General Introduction and Literature review

Estrogen is an important sex hormone produced primarily in ovaries in females. It is now well established that 17β-estradiol, the predominant form of estrogen also plays a critical role in male reproductive function. The importance of estrogen in male reproduction is derived from earlier studies showing extensive presence of estrogen receptors (ER; both α and β) throughout the male reproductive system and male fertility is impaired in mice lacking ERs and aromatase. Presence of circulating estradiol in low concentration, but extraordinarily high level in semen further supports importance of estradiol in male sexual function. Based on recent studies it was suggested that estradiol in men is essential for modulating libido, erectile function and spermatogenesis (Cohen, 1998; O’Donnell et al., 2001). In addition, all the germ cells in different stages of spermatogenesis are dependent upon estradiol, as these germ cells contain aromatase and express ERs (O’Donnell et al., 2001). These findings are important due to recent concerns over reported declines in human sperm counts and speculation that exposure to environmental estrogens may be cause of low sperm counts (Auger et al., 1995). Several recent studies, including the generation of ERα knockout (αERKO) (McPherson et al., 2008) and aromatase knockout (ArKO) mice (O’Donnell et al., 2001), have also changed our understanding of estrogen action. However, these transgenic or knockout mice do not always produce consistent results. The animals treated with estrogen receptor modulator (agonist/antagonist) compounds are better suitable for these studies to reveal physiological action of estradiol in testes.

Despite the abundance of published data about involvement of estrogen in male sexual function, the specific role of estrogen in testicular activity particularly spermatogenesis and steroidogenesis remain unknown. Thus, the primary aim of this dissertation was to elucidate the specific roles and mode of action of estradiol in testicular activities in mice. This overview therefore summarizes the current information about the contribution of estrogen in male reproductive functions.
Biosynthesis of testicular steroids

The biosynthesis of steroid hormones is initiated by endogeneous cholesterol biosynthesis or by the uptake of lipoprotein cholesterol from circulations (Fig. 1). Cholesterol is stored in esterified form as lipid droplets in steroid producing cells. Trophic hormone initiates steroidogenesis by activating a chain of reaction that lead to hydrolysis of cholesterol esters into free cholesterol. The free cholesterol formed is transported into mitochondria by intracellular cholesterol transporters such as steroidogenic acute regulatory protein (StAR), sterol carrier protein 2 (SCP2) and peripheral benzodiazepine receptor (PBR). Inside mitochondria cholesterol is converted into pregnenolone by rate limiting enzyme P450 side chain cleavage enzyme. After this rate limiting step, subsequent biosynthesis steps proceed with the flow of the substrates through the enzyme systems located in the endoplasmic reticulum and mitochondria. After side chain cleavage and using Δ5 or the Δ1 pathway androstenedione is the major intermediary in the synthesis of estradiol.

Figure 1: This diagram depicts the steroidogenic pathway of synthesis of testosterone and estrogen.
The estrogen biosynthesis is catalyzed by a microsomal member of the cytochrome \( \text{P}450 \) superfamily, namely aromatase cytochrome \( \text{P}450 \) (the product of CYP 19 gene). The androstenedione is aromatized to estrone and subsequently to estradiol or androstenedione is converted to testosterone, which subsequently undergoes conversion to estradiol by steroidogenic enzyme \( \text{P}450 \) aromatase. In female most estradiol is produced by the granulosa cells of the antral follicles by the aromatization of androstenedione to estrone which in turn converted to estradiol by 17β-hydroxy steroid dehydrogenase. Smaller amounts of estradiol are also produced by the adrenal cortex. In testis, the Sertoli and Leydig cells are the main site of aromatization and are stimulated by follicle stimulating hormone (FSH) (Yen and Jaffe, 1999). Estradiol is also produced in the fat cells and brain.

The biosynthesis of estradiol like compounds has been observed in leguminous plants, such as *Phaseolus vulgaris* and soyabean (Ososki *et al.*, 2003) where they are termed as phytoestrogens. Thus, consumption of these plant products may have estrogenic effects.

