

Oxidative Stress: A Common Factor in Testicular Dysfunction

Review

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ABSTRACT: Oxidative stress results from the production of oxygen radicals in excess of the antioxidant capacity of the stressed tissue. Many conditions or events associated with male infertility are inducers of oxidative stress. X-irradiation, for example, or exposure to environmental toxicants and the physical conditions of varicocele and cryptorchidism have been demonstrated to increase testicular oxidative stress, which leads to an increase in germ cell apoptosis and subsequent hypospermatogenesis. Such stress conditions can

cause changes in the dynamics of testicular microvascular blood flow, endocrine signaling, and germ cell apoptosis. Testicular oxidative stress appears to be a common feature in much of what underlies male infertility, which suggests that there may be benefits to developing better antioxidant therapies for relevant cases of hypospermatogenesis.

Key words: Oxidative stress, testis, male infertility, apoptosis.
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Oxidative stress is produced by the peroxidation and oxidation of many cell lipids, proteins, carbohydrates, and nucleic acids. The detailed chemistry of oxygen radical generation and the countervailing effect of oxygen radical scavengers have been covered by a number of recent reviews (Pryor et al, 2006; Szabo et al, 2007). The present discussion presents that information briefly but focuses on the conditions that generate oxidative stress in the testis and the effects that stress has on testicular function. It should be mentioned that oxidative stress in semen is commonly studied (Aitken and Clarkson, 1987; Agarwal et al, 2005; Bennetts and Aitken, 2005) but is a different phenomenon than testicular oxidative stress.

Oxygen Radicals and Antioxidants—Oxidative stress in any tissue results from an imbalance between the production of reactive oxygen species (ROS) and their efficient removal by available antioxidant systems. ROS are small, oxygen-based molecules that are highly reactive because of unpaired electrons (Papa and Skulachev, 1997). The most prominent ROS are the superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and the hydroxyl ion (OH^{\cdot}).

ROS can be produced in large amounts by macrophages and neutrophils, but also by spermatozoa

(Aitken and Clarkson, 1987) and other cell types under pathologic conditions. Superoxide anions are largely generated as a result of redox reactions within the mitochondria, but in most situations superoxide is quickly converted to hydrogen peroxide by the enzyme superoxide dismutase (SOD; Mates and Sanchez-Jimenez, 1999; Cadenas, 2004; Table 1). Hydrogen peroxide can undergo reactions with heavy metals like Fe^{++} or Cu^{++} to form ferric or cupric ions and hydroxyl ions or can be detoxified through the glutathione/glutathione peroxidase (GPX) pathway to yield water and reduced glutathione (Table 1). Hydrogen peroxide can also be reduced by catalase to produce oxygen and water (Mates and Sanchez-Jimenez, 1999; Pryor et al, 2006; Table 1). Hydroxyl ions not only are produced from hydrogen peroxide, but also can be generated in other reactions, including the reaction of ionizing radiation with water (Cotran et al, 1994). Hydroxyl ions have nanosecond half-lives, but are damaging inside the cell because they can cause the covalent cross-linking of a variety of biological molecules as well as the propagation of other free radicals through more complex reactions.

Any oxidizing radical is a potential agent of oxidative stress. Some are highly reactive with short half-lives, such as hydroxyl radicals, whereas others are less reactive but with longer half-lives, such as hydrogen peroxide (not a free radical, but an ROS, nonetheless). A consequence of a longer half-life is the potential for a greater diffusion distance, which can allow the reactive species to do damage more remotely from its source. Oxidative damage can occur to many classes of molecules, including lipids,

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Table 1. Common reactions in the production and scavenging of reactive oxygen species

