Chapter-II

Male Reproductive Parameters
Endocrinologists often attempt to describe the roles of neuroendocrine and endocrine factors related to major life history events such as development, sexual maturation, and reproduction while observing animals in their natural habitats. The integration of endocrinology with behavior, ecology, and other aspects of physiology of animals are viewed with an evolutionary perspective, because of a preoccupation with environmental factors that influence neuroendocrine axes and a focus on major life history. Reproduction is one of the important fundamental processes which are under the control of an array of hormones (Harvey and Everett, 2006). During the last few decades, many male reproductive health problems like abnormal male genital development, cryptorchidism, hypospadiasis, erectile dysfunction, feminization of males, poor sperm quality and density, reduced steroidogenesis were observed in humans and wildlife all over the world (Carlsen et al., 1992; Toppari et al., 1996; Olsen and Rachootin, 2003; Slama et al., 2004). It has been reported that many pollutants act as endocrine disrupting chemicals and capable of affecting almost all compartments of hormonally-regulated functions including reproduction (Fisher, 2004).

Reproduction is one of the most characteristic features of living organism. Life would not exist on earth if animals did not reproduce to make their offspring. By reproducing, a living organism can be sure that there is another individual of its kind to take its place when it dies. The offspring of mammals are not born immediately after the parents have mated, a period of development is necessary. This period is called gestation. Gestation takes place in a special organ in the female called the uterus or womb. Mammalian reproduction is the resultant phenomenon of synchronous interplay of exocrine and endocrine secretions.

Several man-made environmental pollutants released into the environment mimic action of hormones and act as endocrine disrupters. Exposure to endocrine-disrupting chemicals in environment has been associated with abnormal thyroid function in fish (Moccia et al., 1981) and birds (Moccia et al., 1986), decreased fertility in birds (Shugart, 1980), fish (Leathereland, 1992) and mammals (Reijnders 1986), alternation of immune
function in birds (Erdman, 1987) and mammals (Martineua et al., 1988). Evidence has also been accumulating which indicates that humans, domestic and wildlife species have suffered adverse health consequences from *in utero* and lactational exposure to chemicals that interact with the endocrine system (Toppari et al., 1996; Moore et al., 2001; Odum et al., 2002;).

Agricultural and industrial chemicals, pollutants and toxicants are now widely dispersed in the environment. These chemicals are known to have a cumulative hazardous effect on humans and animals presumably because of their thermo stability and lipophilicity (Randall and Tus 2002). Follicle stimulating hormone (FSH) and luteinizing hormone are well established as playing separate but complementary roles in regulation of follicle leading to the synergistic actions in stimulating both follicular growth and ovulation (Greep, 1961). A central focus of comparative physiology and endocrinology has been the influence of environmental factors on the development and performance of various systems or whole organisms. Over the last century, it has been clearly established that such factors as temperature, pH, salinity, photoperiod and gas tensions affect the endocrinology of vertebrates (Norris, 1997).

The endocrine-like actions of various chemical contaminants have been a recent focus of much research (Ankley et al., 1997; Guillette and Crain, 2000; McLachlan, 2001). These studies have investigated endocrine disrupting chemicals released from industrial activities, sewage treatment works, and refuse dumps, confined animal feed operations and agriculture fields (Noaksson et al., 2001; Orlando et al., 2004; Soto et al., 2004). In addition, pharmaceuticals and other chemicals with endocrine-like activity have been identified in food products and drinking water (Kolpin et al., 2002; Miyahara et al., 2003).

A number of environmental chemicals have actions that mimic or alter the normal sex steroid hormones. Eroschenko (1981) reported that administration of Kepone to pregnant rats or mice during the main period of fetal organogenesis results in fetal toxicities and malformations. Gellert and Wilson (1979) demonstrated that the female offspring of Kepone-treated dams exhibit persistent vaginal estrus and an ovulation of chronic
administration of synthetic estrogens causes alterations in luteinizing hormone, follicle stimulating hormone, prolactin, in rats (Broockfor and Blake, 1997).

Endocrine disrupting chemicals affect an organism’s physiology through a number of mechanisms. They may mimic naturally occurring steroids, act as hormone receptor agonists or antagonists or alter the enzymes responsible for hormone synthesis and degradation (Crain et al., 2000; Gray et al., 2001; Rooney and Guillette, 2000). Synthetic chemicals such as pesticides, plasticizers or industrial chemicals and naturally occurring heavy metals and plant or fungal compounds have been defined as endocrine disrupting contaminants (EDCs). In the humans and rats, fetal androgen production is required for normal male sexual differentiation (Schardein, 1993). Exposure to environmental contaminants with endocrine-like activity has been hypothesized to be responsible for the increased reproductive and developmental defects in humans and wildlife (Sharpe and Skakkebaek, 1993; Toppari et al., 1996; Gray et al., 1998).

Over the last decade we have become increasingly aware that environment contaminants can induce or suppress normal endocrine responses, through multiple mechanisms to all endocrine functioning of vertebrates and invertebrates (Rodgers-Gray et al., 2000) Mammals are in general are physiologically sensitive to increase the concentration of ammonia in the body. The developing organism is exquisitely sensitive to alternations in hormone function. There is a little data on the toxicity of ammonia on development that has been linked as a primary contributing factor of disease and mortality (Petric et al., 2006). Any stress in the animal causes hormonal changes which decrease the effectiveness of inflammatory response, and stress also impairs production and release of antibodies (Das et al., 2004 and Yean et al., 2005). The earlier works on ammonia toxicity and sub lethal effects on hormonal and hematology work on *Clarias gariepinus* by a number of investigators have reported alterations of intermediary metabolism during ammonia intoxication which suggests that hormone action is also changed (Ajani, 2006).

**Follicular stimulating hormone (FSH) & Luteinizing Hormone LH:**

The developing organism is exquisitely sensitive to alternations in hormones function Each gland assuming a particular role in this elaborate process and being a
necessary component of the establishment of reproductive function for many factors. The organs of the body communicate with each other through the nervous and endocrine systems to coordinate their activities. The nervous system uses neurotransmitters and neurons to convey information to and from the brain. In contrast, the endocrine system uses hormones, which are chemical messengers produced by specific tissues in the body, to transmit information (Sharpe 1993). These hormones travel through the bloodstream to exert their effects on distant target organs. In a similar manner, people communicate with each other by using telephones and the postal service. The body’s nervous system is comparable to the telephone system because its ends fast, direct messages. The endocrine system is comparable to the postal service because the delivery of the message is slower. Like bulk mail, the message is more diffuse (reaches a greater area) and affects more than one person or organ. Although the hormone travels through the body via the blood, it can only affect those cells with receptors for that specific hormone. Hormones are a slower method of communication, but their effects last longer.

Hormones are produced in the body and also involved in the regulation of several vital physiological processes. These include regulation of reproduction, development, metabolism etc. Supra-normal levels of some of the hormones in circulation can be harmful and alter the development of embryo, growth of young ones and also reproductive potential of the adult. These deleterious effects can also occur by interfering with key physiological events during the prenatal period (O’Donnell 2001).

The command center for the endocrine system is the hypothalamus, a small, penny-sized portion of the brain. The hypothalamus acts as an endocrine organ that secretes oxytocin and anti-diuretic hormone (ADH, also known as vasopressin). These hormones travel down the pituitary stalk to the posterior pituitary gland where they are released directly into the blood stream. In addition, the hypothalamus also regulates anterior pituitary gland function through the secretion of releasing hormones: thyroid-releasing hormone (TRH), corticotrophin releasing hormone (CRH), and gonadotropin-releasing hormone (GnRH). These releasing hormones travel through a specialized blood vessel system (known as the hypothalamic hypophysial portal system) that connects the hypothalamus to the anterior pituitary gland. From here, they stimulate the synthesis and secretion of anterior
pituitary hormones, which include thyroid-stimulating hormone (TSH), luteinizing hormone (LH), follicle stimulating hormone (FSH), growth hormone (GH), adrenocorticotropin hormone (ACTH), and prolactin. Each of these hormones is released into the bloodstream to affect specific target organs.

