1.1. Infertility

Today, infertility is one of the major public health problem faced by the people around the world with a significant social, psychological and economic impact. It is estimated that globally, 10-15% of the general population face the incidence of infertility. In general, infertility is defined as the diminished or absent ability to conceive or produce an offspring after at least one year of unprotected sexual intercourse (Suganthi et al., 2014). In countries like India, it is on an alarming rise. It is estimated that 48.5 million couples worldwide in 2010 were infertile and an approximately among 40% of these couples, the male partner has been either the sole or a contributing cause of infertility (Leung and Wong, 2013).

Despite proper diagnostic work-up and as our knowledge of the events involved in normal conception is still limited, still we fail to determine the cause of infertility (Esteves, 2013).

1.2. Overview of male reproductive system in rat

The rat male reproductive system (Fig. 1.1) consists of a pair of testis, accessory organs (such as seminal vesicles and prostate glands) and the penis. Its primary reproductive function is to produce and transport the sperm into the female genital tract.

1.2.1. Testes

The testes are a pair of oval shaped glandular organs present in the scrotum. They are the site of production for several male sex hormones and spermatozoa. Rat testes are similar anatomically to those of other vertebrates. The testes (singular, testis) are located in the scrotum. In the male fetus, the testes develop near the kidneys, and then descend into the scrotum just before birth. Each testis is about 1 1/2 inches long by
1 inch wide. Each testis contains 250-400 lobules. Each lobule contains one to three tightly packed, convoluted seminiferous tubules. The partitions between the lobes and the seminiferous tubules both converge in one area near the anal side of each testis to form called the mediastinum testis. The seminiferous tubules come together and form a thin-walled network called as rete testis. The efferent duct of rete testis opens into duct of epididymis. The testes are made up of two types of cells, supporting cells called Sertoli cells and testosterone-producing cells called Leydig (interstitial) cells.

The Sertoli cells, which are interspersed between the germinal epithelial cells within the seminiferous tubules, are analogous to the granulosa cells in the ovary, and the Leydig cells, which are located beneath the tunica albuginea, in the septal walls, and between the tubules, are analogous to the hormone-secreting interstitial cells of the ovary. Sertoli cells provide nourishment for the developing sperm cells, destroy defective sperm cells, secrete fluid that helps in the transport of sperm into the epididymis and release the hormone inhibin that regulate sperm production. The Leydig cells are irregularly shaped and commonly have more than one nucleus. Frequently they contain fat droplets, pigment granules, and crystalline structures; the Leydig cells vary greatly in number and appearance among the various animal species. They are surrounded by numerous blood and lymphatic vessels, as well as by nerve fibers. Leydig cells constitute the endocrine component of the testis and they secrete the testosterone.

Most of the cells lining the seminiferous tubules undergo a process of maturation from spermatocytes to spermatozoa is known as “spermatogenesis”. Spermatogenesis relies on the coordinated support and interactions of the germ cells, Sertoli cells, Leydig cells, peritubular cells, interstitial macrophages and the blood vasculature. Mammalian spermatogenesis is classically divided into three 3 phases. The first phase is known as proliferative or mitotic phase: the primitive germ cells or spermatogonia undergo a
series of mitotic divisions. In the second phase, the spermatocytes undergo meiotic division to produce the haploid spermatids. Finally, in the third phase spermiogenesis occurs where spermatids differentiate into spermatozoa. The entire process is regulated by paracrine, autocrine and endocrine pathways (Cheng and Mruk, 2010).