Formation of superoxide ion and hydrogen peroxide	
Xanthine	$\xrightarrow{\text{Xanthine oxidase}}$ Hypoxanthine $\xrightarrow{\text{Xanthine oxidase}}$ Uric acid + $\text{O}_2^{\cdot-}$ + H_2O_2
Formation of hydroxyl ions via Fenton reactions	
Fe^{2+}	+ $\text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+}$ + $\text{OH}^{\cdot-}$ + OH^-
Fe^{3+}	+ $\text{H}_2\text{O}_2 \rightarrow \text{Fe}^{2+}$ + $\text{OOH}^{\cdot-}$ + H^+
Formation of peroxynitrite and nitrogen dioxide	
NO	+ $\text{O}_2^{\cdot-} \rightarrow \text{ONOO}^-$
ONOO^-	+ $\text{CO}_2 \rightarrow \text{NO}_2^{\cdot-}$ + $\text{CO}_3^{\cdot-}$
Common oxygen radical scavengers in mammalian systems	
$2\text{O}_2^{\cdot-}$	+ 2H^+ $\xrightarrow{\text{Superoxide dismutase}}$ H_2O_2 + O_2
$2\text{H}_2\text{O}_2$	$\xrightarrow{\text{Catalase}}$ $2\text{H}_2\text{O}$ + O_2
H_2O_2	+ $2\text{GSH} \xrightarrow{\text{Glutathione peroxidase}}$ GSSH + $2\text{H}_2\text{O}$

proteins, nucleic acids, and sugars. This means that cell, nuclear, and mitochondrial membranes, structural and cytoplasmic proteins, complex carbohydrates, RNA, and DNA are all potential victims of oxidative stress (Pryor et al, 2006). In a tissue like the testis, with its high rates of metabolism and cell replication, oxidative stress can be especially damaging, which makes the antioxidant capacity of the tissue very important.

The major antioxidant enzymes in mammals are SOD, catalase, and GPX (Table 1), the latter necessitating a number of other enzymes, such as glutathione reductase, glutathione-S-transferase, and γ -glutamyl transpeptidase, required for the recycling or elimination of glutathione. All of these antioxidant enzymes are expressed in the testis (Zini and Schlegel, 1996, 1997; Maiorino et al, 2003; Ischi et al, 2005). SOD exists in cytosolic, mitochondrial, and extracellular forms, all of which catalyze the dismutation of superoxide anion by successive oxidation-reduction of the transition metal at the enzyme's active site (Hsieh et al, 1998). Catalase exists in only 1 form and is a highly efficient, intracellular enzyme converting hydrogen peroxide to hydrogen and water. GPX exists in 5 different forms, with the predominant form depending on the tissue. GPX IV, also known as phospholipid hydroperoxide GPX, is the predominant form in the mouse testis, whereas GPXs III and V are predominant in the mouse epididymis (Pons et al, 2003; see also the Mammalian Reproductive Genetics data base, <http://mrg.genetics.washington.edu>, from Johnston et al, 2005). In whatever form, this glutamate-based system is a major defense against oxidative stress (Roveri et al, 1992; Pons et al, 2003).

A number of nonenzyme factors also function as antioxidants in the testis. Among them, vitamin C, vitamin E, resveratrol (a botanical antioxidant), and melatonin have each proven efficacious in reducing

testicular oxidative stress under different circumstances (Narra et al, 1993; Gavazza and Catala, 2003; Uguralp et al, 2005; Kutlubay et al, 2007). Lipocalins like prostaglandin D₂ synthase may also have a protective function, because in some systems they sequester lipid peroxidation products and reduce oxidative damage (Lechner et al, 2001).

Nitrosylated Oxygen Radicals—NO, a potent vasodilator and cell-signaling molecule, can play its own role in amplifying testicular injury, but through interaction with superoxide radicals it forms peroxynitrite (ONOO⁻), another potent oxidizing agent (Pryor et al, 2006; Szabo et al, 2007; Table 1). NO can also react with CO₂ to form nitrogen dioxide ($\cdot\text{NO}_2$), a radical of less activity than peroxynitrite but of longer diffusion distance (Pryor et al, 2006). Peroxynitrite can modify proteins with thiol groups to generate nitrosothiols, which can disrupt metal-protein interactions and lead to the generation of other metal-derived free radicals (Pryor et al, 2006; Szabo et al, 2007).

NO is synthesized by nitric oxide synthase (NOS), which exists in 3 known forms: endothelial NOS (eNOS), inducible NOS (iNOS) and neuronal NOS. The latter appears to exist only in a truncated form in the testis (Wang et al, 1997) and is likely inactive there. NOS and/or NO have been found to be up-regulated in a number of experimental conditions known to induce testicular oxidative stress, such as cryptorchidism (Ishikawa et al, 2005), testicular torsion (Shiraishi et al, 2001), obstructive azoospermia (Basar et al, 2006), and varicocele (Shiraishi and Naito, 2007).

