Preface and Consolidated abstract

Estrogen is a sex hormone with profound effects on reproductive function of both male and female. In males, estrogen is present in low concentrations in blood, but can be found in high concentration in semen, and in rete testis fluids, which may be higher than serum estradiol level in the female. The importance of estrogen in male reproduction was greatly enhanced based on earlier studies clearly demonstrated that male fertility is impaired in mice lacking estrogen receptor alpha (ERα) or aromatase, together with discovery of a second estrogen receptor beta (ERβ), which was expressed mainly in the male reproductive tract. Studies have further demonstrated that targeted deletion of the aromatase, ERα, and/or ERβ gene caused a variety of testicular anomalies including infertility in mutant mice. However, clearly more studies are required to resolve the confusion persisting regarding the potential effects of estrogen on testicular activity and its mechanism of action. The role of estrogen in male reproduction has been mostly studied during reproductively active condition; however its role during different stages of aging has not yet been studied. Another promising but as yet poorly explored aspect requires detailed investigation includes “anti-diabetic effects of estradiol on male reproductive functions”. Therefore, this dissertation aims to elucidate the role played by estrogen in regulation of testicular activities during aging, spermatogenically active and diabetic conditions in male mice.

The present thesis is divided into four chapters. Chapter I describes the significance of estrogen in testicular activity of mice during aging. An attempt is also made to find out whether nitric oxide mediates the effects of estrogen on testis. Chapter II describes the effect of Letrozole, a selective non-steroidal aromatase inhibitor, associated decline in estradiol on various testicular activities in reproductively active mice. Additional aim of this study was to evaluate, whether estradiol associated suppression in testicular activities is mediated through changes in insulin sensitivity. Chapter III describes the pathway and mechanism by which tamoxifen, selective estrogen receptor modulator (SERM), affects testicular functions, using both in vivo and in vitro studies, of reproductively active mice and Chapter IV describe effects of short- and long-term treatment of 17β-estradiol and phytoestrogen on testicular activities of steptozotocin-treated and high fat
fed type II diabetes-induced mice. This study was undertaken to find out whether treatment with phytoestrogen (genistein) can be as effective as treatment with estrogen in ameliorating reproductive and metabolic abnormalities of diabetes-induced male mice.
Chapter 1

Alteration in expression of estrogen receptor isoforms alpha and beta, and aromatase in the testis and its relation with changes in nitric oxide during aging in mice

The aim of present study was to investigate the changes in the testicular expression of aromatase, estrogen receptor alpha (ERα), estrogen receptor beta (ERβ) and iNOS protein and correlate these with serum testosterone and nitric oxide levels, to elucidate the role of estrogen and nitric oxide in the testis during aging. This study showed localization of aromatase and ERα mainly in the Leydig cell and showed close correlation of testicular aromatase level with circulating testosterone level suggesting that estrogen may be modulating testicular steroidogenesis. Localization of ERα mainly in the mitotically active germ cell suggest possible role of estrogen in germ cell proliferation. This study showed basal level of nitric oxide during reproductively active period, whereas increased serum nitric oxide coincides with decreased testicular activity in old age. This study showed inverse correlation between aromatase and nitric oxide (NO) level. Treatment with either SNP or L-NAME on testicular steroidogenic factor (3β-HSD/ StAR) or germ cell survival factor (Bcl2) showed that increased NO causes decreased steroidogenesis and increased germ cell apoptosis. In conclusion this study suggest that estrogen modulate steroidogenesis and germ cell survival in reproductively active period whereas in old age decreased estrogen concentration causes increased nitric oxide which in turn decreases testicular steroidogenesis and germ cell apoptosis.
Chapter 2

Effect of Letrozole, a selective aromatase inhibitor, on testicular activities in adult mice: Both in vivo and in vitro study

