INTRODUCTION

Spermatogenesis is a paradigm of development that continues throughout adult life in most mammals. This process occurs in seminiferous tubules containing an epithelium populated by a mixture of germ cells and sertoli cells, surrounded by a thin wall of peritubular cells. Sertoli cells serve a crucial nurturing role for germ cells and are believed to help coordinate important events of spermatogenesis (Griswold, 1998). Sertoli cells also divide the seminiferous epithelium into two compartments: a basal compartment where cells are exposed to the surrounding milieu and a luminal compartment where cells are sequestered behind a "blood-testis" barrier formed by junctional complexes between sertoli cells (Eddy, 2002).

Germ cell development occurs in successive mitotic, meiotic, and post-meiotic phases, with the germ cells moving from the periphery to the lumen of the seminiferous tubule during this process. The mitotic phase occurs in the basal compartment, while the meiotic and post-meiotic phases occur in the luminal compartment (Eddy, 2002).

For normal fertility, a man requires normal spermatogenesis, successful epididymal storage and normal sperm transport. Partial or complete disruption in any of these processes results in spermatogenic arrest and decline in sperm counts leading to infertility (Dada et al., 2001).

Infertility is a major health problem that affects ~10-15% of couples; ~40% of infertility cases are attributed to the male partner (Dunson et al., 2004). Causes of male infertility can also be divided into four general categories: defective sperm production, obstruction or physical blockage of the reproductive tract, inflammation or immunological dysfunction or sexual disorders such as impotence (Namiki, 2000).

Spermatogenic failure, including azoospermia and oligospermia, is a major cause of male infertility (Shinka et al., 2004). Other factors include, genetic factors (Krausz and Giachini, 2007), environmental and occupational exposure (Mendiola et al., 2008) and physiological factors (Ingman and Robertson, 2007).

It is proposed that oxidative stress precipitates the range of pathologies that currently are thought to afflict the reproductive function (Sharma and Agarwal, 1996). Recent reports have indicated that high levels of ROS are detected in semen samples of 25% to 40% of infertile men (Padron et al., 1997). The generation of ROS has
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become a real concern because of their potential toxic effects at high levels on sperm quality and function (Sikka, 1996). Spermatozoa are particularly susceptible to oxidative stress-induced damage because their plasma membranes contain large quantities of polyunsaturated fatty acids (PUFA) (Alvarez and Storey, 1995) and their cytoplasm contains low concentrations of scavenging enzymes (de Lamirande and Gagnon, 1995). Oxidative stress-mediated damage to the sperm plasma membrane may account for defective sperm function observed in a high proportion of infertility patients (Iwasaki and Gagnon, 1992; Aitken, 1994). Oxidative stress attacks not only the fluidity of the sperm plasma membrane but also the integrity of DNA in the sperm nucleus (Aitken, 1999). Oxidative stress-induced DNA damage may accelerate the process of germ cell apoptosis leading to the decline in sperm counts associated with male infertility and the apparent deterioration of semen quality observed over the past 4 to 5 decades.

The seminal plasma is well endowed with an array of antioxidant defense mechanisms to protect spermatozoa against oxidative stress (Marti et al., 2007). These mechanisms compensate for the deficiency in cytoplasmic enzymes in sperm (Donnelly et al., 1999). Seminal plasma contains a number of enzymatic antioxidants such as superoxide dismutase, SOD (Alvarez et al., 1987), the glutathione peroxidase/glutathione reductase (GPX/GRD) system (Chaudiere et al., 1984), and catalase (Jeulin et al., 1989). In addition, seminal plasma contains a variety of non-enzymatic antioxidants such as ascorbate (Fraga et al., 1991), urate (Thiele et al., 1995), a-tocopherol (Aitken and Clarkson, 1988; Moilanen et al., 1993), pyruvate (de Lamirande and Gagnon, 1992), glutathione (Lenzi et al., 1994), taurine and hypotaurine (Alvarez and Storey, 1983).