The rate of peroxynitrite formation from NO in the stressed testis is unknown, but it has a relatively short half-life. The molecule is highly diffusible, however, and has been reported to have oxidative effects as far as 2 cell diameters from its point of origin (Szabo et al, 2007). The effects of peroxynitrite, as with other superoxides, are deleterious to many cell processes (Table 2).

Conditions That Induce Testicular Oxidative Stress

Testicular oxidative stress plays a role in a number of conditions known to be detrimental to male fertility. These conditions vary from toxicant exposure to aging and from varicocele to testicular torsion. The involvement of oxidative stress in these and other pathologic conditions is briefly summarized below.

Toxicant Exposure—Multiple studies have shown that environmental toxicants can cause oxidative stress in the testis with resulting disturbance in spermatogenesis. This review cannot cover all these compounds, but several examples will serve. Rats exposed to the pesticide hexachlorocyclohexane, for example, exhibit a

Table 2. *Effects of peroxynitrite and other oxygen radicals on various cell functions (Szabo, 2007)*

Action	Consequence
Inhibition of antioxidant enzymes	Diminished ability of the cell to protect itself against oxidant and free radical damage
Inhibition of various other cytosolic enzymes	General cell function impairment
Inhibition of membrane channels	Impairment of cellular ionic balance and cellular calcium handling
Intracellular calcium imbalance	Cell energy depletion, enzyme dysfunction, and promotion of cell death
Aggregation of proteins	Lewy body ^a formation
Impairment of enzyme cofactors	Impairment of BH4 ^b -mediated enzymes and inhibition of NAD-dependent enzymes
Depletion of antioxidants	Diminished ability of the cell to protect against oxidant damage
Dysregulation of cell adhesion molecules	Enhanced inflammatory responses
Lipid peroxidation and nitration	Oxidation and nitration of fatty acids, membrane destabilization, and thiol modification of proteins
Mitochondrial dysfunction	Release of mitochondrial death factors and generation of mitochondrial oxidants and free radicals
DNA damage	Genotoxic damage and cell death

^a Spherical bodies in nerve cells displacing other cell components.

^b Tetrahydrobiopterin, an enzyme cofactor.

significant increase in testicular oxidative stress leading to an increase in damaged and apoptotic germ cells (Samanta and Chainy, 1997). Other industrial pollutants such as 1,3-dinitrobenzene (Jacobson and Miller, 1998) or nonylphenol (Han et al, 2004; McClusky et al, 2007) have the same effect. Methoxyethanol, a glycol ether used in paints, brake fluids, and other industrial products, along with its primary metabolite, methoxyacetic acid, also causes an increase in oxidative stress (Syed and Hecht, 1998) with subsequent testicular atrophy (Hardin, 1983). Other industrial toxicants such as 2,4,6-trinitrotoluene from explosives manufacturing and sulfur dioxide from the burning of petroleum products and coal also have a pro-oxidant effect in the testis (Homma-Takeda et al, 2002; Ming and Bai, 2004). 2,5-hexanedione, another organic toxicant known to induce germ cell apoptosis (GCA; Allard and Boekelheide, 1996) is an example of a compound that may produce its effect via oxidative stress, but other routes to apoptosis are possible. Aitken et al (2004) have reviewed the effects of xenobiotics, generally, on male reproduction.

Exposure to high concentrations of certain metals has also been shown to cause oxidative stress. For example, high iron doses increase oxidative damage and deplete antioxidants in the testes of rats (Lucesoli and Fraga, 1995; Wellejus et al, 2000). Cadmium also increases testicular oxidative stress (Koizumi and Li, 1992; Oteiza et al, 1999) and high lead exposures decrease rat testicular sperm output, increase epididymal sperm ROS production, and decrease epididymal sperm motility (Hsu et al, 1997) as well as lowering antioxidant capacity of the testis and increasing lipid peroxidation (Marchlewicz et al, 2007).

Finally, lifestyle choices such as excessive alcohol consumption or cigarette smoking increase free-radical production in all tissues and have on multiple occasions

been associated with male infertility or with conditions contributing to that infertility (Mattison, 1982; Peltola et al, 1994; Wu and Cederbaum, 2002).