**Hypothalamic-pituitary and gonadal axis and regulation of estrogen synthesis in males**

The gonadal axis is regulated by a nexus of hypothalamic neurons (hypothalamic pulse generator) in the mediobasal hypothalamus that responds to stimulation by the peptide hormone kisspeptin. Gonadotropin-releasing hormone (GnRH) is released in pulses every 60–90 minutes (Crowley *et al.*, 1985; Wetsel *et al.*, 1992) which stimulates the pulsatile release of the gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH) into the bloodstream. LH stimulates Leydig cells to produce testosterone, and FSH, in conjunction with intratesticular testosterone, acts on Sertoli cells and seminiferous tubules to stimulate spermatogenesis. Testes produce 3–10 mg of testosterone daily, about 10.4–34.7 nmol/L in serum that peak in the morning. Testosterone acts directly via androgen receptors and via its conversion into two active metabolites, dihydrotestosterone by the enzyme 5-α reductase or estradiol by the enzyme aromatase. Testosterone and estradiol feedback negatively to the hypothalamus and pituitary to suppress gonadotropin secretion (Fig. 2).
Testis synthesizes 15% of total circulating estrogen (Basaria, 2014). There is a high concentration of estrogen in rete testis fluid (Free and Jaffe, 1979) and, in the rat, the concentration of estrogen in the caput epididymis is approximately 25 times the level measured in plasma (Kumari et al., 1980). It is found that the concentrations of estrogen in the testis and rete testis fluid far exceeds the concentration in female serum in various species (Hess, 2000), thus suggesting a major function of estradiol in testicular and epididymal activity. In testicular tissue from adult rats, the concentration of estrogen in interstitial tissue was several times higher than that in the seminiferous tubules (De Jong et al., 1974). However, it is now becoming clear that the level of aromatase activity in germ cells of the adult rodent is equal to or higher than the aromatase activity in Leydig cells (Levallet et al., 1998; Nitta et al., 1993). Although Leydig cells have previously been considered to be the primary source of estrogen in the testis, germ cells must now be considered to have an important role also (Carreau, 2000; Hess et al., 1995). Thus the
source of the high concentration of estrogen in fluid leaving the testis may be due largely to the high concentrations of aromatase in testicular germ cells, particularly in spermatids (Levallet et al., 1998; Janulis et al., 1996a; Janulis et al., 1996b; Tsubota et al., 1997; Kwon et al., 1995).

**Estrogen receptors**

Estrogens modify cell function by binding to high-affinity estrogen receptors (ER). Two subtypes α and β have been identified. ER is a protein belongs to the steroid hormone superfamily of nuclear receptors that functions as a major component in the mechanisms of estrogen action. ER acts as transcription factors after binding to estrogens. Estrogen signaling is selectively stimulated or inhibited depending upon a balance between ERα and ERβ activities in target organs. The human ERα gene is located on chromosome 6 while the ERβ gene is on chromosome 14 (Kong et al., 2003). ERα is highly expressed in the uterus, prostate stroma, ovarian theca cells, Leydig cells in testes, epididymis, breast, and liver (Lane, 2008). ERβ is highly expressed in prostate epithelium, testes, ovarian granulosa cells, bone marrow, and brain (Weiser et al., 2008). ERα and ERβ have different downstream transcriptional activities, resulting in different biological functions. The full-length human ERα protein has 595 amino acids and a molecular size of 66 kDa while the full-length human ERβ protein has 530 amino acids and a molecular size of 54 kDa. Similar to other NRs, ERs have five domains with distinct functions (Fig. 3) (Swedenborg, 2009).