LH is released from the anterior pituitary gland in response to GnRH secreted from the hypothalamus. LH is seen in both males and females but has different functions. In the male, LH travels to the Leydig cells that are located in the connective tissue between the seminiferous tubules of the testes. The Leydig cells release testosterone (Amer et al., 1997), which is responsible for the male sex drive and secondary sex characteristics, such as increased body hair and a deeper voice. An excess of testosterone can cause an increase (anabolic) in muscle mass. Negative effects of testosterone are male pattern baldness and increased secretion of the sebaceous glands, which can lead to acne presents the relative anatomy of the male reproductive tract. In the female, LH causes the follicle (developing egg) in the ovary to secrete estrogen. Estrogen participates in either a positive or negative feedback loop, depending on the stage of the menstrual cycle. In the preovulatory and postovulatory phases, estrogen regulates the release of LH through negative feedback. However, there is a large rise in levels of LH just before ovulation (release of the egg from the ovary) due to a positive feedback mechanism. During this interval, the secretion of estrogen from the follicle further stimulates the release of LH from the anterior pituitary gland. The increased levels of LH are essential for ovulation to occur.

Female reproduction could also be affected by estrogenic chemicals at a number of target sites including the brain, pituitary, gonad, liver and oviduct. Gonadal effects of estrogens mimicking chemicals have considerable potentials to impair the reproduction. (Gullette et al., 1994). Estrogen causes the development of female secondary sex characteristics and sustains the female reproductive tract. A woman who lacks ovaries (and therefore follicles will not produce estrogen. However, the pituitary gland will secrete excess LH because the feedback inhibition no longer exists. Excess levels of estrogen cause early sexual development in the female as do high levels of testosterone in males. To simplify the relationship between the reproductive and endocrine systems, the female
reproductive system is more difficult to study than the male reproductive system because it is continuously cycling (Kavolock et al., 1996; Anway et al., 2005).

Results:

In the present study the follicle stimulating hormone and luteinizing hormone levels were estimated in control, ammonium sulphate, and ammonium sulphate treated rats with vitamin -C. In the present study the serum follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels were significantly decreased in adult male rats exposed to ammonia when compared to the corresponding group of control rats. The decrement in FSH and LH is more in ammonium sulphate rats when compared with ammonium sulphate treated rats with vitamin-c.
Table 2.1: Follicle stimulating hormone (FSH), and luteinizing hormone (LH) levels in serum of male rat in animals receiving ammonium sulphate and ammonium sulphate with vitamin-c.

<table>
<thead>
<tr>
<th>Hormone levels</th>
<th>Control</th>
<th>Ammonium sulphate</th>
<th>Ammonium sulphate+vitamin-c</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FSH(ng /ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>14.18±0.372</td>
<td>9.59±0.395</td>
<td>11.16±0.406</td>
</tr>
<tr>
<td>%Change over control</td>
<td></td>
<td>-32.36</td>
<td>-21.29</td>
</tr>
<tr>
<td>% Change over ammonia sulphate</td>
<td></td>
<td></td>
<td>+16.37</td>
</tr>
<tr>
<td><strong>LH(ng /ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>11.69±0.338</td>
<td>7.393±0.432</td>
<td>8.44±0.48</td>
</tr>
<tr>
<td>%Change over control</td>
<td></td>
<td>-36.75</td>
<td>-27.80</td>
</tr>
<tr>
<td>% Change over ammonia sulphate</td>
<td></td>
<td></td>
<td>+14.16</td>
</tr>
</tbody>
</table>

All the values are mean of six individual observations, % - Percent change over control, % - Percent change over Ammonium sulphate, SD – Standard deviation

Values are significantly over control at P<0.000*, Values are significantly over Ammonium sulphate at P<0.05.

ONE WAY ANOVA:

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degree of freedom</th>
<th>FSH</th>
<th>LH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sum of Squares</td>
<td>Mean Squares</td>
<td>Sum of Squares</td>
</tr>
<tr>
<td>Between Groups</td>
<td>2</td>
<td>63.36</td>
<td>31.68</td>
</tr>
<tr>
<td>Within Groups</td>
<td>15</td>
<td>9.696</td>
<td>0.646</td>
</tr>
<tr>
<td>F Value</td>
<td></td>
<td>49.013</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>73.060</td>
<td>74.323</td>
</tr>
</tbody>
</table>

All the values are Significant at p<0.05
Fig 2.1 Follicle stimulating hormone (FSH), and luteinizing hormone (LH) levels in serum of male rat in animal receiving ammonium sulphate and ammonium sulphate with vitamin-C.

All the values are mean ±S.D of six individual observations

% - Percent change over control,

Values are significantly different from control p<0.000*
Discussion:

The reproductive functions in various species may be assessed from the alterations in plasma/or serum sex hormonal levels. Therefore, quantitative and qualitative estimations of both tissue and serum sex hormonal levels are a good assessor of reproductive integrity in both animals and man (Manassaram, 2006). Significant decreases in the levels of serum sex hormones are known to be associated with suppressed reproductive functions. Exposure to several chemical agents has been reported to cause reproductive dysfunctions in different subjects (Yarube et al., 2009).

Mammalian reproduction is regulated by a set of hormone signals that act in a finely tuned and coordinated manner. These hormones act upon multiple independent target cells, directing the development of gametes as well as their release, transport, fertilization, implantation and gestation. This process produces offspring and continues indefinitely, maintaining the evolutionary survival of the species. Exposure to certain environmental contaminants can adversely influence reproduction and development through a variety of mechanisms. The balance of estrogens and androgens is critical for normal development, growth and function of the reproductive system. Although they are important during development, this balance is also important throughout life for preservation of normal feminine or masculine traits.

The quantitative and qualitative effects of ammonia on the hormonal effects were studied under this ammonia stress. The earlier works of Lobert et al., (1993) reported hormonal depressions and inhibitions which were positively correlated to the histological damage on uterus ovaries and oviduct showing that the histological destruction resolved in pathophysiological decrease in hormonal production and function may lead to infertility (Lafuenta 2000, Gupta et al., (2004) Uche & Obianime 2008).

Ait Hamadouche N. et al., (2013) reported serum LH and FSH were decreased in lead acetate treated groups of animals as compared to their respective control. Significant alterations in LH and FSH levels have been reported after exposure to certain heavy metals (Gabuchyan, 1987; Chattopadhyay et al., 2005; Atef Al Attar, 2011). In our study, the FSH and LH levels in Ammonia stress rats were significantly lower than the levels in the control
rats at the end of the 30 days. However, treatment with vitamin-C has a protective effect on FSH and LH levels.

The reproductive hormone LH and FSH were significantly decreased in treated rats when compared to the serum levels in control while causing synergistic effects in the reduction of the serum levels (Aprioka and Obrianime 2009). Furthermore, the ability of vitamin-C to restore pituitary AChE activities may have improved neuronal activity, resulting in increased synthesis and/or release of the FSH and LH. In this present study, a similar situation might have restored the serum FSH and LH levels.

Ammonium metavanadate caused a decrease in the serum levels of Luteinizing hormone (LH), Follicle stimulating hormone (FSH) in rats reported by A.W.O. Bianime et al., (2009). These might result in the normal feedback inhibitory mechanisms which will reduce hormonal productions. Further more studies with oxidizing agents like Cd, oxolinic acid, lansoprazole and procymedone have shown that these agents may in addition had direct effects on the pituitary axis (Fort et al., 1995). In the present study, a similar situation might have decreased the serum FSH and LH levels. However treatment with vitamin-C has a protective effect on FSH and LH levels.