Chemotherapy—Cancer chemotherapy is gonadotoxic (Arnon et al 2001). That effect may result from factors ranging from endocrinopathy (Maines et al, 1990; Brennemann et al, 1997) to generalized cell-stress responses mediated by heat shock proteins (Tilgada, 2006), but it is widely recognized that many chemotherapy agents like doxorubicin (Asmis et al, 2006; Wolf and Baynes, 2006), cyclophosphamide (Sudharsan et al, 2005), and cisplatin (Santos et al, 2008) induce oxidative stress in a variety of tissues and cell types. Although studies of chemotherapy agents on oxidative stress in the testis, specifically, have not been found, given the acknowledged sensitivity of the testis to the effects of oxidative stress, it is likely an important factor in the loss of testis function after chemotherapy.

Ionizing Radiation—The testis is very sensitive to x-irradiation, which induces oxidative stress (Sohal et al, 1995; Manda et al, 2007) and results in GCA (Hasegawa et al, 1997; De Rooij et al, 2002). Not all cells in the testis are equally sensitive to irradiation, however, with Sertoli and Leydig cells being relatively radiation-resistant. This may be caused by the increase in antioxidants also noted in those cells after irradiation (Lee et al, 2002).

Orchitis/Inflammation—Localized infections or systemic inflammations may have transient or even permanent effects on male fertility, but because not all infections/inflammations are the same, how they impact male fertility can vary. In the laboratory setting, testicular inflammation has been associated with a significant decrease in testosterone production, a disruption of spermatogenesis, and an increase in GCA. For example, using a rat model of systemic inflammation, Reddy et al (2006) noted a rise in testicular iNOS,

interleukin (IL)-1 β , and cyclooxygenase-2, which occurred contemporaneously with a decrease in antioxidant enzymes and germ cells. Allen et al (2004) reported that a single injection of the inflammatory agent lipopolysaccharide in mice resulted in an increase in lipid peroxidation of Leydig cell membranes, a marked reduction in mitochondrial membrane potential, and a reduction in steroidogenesis, which is, itself, associated with GCA (see section V). Interestingly, a recent microarray analysis of human testicular gene expression in 69 infertility patients demonstrated an increase in the expression of inflammatory-response genes, generally, in those testes (Spiess et al, 2007). Those data suggest that inflammation or inflammatory-like conditions with its associated oxidative stress is a common underlying factor in male infertility.

Varicocele—Varicocele, or dilation of the spermatic vein, typically occurs on the left side only and is associated with an increase in male infertility (Fretz and Sandlow, 2002). Experimental left varicocele bilaterally increases testicular blood flow and temperature in lab animals and causes a reduction in testicular sperm output (Turner, 2001). The unilateral lesion in humans also bilaterally increases testicular temperature (Goldstein and Eid, 1989) and establishes a trend toward increased blood flow (Ross et al, 1994). Both the increased blood flow and the increased temperature may play a role in the oxidative stress evidenced in the testes (Santoro and Romeo, 2001) and semen (Hendin et al, 1999; Smith et al, 2006) of varicocele patients. Varicocele is also associated with a decrease in antioxidant capacity of the rat testis (Ozdamar et al, 2004) and human semen (Hendin et al, 1999). Interestingly, NO has been linked to an increase in lipid peroxidation in both human varicocele patients (Romeo et al, 2003) and rats with experimental varicocele (Ozdamar et al, 2004). This implies a role for peroxynitrites in the oxidative stress of varicocele. Although much remains to be understood about the basic pathobiology of varicocele, it does appear that testicular oxidative stress is an associated factor.

Cryptorchidism—The increases in testicular temperature implicit in cryptorchidism have long been associated with increases in testicular oxidative stress (Ahotupa and Huhtaniemi, 1992; Peltola et al, 1995; Misro et al, 2005). Li et al (2006) examined ROS production and gene expression patterns after the induction of cryptorchidism in adult mice. Those investigators reported that cryptorchidism induced an increase in ROS, which was correlated with increased GCA and alterations in the expression of a number of genes associated with energy and lipid metabolism, stress response, and redox reactions. Testis tissue under increased temperature *in vitro* also shows an increased susceptibility to oxidative

stress and GCA (Ikeda et al, 1999). The increase in ROS during cryptorchidism has also been correlated with a decline in testosterone (Chaki et al, 2005), and oxidative stress, specifically an increase in NO subsequent to eNOS overexpression, has been linked to germ GCA in a mouse model of cryptorchidism (Ishikawa et al, 2005).

