testis to heat for 30 min, there was a major and progressive decline in testicular weight throughout the experimental period, reaching 39% of control values by 42 days. In these rats, the serum concentrations of FSH were significantly increased (P < 0.01) throughout the period of study. Furthermore, the activity of the interstitial fluid factor(s) can be increased by inducing severe but selective disruption of spermatogenesis, where as moderate disruption has no effect. Moreover, as ABP secretion in interstitial fluid was increased after severe disruption suggesting that in such animals proportionately more ABP may be secreted via the base of the Sertoli cell. The parallel changes in activity of the interstitial fluid factor(s) and concentration of ABP in interstitial fluid also provides further circumstantial evidence that these products may have a common (Sertoli cell) origin.

Hardy et al (1989) used stereological methods to show that in testis the mesenchymal cells are comprised of 44% of the total interstitial cell population and Leydig cells are only 16% at 2 day PN, whereas by day 56 postpartum the
relationship reverses; then the mesenchymal cell comprise 3% and Leydig cells 49%. These results suggested a precursor-product relationship between mesenchymal and Leydig cells because no such reciprocal relationship was observed between Leydig cells and macrophages, pericytes, endothelial or myoid cells.

When spermatogenesis is disrupted, following heating of the testis and the gonadotropic stimulation is kept constant, the Leydig cells undergo hypertrophy, presumably because of a change in the secretion of paracrine factor(s) by the tubules. However, there is also a decrease in the testosterone secretion which is closely related to decrease in blood plasma though the testis bear little or no relation to the number, total volume, size of the Leydig cells, or the concentration of testosterone in the testicular venous blood (Setchell et al, 1990).

Immobilization and lead poisoning both are known to induce stress. Saxena et al (1990) reported the effect of the combination of these two stressors. They have shown reduction in cholesterol and ascorbic acid contents, reduced sperm counts and marked histopathological changes in testes of rats. The changes can be attributed to disturbances of testicular androgen synthesis and responsible for enhanced testicular injury in lead induced stressed rats.

Small to young (1-9 weeks) age rabbit testes were studied by morphometric methods (Lczkowski et al, 1991). The report showed a marked, steady increase in testis volume and tubular length and volume over the prepubertal period; but the
tubular diameter showed no significant increase and in fact decreased until week 4. Overall tubules lengthened by 40-fold and testis volume increased by 25-folds. The ratio of germ cells to total tubular (germ and sertoli) cells was lowest at 3 weeks. However, total germ cells increased little until 3 weeks, after which it rose at a sharp rate commensurate with testis volume. Germ cell degeneration remained relatively constant during week 1 through 6, with an increase at 7 weeks.

Vergouwen et al (1991) reported in mice the Leydig cells featured a very low proliferative activity up to D21 pp. At D29 pp there was a peak of 7.4% followed by a sharp decrease to 0.35% at D35 pp. The mesenchymal cells decreased from 11.4% at D14 pp to 1.1% at D14 pp and remained more or less constant thereafter. The proliferative activity of myoid, endothelial and perivascular cells followed a similar course to that of mesenchymal cells. There was an increase in the relative number of Leydig cells from ~ 4% of the total interstitial cell number at D14 pp to 29.5% at D35 pp. At the same time, the relative number of mesenchymal cells decreased from 55 to 13%. The diameter of the seminiferous tubules showed a peak of 92 μm at D16, decreased to 44 μm at D1 pp and increased again to 204 μm at D33 pp.

These results show that except for the Leydig cells, the proliferative activity of testicular cell types is highest during the pre- and early postnatal period. The major outgrowth of the Leydig cell population occurs around the fourth week after birth. The results are in accordance with the hypothesis that the mesenchymal cells are the progenitors of Leydig cells.
Hess et al (1993) use a goitrogen 6-propyl 2-thyourocil (PTU) and observed that the adult seminiferous tubule, accounts for the increase in sperm production. Using this model, it has been demonstrated experimentally that by increasing the number of Sertoli cells in the rat testis it is possible to nearly double the number of germ cells. After treatment the differentiated Sertoli cells were compacted within the seminiferous epithelium, thereby producing an increased efficiency of spermatogenesis (i.e. increased number of Sertoli and germ cells per g testis).

Vergouwen et al (1993) noted in mice the body weight increased from 1.3 gm at day 1 to 22.5 gm at day 25. Over the same period the testis weight showed a faster increase from about 1 mg to almost 60 mg. Spermatogenesis was found to be complete by day 35. All cell types in the mice testis were studied, the population was noted. The major cell populations in the mouse testis reach their adult size by the end of 5th week after birth. The changes in the number per testis of various cell types during development were found to be closely related to their proliferative activity. The maturation of tubular cell population precedes that of the interstitial cell population by about 1 week and that the development of most of the cell populations can be explained by their proliferative activity during development.