Muftau Shittu et al., (2012) reported in albino rats that Pretreatment with vitamin C apparently restored the FSH and LH concentrations. This shows that oxidative stress is partly involved in the low gonadotropin concentrations in the chlorpyrifos group, perhaps due to protection of the hypothalamus and the pituitary glands from Chlorpyrifos-induced oxidative damage (Shittu et al., 2012) The ability of vitamin C to protect brain neurons and glial cells from CPF-induced lesions, may have improved the activity at the hypothalamo-pituitary axis, thereby aiding the gonadotropins synthesis and release (Ambali 2011).

Palaniappan Murugesan et al., 2005 reported that the protective role of vitamin-C and E against Aroclor 1254 for 30 days induced changes in LH. Aroclor1254 significantly reduced the serum LH levels. However the simultaneous administration of vitamin-C and E on Aroclor 1254 exposed rats resulted a significant restoration of LH levels to the control level. Vitamin-C might have ameliorative role against adverse effects of PCB (Aroclor 1254). In this present study, a similar situation might have restored the serum LH levels. In
ammonia induced stress and the ability, of vitamin –C to restore the activities may have improved, resulting increased synthesis and/or release of the LH.

**TESTOSTERONE:**

Testosterone plays an important role throughout the development of male organism. It is necessary in the sexual differentiation during embryonic development. During puberty, testosterone is necessary for sperm production, proper sexual function (Sanderson, 2006), and development of accessory sex organs and sexual character (Krasnow and Steiner, 2006). In adulthood, testosterone effects on muscle, fat, bone, hematopoiesis, coagulation, psychosexual and cognitive behaviour is well established (Christiansen, 1998). Testis secretes about 95% of circulating testosterone in human males, with a production rate of 6-7 mg per day (Meikle et al., 1992; Rommerts, 2004). The first step in testosterone synthesis is conversion of the cholesterol to pregnenolone. Delivery of the cholesterol to the inner mitochondrial membrane, where this conversion occurs, is controlled by LH. Pregnenolone is then converted to testosterone through one of two pathways. In the predominant testicular pathway, pregnenolone is converted to 17α-hydroxypregnenolone than testosterone. In the secondary pathway, pregnenolone is converted to progesterone, 17α-hydroxyprogesterone, androstenedione and then testosterone (Payne and Hales, 2004).

As ammonia is a fast acting neurotoxin; it initiates a cascade of intracellular reaction leading to the functional impairments of neurons and ultimately, to the death of the intoxicated animal. Its cytotoxicity is caused by its ability to increase the mitochondrial level of Ca\(^{2+}\) (Kosenko, E., et al., 2000), which, in its turn, activates a number of bio-chemical reactions resulting in generation of superoxide and nitroxide radical (Kosenko, E., et al., 1998) and exhaustion of the mitochondrial anti-oxidative system (Kosenko, E., et al., 1997). Ammonia induced oxidative stress in the mitochondria is one of initial stages of cell death. Mitochondria are known to be able to integrate various prosuicide stimuli (Cande, C., et al., 2002). The decrease in serum FSH and LH levels could be due to the impairment of spermatogenesis in the spermatogenic compartment in the ammonia exposed rats or through the inhibition of testosterone production or direct effects of the ammonia on brain.

Hormones are well appreciated chemical messengers which play an important role in the regulation of a wide array of biological processes in animal systems. Classically, it is
believed that hormones coordinate in a cascade of intact circuits and thus influence vital processes including reproduction. In mammals the reproduction, is mainly under the control of a range of hormones and among them, gonadotropins like Follicle stimulating hormone (FSH) and Leutinizing hormone (LH) and steroids are fundamentally involved. In males, FSH and LH mainly target testicular Sertoli cells and Leydig cells, respectively and sustain the processes like spermatogenesis and steroidogenesis. The action of FSH on the germ cells is indirect and mediated by a paracrine signal(s) of Sertoli cell origin that acts as a survival factor for differentiated spermatogonia and therefore amplifies a basal level of spermatogenesis that is maintained by testosterone (T). Testicle usually performs two important functions: production of testosterone and formation of germ cells. For these processes to occur, an intact circuit of FSH-Sertoli cell and LH-Leydig cells is considered important.

The pituitary hormones, follicle stimulating hormone and luteinizing hormones are responsible for the stimulation of spermatogenesis and steriodogenesis respectively (Greep and Fevold 1973). Testosterone plays an important role in maintaining spermatogenesis, accessory organs and secondary sexual characters. Testosterone acts via androgens receptors (ARs) on sertoli leyding cell and per tubular cells. The fact that testosterone exerts its effects on somatic cells rather than germ cells was highlighted by recent germ cell transplantation studies (Johnson et al., 2001) in which spermatogonia from AR-deficient animals development into spermatozoa in wild type recipients. The pituitary hormones luteinizing hormones (FSH) also secreted by the anterior pituitary gland plays a key role in the development of the immature testis, especially by controlling steroli cells proliferation (Orth, 1993). Without this stimulation the conversion of the spermatids to sperm will not occur. So, both these pituitary hormones are responsible for stimulation of spermatogenisi and steroidogenesis respectively (Greep and Fevold, 1973).

The testosterone is secreted by the testes in response to LH. The reciprocal effect of turning off anterior pituitary secretion LH does results from the direct effect of testosterone on the hypothalamus by decreasing the secretion of GnRH. This in turn causes a corresponding decrease in the secretion of both LH and FSH by the anterior pituitary and the decrease in LH decreases the secretion of testosterone by the testes. Thus, whenever the
secretion of testosterone becomes too high, this automatic negative feedback effect, operating through the hypothalamus and anterior pituitary gland, reduces the testosterone secretion back toward its normal operating level. Conversely, too little testosterone allows the hypothalamus to secrete large amounts of GnRH, with a corresponding increase in anterior pituitary LH and FSH secretion and an increase in testicular testosterone secretion (Guyton, 1991). Chronic administration of synthetic estrogens results in testosterone secretion in male rats (Brockfor and Blake, 1997) and in female rats (Katsuda et al., 2002).

In the peripheral tissues (including skin and adipose tissue), circulating testosterone is enzymatically converted by 5α-reductase and aromatase into its active metabolites, dehydrotestosterone (DHT) and estradiol (E2). In normal men, the resulting plasma levels ratios of DHT/testosterone and E2/testosterone are 1:10 and 1:200.

\[ \text{Steroid biosynthesis} \ (\text{Nieschlag and Behre, 2004}) \]

1. Cholesterol side chain cleavage cytochrome P-450 complex
2. 3β-hydroxysteroid dehydrogenase
3. 17α-hydroxylase
4. 17, 20-lyase
5. 17β-hydroxysteroid dehydrogenase
6. Aromatase
7. 5α-reductase.
Metabolism of testosterone

Testosterone can mediate effects via active metabolites. The conversion of testosterone to DHT is catalyzed by 5α-reductases. The enzyme 5α-reductase is present in many of the androgen target tissues, for example the male accessories sex glands, the skin and tissues of the external genitalia. This enzyme converts testosterone to DHT, which has a much higher affinity for the androgen receptor in these target cells, and it is much potent androgen than testosterone (Grino et al., 1990).

Conversion of testosterone to estradiol involves a P450-dependent aromatase enzymes (CYP 19) and acts to diversify androgen action, since estradiol binds to the estrogen receptor (ER), thereby regulating the expression of a completely different set of genes (Simpson et al., 2002).