1.2.2. Epididymis

The epididymis is one of the important accessory sex organs. It is a single, narrow, tightly-coiled tube about 2 inches long connecting the efferent ducts from the rear of each testicle to its vas deferens. On arrival of the mature sperm from the testis to the epididymis, the sperm undergoes further maturation processes which are under the control of hormonal-dependent secretory, resorptive, and storage functions. The epididymis consists of three parts: the head (cap-out), the middle (corpus) and the tail (cauda) part. The spermatozoa are stored in the tail of epididymis where they remain viable for a month and they become motile and acquire the capacity to fertilize. The epididymis serves as a reservoir of sperm for a long period. Most of the maturation process of sperm involves reorganization of the molecular architecture of the plasma membrane. These modifications take place as the sperm progress from caput to caudal region. The secretory and reabsorptive function of the epididymal epithelium provides an appropriate microenvironment for proper maturation of sperm (Kirchhoff, 1998). Thus, the resorption of fluid through the efferent ductules, as well as maturation of sperm during their passage through the epididymis, is fundamental for adequate sperm content of the ejaculate and for fertilizing capabilities.

1.2.3. Vas deferens

The vas deferens also known as the ductus deferens or sperm duct, or, spermatic deferens connects the epididymis with the prostate. It extends from the epididymis in the scrotum on its own side into the abdominal cavity through the inguinal canal. The
inguinal canal is an opening in the abdominal wall for the spermatie cord (a connective tissue sheath that contains the vas deferens, testicular blood vessels, and nerves). Vas deferens is a thick-walled tube that transports sperm cells from the epididymis, where the sperm are stored prior to ejaculation. Each vas deferens ends in an enlarged portion, an ampulla, which acts as a reservoir. Each vas deferens terminus expands to form the terminal ampulla. Terminal ampulla unites with the seminal vesicle duct and forms the ejaculatory duct, which enters the prostate gland and ends with the prostatic urethra. The ejaculatory ducts serve as a passageway for semen and fluid secreted by the seminal vesicles. The main function of the vas deferens is to transport and ejaculate the mature sperm. The smooth muscle layer of the vas deferens contracts in waves of peristalsis during ejaculation.

1.2.4. Seminal Vesicles

Seminal vesicles are paired, bag shaped glands and the internal surface consists of intricate system of folds to form irregular diverticula. They are two glands composed of many lobes and are located posterior to the urinary bladder. They secrete fructose to provide an energy source for sperm and alkalinity to enhance sperm mobility. The duct of each seminal vesicle joins the ductus deferens to form the ejaculatory duct.

1.2.5. Prostate gland

The prostate gland is a muscular gland that surrounds the anterior part of the urethra as it emerges from the bladder. The smooth muscle of the prostate gland contracts during ejaculation to contribute to the expulsion of semen from the urethra. It secretes a thin, milky, alkaline fluid containing high levels of zinc, calcium, citric acid, and acid phosphatase. This fluid protects the sperm from the acidic environment of the vagina and the male urethra, which could be spermicidal.
1.2.6. Cowper’s gland

Bolbo urethral (Cowper’s) glands are a pair of small round structures on either side of the urethra located below the prostrate gland. The glands secrete a clear, thick, alkaline fluid rich in mucoproteins. These secretions serve as a lubricant and their alkalinity helps to protect the sperm from the acid present in the male urethra and female vagina and thereby increases sperm motility.

The formation of the copulatory plug, which retains the sperm in the female vagina, requires the additional secretions of the coagulating gland. Ejaculation and emission of semen into the female genital tract requires effective mounting, penile erection, and intromission. These functions are dependent on a mixture of hemodynamic and androgenic factors as well as central and autonomic nervous input. Interference with any of these processes has the potential to reduce fertility of the male.
**Figure 1.1.** Male reproductive system of rat

LT: Left testis  
RT: Right testis  
E: Epididymis  

CP: Cap out  
CD: Cauda part  
VD: Vas deferens  

SV: Seminal vesicles  
PG: Prostate gland  
P: Penis  

**1.3. Hormonal regulation of male reproduction**
Hormones control several complex physiological processes in animal systems including reproduction. In mammals, male fertility is mainly regulated by hormones of hypothalamo-pituitary-testicular (HPT) axis.

1.3.1. Hypothalamus

Hypothalamus is the master controlling gland of HPT axis. It encompasses at the ventral portion of the diencephalon, just below the thalamus (Maffucci and Gore, 2009). It synthesizes gonadotropin releasing hormone (GnRH), a decapeptide in a pulsatile manner, which is mainly responsible for the production and release of gonadotropins from anterior pituitary gland.