Deng et al (1993) stated that high-energy shock waves on the testis of Wistar rats, although produce temporary histologic changes in the testicular tissue, the rat testis appears to be functionally resistant to the high energy shock waves.
Ohmori et al (1993) using shock waves on the male reproductive system of rats, reported that in proportion to an increasing number of shock waves, sperm motility and percentage of viable sperm decrease while the percentage of short-tailed sperm increases. After 500 or 1000 shock waves to the testis of infant and adult rats it was found histologically that basement membrane was ruptured and outflow of spermatogenic cells. After 5 weeks, the testes appeared atrophic, with absence of spermatogenic cells in seminiferous tubules except for the sertoli cells. This led to the conclusion that in the testis spermatozoa appears to be structurally damaged by shock waves.

Hochereau-de Reviers et al (1993) exposed testes of ram by a single dose of heat for 45 min, hypophysectomised the animals and treated with ovine pituitary extract. Authors observed that pituitary extract significantly increased the testis weight and spermatogonial multiplication from A1 spermatogonia onwards. Twenty days after a single heat treatment testis weight was significantly reduced by heating; both tubular and intertubular tissues were affected. Mean seminiferous tubule diameter was significantly reduced.

Van Haaster et al (1993) stated that a short prenatal photoperiod delay start of spermatogenesis and alter proliferation pattern of Sertoli cells. The Sertoli cells supports more germinal cells from Day 20 postnatal. However, the duration of the prenatal and postnatal photoperiod did not affect the ultimate number of Sertoli cells.
Ultrasound exposure in utero is capable of disrupting foetal development and have potential subsequent effect on the fertility in male. In male mice, the exposure of ultrasound in utero decreased testis size and daily sperm production ranging from 9% to 30% (Cames et al, 1995).

De Franca et al (1995) studied spermatogenesis largely at the ultrastructural level. The authors recorded volume and surface area parameters for virtually all cellular and subcellular features for nine periods during the spermatogenic cycle. Virtually, all germ cell components showed dynamic properties associated with specific phases of their development. The data provided can be used in an objective way to characterize structural changes taking place during spermatogenesis to relate those structural changes to functional properties of germ cells.

Edmonds and Stetson (1995) stated that prepubertal male rats are responsive reproductively to photoperiod early in postnatal development. Testicular growth was inhibited in rats gestated and reared to 28 days of age on photoperiods ranging from 8 to 13 hr of light per day, and stimulated in animals reared on 14 hr or more of light per day, suggesting a critical photoperiod between 13 and 14 hr for testicular growth. Postnatal exposure to constant light unmask effects of the prenatal photoperiod on testicular development. Exposure to short or long photoperiods during the period of lactation (D 1-14 of life) affect testicular maturation. Alteration of photoperiod experienced after D14 of life markedly
affected testicular development and was the primary factor determining whether maturation would occur.

McLachlan et al (1995) studied the effect of recombinant FSH on the restoration of spermatogenesis after gonadotropin withdrawal by GnRH immunization. GnRH immunization severely reduced germ cell number, which were then significantly restored in all cell types, except elongated spermatids, by 7 days of FSH. At no stage did FSH increase elongated spermatid number above 1% of control level. Sertoli cell number was not altered by any treatment, however, sertoli cell nuclear volume was significantly decreased from control value by GnRH immunization and increased after 7 and 14 days of FSH treatment. FSH treatment restored serum inhibin levels to normal but did not increase serum or testicular androgen levels. It was concluded that recombinant FSH partially restores spermatogenesis in the gonadotropin-deficient rats by increasing the number of spermatogonia and promoting subsequent steps upto to round spermatid stage. Spermatid elongation was not restored by FSH, indicating the need for an additional factor(s), most likely testosterone.

Sinha Hikim and Swerdloff (1995) reported that rats GNRH-A treatment for 1 week to rats led to significant reduction in testis weight (26.6%) and in the number of specific germ cells involving preleptotene (27.7%) and Pachytene spermatocytes (36.7%) and step 7 spermatids (30.3%) at Stage VII of seminiferous epithelial cycle. The number of advanced spermatids declined by 44.3%. Concomittant administration of rhfsh for 1 week resulted in a significant increase in testicular
weight, tubular areas at Stage VII-VIII, and in the absolute volumes of seminiferous tubules and their lumens compared to GnRH-A alone. FSH replacement to GnRH-A treated rats was able to increase the number of B spermatogonia for entry into meiosis and maintain the number of preleptotene spermatocytes. The results demonstrated a positive role and relative contribution of FSH on the maintenance of spermatogenesis in the adult rats and provide a basis for further studies involved in the interaction between the target cell (Sertoli) and stimuli and between sertoli and germ cells, which in turn regulate the proliferation and differentiation of germ cells.