Selenium (Se) is a dietary essential trace element. The biological roles ascribed to selenium include the prevention of cancer (Combs and Lu, 2001), cardiovascular disease (Rayman, 2002; Beckett et al., 2004) and viral mutation (Beck, 2001). In addition, the trace element is essential for optimal endocrine and immune function and moderating the inflammatory response (McKenzie et al., 2002; Arthur et al., 2003).

The reproductive organ appears to be a priority tissue for selenium accumulation (Behne et al., 1988) and nutritional studies indicate that selenium is essential for male fertility (Wu et al., 1973; Behne et al., 1982). The selenium content of male gonads increases during pubertal maturation (Behne et al., 1986) and its main
target cells are spermatozoa, which show highest retention of selenium (Calvin et al., 1981). Selenium deficiency gives rise to testicular structural and functional disturbances (Behne et al., 1996). Both low and high concentration of selenium in the seminal plasma is harmful to male fertility (Spallholz, 1994). Excess selenium supplementation is toxic and is associated with increased abnormalities in the mid-piece region of the rat spermatozoa (Kaur and Parshad, 1994).

These biological actions of selenium are mediated in most cases through the expression of at least 30 selenoproteins coded by 25 selenoprotein genes in humans (Kryukov et al., 2003). Most of the selenoproteins have reactive oxygen species (ROS) scavenging activities, so that the action of selenium has been known as an antioxidation system in cell survival. In these aspects, selenium is recommended for nutritional additive in sustaining a long healthy life (Kim et al., 2004).

Phospholipid hydroperoxide glutathione peroxidase (PHGPx or GSH-Px4) is selenium-dependent glutathione peroxidase. It is reported that the relative PHGPx mRNA levels are much higher in the testis than in the other tissues (Mizuno et al., 2000) and it accounts for almost the entire selenium content of mammalian testis. Male infertility in selenium-deficient animals, which is characterized by impaired sperm motility and morphological midpiece alterations, is considered to result from insufficient PHGPx content (Foresta et al., 2002).

Recently another selenoenzyme, sperm nucleus glutathione peroxidase (snGSH-Px) which has properties similar to GSH-Px4 has been identified. It is present in rat spermatids where it forms cross-linked disulfides and aids in the process of chromatin condensation which is crucial in order to protect sperm DNA against oxidation (Pfeifer et al., 2001). The major selenoenzyme, which acts as peroxidase, is cellular cGSH-Px (GSH-Px1). Further it has been shown that it is GSH-Px1 mRNA, which is readily affected by selenium status while GSH-Px4 and other selenoproteins are less regulated by it (Lei et al., 1995; Weiss et al., 1997; Sachdev and Sunde, 2001). This suggests that besides the above mentioned facts, there might be other possible mechanisms by which selenium regulates spermatogenesis.

Selenium has been linked to regulatory functions in cell growth, survival, cytotoxicity and transformation (Medina et al., 1983; Medina and Oborn, 1984; Shamberger, 1985; Bansal et al., 1990). Selenium appears to modulate such cellular activities presumably by acting on proteins important for signal transduction (Handel et al., 1995; Spyrou et al., 1995; Kim and Stadtman, 1997). On the other hand, the
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cytotoxic activity of selenite is suggested to be associated with the oxidative stress (Shen et al., 2001).

The transcription factors activator protein 1 (AP-1) and nuclear factor κB (NFkB) are sensitive to oxidants, antioxidants and conditions which affect the intracellular redox state (Ginn-Pease and Whisler, 1998; Li and Karin, 1999). Recent studies have also shown that both endogenously produced and exogenously added ROS can regulate the activity of kinases that regulate the activation of these transcription factors; the mitogen-activated protein kinases (MAPks) for AP-1 and the kinases for NFkB, that may be involved in the cellular responses including proliferation, differentiation and apoptosis (Sano et al., 2001; Jang et al., 2002). MAPks include three major kinases; extracellular signal-regulated kinase (ERK), p38 kinase and c-Jun N-terminal kinase (JNK), and the kinases for NFkB include the NFkB inducing kinase (NIK) and the Inhibitory κB Kinase (IKK).