The aim of present study was to evaluate the significance of estradiol (E2) in testicular activities and to find out the mechanism by which E2 regulates spermatogenesis in mice. To achieve this, both in vivo and in vitro effect of Letrozole on testis of adult mice was investigated. Letrozole-induced changes in testicular histology, cell proliferation (proliferating cell nuclear antigen; PCNA), cell survival (B cell lymphoma factor-2; Bcl2), apoptotic (cysteine-aspartic proteases; caspase-3), steroidogenic (side chain cleavage; SCC, 3β-hydroxy steroid dehydrogenase enzyme; 3β-HSD, steroidogenic acute regulatory protein; StAR, aromatase and luteinizing hormone receptor; LH-R) markers, glucose level, and rate of expression of glucose transporter (GLUT) 8 and insulin receptor (IR) proteins in the testis along with changes in serum E2 and testosterone (T) levels were evaluated. Letrozole acts on testis and caused significant decrease in E2 synthesis, but increase in testosterone level and showed regressive changes in the spermatogenesis. Letrozole-induced changes in various testicular markers were compared with the changes in serum E2 level. The correlation study showed that decreased circulating E2 level may be responsible for decreased insulin receptor (IR) level in the testis. The decreased effects of insulin inhibited the glucose transport in the testis by suppressing GLUT8. The decreased level of testicular glucose may produce less lactate as energy support to developing germ cells consequently resulting in decreased cell proliferation and cell survival, but increased apoptosis. Thus, Letrozole suppresses spermatogenesis by reducing insulin sensitivity and glucose transport in the testis, but significantly increased testosterone level by promoting gonadotrophin release by decreased E2.
Chapter 3

Effect of tamoxifen on spermatogenesis and testicular steroidogenesis

The aim of this study was to evaluate the effects of in vivo and in vitro treatments with selective estrogen receptor modulator (SERM), tamoxifen on testicular functions. The testis treated with tamoxifen, in vivo or in vitro, showed dose-dependent regressive changes in spermatogenesis. This study showed that the decreased estrogenic effect due to tamoxifen may be directly responsible for decreased testicular expression of aromatase, which in turn may be responsible for decreased synthesis of estradiol in the testis. The decreased endogenous estradiol through cAMP-CREB signaling mechanism may decline germ cells proliferation (PCNA) and survival (Bcl2). The tamoxifen-induced decreased estrogenic effect may also be responsible for increased expression of testicular NOS and consequently increased production of NO, which may cause increased germ cells apoptosis (Caspase-3) and impaired spermatogenesis. Both in vivo and in vitro studies showed the inhibitory effect on testicular steroidogenic factors. Thus, tamoxifen inhibits testicular spermatogenesis and steroidogenesis either directly acting on testis or indirectly through gonadotropin release.
Chapter -4

Comparative effects of estrogen and phytoestrogen on testicular activities of streptozotocin-induced type II diabetic mice

The aim of this study was to compare the effect of synthetic estrogen with a phytoestrogen, genistein in ameliorating Type II Diabetes mellitus (T2D)-mediated testicular dysfunction in mice. The T2D model was developed by treatment of streptozotocin (STZ) to mice fed with high fat diet. The STZ-induced T2D-mice were treated exogenously with either synthetic estradiol or genistein (phytoestrogen) for either short- (14 days) or long- (28 days) term and compared their effects on the steroidogenesis, spermatogenesis and metabolic changes in glucose together with changes in insulin secretion and sensitivity in the testes. The diabetic mice showed a marked regression in testicular histology due to significant decline in circulating estrogen and testosterone levels. The diabetic mice also showed hyperglycemia and reduced insulin sensitivity together with increased oxidative stress; these changes may be responsible for testicular impairments by suppressing germ cell proliferation and survival but increased rate of apoptosis. The short term (14 days) treatment of synthetic estradiol improved testicular dysfunction by stimulating testosterone synthesis and by improving insulin sensitivity within the testis of type 2 diabetic mice, whereas long term (28 days) treatment of synthetic estrogen failed to improve T2D mediated testicular dysfunction. The treatment of genistein for both short (14 days) and long (28 days) term was beneficial in improving type 2 diabetes mediated testicular dysfunction in mice. Genistein treatment reduces glucose level by increasing insulin sensitivity, which in turn increases production of antioxidants and lactate, these changes finally contribute to decreased germ cell loss and therefore, improves steroidogenesis and spermatogenesis in Type 2 diabetic mice.

Supervisor
(Prof. Amitabh Krishna)

Student
(Rachna Verma)