Metabolism of testosterone (Nieschlag and Behre, 2004)
Molecular mechanisms of androgen action:

The molecular mechanism mediating cellular responses to androgens is complex and involves both genomic and non-genomic effects. Genomic effects of androgens are mediated by a specific receptor, the androgen receptor (AR). In response to the binding of androgens to AR, it switches to transcription factor that regulates target gene expression (Davison and Bell, 2006). Non-genomic effects of androgens occur independently of AR. Instead, membrane-bound receptors have been proposed to trigger rapid effects of androgens that lead to 2nd messenger signaling (Benten et al., 1999). This, in turn, triggers a variety of cell responses (Wierman, 2007).

Testosterone and male sexual differentiation

Testosterone plays a crucial role in sex differentiation in mammals (Wilson, 1978). The fetal testis produces two hormones: testosterone and anti-muellerian hormone (AMH). Testosterone produced from 8 to 37 weeks gestation in humans (Embryonic day 15.5 to 21.5 in rats) (Welsh et al., 2008). During this period, masculinization of fetal reproductive tract occurs. Presence of testosterone is very much needed for the development of the Wolffian ducts, differentiation of the internal (epididymis, vas deferens, seminal vesicles) and external (Penis, scrotum) genitalia (Hernández-Valencia and Zárate, 2010).

Testosterone and spermatogenesis:

Spermatogenesis is well known androgen dependent process. Testosterone action is crucial for the completion of meiosis in the spermiogenesis (Singh et al., 1995; Meachem et al., 1999; McLachlan et al., 2002; Haywood et al., 2003) and germ cell survival particularly during stage VII (Russell et al., 1981; Henriksen et al., 1995). Testosterone is also essential for the conversion of round spermatids between stages VII and VIII (O’Donnell et al., 1994, 1996). Testosterone is required for the completion of the first wave of spermatogenesis during puberty (Marathe et al., 1995). In addition, testosterone alone facilitated the completion of meiosis in adult hypogonadal mouse, as evidenced by the production of round spermatids from spermatocytes (Haywood et al., 2003).
Hypogonadism and male infertility

Low testosterone or increased levels of LH are present in 20-30 % of male infertility cases (Nieschlag and Behre, 2004; Cheng and Mruk, 2010). Hypogonadism can be caused by testicular insufficiency, androgen resistance in the target organ or failure of the hypothalamic-pituitary-gonadal axis. The symptoms of androgen deficiency can be prevented or reversed by testosterone treatment. Testosterone treatment was used for the sexual thoughts and fantasies, sexual interest and desire, satisfaction with sexuality, frequency of erections and number of morning erections and ejaculations (Lee et al., 2003). These clinical studies are confirmed by studies on hypogonadal men after testosterone treatment (Morales et al., 1997; Jain et al., 2000)

Results:

In the present study the serum testosterone levels were determined. The levels of serum testosterone were significantly decreased in adult rats exposed to ammonium sulphate when compared control rats .The decrement in serum testosterone is more in ammonium sulphate rat (43.56%).When compared with ammonium sulphate treated with vitamin –c treated rat (26.48%).
Table 2.3: Testosterone levels in ammonium sulphate exposed and ammonium sulphate along with vitamin-c experiments in male albino rats.

<table>
<thead>
<tr>
<th>Hormone levels</th>
<th>control</th>
<th>ammonia</th>
<th>Ammonia+ vitamin -c</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Testosterone</strong> (ng/ml)</td>
<td>9.38±0.439</td>
<td>5.23±0.362</td>
<td>6.651±0.430</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>-44.24</td>
<td>-29.093</td>
</tr>
<tr>
<td>%Change over control</td>
<td></td>
<td></td>
<td>+27.17</td>
</tr>
<tr>
<td>%change over ammonia sulphate</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All the values are mean of six individual observations. % - Percent change over control, % Percent change over Ammonium sulphate, SD – Standard deviation, Values are significantly over control at P<0.000, ** Values are significantly over Ammonium sulphate at P<0.05.

**ONE WAY ANOVA:**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Sum of squares</th>
<th>Degree of freedom</th>
<th>Mean squares</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>48.435</td>
<td>2</td>
<td>24.21</td>
<td></td>
</tr>
<tr>
<td>With in groups</td>
<td>5.200</td>
<td>15</td>
<td>0.347</td>
<td>69.85</td>
</tr>
<tr>
<td>total</td>
<td>53.635</td>
<td>17</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are significant at P<0.000*
Fig 2.3: **Testosterone** levels in ammonium sulphate exposed and ammonium sulphate along with vitamin-c experiments in male albino rats.

All the values are mean ± S.D of six individual observations.
% - Percent change over control,
Values are significantly different from control p<0.000*
Discussion:

The risk of chemical toxicity is recognized to be greatest in this rapidly industrialization and restricting developing countries (WHO, 1992; Uzma et al., 2008) and this is the remarkably at variance with the developed countries with ample facilities to reduce over exposure and the toxic effects of chemicals on Testosterone production and over stimulation of Luteinizing hormone (Yamada 1994; Fort et al., 1995, Murakami 1995). The weight of the testis in the present study was also significantly decreased when compared to controls, thus, the decrease in the weights of testis may reflect loss of germ cells and also improper functioning of Leydig cells. In the present study, ammonia intoxication negatively affects the serum levels of both these hormones (FSH and LH) in the rats. This suggests that ammonia stress might have affected the brain of treated animals. Earlier it was suggested that toxic does of ammonia induces pro-apoptotic characters in the rat brain cells. (Yu. G. Kaminsky et al., 2007).

Palaniappan Murugesan et al., 2005 reported that the protective role of vitamin-C and E against Aroclor 1254 for 30 days induced changes in testosterone level. Aroclor1254 significantly reduced the serum testosterone levels .However the simultaneous administration of vitamin-C and E on Aroclor 1254 exposed rats resulted a significant restoration of testosterone levels to the control level. Vitamin –c have ameliorative role against adverse effects of PCB (Aroclor 1254). In this present study, a similar situation might have restored the serum testosterone levels. The present study demonstrates the adverse effect of ammonia stress on spermatogenesis and steroidogenesis and protection caused by vitamin-c co-administration in rats. A significant decrease in the activity levels of testosterone was observed which clearly indicates the impairment of steroidogenesis .The decreased steroidogenic enzyme activity levels indicate decreased androgen production in experimental rats which in turn lead to decreased reproductive activities in male rats. It seems ammonia might acted on Leydig cells and inhibits the testosterone production which was evident by decrease in the activity levels of 3β-HSD and 17β-HSD enzymes in the testes of experimental rats .The activity levels of testosterone were significantly increased in ammonia and vitamin-C treated rats when
The decreased levels of LH together with decreased levels of serum testosterone are indicative of decreased steroidogenic ability of the testes of the rats exposed to ammonia. In addition, the levels of testosterone were also decreased during the ammonia stress. In the present study the reproductive hormone which were investigated are LH and FSH were found to decrease significantly in a treated animals when compared to the serum levels in the control animals. It is reported that Cd metal has also been associated with reproductive dysfunctions in both male and female experimental animals (Zhang et al., 2008; Aprioku et al., 2009), and causes alterations in the serum levels of androgens and other reproductive organs (Massanyi et al., 2007, A.W.Obianime et al., 2010).

Testosterone is the most important sex hormone produced in the male. It is the hormone that is primarily responsible for producing and maintaining the typical adult male characteristics. Testosterone is produced mostly in the outer layer of the adrenal gland. The hypothalamus controls hormone production in the pituitary gland by means of gonadotropin releasing hormone (GnRH). This hormone stimulates the pituitary gland to synthesize follicle stimulating hormone (FSH) and luteinizing hormone (LH). LH signals the testes to produce testosterone. Testosterone supports spermatogenesis, sperm maturation, and sexual function (Steinberger and Duckett; 1965, Ewing and Keeney, 1993; Weinbauer and Nieschlag, 1993).