1.3.2. Pituitary gland

Pituitary gland plays an important role at least in part in all biological processes in the body. The hypothalamus produces luteinizing hormone-releasing hormone (LHRH), which is released in pulses into a system of blood vessels connecting the hypothalamus and the pituitary gland. In response to the LHRH signal, the pituitary gland produces two to produce two classical gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). These two hormones (LH and FSH) are then released into the body's general circulation and act primarily at the level of the gonads (testis). LH and FSH through signal transduction pathways affect the Leydig cells and the Sertoli cells, respectively. LH binds to receptors on the surface of Leydig cells in the testis and stimulates the production of testosterone, a steroid hormone that diffuses into the seminiferous tubules. Within the seminiferous tubules only Sertoli cells possess receptors for testosterone and FSH and thus these cells are the major targets of the ultimate hormonal signals that regulate spermatogenesis (Walker and Cheng, 2005).

1.4. Hormonal regulation of spermatogenesis
Regulation of the spermatogenesis by hormones varies from species to species. The instigation of spermatogenesis occurs at puberty due to the interaction of the hypothalamus, pituitary gland and Leydig cells. The successful initiation of testicular function depends upon the hypothalamic secretion of GnRH which in turn stimulates the pituitary gland to release FSH and LH to act on the testis. These interdependent actions initiate spermatogenesis and testosterone production. Removal of the pituitary gland can still initiate the spermatogenesis through follicle stimulating hormone and testosterone. Though, the precise mechanism of the regulation of spermatogenesis is not clearly understood, the role of pituitary gonadotropins in the regulation of spermatogenesis has been unequivocally demonstrated.

Both the production of androgen binding protein by Sertoli cells, and the formation of the blood-testis barrier is stimulated by Follicle stimulating hormone. In order to concentrate testosterone in levels high enough to initiate and maintain spermatogenesis, which can be 20-50 times higher than the concentration found in circulation androgen binding protein is very essential. Follicle stimulating hormone may initiate to sequester the testosterone in the testes, but once developed only testosterone is required to maintain spermatogenesis. However, increasing the levels of follicle stimulating hormone will increase the production of spermatozoa by preventing the apoptosis of type A spermatogonia. The hormone inhibin acts to reduce the levels of follicle stimulating hormone. Gonadotropin hormones (both LH and FSH) support the process of spermatogenesis by suppressing the proapoptotic signals and thereby promoting the spermatogenic cell survival (Pareek et al., 2007). The Sertoli cells themselves mediate the part of spermatogenesis through hormone production.

1.5. Steroidogenesis
The synthesis of steroid hormones is essential for various functions of the body. The adrenal gland synthesizes glucocorticoids and mineralocorticoids which regulate the metabolism and water balance, and also small amounts of sex hormones such as androgens. The main site of androgen synthesis is the Leydig cells in the testes and the oestradiol in the ovary. The testes and ovaries are collectively called gonads and gonadal steroids that are essential for the reproductive function.

The production of steroid hormones by Leydig cells involves the activity of several enzymes. The first rate-limiting step of steroidogenesis is the transport of cholesterol from the outer to the inner mitochondrial membrane by steroidogenic acute regulatory protein (StAR) (Privalle et al., 1983; Christenson and Strauss, 2000; Stocco, 2001). The total steroid synthesis in the steroidogenic tissues, the rate-limiting step is the side-chain cleavage of cholesterol to pregnenolone. Human, animal and cell systems based studies show that the rate-limiting step in steroidogenesis is the conversion of cholesterol to pregnenolone. In the adrenal glands and gonads, this step is subject to both acute and chronic regulation. Chronic regulation is primarily, but not exclusively at the level of gene transcription, leading to the production of more steroidogenic machinery and thus increasing the cellular capacity for steroidogenesis. Chronic regulation can be inhibited by inhibiting protein synthesis with cycloheximide, but this response varies among various cell types and species. Though the P450scc enzyme system converts the cholesterol to pregnenolone it is inherently very slow, the principal site of acute regulation is at the delivery of free cholesterol to mitochondria, rather than at the delivery of reducing equivalents to P450scc.