![Diagram of nuclear receptor domains](image)

**Figure 3:** Diagrammatic representation of the domain structure of nuclear receptors. The A/B domain at the NH2 terminus contains the AF-1 site where other transcription factors interact. The C/D domain contains the two-zinc finger structure that binds to DNA, and the C/F domain contains the ligand binding pocket as well as the AF-2 domain that directly contacts co-activator peptides. Adopted from Nilsson et al., 2001.
**ER and aromatase in male reproductive system**

Several studies have demonstrated the presence of ERα, ERβ, and aromatase in the adult testis. The expression and localization of ER subtypes have been demonstrated in the adult testis of mice (Couse et al., 1997; Rosenfeld et al., 1998), rats (Saunders et al., 1998; Fisher et al., 1997; Van Pelt et al., 1999; Pelletier et al., 2000), primates (Fisher et al., 1997; Pelletier et al., 2000; West and Brenner, 1990), and humans (Enmark et al., 1997; Pentikainen et al., 2000; Saunders et al., 2001). These studies showed expression of ERα mainly in the Leydig cells of rats and mice (Fisher et al., 1997; Pelletier et al., 2000; Saunders et al., 2001), however, the localization of ERα in Leydig cells of primates and humans is more controversial. In the adult mouse ERβ protein is expressed in the Leydig cells (Rosenfeld et al., 1998), but this does not seem to be the case in the adult rat (Van Pelt et al., 1999; Pelletier et al., 2000). Leydig cells contain a high level of aromatase in adult rodents (Carreau et al., 1999; Levallet et al., 1998; Nitta et al., 1993; Janulis et al., 1998), which is stimulated by LH and steroids (Genissel et al., 2001). In fact, aromatase activity is higher in the adult than at any other age (Tsai-Morris et al., 1985) and is higher in the adult Leydig cells than in the Sertoli cells (Levallet et al., 1998). The presence of aromatase in the Leydig cells of primates and humans is well established (Carreau et al., 1999). Sertoli cells in primates and humans contain ERβ but not ERα (Saunders et al., 2001). There is now considerable evidence that germ cells also contain both ERs and aromatase. Earlier studies showed the presence of ERβ is present in rat type A spermatogonia (Van Pelt et al., 1999) as well as in intermediate and type B spermatogonia (Saunders et al., 1998), and is also seen in spermatogonia in monkeys and humans (Saunders et al., 2001). ERβ is found in pachytene spermatocytes and round spermatids, but not elongating spermatids in rats (Saunders et al., 1998; Van Pelt et al., 1999) and in primates and humans (Enmark et al., 1997; Pelletier et al., 2000; Saunders et al., 2001). Other studies in rats and humans found ERβ in Sertoli cells but not in germ cells (Pelletier et al., 2000). A further complexity to the testicular localization of ERβ is that one study in mice could not detect ERβ mRNA at all in mouse testis by RNase protection assay (Couse et al., 1997). Therefore, considerable conflict in the literature exists in terms of ERβ localization in the testis. Aromatase mRNA and activity is found in germ cells from the pachytene spermatocyte stage in both rats and mice, and aromatase
remains in the germ cells as they mature into round spermatids (Levallet et al., 1998; Nitta et al., 1993; Janulis et al., 1998). Aromatase localization is observed to move from the Golgi apparatus to the cytoplasm during spermatid development when round spermatids begin the morphological transformation into elongated spermatids, aromatase continues to be found in these cells and is immunolocalized to the flagella of the developing spermatid (Nitta et al., 1993). Aromatase appears to be present in higher levels in mature spermatids of the rat as compared to earlier stages of germ cells (Levallet et al., 1998; Carreau et al., 1999). Aromatase mRNA and activity was higher in the germ cells of the mouse and rat when compared with Leydig cells (Nitta et al., 1993; Janulis et al., 1998; Carreau et al., 1999), suggesting that the germ cells are an important source of estrogen in the testis (Fig. 4).

![Diagram of Aromatase and ERs expression in testicular cells](Adopted from O’Donnell 2001)

When elongated spermatids are released from the epithelium, during the process of spermiation, aromatase remains in the residual body that is subsequently phagocytosed by the Sertoli cell (Nitta et al., 1993; Janulis et al., 1996a). However, not all the cytoplasm is phagocytosed, and aromatase activity remains in the cytoplasmic droplet that is still attached to the flagellum as the sperm make their way through the epididymis (Janulis et
al., 1998; Janulis et al., 1996b). Thus it appears as if mature sperm are able to synthesize their own estrogen, as they traverse the efferent ducts (Hess et al., 1995). The ability to synthesize estrogen gradually decreases as the droplet slowly moves to the end of the tail during epididymal transit until it is finally lost (Janulis et al., 1996b).