Fernandes, et al., 2011 reported that vitamin C plays key roles in the synthesis of testosterone and in their study vitamin C-treated hyperglycemic rats showed partial recovery of testosterone level. In the present study, the reduced LH level corroborates previous studies that reported diminished LH release from pituitary gland in hyperglycemic male rats (Scarano et al., 2006). The partial recovery of LH levels in the vitamin C treated group may
be related to the fact that ascorbic acid can be a vitaminergic transmitter that activates the release of LH and FSH from the anterior pituitary gland (Karanth et al., 2001). which are similar to our present study. The present study showed that vitamin C supplementation minimized some alterations in the male reproductive system caused by ammonia stress such as reduction of testosterone. However, questions about vitamin supplementation, or any other nutrient, have been asked since excessive doses can be harmful to the body. Thus, it is possible that the beneficial effects of vitamin C supplementation are only relevant to those individuals with high levels of oxidative stress that occur in ammonia condition.

Low level of testosterone in the serum observed in the ammonia exposed animal in comparison to control indicates either decreased synthesis of testosterone or increased breakdown of the hormone. The decreased level of LH with a decreased serum testosterone levels in experimental rats indicates diminished responsiveness of Leydig cells to LH thereby decrease in testosterone levels. The decreased steroidogenic effect also indicates the decreased production of testosterone (Pushpalatha et al., 2003). Exposure to chemicals depot during embryonic development also results in atrophy and hyperplasia in the Leydig cells of corresponding groups as compared to the control rats and also indicated decreased steroidogenic ability of testis in the experimental rats (Atanassova et al., 2000; Goyal et al., 2003; Pushpalatha et al. 2004).

The present observations of reduced testosterone are in agreement with those of previous studies, which also reported lower testosterone in adult animals as a consequence of estrogen treatment of neonatal animals (Sharpe et al. 1998; Cook et al., 1998; Atanassova et al., 1999; 2000; Goyal et al., 2003).

Ait Hamadouche N. et al., 2013 reported that serum testosterone levels were decreased in lead acetate treated groups of animals as compared to their respective control. Significant alterations in testosterone levels have been reported after exposure to certain heavy metals (Gabuchyan, 1987; Chattopadhyay et al., 2005; Atef Al Attar, 2011).

Many studies in rodents and humans suggest that inappropriate exposure to estrogens in utero and during the neonatal period impairs reproduction (Stillman, 1982; Arai et al. 1983; Sharpe and Skakkebaek, 1993; Toppari et al., 1996; Khan et al., 1998). A single
injection of 2 mg aqueous estradiol reduced plasma testosterone without affecting serum LH levels in young men. Low doses of estradiol administered mice during neonatal period exhibited decreased testosterone content in serum (Cooke and Eroschenko, 1990). Recent studies (Tohei et al. 2001) also demonstrated a decreased serum testosterone in BPA administered rats and mice.

Muftau Shittu et al., 2012 reported that the significant reduction in the testosterone concentration in the CPF group agreed with the result obtained by Joshi et al 2007 and Kang et al 2004. The low serum testosterone concentration may be linked to the inhibitory effect of OP insecticides on the secretion of pituitary gonadotropins (FSH and LH), which are involved in testosterone biosynthesis (Joshi et al., 2007). Reduced testosterone concentration may also occur due to direct damage to the Leydig cells (Zidan, 2009). Indeed, oxidative and degenerative changes in the testes of rats chronically exposed to CPF have been previously demonstrated in by Shittu et al., 2012. Thus, the low testosterone concentration in the CPF group may be partly due to oxidative damage to the pituitary gland and the testicular tissues. The improvement in testosterone concentration in group pretreated with vitamin C showed the ameliorating effect of the antioxidant vitamin. The effect may be related to the enhancement of gonadotropin secretion and release due to reduced peroxidative damage to the pituitary gland and improvement in testicular tissue integrity, which were similar and observed in the present study.

Testosterone is the principal androgen in males. Leydig’s cells are responsible for testosterone production which depends upon stimulation of these cells by Luteinizing Hormone (LH). Decreased testosterone, and the fall of ratio between testosterone levels is thought to happen in stress. Decline in testosterone levels as mentioned before is indicative of catabolic tendency in body and increasing this ratio is useful to body in various ways. The literature shows that the deficiency of ascorbic acid may lead to diminished testosterone production with consequent impaired fertility (Chitra et al., 1999).

**STEROID MARKER ENZYMES:**

Endocrine disrupting substances can cause adverse effects by interfering within the body's hormones or chemical messengers. These substances are called hormone disruptors
or endocrine disruptors, the endocrine glands secrete the hormones (Waissmann, 2002). Endocrine disruptors can exert their effects in several different ways, either bind to the hormone’s receptor, or block the action of the hormone. Alternatively, they can stimulate or inhibit the enzymes responsible for the synthesis or clearance of a hormone, and thereby give rise to an increased or decreased action of the hormone. Endocrine disruptors can cause reproductive anomalies and congenital malformations (Lemos, 2001; Waissmann, 2002; Nelson, 2003). Alterations caused by endocrine disruptors can be temporary or permanent (WHO, 2001; Waissmann, 2002). The principal effects of endocrine disruptors on male fertility are temporal reduction in sperm concentration and quality (Bruss et al., 2004).

The two key enzymes involved in the biosynthetic pathway of testosterone are 3β-hydroxysteroid dehydrogenases (HSD) and 17β-hydroxysteroid dehydrogenases (HSD). The activity levels of 3β-HSD and 17β-HSD have been used to study the testicular steroidogenesis of rats’ in different experimental conditions. These two enzymes are having regulatory functions in the maintenance of steroidogenesis and also involves in the synthesis of testosterone. Male reproductive function is directly related to the structure and function of the testis Wan et al., (2006) which is regulated by the hypothalamic pituitary testicular axis. Testosterone is produced by Leydig cells in the testis, plays an important role in this regulation process (Holstein et al., 2003).

The enzymes 3β-HSD and 17β-HSD are important in the synthesis of most biologically active steroids (Jana and Samanta, 2006). The activity levels of 3-HSD and 17-HSD have been used to study the testicular steroidogenesis of rats in different experimental conditions (Gosh et al., 1990; Srivastava and Srivasthava, 1991: Gosh et al., 1991; 1995). The biosynthesis of active androgens and estrogens from DHEA is achieved by three steroidogenesis-related enzymes (Labrie, 2004). Testosterone is synthesized through metabolism of DHEA by 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-HSD. The enzyme 3β-HSD is essential for the biosynthesis of all steroid hormones.3β-HSD is an enzyme which catalyses the synthesis of progesterone from pregnenolone, 17-hydroxyprogesterone from 17-hydroxypregnenolone, and androstenedione from dehydroepiandrosterone in the adrenal gland.
Determination of activity levels of 3β-HSD and 17β-HSD in testis reflected the status of steroidogenesis in testis. Masculation of male fetus is dependent upon secretion of androgens by the fetal testis. Androgens are synthesized from cholesterol through the actions of the steroidogenic enzymes viz., cytochrome p450 side chain cleavage (p450 SCC), 3β-hydroxy steroid dehydrogenase (3β-HSD), Cytochrome P450 17α-hydroylase (p450c17) and 17β-hydroxy steroid dehydrogenase /17-keto steroid reductase (17β-HSD/17KSR) each of these enzymes is expressed in the testis (Baker et al., 1999) and localization studies in fetal/neonatal animals have shown that P450SCC, 3β-HSD and p450c17 are expressed specifically within the Leydig cell compartment (Ikeda et al., 1994). In the testis it is clear that 17β-HSD type 3 is the major isoform involved in testosterone biosynthesis because loss of this form leads to a failure of normal masculinization during development and a rise in circulating androstenedione with a reduction in circulating testosterone in adult (Androsson et al., 1996).