CYP11A1 initiate the steroid synthesis by converting the cholesterol to pregnenolone (Miller, 1995). Two different pathways are involved in the biosynthesis of testosterone and estradiol; Δ4 pathway via progesterone and 17α-hydroxyprogesterone, or by Δ5 pathway via 17α-hydroxypregnenolone and dehydro-epiandrosterone (DHEA) (Conley et al., 1996). The enzymes cytochrome P450 c17 (CYP17A1) (Sasano et al.,
1989) and 3β-hydroxysteroid dehydrogenase (3β-HSD) (Clark et al., 1996) are involved in the Δ5 pathway, and CYP17A1 catalyzes both 17α-hydroxylase and 17,20 lyase activity (Nakajin et al., 1981) to convert pregnenolone into testosterone and estradiol precursors. Cytochrome b5 (CYB5) and CYP17A1 make up the andien-β synthase enzyme system which catalyze the metabolism of pregnenolone to androstenedione (Meadus et al., 1993). The interaction between CYP17A1 and CYB5 is also important to the 17, 20 lyase activity (Hall, 1991). The last step in formation of testosterone is catalyzed by 17β-hydroxysteroid dehydrogenase (17β-HSD) enzymes. The synthesis of estrogens from androgens is catalyzed by the enzyme aromatase (CYP19A1) (Conley et al., 1996).

1.6. Overview of female reproductive system

The organs of the female reproductive system are specialized to produce ova (eggs), transport the egg cells to the site of fertilization, to provide a favorable environment for developing embryos, and to move offspring outside of the body (birth) at the appropriate time. The reproductive system also supplies nourishment for the offspring after birth and produces female sex hormones.

The female reproductive system consists of the two ovaries and the female genital tract. In the mammals, the female genital tract arises from the Mullerian ducts, commencing with the ostium of the oviduct. In the rat, this ostium forms a complete capsule known as ovarian bursa, which envelops the ovary. The oviducts are small, highly coiled tubes. The uterus consists of two separated uterine horns, enabling the rat to have multiple offsprings. The vagina of the rat opens directly to the exterior (Kent GC and RK, 2001). The female reproductive system consists of vagina, cervix, uterus, fallopian tubes and ovary (Fig. 1.2).
1.6.a. Vagina

The vagina is a short muscular, hollow tube that extends from the vaginal opening to the cervix of the uterus. It is located between the urinary bladder and the rectum. It is also known as the birth canal. The vaginal walls are lined with mucous membranes which keep it protected and moist. The vagina serves as the birth canal and also orifice for acceptance of sperms during mating.

1.6.b. Cervix

The cervix is cylindrical and lower, narrow portion of the uterus. Each uterine horn has its own cervix. The cervixes are located where the uteri connect to the vagina. Each cervix has strong thick walls. The opening of the cervix is very small but expands to allow birth. Its purpose is to protect the uterus.

1.6.c. Uterus

The uterus is a hollow, pear-shaped organ that is the home to a developing fetus. It is located near the floor of the pelvic cavity, with a thick lining and muscular walls. It is hollow to allow a blastocyte to implant and grow. Major function of the uterus is to provide mechanical protection, nutritional support, and waste removal for the developing embryo. In addition, contractions in the muscular wall of the uterus are important in ejecting the fetus at the time of birth. Rat has a uterus consisting of the right and left cornua (horns) referred to as a bicornuate uterus. This structure enables the rat to have multiple offspring.

1.6.d. The Fallopian tubes
There are narrow tubes that are attached to the upper part of the uterus and serve as tunnels for the ova (egg cells) to travel from the ovaries to the uterus. Conception, the fertilization of an egg by a sperm, normally occurs in the fallopian tubes. The fertilized egg then moves to the uterus, where it implants into the lining of the uterine wall.