The demonstration of aromatase in sperm is important as it suggests that the sperm themselves could control the levels of estrogen present in the luminal fluid, directly modulating functions such as the reabsorption of fluid from the efferent ductules (Hess et al., 1995). A very high level of expression of ERα is seen in the efferent ductules of the rat (Fisher et al., 1997). In fact, it has been found that it is the efferent ductules that possess the highest level of ERα immunostaining, relative to the testis, excurrent ducts, and epididymis (Fisher et al., 1997). In addition, the efferent ductules appear to be the first male reproductive structure to express the ER in fetal development (Cooke et al., 1991), suggesting a role for estrogen in the development of this tissue.

The studies described above suggest that the testis is capable of synthesizing and responding to estrogens throughout all stages of development. The localization of ERα, ERβ, and aromatase demonstrates that estrogen action is likely to be important for Leydig cell, Sertoli cell, and germ cell development and function, as well as in the development and function of the efferent ductules and epididymis. In particular, germ cells are capable of local estrogen synthesis and response, via ERβ, suggesting that paracrine and intracrine actions of estrogens may be important in male germ cell development. The localization of aromatase in sperm in the testis, and as they traverse the efferent ductules and epididymis, together with the demonstration of high levels of ERα and ERβ in the efferent ductules, support the hypothesis that estrogen in sperm acts on ER in the efferent ductules. Even though numerous studies have demonstrated the synthesis of estrogen in significant amount in the testis of several mammals through selective expression of aromatase and ERs, in different testicular cells, but the specific role of estrogen in male reproduction has remained unclear.
Role of Estrogen in male reproduction

It was believed in the beginning that estrogen in male was produced primarily by the accessory sex glands. Based on this, the role of estrogen in male was proposed to influence the female reproductive tract following ejaculation (Willenburg et al., 2003). The testis was suggested to be responsive to estrogen as early as 1935 by Wolf. In early 1990’s, many scientists showed the presence of estrogen receptor in male reproductive tract but suggested only a residual of embryological differentiation (Greco et al., 1993). Subsequent studies showed remarkably high levels of estrogen in the semen (Free and Jaffe, 1979; Setchell et al., 1983; Claus et al., 1992). Estrogen levels within the testes and in semen were found to be higher than circulating level in female (Free and Jaffe, 1979; Claus et al., 1992). Estrogen synthesis in the male reproductive tract was first demonstrated in Sertoli cells during development, but in Leydig cells of adult testes (O'Donnell et al., 2001; Sharpe, 1998). Later study failed to demonstrate the presence of aromatase in rete testis, efferent ductules, epididymis or vas deference. Subsequent studies provided plenty of evidences suggesting that germ cells and sperm also produce estrogen (Nitta et al., 1993; Kwon et al., 1995) and have demonstrated aromatase expression and activity in human sperm (Hess, 2000).