**Results:**

The 3β-HSD and 17β-HSD enzyme activities decreased significantly in the testis of ammonia sulphate treated rats when compared to the control. In general 3β-hydroxysteroid dehydrogenases (HSD) activity was greater than 17β-hydroxysteroid dehydrogenases (HSD) activity. A significant decrease in activity levels of 3β-hydroxysteroid dehydrogenases (HSD) and 17β-hydroxysteroid dehydrogenases (HSD) was observed in the testis of both the experimental rats. But the decrement was less in ammonium sulphate treated with vitamin-c.
Table 2.4: β-hydroxysteroid dehydrogenases (HSD) and 17β-hydroxysteroid dehydrogenases (HSD) activity levels in male rats of control and ammonium sulphate exposed along with ammonium sulphate with vitamin-C exposed.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>control</th>
<th>Ammonium sulphate</th>
<th>Ammonium sulphate +vitamin-c</th>
</tr>
</thead>
<tbody>
<tr>
<td>3β-HSD</td>
<td>0.863±0.086</td>
<td>0.346±0.0920</td>
<td>0.500±0.091</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td></td>
<td>%Change over control</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-59.90</td>
<td>-42.06</td>
</tr>
<tr>
<td>% change over ammonia sulphate</td>
<td>0.436±0.028</td>
<td>0.321±0.188</td>
<td>0.375±0.126</td>
</tr>
<tr>
<td>17β-HSD</td>
<td></td>
<td>%Change over control</td>
<td></td>
</tr>
<tr>
<td>Mean ±SD</td>
<td></td>
<td>-26.37</td>
<td>-13.99</td>
</tr>
<tr>
<td>% change over ammonia sulphate</td>
<td></td>
<td></td>
<td>+16.82</td>
</tr>
</tbody>
</table>

All the values are mean ±S.D of six individual observations

% - Percent change over control,

Values are significantly different from control p<0.000*

ONE WAY ANOVA:

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degree of freedom</th>
<th>3β-HSD</th>
<th>17β-HSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sum of Squares</td>
<td>Mean Squares</td>
</tr>
<tr>
<td>Between Groups</td>
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<td>.799</td>
<td>.400</td>
</tr>
<tr>
<td>Within Groups</td>
<td>15</td>
<td>.295</td>
<td>.020</td>
</tr>
<tr>
<td>F Value</td>
<td></td>
<td>20.31</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>1.095</td>
<td>0.304</td>
</tr>
</tbody>
</table>

All the values are Significant at p<0.05
Fig 2.4: 3β-hydroxysteroid dehydrogenases (HSD) and 17β-hydroxysteroid dehydrogenases (HSD) activity levels in male rats of control and ammonium sulphate exposed along with ammonium sulphate with vitamin-C exposed.

All the values are mean ±S.D of six individual observations

% - Percent change over control,

Values are significantly different from control p<0.000*
Discussion:

In steroidogenesis, 3β-HSD and 17β-HSD are the key regulatory enzymes (Hineshelwood et al. 1994). A significant decrease in the activity levels of 3β-HSD and 17β-HSD in the testes of the experimental rat indicates decreased steroidogenesis. The decrease in testicular 3β-HSD and 17β-HSD activity levels with decrease in male reproductive potential has been reported following exposure to several xenobiotics in mammals (Badri et al., 2000; Sujatha et al., 2001; Mani et al., 2002; Pant and Srivastava, 2003).

The testicular steriogenic marker enzymes play a key role in testosterone biosynthetic pathways and as such are frequently targets of endocrine-disrupting chemicals (Sanderson 2006). The results of this investigation demonstrate the adverse effect of ammonia on testicular steriodogenesis. The suppression of testicular 3β-HSD and 17β-HSD in treated animals has been reported by other investigators (Jana et al., 2006). 3β-HSD and 17β-HSD play a regulatory role as these are the prime enzymes in testicular androgenesis (Ghosh et al., 1990; Jana and Samanaha 2006). The diminution in these enzymes activities after ammonium sulphate treatment is in agreement with the findings of Sarkar et al., 2003 where treatment of ammonia was associated with the inhibition of testicular androgenesis. Puspaltha 2007 reported that injection of testostoviron depot increases activity levels of steriodogenic marker enzymes 3β-HSD and 17β-HSD in experimental animals this leads to increase the steridogenesis to induce androgen production since these enzymes activities are important in steriod synthesis (wiebe, 1969) and the increased testosterone in turn increases the male fertility in treated rats. In similar way vitamin –C restored the activity levels of 3β-HSD and 17β-HSD levels in induced ammonia sulphate treated rats. From the results it is evident that the administration of vitamin-c in to exposed rats prevents the induction of most of the reproductive abnormalities and treatment with vitamin-c resulted in partial/complete recovery of these reproductive changes.

From the results it can be concluded that decrease in testicular 3β-HSD and 17β-HSD enzyme activity levels may lead to decrease in steridogenisis which in turn may suppress the reproductive activities. Determination of 3β-HSD and 17β-HSD activities can be used as sensitive bio markers to monitor the action of reproductive toxicants. Pretreated...
with vitamin C showed the ameliorating effect of the antioxidant vitamin. The effect may be related to the enhancement of gonadotropin secretion and release due to reduced peroxidative damage to the pituitary gland and improvement in testicular tissue integrity, which were observed in the present study.

Palaniappan Murugesan et al., 2005 reported that the protective role of vitamin-C and E against Aroclor 1254 for 30 days induced changes in leyding cell steridogenesis and antioxidant system. Aroclor1254 significantly reduced the $3\beta$-HSD AND $17\beta$-HSD levels. However the simultaneous administration of vitamin-C and E on Aroclor 1254 30 days exposure to rats resulted a significant restoration of $3\beta$-HSD & $17\beta$-HSD levels to the control level. Vitamin -C have ameliorative role against adverse effects of PCB (Aroclor 1254). In this present study, a similar situation might have restored the steriod marker enzymes. The present study demonstrates the adverse effect of ammonia stress on spermatogenesis and steroidogenesis and protection caused by vitamin-C co-administration in rats’ exposure ot ammonium sulphate. The decreased steroidogenic enzyme activity levels indicate decreased androgen production in experimental rats which in turn lead to decreased reproductive activities in male rats. It seems ammonia might act on Leydig cells and inhibits the testosterone production which was evident by decrease in the activity levels of $3\beta$-HSD and $17\beta$-HSD enzymes in the testes of experimental rats. The activity levels of testosterone were significantly increased in ammonia + vitamin -c treated rats when compared with ammonia sulphate treated rats. This increase in testosterone activities levels in testis indicates the restoration of steroidogenesis and leads to normal fertility in ammonia +vitamin-c treated rats.

In ammonia treated rats, a significant decrease in the activity levels of $3\beta$-HSD and $17\beta$-HSD was observed which clearly indicates the impairment of steroidogenesis. The decreased steroidogenic enzyme activity levels indicate decreased androgen production in experimental rats which in turn lead to decreased reproductive activities in male rats. The activity levels of $3\beta$-HSD and $17\beta$-HSD were significantly increased in vitamin-c +ammonia treated rats when compared with ammonia treated rats. This increase in $3\beta$-HSD and $17\beta$-HSD activities levels in testis indicates the restoration of steroidogenesis and leads to normal fertility in vitamin-c + ammonia treated rats. Vitamin-c significantly increased the
steroidogenic marker enzyme (3β-HSD and 17β-HSD) activity levels in the testis of ammonia treated rats. This may result in increased androgen production which in turn enhances the male reproductive efficiency.