1.6.1. Estrous cycle

An estrous cycle is a rhythmic reproductive cycle occurring in sexually mature female non-primate mammals which depend upon the periodic release of gonadotropic releasing hormones, gonadotropins and sex hormones. In rodent, estrous cycle takes for 4 to 5 days, which is divided by proestrous, estrus, metestrus and diestrus (Freeman, 1988). The rat estrous cycle occurs throughout the year with no seasonal effect. Proestrous phase occurs within the first 12 hours of the cycle that estrogen is in peak at the end of this cycle and confirmed by the presence of predominantly nucleated epithelial cells. Estrus phase occurs 12 hours after proestrous which is indicated by the presence of cornified cells in the vaginal smear. Ovulation usually occurs from the beginning of proestrus to the end of estrus. Estrus is defined as the period when the female accepts the male and allows copulation. Several behavioral changes occur during the estrus phase, including the increase in the running activity, lordosis and ear quivering. During estrus, dry vaginal wall and a swollen vulva can be observed (Baker, 1979). Combination of leucocytes, cornified, and nucleated epithelial cells in the vaginal smear, indicate metestrous phase. This phase occurs within 21 hours after the estrous phase.

The diestrous phase has the longest interval time of 57 hours and during this time, the vaginal cells consisted primarily leucocytes. Corpus luteum activity is in metestrous and diestrous phases that produce progesterone hormone. Due to its short estrous cycle length, rat is a perfect animal model for the investigation of changes occurring during the reproductive cycle. The length of estrous cycle increases slightly
with age and lasts about 6 days near the end of the reproductive life span (Lu et al., 1979; Maeda KI et al., 2000). Hormones play critical role in the estrous cycle. Gonadotrophins that are secreted by the anterior pituitary, regulate the estrous cycle through luteinizing hormone (LH) and follicle stimulating hormone (FSH). Hormonal fluctuations result in the ovaries and the follicles, as well as changes in vaginal cytology. FSH stimulate the follicle growth whereas LH stimulates the follicles to ovulate and form the corpus luteum. Progesterone is secreted by the corpus luteum during metestrus and decline during diestrus. During follicular development, the level of estradiol-17β increases. The cycle ends when estrogen peaks during proestrus, stimulating gonadotropin release to trigger ovulation (Freeman, 1988). The female accepts the male at the end of proestrus, while during metestrus and diestrus, it does not accept the male (Lohmiller J and SP, 2006).

The phases of estrous cycle can be detected by observing the behavioral changes (Lohmiller J and SP, 2006) or by examining the vaginal cytology (Lohmiller and Swing, 2006). This method is widely used parocedure and is considered as a rapid and practical way to determine the different phases of the estrous cycle (Marcondes et al., 2002). The accurate determination of the phase depends upon the smears taken at fixed times in the day, as the cell populations vary throughout a 24-h period. The estrous cycle in the rat is generally affected by various environmental factors, such as temperature, photoperiod, noise, restraint, immobilization, handling and research procedures. High ambient temperatures increase the duration of the cycle and thus reduce the number of estrous cycles occurring in a given period of time (Sod-Moriah, 1971). Changes in photoperiod (Clough, 1982), extended light period (Hardy, 1970) and experimental procedures (Sharp et al., 2002) may increase the estrous cycle in female rats.
**Figure 1.2.** Female reproductive system of rat

A: Ovary  B: Fallopian tube  C: Uterus  D: Cervix
Mating behaviour in females is controlled by both estrogen and progesterone; in males, it is controlled by testosterone. Copulation in rats mostly occurs during the last third of the dark cycle (Maeda KI et al., 2000).