O'Donnell and his colleagues (2001) suggested only minor role of estrogen in adult testicular physiology. Further study showed that treatment of antiestrogen in vitro inhibits Leydig cells activity, but treatment with estrogen alone was unable to stimulate Leydig cells steroidogenesis (Akingbemi et al., 2003). In the developing testis, estrogen was shown to have significant role in Sertoli cell activity and help in establishing Sertoli-germ cell interaction (MacCalman et al., 1997). Estrogen signaling plays significant role in maintaining the male reproductive system. Analysis of ERα and ERβ using knockout mouse models has demonstrated the general roles of estrogen signaling. The transgenic mice have been useful for understanding gene-specific ER functions (Shao et al., 2010). Many experiments with estrogen receptor knockout (ERKO) mice were able to elucidate ERs function in specific pathological conditions (Sun et al., 2006). Surprisingly, both male and female αERKO mice are infertile, whereas fertility differs among βERKO mice according to gender. An appropriate balance between ERα and ERβ is required for
normal development of male reproductive tissues (McPherson et al., 2008). Low fertility in αERKO male mice is due to reduced sperm counts and low sperm quality. In contrast, the βERKO males have been shown to produce a sufficient number of sperm to maintain fertility. These findings imply that ERα is more important than ERβ for reproduction system development and sperm maturation in male mice. The knockout mice for aromatase, ERα and ERβ genes, completely lacking endogenous estradiol were fertile initially, but later on showed severe impairments in spermatogenesis resulting in decreased fertility. However, in the total absence of estrogen synthesis, the aromatase knockout (ArKO) male shows normal spermatogenesis at the beginning of puberty and impairs only with aging (O'Donnell et al., 2001; Robertson et al., 2002). Spermatogenesis is dependent upon estradiol to some extent, as all cells involved in the process of sperm production contain aromatase and express ERs. Presence of an optimum level of estradiol is essential when treating men with testosterone, as estradiol levels below 5 ng dl$^{-1}$ correlate to a decrease in libido. Considering the complexity and taking into account some conflicting data, more research is necessary so that when better understood, estradiol can become clinically useful in treating diminished libido, erectile dysfunction, and perhaps even oligosperma. The ERαKO male showed elevated concentrations of testosterone (Eddy et al., 1996), this is due to the disruption in feedback regulation at the hypothalamus (Akingbemi et al., 2003). This finding thus suggests that ER is not necessarily essential for spermatogenesis; but shown to have an important function in Leydig cells. Estrogen is shown to have a regulatory role in the testis because the absence of ERs caused adverse effects on spermatogenesis and steroidogenesis.

Despite the abundance of published data on the response of the testis and spermatogenesis to either estrogen deprivation or estrogen treatment, the specific roles of estrogen in spermatogenesis remain unknown. The mechanism by which estrogen affects spermatogenesis and steroidogenesis is also incompletely known.

**Estradiol receptor modulator (agonist/antagonist) or aromatase enzyme inhibitors**

Exogenous supplementation of estradiol has been utilized by male reproductive and sexual medicine specialists to treat conditions such as infertility and hypogonadism. The compounds that modulate estradiol levels in these clinical conditions are referred to as
selective estrogen receptor modulators (SERMs) and aromatase inhibitors (AIs). It has previously been reported in some studies that in a certain subset of infertile men, particularly those with hypogonadism, or those who have a low serum testosterone to estradiol ratio, there are some evidences suggesting that SERMs and AIs can reverse the low serum testosterone levels or the testosterone to estradiol imbalance and may improve any associated infertile or subfertile state (Rambhatla et al., 2016). Further studies suggested that the animals treated with estradiol receptor modulator (agonist/antagonist) or aromatase enzyme inhibitors are better model to effectively demonstrate the significance of estradiol for testicular activities in reproductively adult condition.

Both testosterone and estradiol in men may act on hypothalamus and modulate the negative feedback inhibition of LH and FSH from the pituitary gland (Arevalo et al., 2015) as shown in figure 5.

It is primarily the modulation by SERMs and AIs of this mechanism of action of testosterone and estradiol on the hypothalamic-pituitary-testicular axis that forms the clinical basis for trying to increase testosterone production and/or increase spermatogenesis within the testes (Gooren et al., 1984).
Selective Estrogen Receptor Modulators (SERM)

SERMs are compounds that are used to modulate estradiol level in various experimental and clinical conditions. Estrogen receptor agonist or antagonist such as clomiphene and tamoxifen, are most commonly used SERMs (Komm and Mirkin, 2014). Clomiphene is a SERM that exists as two isomers, zucloimiphene and enclomiphene. Clomiphene citrate works as an estrogen receptor antagonist at the level of the pituitary gland and thus stimulates the release of LH and FSH, which consequently regulates various testicular activities.