Ravi Sekhar 2010 reported that a significant decrease in the activity levels of 3β-HSD and 17β-HSD activity levels in the testis of the experimental mice treated with cypermithane indicated decreased steriogenisis. The decrease in the testicular 3β-HSD and 17β-HSD activity levels with decrease in male reproductive potentials has been reported following exposure to several xenobiotics in mammals (Sujatha et al., 2001; Mani et al., 2002; Pant and Srivastava 2003).

3β-HSD and 17β-HSD are important in the synthesis of most biologically active steroids (Wiebe, 1969). Determination of the activity levels of these enzymes can be an invaluable aid in subjectively assessing the state of the steroid biosynthesis. The activity levels of 3β-HSD and 17β-HSD have been used to study the testicular steroidogenesis of rats’ the present experimental conditions. The decreased steroidogenic enzyme activity levels may lead to decreased steroidogenesis in experimental rat which in turn may suppress the reproductive activities in the male rat.

It is well known that androgens are essential for normal spermatogenesis (Sharpe, 1987). The decrease in serum testosterone levels in experimental rat may occur due to inhibition of testicular steroidogenic enzyme (3β-HSD and 17β-HSD) activity, because these enzymes are responsible for the regulation of testicular testosterone synthesis. Ramadan, 1988 reported that decreased testosterone levels might also be responsible for decreased spermatogenesis in mice after exposure to cypermethrin and sodium fluoride. Cypermethrin could disrupt the normal functioning of sex hormones The testicular steroidogenic marker enzymes play a key role in testosterone biosynthetic pathways and as such are frequently targets of endocrine-disrupting chemicals (Sanderson, 2006).

The results of this investigation demonstrate the adverse effect of ammonium sulphate on testicular steroidogenesis. The suppression of testicular 3β-HSD and 17β-HSD in ammonia treated animals has been supported by studies by other investigators (Pushpalatha et al., 2005; Ghosh et al., 2002). 3β-HSD and 17β-HSD play a regulatory role,
as these are the prime enzymes in testicular and rogenesis (Ghosh et al., 1990, Jana and Samanta, 2006). Sodium fluoride treatment at 20mg/kg/day for 29 days by oral gavage resulted in significant diminution in the testicular 3β-hydroxysteroid dehydrogenase (HSD), 17β-HSD activities reported by Ghosh et al., 2002.

Chattopadhyay et al., 2001 reported that 3β-HSD & 17β-HSD were decreased significantly in arsenic treated rats and the co-administration of L-asborate(vitamin-c) restored the steroid marker enzymes. The L-asborate (vitamin-c) plays a pivotal role in maintain normal activities and brain monoamines in arsenic treated rats. Vitamin-c significantly increased the steroidogenic marker enzyme (3β-HSD and 17β-HSD) activity levels in the testis of ammonia treated rats. This may result in increased androgen production which in turn enhances the male reproductive efficiency.

**SPERM COUNT:**

Male reproductive health has deteriorated in many countries during the last few decades (Androsen et al., 2000). The incidence of testicular cancer has increased during the same time. Incidences of hypospadias and cryptorchidism also appear to be increasing (Carlesen et al., 1995; Sakakkebaek et al., 1998). Similar reproductive problems occur in many wild life species. Mean sperm counts in men had declined by around 40-50% over the past 50 years the most comprehensive of these studies concluded that sperm counts in fertile men have declined since few decades. Carlsen et al., 1992 reported that decline in sperm counts might be related to an increasing incidence of other disorders of development of the male reproductive system and that this could have arisen because of increased exposure to different environmental toxicants. Reactive oxygen species are involved in the peroxidative damage of human spermatozoa seen in many cases of male infertility (Aiten 1994). These free radicals may arise from defective spermatozoa and from leukocytes (Aitken and west 1990).

The follicle stimulating hormone (FSH) also secreted by the anterior pituitary gland, plays a key role in the development of the immature testes, especially by controlling sertoli cell proliferation. Without this stimulation, the conversion of the spermatids to sperm will not occur. So, both these pituitary hormones are responsible for stimulation of
spermatogenesis and steroidogenesis respectively (Orth, 1993). The quality and quantity of sperm was used as an index for male reproduction. Sperm motility assessments are integral part of some reproductive toxicity test guidelines. A physiological capacity of sperm (Gray et al., 1988). Determination of sperm count were performed to assess the quality and functional status of sperms which play an important role in male fertility determination.

Spermatogenesis depends on the action of testosterone (Sharpe et al., 1986). Testosterone is produced almost exclusively by Leydig cells in the testis. The sertoli cells play a central role in development of functional testis, and hence in the expression of a male phenotype. Sertoli cells are the first cells to differentiate recognizably in the indifferent fetal gonad, an event which enables seminiferous cord formation, prevention of germ-cell entry into meiosis and differentiation and function of the leydig cells (Mackay, 2000).

The secretions of the leydig cells (testosterone) then play vital roles in downstream masculization events and in descent of the testis into scrotum (Sharpe, 2001). Moreover, the number of sertoli cells will determine the number of germ cells that can be supported through spermatogenesis and hence will numerically determine the extent of sperm production (Sharpe, 1994; 1999).

The living organisms are exposed everyday to several substances in our environment. Toxic substances that can damage the cells of the various organs of reproduction and impair the hormonal responses affect the quality and quantity of sperm cells. The chemical compounds could get easily accumulated through repeated intake via food chain or direct chemical intake via medicine to levels high enough to affect their physiological state. Walsh et al. (1993) have studied the variation in the activity of oxidative metabolism of various animals during exposure to a wide range of chemicals that are harmful to humans, by affecting the male reproductive system (Lue et al., 1999; Bryan et al., 2000; Kasahara et al., 2002). There has been growing concern about toxicity of a number of chemicals, including pesticides, on the male reproductive system (Murray et al., 2001; Sharpe, 2001).

In 1992, four Danish scientists published a research study suggesting that sperm counts have declined about 50 percent since 1940 worldwide (Carlsen et al., 1992). The
study analyzed the results of over 60 studies of sperm counts published between 1938 and 1991 as a statistical analysis that linked results of a large number of independent studies. Using a model which assumed that sperm counts changes over time in a linear way, the results of Carlsen et al., (1992) on the meta-analysis indicated average sperm counts declined from 113 million to 66 million per ml of semen during the half century.

Sperm quality is one of the important indexes of male reproductive function. Good quality semen is essential for reproductive success. This quality appears to have been directly affected in recent years, and evidently have unfavorable trends in male reproductive health (Guillette and Crain, 2000). Since 1970s, various authors have reaffirmed the possible significant drop in sperm quality and consequently an increase in male infertility rates (Pasqualotto et al., 2003; Bruss et al., 2004).

RESULTS:

The average sperm count was found to be 37.17±0.607 millions /gm testis in control rats. Ammonium exposed male rat showed a significant depletion in sperm count than the control rats. The changes were more in rats exposed to ammonia (48.87%) when compared with corresponding ammonia treated with vitamin –c group.
Table 2.5: Effect of ammonium sulphate induced stress and vitamin –C along with ammonium sulphate on sperm count in male albino rats.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>control</th>
<th>ammonia</th>
<th>Ammonia+ vitamin -c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm count (million /ml)</td>
<td>37.17±0.639</td>
<td>19.13±0.603</td>
<td>25.76±0.540</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td></td>
<td>-48.53</td>
<td>-30.69</td>
</tr>
<tr>
<td>%Change over control</td>
<td></td>
<td></td>
<td>+34.65</td>
</tr>
<tr>
<td>% change over ammonia sulphate</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All the values are mean ±S.D of six individual observations

% - Percent change over control,

Values are significantly different from control p<0.000*

ONE WAY ANOVA:

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Sum of squares</th>
<th>Degree of freedom</th>
<th>Mean squares</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
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<td>2</td>
<td>488.392</td>
<td>799.685</td>
</tr>
<tr>
<td>With in groups</td>
<td>9.161</td>
<td>15</td>
<td>.611</td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>985.944</td>
<td>17</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are significant at P<0.000*
Fig 2.5: Effect of ammonium sulphate induced stress along with vitamin –C on sperm count in male albino rats.