1.7. **Fertilization**

In mammals, fertilization takes place in the oviduct. A successful fertilization requires complex spermatozoa-ova interactions. Fertilization involves several sequential steps, beginning with the binding of spermatozoa to the zona pellucida, followed by the acrosome reaction and penetration of spermatozoa through the zona pellucida, then the spermatozoa bind to and fuse with the egg, leading to egg activation (R, 1994). Sperm migration through the rat oviduct depends upon both estradiol and progesterone (Orihuela PA et al., 1999). In mammals, fertilization steps are thought to be regulated by proteins located in the acrosome of the spermatozoa whereas fertilization in rats is regulated by rat epididymal 37 kDa protein (DE) (Cohen et al., 2000). After fertilization, a single cell embryo (zygote) doubles to two cells, then undergoes a series of mitotic divisions into four cells, eight cells, and a morula. Several more rounds of mitotic division form the blastocyst. The blastocyst is composed of differentiated tissues: a layer of trophectoderm cells, which give rise to the placenta, and the inner cell mass (ICM), which gives rise to the embryo. The blastocyst becomes competent for implantation after shedding the zona pellucid (Lee and DeMayo, 2004).

In rats, after fertilization the zygote develops into two and four cells on the first day, to eight cells on the second day, and to a sixteen cell embryo on the third day after fertilization. The embryo develops to the morula stage on day four and to the blastocyst stage on day five of pregnancy (Agca Y and JK, 2006). Pre-implantation rat embryos can be collected from the female reproductive tract and used in basic research, embryo
culture studies, genome banking, and establishment of stem cells (Jiang et al., 1999). However, time of embryo collection depends on the embryo stage needed.

1.8. **Endocrine disrupting chemicals**

Endocrine disrupting chemicals (EDCs) are nothing but, chemicals that are thought to mimic natural hormones, inhibit the action of hormones, or alter the normal regulatory function of the immune, nervous, and endocrine systems. Possible health hazards affected by these agents include breast cancer and endometriosis in women, testicular and prostate cancers in men, abnormal sexual development, reduced male fertility, alteration in pituitary and thyroid gland functions, immune suppression, and neurobehavioral effects (Crisp et al., 1998). EDCs can alter the endocrine function by i) mimicking the natural hormones ii) inhibiting the action of hormones and/or iii) altering the normal regulatory function of the endocrine system. EDCs act at the level of the hypothalamo-pituitary-gonadal (HPG) axis by an antiandrogic or antiestrogenic mechanism. People are exposed to EDCs in their everyday life, because EDCs are found in low doses in literally thousands of products. Dioxins, polychlorinated dibenzofurans (PCDFs), tributyl tin, ethyl oestradiol (EE), alkylphenols, plant sterols, fungal oestrogens, chlordecone, dibromochloropropane (DBCP) are some of the chemicals that are known to affect reproduction in humans, domestic animals or wildlife via endocrine mechanisms (Migliarini et al., 2011).

1.9. **Andrographis paniculata**

Since ancient times, herbal preparations/medicaments have been used as a treatment strategy in several parts of the world. Several plant products are known to inhibit the male fertility which can be developed into male contraceptives (Akbarsha and Murugaian, 2000). From the last two decades, usage of herbal products as natural cosmetics and for self-medication by the general public to the scientific investigations
of plants for their biological effects in human beings has grown enormously. There is a wide tendency to utilize these herbal products to supplement the diet, mainly for improving the quality of life and preventing the diseases of elderly people (sharma and joshi, 2011).

*Andrographis paniculata* is one such plant belonging to the family Acanthaceae known to be one of the important herbal medicines used for centuries in Asia to treat several diseases such as gastro-intestinal tract and upper respiratory infections, fever and herpes. It is known to a predominant constituent in 26 Ayurvedic formulations (Sattayasai et al., 2010). It is an annual herbaceous plant widely cultivated in Southern Asia, India, China and some parts of Europe. Its leaves and roots are traditionally been used over centuries in the regions of Asia and Europe as a folklore medicine for a wide variety of ailments or as herbal supplements for health promotion. The plant has Ayurvedic properties like Rasa-Tikta, Guna-Laghu, Veerya-Ushna, Vipaka-Katu, Doshaghnata-Kaphapittashamaka. Traditionally it is used as carminative (Deepana), Liver stimulant (Yakriduttejaka), Pittasaraka, Laxative (Rechana), anthelmintic (Krimighna), blood purifier (Rakta shodhaka), anti-inflammatory (Shothahara), sweđajanana, antileprotic (Kusthaghna), antipyretic (Jwaraghana) and preventive major for malaria (vishmajwara-pratibandha) etc. (Dey et al., 2013).