MAPks are a family of conserved protein kinases that phosphorylate specific serine and threonine residues of target protein substrates and regulate a number of cellular activities (Kuida and Boucher, 2004).

ERK is generally activated by mitogenic and proliferative stimuli such as growth factors and involved in cellular proliferation and differentiation. JNK and p38 kinase are mainly activated by intracellular stresses, such as UV irradiation, inflammatory cytokines, heat and arsenic trioxide (Kyriakis and Avruch, 2001; Chung et al., 2003; Kang et al., 2003). JNK is believed to be regarded as signaling pathways for apoptosis, transformation, development, immune activation, inflammation and adaptation to environmental changes (Davis, 2000). JNK binds to and phosphorylates the DNA binding protein c-Jun and increases its transcriptional activity. c-Jun is a component of the AP-1 transcription complex, which is an important regulator of gene expression. AP-1 contributes to the control of many cytokine genes and is activated in response to environmental stress, radiation, and growth factors-all stimuli that activate JNKs (Johnson and Lapadat, 2002).

The p38 and JNK pathways are collectively termed SAPKs because of their role in the way cells meter their response to cytotoxic stimuli such as UV irradiation, proinflammatory cytokines, and osmotic shock (Karin M, 1998). Many p38-dependent effects may be mediated through modulation of Activator protein-1 (AP-1), a transcription factor composed of Jun:Jun homodimers or Fos:Jun heterodimers (Karin et al., 1997).
MAPKs are implicated in regulating cell division processes. Recent evidence suggests a role for MAPKs in chromatin condensation during the first meiotic division of male germ cells (Sette et al., 1999; Agostino et al., 2002). The MAPKs and their activating kinases, the MEKs are expressed in all pre-meiotic germ cells and spermatocytes (Inselman and Handel, 2004). ERK contributes to the mitotic proliferation of primary spermatogonia and later to the acquisition of sperm motility (Lu et al., 1999).

The transcription factor NFκB participates in the regulation of pro-inflammatory and immune cellular responses, the regulation of cell proliferation, and apoptosis (Collins and Cybulsky, 2001; Yamamoto and Gaynor, 2001). There is substantial evidence for redox regulation of this stress-activated transcription factor by two of its upstream kinases; the Inhibitory kappaB kinase alpha (IKKα) and NFκB inducing kinase (NIK). NIK is an upstream kinase that phosphorylates IKK, and there is evidence that NIK is the actual target of redox regulation (Zhang et al., 2001; Li and Engelhardt, 2006). NFκB expression has been found to be regulated during mammalian spermatogenesis (Delfino and Walker, 1998). Its expression is stage specifically controlled which suggests that it plays an important role during the development of germ cells (Lilienbaum et al., 2000). NFκB is an interesting candidate to study spermatogenesis since there is growing evidence that this transcription factor is involved in cell proliferation and apoptosis (Barkett and Gilmore, 1999; Pahl, 1999).

Since, the progression of spermatogenesis is regulated by a highly orchestrated pattern of expression of genes that control cell division, cell interactions, and morphogenetic changes in both the somatic and germinal cell lineages. Understanding how the expression of these genes is modulated at the level of transcription is of interest at many levels, from identifying the unique regulatory mechanisms characteristic of this specialized system, to providing insight into the origin of infertility or tumorigenesis, to suggesting novel approaches to contraception. This further requires understanding of the transcription factors involved in the entire process and of the various kinases that regulate their activation. Studies on transcription factors AP-1 and NF-κB have been undertaken previously (Shalini and Bansal, 2006; 2007) under selenium status conditions. Thus, seeing the role of selenium action in multiple ways, present study is aimed to investigate the effect of different selenium status on testicular development and spermatogenesis by
evaluating the changes in the expression of redox active enzymes, and related MAPKs. These studies are hoped to help in elucidating the role of selenium in regulating spermatogenesis at molecular level. The MAPKs modulation by selenium may be relevant to gene expression and drug development, as the importance of MAPKs in controlling cellular responses to the environment and in regulating gene expression, cell growth, and apoptosis has made them a priority for the research related to many human diseases