Horn et al (1996) studied testis of hamster aged 6, 12 and 18 months and observed a progressive involution of the seminiferous tubules, beginning to be perceptible at 12 months with slight hypospermatogenesis and desquamation. At 18 months age degeneration was more significant in pachytene spermatocytes. Although morphometrically, the decrease in the germ cells was detectable at 12 months but severe changes were found around 18 months. Leydig cell numbers were significantly reduced in the 18 month age. Hypospermatogenic tubules in 18 month old, hamsters were more prominent. A significant increase in the size of tubular lumen with age was seen, probably due to diminished number of various cell types in the seminiferous epithelium. No significant change in the diameter of tubule were noticed. A diminution in the sperm number in the vas deferens was observed. Age-related diminution in the sertoli cells were attributed to reduced germ cell maturation and spermatozoal production.
Chen et al (1996) used Brown-Norway rats in which it was known that the capacity to produce testosterone by the Leydig cells decreases with age. Authors quoted that previous studies have shown that a single injection of ethane dimethane sulfonate (EDS) specifically destroyed all Leydig cells in young rat testis and that subsequently, under the influence of LH, a new population of fully functional Leydig cells are restored. In the present report, authors stated that the enhanced ability of the Leydig cells restored to the aged testes to produce testosterone was not a consequence of exposure to increased levels of LH. Thus situated in an aged testis and in the environment of an aged hypothalamic-pituitary axis, the steroidogenic function of the Leydig cells restored to aged rat testis was equivalent to that of young rat Leydig cells.

In postnatal development of monkeys, immature Leydig cells increased 7-fold from the neonatal to early pubertal period and increased at a lower rate during puberty, mature Leydig cells remained stable until early puberty and increased significantly during late pubertal development (Rey et al, 1996).

Maternal separation for 6 hours a day between D4 to D21 Pn have produced delay in vagina opening in females, production of sperm in males of Sprague dawley rats. In addition, there were changes in the reproductive tissue, and in females estrous cycle was delayed (Lau et al, 1996).

Mon et al (1997) conducted morphological analysis of germ cell apoptosis during postnatal testis development in normal and Hsp 70-2 knockout mice. The
authors reported that Hsp 70-2 knockout males are infertile. In this study, the authors stated that (1) the percentage of apoptotic cell death is higher in mice from day 8 to 22 than in younger or older mice, (2) the majority of apoptotic cells are spermatogonia and less frequently are spermatocytes and (3) the degenerative cell death of spermatogonia are primary spermatocytes involves apoptosis with fragmentation of DNA. The analysis of apoptotic cell death in the testes of juvenile Hsp 70-2 knockout mice showed an additional increased level of apoptosis at day 17, during the first wave of spermatogenesis, in pachytene spermatocytes.

Sailer et al (1997) found exposure of mice to higher environmental temperatures several degrees above normal physiological temperature results in decreased testicular weight and disruption of spermatogenesis. Where as mice exposed to 42°C have shown absence of sperms in cauda epididymis. Mice exposed to 38°C temperature showed some minor sperm chromatin abnormalities.

Nelson et al (1997) have reviewed and stated that short day lengths inhibit breeding in many nontropical rodent species. Restricted food availability can also inhibit reproductive function among some individuals in these so-called photoperiodic populations. Rodents born at the end of the breeding season typically delay sexual maturation until the following spring. Prepubertal rodents exposed to day lengths that are <12 h light/day will not undergo puberty for 4-7 months in the laboratory. Food restriction can also affect the timing of puberty onset. Reproductive function of food restricted juvenile mice may remain inhibited until food availability improves. Alternatively, reproductive function of food-
restricted juvenile mice might eventually develop despite restricted food intake. Taken together, the authors state that retardation of reproductive development by food restriction is only superficially similar to the delay in reproductive maturation imposed by short day exposure.

Increased ACTH secretion in stressed rats is probably due to hypersecretion of CRH from the hypothalamus which suppresses the gonadotropin secretion via the inhibition of LHRH. The decreased level of testosterone may be caused by stress induced decrease in the plasma LH concentration and increased secretion of corticosterone in stressed rats. The low levels of plasma inhibin in stressed rats is also probably due to direct effect of corticosterone on Sertoli cells (Tohei et al, 1997)

Experiments of Loudon et al (1998) have shown that Syrian hamsters exposed to short photoperiod or constant darkness induce a decrease in gonadotropin secretion and gonadal regression. However, after 10-12 weeks the animals undergo spontaneous reactivation, gonadotropin secretion rises, and in males, testes size increases and spermatogenesis resumes. Thus, it is inferred that once initiated, the rate of spontaneous reactivation may be independent of the circadian axis.

Pellagrini et al (1998) stated that repeated stress proved to bring the morphological and histochemical changes in the adrenal cortex and testis of rats. Consequent upon the stress, corticosterone and progesterone in plasma levels