*Andrographis paniculata* contains a number of diterpenoids like andrographolide and several flavonoids such as 5, 7, 2’, 3’-tetramethoxyflavonone and 5-hydroxy-7, 2’,3’-trimethoxyflavone (sharma et al., 2011). The other chemical constituents present in *A.paniculata* are andrographin, panicolin, andrographolide, deterpene glucosideneandrographolide, andrographidihnes, neoandrographolide, chlorogenic, myristic acide, homoandrographolide, andrographiside andropanoside, etc (Dey et al., 2013). Andrographolide, neoandrographolide, and 14-deoxy-11,12-didehydroandrographolide are known to be viricidal against herpes simplex virus 1
(HSV-1) without having any significant cytotoxicity at viricidal concentrations (Akbar, 2011). Among the several components of *A. paniculata*, Andrographolide is a major component of *A. paniculata*, known to have multiple pharmacological properties, such as antipyretic, anti-inflammatory, anti-allergic, anti-platelet aggregation, antiviral, anti-HIV, antithrombotic and antidiabetic activities. It has been widely used for the treatment of fever, cold, inflammation, diarrhea and other infectious diseases (Sattayasai et al., 2010). Andrographolide (2.39%) is known to be present at the highest amount in the leaves and lowest in seeds of *A. paniculata*. It is the primary medicinal component of Andrographis. It has a very bitter taste, is a colorless crystalline in appearance, and is called a "diterpene lactone"- a chemical name that describes its ringlike structure (Yadav and Singh, 2012).

Several animal studies showed that *A. paniculata* leaf arrests the spermatogenesis with a decrease in sperm count, disruption of seminiferous epithelium, seminiferous tubules, spermatozoa, sertoli cells (Akbarsha and Murugaian, 2000), weights of the testis, epididymes, sperm motility and seminal vesicle (K. Sathiyaraj et al., 2011b), lower level of hormone, female rats have promising percentage of infertility (Sakila et al., 2009) and showing antifertility (Gupta and Sharma, 2006). Another study showed that 50% ethanolic extract of *A. paniculata* did not affect the body weight, reproductive and other internal organs weight changes (M.S. et al., 2013). Andrographolide on the other hand known to effect sexual functions, vascular reactivity and serum testosterone level and in another study it is showed no significant effects on sperm morphology and motility (Sattayasai et al., 2010). Since, previously several controversial results were observed on the antifertility efficacy of *Andrographis paniculata*, in this study we have undertaken to investigate the effect of *Andrographis paniculata* leaf extract and Andrographolide on the reproductive system of male rats.
1.10. Significance of the parameters selected

Several scientists used different reproductive end points to analyze the reproduction in animals. The body weight gain indicates the general metabolic conditions of the animal. In the present study weight of the animal was recorded to assess the metabolic activity of the animal. The weight of the testis is largely dependent on the mass of differentiated spermatogenic cells and it has been used as a crude measure of the damage to spermatogenesis (Schlappack et al., 1988). A strong correlation exists between weight of the testis and the number of germ cells (Sinha Hikim et al., 1988). The reduction in the weight of the testis has been shown to occur due to the loss of germ cells (Setchell and Galil, 1983). In the present study weight of the testis was taken to assess the maintenance of testicular functions.