Tamoxifen citrate is an oral SERM that was approved in 1970s for the treatment of breast cancer. It has tissue specific action and acts as an estrogen receptor blocker in breast tissue and exhibits agonistic properties in the bone and uterus (Komm and Mirkin, 2014). Treatment of tamoxifen causes inhibition of estrogenic effects at the hypothalamus and pituitary gland, which through negative feedback action results in increased release of LH and FSH, which consequently may increase biosynthesis of testosterone. Tamoxifen also alters the effect of estradiol and expression of estradiol dependent gene. In earlier studies the effect of SERMs tamoxifen, toremifine, and raloxifene on the hypothalamic-pituitary axis in men with oligospermia was observed after 3 months of treatment. The result showed statistically significant increase in serum gonadotropins, testosterone, and semen parameters (Tsourdi et al., 2009).

The effect of tamoxifen on male reproductive system, particularly its effects on testicular spermatogenic and steroidogenic activities are not well elucidated and needs further investigation.

Aromatase Inhibitors (AIs)

AIs are drugs that lower estrogen levels by blocking the aromatase enzyme, the enzyme that converts testosterone to estradiol (Tsourdi et al., 2009). AIs are classified as either steroidal or nonsteroidal, or as first, second or third generation. Steroidal inhibitors such as formestane and exemestane inhibit aromatase activity by mimicking the substrate androstenedione. Nonsteroidal enzyme inhibitors such as anastrozole and letrozole inhibit
aromatase enzyme activity by binding with the heme of cytochrome P450 enzyme. First-generation AIs such as aminoglutethimidine are relatively weak and non specific; they can also block other steroidogenic enzymes necessitating adrenal steroid supplementation. Third-generation inhibitors such as letrozole and anastrozole are potent inhibitor of aromatase and do not inhibit related steroidogenic enzymes. They are well tolerated and apart from their effect on estrogen metabolism their use does not appear to be associated with important side effects in postmenopausal women (Bhatnagar, 2007). The aromatase inhibition by anastrozole and letrozole do not suppress plasma estradiol levels completely. In men third-generation AIs will decrease the mean plasma estradiol/testosterone ratio by 77% (Bhatnagar, 2007; Bhatnagar et al., 2001). This finding probably relates to the high plasma concentrations of testosterone, a major precursor for estradiol synthesis in adult men. As aromatase inhibition is dose dependent, it has been suggested that aromatase is less suppressed in the testis compared to the adipose and muscle tissue, explaining the incomplete efficacy of aromatase inhibition in men. Aromatase activity is high in the testes and the molar ratio of testosterone to letrozole is much higher in the testes compared with the adipose tissues and muscles.

AIs were initially used for the treatment of metastatic breast cancer. The use of AIs has recently broadened to include conditions require to decrease serum estradiol or increase serum testosterone level (Schlegel, 2012). Modulation of plasma estradiol level within the male physiological range is associated with strong effects on plasma levels of LH through an effect at the level of pituitary gland (Burnett-Bowie et al., 2009). Lowering estradiol level by administering an aromatase inhibitor is associated with an increase in the levels of LH, FSH and testosterone (Schlegel, 2012; Zumoff et al., 2003). AIs, therefore, have been suggested as a tool to increase testosterone levels in men with low testosterone levels. Due to their mode of action, the use of AIs is limited to men with at least some residual function of the hypothalamo-pituitary-gonadal axis. Therefore, AIs have been tested in aging men suffering from so-called late onset hypogonadism or partial androgen deficiency.

AIs may be an alternative for traditional testosterone substitution in elderly men because these compounds can be administered orally, once daily and may result in physiological testosterone profiles. Additionally, misuse of AIs is unlikely since testosterone levels
may not be stimulated to vastly supraphysiological levels. A small, controlled study demonstrated that anastrozole in a dose of 1 mg daily during 12 week may result in doubling of the mean bioavailable testosterone level in older men (Bharti et al., 2013).

Letrozole prevents aromatase from producing estrogens by competitive, reversible binding to the heme of its cytochrome P450 unit. Action of letrozole is specific and does not affects production of mineralocorticoids or glucocorticoids. The SERMs and AIs drug led scientists to re-evaluate their importance as estrogens or as anti-estrogens on the male reproductive tissues. Many recent studies suggested the use of SERMs or AIs instead of treating directly the exogenous testosterone (Table 1).