All the values are mean ±S.D of six individual observations

% - Percent change over control,
Values are significantly different from control p<0.000*
Discussion:

In the last few years, a marked decrease in the quality of semen has been reported (Carlsen et al., 1992). Infertility is one of the major health problems in couples’ lives; approximately 30% of couple’s infertility is due to male factors (Isidori et al., 2006). Several conditions can interfere with spermatogenesis and reduce sperm quality and production. Many factors such as drug treatment, chemotherapy, toxins, air pollution, and insufficient vitamin intake may have harmful effects on spermatogenesis and the normal production of sperm. Researchers have reported that using antioxidants and vitamins A, B, C, and E in the daily diet can protect sperm DNA from free radicals and increase blood testis barrier stability (Lu et al., 2003; Jedlinska-Krakowska et al., 2006).

Khaki et al., 2009 reported that Onion and garlic contain a wide variety of phytochemicals and micro constituents such as trace elements, vitamins, fructans, flavonoids, and sulphur compounds, which may have a protective effect against free radicals. Recently, much attention has been focused on the protective effects of onion against colon cancers in rats (Fukushima, 1997; Ross, 2006). Results clearly indicate that Allium cepa (onion) has a good effect on spermatogenesis in rats. Results showed that administration of onion juice (1 g/rat/day) for 20 consecutive days a marked increase in sperm count, viability, and motility, as compared to respective controls. In the present study ammonia has reduced sperm count. But when treated with vitamin –c, we observed increase in sperm count. Vitamin-C might have a protective effect against free radicals and restored sperm counts in rats.

Vitamins C and E are well known antioxidants that can ameliorate oxidative stress-related testicular impairments in animal tissues (Gosh 2002; kujo 2006). Vitamin C may execute its role by modulating testicular free radical production and/or stimulating testicular androgenesis and is essential for testicular differentiation, integrity, and steroidogenic functions (Dawinson 1990; Luck 1995; Marchlewicz 2007)

The results clearly indicate that the exposure to ammonia during gestational period impairs the titer of serum FSH, LH and testosterone in adult rats. The decreased testosterone levels might be responsible for decreased sperm count, and impairment of the male
reproductive performance in experimental rats. Vitamin –c has altered the levels of FSH, LH and testosterone levels in experimental rats and it might be responsible for increased sperm count.

Muftau shittu 2012 reported that the relatively lower FSH concentration recorded in the CPF group in their study was in agreement with established findings of other workers Zidan, 2009. The apparently lower FSH concentration may have been partly responsible for the low sperm count in the CPF group since FSH is actively involved in spermatogenesis (Plant, 2001). Pretreatment with antioxidant vitamin C has been shown in the present study to cause a relative increase in sperm count. This may be partly due to the antioxidant properties of the vitamin, which may have aided in maintaining the integrity of testes, especially the seminiferous tubules, Sertoli cells and the epididymis, thereby providing favorable environment for spermatogenesis and sperm maturation. These reports are similar to our present study.

The number of pachytene spermatocytes was significantly reduced in rats after exposure to ammonia prenatally as compared to the corresponding group of control rats. The number of sertoli cells reduced significantly in exposed rats with respect to the corresponding group of control rats.

Takhshid et al., 2012 reported that Exposure to pesticides could cause male infertility by causing a significant decrease in sperm quality and quantity. The results of the Srinivasa, 2007 present study clearly indicate that endosulfan at a daily dose of 10 mg/kg significantly reduces the quality and quantity of sperm production and sperm count. The result also shows the protective role of vitamin E and C on endosulfan induced sperm toxicity by decreasing further proved by improvements in qualitative and quantitative sperm parameters in vitamin-treated rats compared to endosulfan treated ones. Vitamins C and E are known antioxidants that are effective in preventing oxidative stress-induced testicular damages (Luck, 1995; Krishnamoorthy, 2007).

The size of the seminiferous tubules are androgen dependent Goyal et al., (2001) observed a decrease in the diameter of seminiferous tubules during exposed rats and attributed to the possible decrease in androgen circulation. Several lines of evidence
indicate that estrogenic chemicals cause degeneration of seminiferous tubules, a reduction in the thickness of the seminiferous epithelium, accompanied by arrest of spermatogenesis increased interstitial spaces suggesting the possible accumulation or water content in the tissue (Kim et al., 1999; Limanowski et al., 1999). Similarly the increased lumen diameter and decrease in number/disintegration of Leydig cells, suggests hypertrophy of these cells. Such observations on hypertrophy of Leydig cells have been reported under testicular dysfunction (Abney and Myers, 1991; Atanassona et al., 1999).

There is substantial evidence showing adverse effects of fetal/neonatal estrogen exposure on spermatogenesis and sperm output in adulthood in rodents (Brown –Grant et al. 1975; Arai et al., 1983; Bellido et al., 1990; Khan et al., 1998; Aceitero et al., 1998; Sharpe et al., 1998; Fisher et al., 1998). Few studies are also available on the histological changes in the testis after in utero exogenous estrogen exposure and concluded that the fetal testis is directly affected by estrogen during fetal and / or neonatal testis differentiation (Parks et al. 2000; Mylchreast et al. 2002; Fisher et al., 2003).

Besides decreased sperm counts percentages were also adversely affected in experimental animals. These changes were greater in rats exposed to higher dose of ammonia. There are few other reported studies that have examined the estrogen effects of sperm motility. Ethynil estradiol treatment reduced the percentage of motility (Kaneto et al., 1999). Goyal et al., (2001) observed reduced motility of sperm. The reduced sperm count observed in the present study, might also be a causative factor for reduction in fertility in rats exposed to ammonia. The reported reproductive toxic effects include increase in numbers of abnormal spermatozoa (Pati and bhunya, 1987), loss of spermatogenesis (Kour and Sing, 1980) and interference with steroidogenesis (Narayana and Chinoy, 1994). Many studies indicated that insecticides have harmful influence on the male reproduction (El-Samannody et al., 1986; Alhazza and Bashandy, 1998). Exposure to the higher dose of fenvalerate was toxic to testis and epididymis as shown by a decrease in the absolute weights and sperm counts in both organs of rats (Arielle et al., 2008).

It is well known that androgens are essential for normal spermatogenesis. Testosterone deprivation is known to impair the reproductive behavior and fertilizing ability
Neonatal exposure to low doses of β-estradiol 3-benzoate induces the changes in the reproductive tract of male rats ultimately results in reproductive potential (Putz et al., 2001; 2001). Suppression of testosterone production and in the testicular testosterone has been reported in rats following estrogen exposure (Barlow et al., 2003).

These results indicate that the exposure to ammonia not only impairs the quality and quantity of sperm but also affects the male fertility in rats. The data also suggested that the observed impairment of male reproductive performance could be due to decreased testosterone levels in prenatally exposed rats. It is well known that testosterone is essential to maintain spermatogenesis quantitatively.

This experiment thus demonstrates that ammonia intoxication not only targets brain (pituitary) but also testis indicating alterations in the intact hormonal circuits of pituitary-testicular axis. Knowledge pertaining to the ammonia intoxication and the levels of FSH, LH and T were little explored. The present study showed that ammonia intoxication has an effect on male reproduction in rats. Further, some more experimental data is necessary to provide direct evidences that ammonia intoxication decreases male reproductive health.