The weights of the accessory sex organs are dependent on the availability of androgens as castration causes reduction of weights due to absence of testosterone. Increase in the availability of testosterone leads to hypertrophy and consequent increase in the weights of accessory sex organs. The organ size may even exceed that of its precastrational state with the administration of higher dose of testosterone (Neumann and Steinbeck, 1974). The weights of the accessory sex organs in castrated rats have been widely used as a bioassay for androgenic and anti-androgenic compounds (Neumann and Steinbeck, 1974). The measurement of the weights of the accessory sex organs in intact rats has been shown to reflect the estimation of the cumulative effect of biologically active testosterone over a period of time (Mathur and Chattopadhyay, 1982). In the present study the weights of epididymis, seminal vesicles and ventral prostate were taken to assess the bioavailability of androgen and the cumulative effect of androgenic activity.
Sperm analysis is the initial and most essential step of the infertility evaluation. A combination of sperm characteristics, such as daily sperm production, sperm count, and the percentage of motile and viable sperm, morphology and membrane integrity provides the best diagnostic profile that could determine the fertile from the suspected subfertile/infertile male groups. Sperm analysis is also considered a cornerstone of the laboratory evaluation of the infertile male and helps define the severity of male factor infertility. Testicular daily sperm production and epididymal sperm count are considered to be useful indicators to detect adverse effects on spermatogenesis quantitatively (Ban et al., 1995). Sperm motility is considered a critical indicator of semen quality and fertility potential, because it is required for penetration in cervical mucus, transport through the female genital tract, and penetration through the corona radiata and zona pellucida before oocyte fertilization (McConnell, 1997). Several studies in the literature have reported that percentage normal morphology is an essential characteristic for in vivo fecundity and in vitro fertilization (Coetzee et al., 1998). In the present study, all the above sperm parameters were evaluated to determine the sperm fertility potential.

Testosterone plays an important role in maintaining spermatogenesis, growth and functioning of accessory sex organs and secondary sexual characters. The pituitary hormones, follicle stimulating hormone, and luteinizing hormone are responsible for the stimulation of spermatogenesis and steroidogenesis respectively (Sofikitis et al., 2008). In the present study, the circulatory levels of testosterone, follicle stimulating hormone and luteinizing hormone were determined to assess the availability of these hormones in extracellular fluid for the action on their target sites.
The two key enzymes involved in the biosynthetic pathway of testosterone are 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase. The activity of delta-5-3β-hydroxysteroid dehydrogenase has been shown to respond to hCG (Human Chorionic Gonadotropin) in neonatal interstitial cells in culture (Meidan et al., 1985). Stimulation of 3β-HSD activity in testes of immature, hypophysectomized rats by administration of hCG in vivo has been reported (Murono and Payne, 1979). Suppression in the activities of 17β-HSD has been reported in the rat testis after administration of hCG or testosterone (Inano et al., 1973). The activities of 3β-HSD and 17β-HSD have been used to study the testicular steroidogenesis of rats in different experimental conditions (Ghosh et al., 1995). Determination of activities of 3β-HSD and 17β-HSD in the testes reflected the status of steroidogenesis.

Histological studies on the testis provide the strongest evidence to see the difference in the architecture between control and experimental tissues (Russell et al., 1990). A positive relationship exists between the tubular diameter and the spermatogenic activity of the testis (Sinha Hikim et al., 1989). Tubular diameter measurements have been reported to discriminate between varying levels of spermatogenic damage (Russell et al., 1990). Johnson et al. (1983) have shown that late spermatocytes can also be used to evaluate daily sperm production which indicates the cell loss during meiotic divisions and also during spermiogenesis. In order to evaluate the status of spermatogenesis the architecture of testis was examined.

Another reproductive endpoint that was tested was the male’s ability to sire offspring in a fixed period of time. Fertility analysis is of central importance as births are a vital component of population growth. Fertility related studies also provides important information about reproductive behavior and attitude and will help to analyze
the relationship, if any, with testicular and epididymal sperm quantity and quality and/or sexual desire of the animal. In the present study, mating index, fertility index, pre- and post-implantation loss were determined to assess the effect of RS, OP and AL alone or in combination on male fertility.

1.11. Effects of *Andrographis paniculata* on fertility

1.12. Aims and Objectives of the study

The specific objectives of the present study were to evaluate:

- the effect of ethanolic extract of *A.paniculata* on the reproductive system of male rats.

- the effect of Andrographolide on the reproductive system of male rats.

- the induced oxidative stress and changes in enzyme activities.

- the fertility ability in male rats after ethanolic extract of *A.paniculata* and Andrographolide treatment.

- the histopathological studies in testis and epididymis by Light microscopy.