**Table1: SERMs and AIs Clinically Used in Men’s Health**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Mechanism of action</th>
<th>Effect on testosterone</th>
<th>Effect on LH</th>
<th>Effect on Estradiol</th>
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<tbody>
<tr>
<td>Clomiphene</td>
<td>E2_RA</td>
<td>↑</td>
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<tr>
<td>Tamoxifen</td>
<td>E2_RA</td>
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<tr>
<td>Anastrazole</td>
<td>A_I</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Letrozole</td>
<td>A_I</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
</tr>
</tbody>
</table>

AI, aromatase inhibitor; E2_RA, estrogen receptor antagonist; LH, luteinizing hormone; SERM, selective estrogen receptor modulator

**Diabetes and Estrogen**

Diabetes mellitus (DM) is one of the most prevalent metabolic disorders characterized by hyperglycemia and insulin resistance. The worldwide cases of DM increased upto 171 millions in year 2000 and expected to attain 366 millions by 2030 (Wild et al., 2004). Sustained hyperglycemia in DM consequently may cause deleterious effect in many organs such as brain (neuropathy), eyes (retinopathy), kidney (nephropathy) and heart (cardiovascular diseases) (Baccetti et al., 2002; Barros et al., 2006) and also found to be a major cause of male impotency and infertility. Several studies from experimental animals and diabetic men showed that sustained hyperglycemia results in deleterious effect on reproductive system. The high blood glucose level possibly lead to increase in oxidative stress and cell apoptosis, which result in structural and functional impairments of reproductive tissues and finally contribute to infertility (Long et al., 2015). Particularly
increasing trends of diabetes have been reported recently in young persons; therefore DM-induced reproductive complication is emerging as a global health challenge (Alberti et al., 2005). Earlier studies clearly demonstrated that DM causes hypogonadism, retrograde ejaculation and impotency resulting in infertility (Jain and Jangir, 2014). DM may cause various abnormal changes in male reproductive functions such as decline in sperm quality, altered spermatogenesis, histomorphological changes in testes, altered glucose metabolism in Sertoli-blood testes barrier, reduced testosterone, ejaculatory dysfunction and reduced libido (Jain and Jangir, 2014). Recent studies also reported increased DNA damage in sperm as a major complication of DM in men whose developing sperm are exposed to supraphysiological levels of glucose and thus oxidative stress. Endocrine disorders, neuropathy and increased oxidative stress are major factors responsible for DM-induced alteration in male reproductive potential.

As, insulin plays a key role in maintaining blood glucose homeostasis, the diabetic subjects found to have insulin resistant condition along with loss of functional pancreatic β-cell mass (Zhuo et al., 2013). Insulin is known to play a pivotal role in facilitation of anabolic processes and a potent regulator of sex steroid hormone synthesis. In diabetic condition the sex steroid level get severely disturbed (Livingstone and Collison, 2002). The contribution of estrogen deficiency in the pathophysiology of DM in women is emerging as a new therapeutic possibility (Mauvais-Jarvis et al., 2013). Previous studies provided sufficient evidences suggesting antidiabetic action of estrogen in both human and in rodent model (Louet et al., 2004; Barros et al., 2006). Decline in estrogen is shown to impair glucose homeostasis and develops insulin resistance. Estrogen via ERα may also have anti-diabetic function due to its role in protection of pancreatic beta-cell apoptosis induced by oxidative stress, thus estrogen prevents DM by increasing production of insulin (LeMay et al., 2006). The other anti-diabetic action of estrogen seems to involve 1) the stimulation of fatty acid oxidation and prevention of lipid accumulation in liver and 2) the improvement of pancreatic β-cell function and survival, in various conditions of oxidative injury (Louet et al., 2004). Estrogens are important modulator of metabolic syndrome including glucose homeostasis (Gupte et al., 2015). Diabetes risk was reduced by 62% in women used estrogen as hormone replacement therapy (HRT) as compared with individuals never used HRT (Pentti et al., 2009). HRT
also improved glucose control in women with pre-existing diabetes. These and other evidences suggest that estrogen has remarkable potential to treat diabetes and its associated complications. Estrogen is also found to play an important role in regeneration of pancreatic β-cell mass (Godsland, 2005). That’s why estrogen is widely used in treating diabetes in post menopausal women (Cederroth and Nef, 2009). But as, long term treatment of estrogen might cause harmful effects like cancer due to its proliferative action, use of plant products mimicking estrogen action is preferred and require further investigation.

**Diabetes and Phytoestrogen**

Interest in the physiological role of bioactive compounds present in plants has increase dramatically over the last two decades. Of particular interest in relation to human health are the classes of compound known as phytoestrogens. The phytoestrogens constitute a group of plant-derived estrogenic compounds which include mainly isoflavones, lignans, coumestanes, stilbenes, flavonoids and quercetin. These estrogenic compounds interact with the nuclear ERs isoforms α and β and exhibiting either agonistic or antagonistic effects. The phytoestrogens have been shown to be cell/ tissue specific and also known as “Natural Selective Estrogen Receptor Modulator”. Recently, some newly discovered novel estrogen-like compounds are being identified suggesting that our knowledge about the phytoestrogen in nature is expanding. Currently, phytoestrogen, are widely used for the treatment of diabetes because of its hypoglycemic and anti-oxidative properties. It acts as a weak estrogen and competes with 17β-estradiol to bind with the intranuclear estrogen receptor protein to modulate gene transcription (Markiewicz et al., 1993). Phytoestrogens can exert their biological effects via non-estrogen receptor-mediated mechanism based on inhibiting the activity of several enzymes (Linassier et al., 1990) and are known to have potent antioxidative activity (Vedavanam et al., 1999). Furthermore, evidence has also emerged that dietary phytoestrogens play a beneficial role in diabetes. Phytoestrogens mainly belong to a large group of substituted natural phenolic compounds: the prenylflavonoids, coumestans and isoflavones are three of the most active in estrogenic effects in this class. The best-researched phytoestrogens are
isoflavones, having similar structure to the mammalian estrogen 17β-estradiol (Ososki and Kennelly, 2003) and which are commonly found in soy and red clover. 

The major bioactive isoflavones are genistein and daidzein (Bhathena and Velasquez, 2002). The plant-derived isoflavone genistein is one of the most commonly used phytoestrogen. Genistein (4,5,7-trihydroxyisoflavone), the most abundant isoflavone in soybean is also present in other plants which represent excellent source of phytoestrogens such as lupine, fava bean, Kudzu and psoralea (Kaufman et al., 1997). Genistein reduces the low-grade inflammation and reactive oxygen species production and therefore may improve the insulin resistance (Behloul and Wu, 2013). Numerous studies have demonstrated that genistein has direct effects on β-cell proliferation, glucose stimulated insulin secretion and protection against apoptosis, independent of its functions as an estrogen receptor agonist, antioxidant, or tyrosine kinase inhibitor (Zhuo et al., 2013). Genistein reduces insulin resistance index in ovariectomized rats by altering lipid metabolism (Choi et al., 2012). These studies suggest that genistein may be used as an anti-inflammatory, antioxidant and hypoglycemic agent in diabetic animals (Park et al., 2006; Chang et al., 2004). Thus genistein may be used as a promising therapeutic agent for ameliorating diabetes induced reproductive dysfunction.

Based on current knowledge about the significance of estrogen in male reproductive processes, this dissertation aims to investigate the specific roles and mode of action of estradiol particularly during aging and spermatogenically active conditions. An attempt is also made to determine the efficacy of estradiol in diabetes associated testicular dysfunction. To achieve the above mentioned aim, the present thesis is divided into four chapters: Chapter I describes the role of estrogen in testicular activities of mice during aging; Chapter II describes the effect of Letrozole, a selective non-steroidal associated decline in estradiol, on various testicular activities in reproductively active mice; Chapter III describes the pathway and mechanism by which a selective selective estrogen receptor modulator, tamoxifen affects testicular function using both in vivo and in vitro studies of reproductively active mice; and chapter IV describes effects of short and long term treatment of estrogen and phytoestrogen on testicular activities of streptozotocin-induced type II diabetic mice.