Aging—Aging results in eventual declines in steroidogenesis (Zirkin and Chen, 2000), which has been suggested to be caused by an increase in testicular oxidative stress (Syntin et al, 2001). Further, Cao et al (2004) and Luo et al (2006) have shown that Leydig cells from aged rats show a reduced expression of key enzymatic and nonenzymatic antioxidants, which leads to enhanced oxidative damage. Elements of the glutathione-dependent antioxidant system are also reduced in the aged rat testis (Mueller et al, 1998; Luo et al, 2006). These results from the testis are consistent with what is known about increased oxidative stress and aging, generally (Sastre et al, 2000), but the detailed relationships between aging, oxidative stress, and testis function remain to be clarified.

Testicular Torsion—The incidence of testicular torsion has been estimated to be as high as 1 in 158 males by the age of 25 (Anderson and Williamson, 1988) with >35% of these having poor ejaculate quality (Anderson and Williamson, 1990). Numerous studies have reported increases in oxidative stress in the testis after repair of testicular torsion (Turner et al, 1997; Da Ros et al, 1998; Lysiak et al, 2001, 2007; Ozkurkcugil et al, 2004; Anim et al, 2005; Rodriguez et al, 2006) and all have reported its adverse effects on testicular function, including germ cell loss and disruption of the seminiferous epithelium. As might be expected, inhibitors of oxidative stress provide significant testicular salvage after torsion repair and reperfusion of the organ (Lysiak et al, 2002; Romeo et al, 2004; Turner et al, 2004; Dokmeci, 2006). Thus, testicular torsion, when repaired before infarction and necrosis, causes an ischemia-reperfusion (IR) injury that is a classic inducer of oxidative stress.

In many of the conditions or exposures mentioned in previous sections, such as toxicant exposure, cryptorchidism, or varicocele, it has been established that testicular oxidative stress occurs. Commonly, however, little research has been done on the chemical and cellular events that cause the oxidative events or the tissue, cell, or molecular consequences of those events. A number of laboratories have used testicular torsion as a model of acute oxidative stress and have evaluated testicular cell and molecular responses ranging from the microvascular endothelium to the seminiferous epithelium. This multilevel approach has allowed a broad understanding both of what happens in the testis under oxidative stress and how alterations of one cell type may influence

others. The following discussion borrows heavily but not exclusively from those studies.

Vascular Events That May Contribute to or Result From Testicular Oxidative Stress

In the normal rat testis, variation in microvascular blood flow is caused by vasomotion or cyclic vascular contraction/relaxation under complex regulation (Collin et al, 1993; Turner et al, 1996; Figure 1A). Vasomotion is altered after testicular IR (Figure 1B) and only returns days later (Figures 1C and 1D; Lysiak et al, 2000a). As mentioned previously and in further detail below, intratesticular testosterone declines under the oxidative stress induced by IR and reduced testosterone concentrations have been shown to eliminate vasomotion (Collin et al, 1993). Vasomotion may also be influenced by the vascular relaxing effects of NO, which increases in the testis after IR (Zini et al, 1998; Ozokutan et al, 2000; Shiraishi et al, 2001; Ozturk et al, 2003). For example, injection of 20 μ L of 5 mM SIN-1, an NO donor, into the testicular artery eliminates vasomotion in vivo (Figure 2). NO is also active in cell processes other than those inducing vascular relaxation and may participate in other events leading to testicular injury. As a case in point, NO has been reported to be a regulator of the expression of cell adhesion molecules (CAMs) on the luminal surface of the vascular endothelium (Kribben et al, 1999; Galley and Webster, 2004). CAMs play a key role in IR injury in the testis as well as in other tissues because they are key modulators of leukocyte recruitment. The recruitment of leukocytes is the forerunner of much of the subsequent IR pathology in organs, generally (Laroux et al, 2000; Galley and Webster, 2004), and in the testis, specifically (Lysiak et al, 2001); thus, it would be of interest to know more about the role of NO and its key regulatory molecules, iNOS and eNOS, in modulating vasomotion and endothelial CAMs during periods of testicular oxidative stress.

Endocrine Events That May Contribute to or Result From Testicular Oxidative Stress

Previous studies have indicated that testicular testosterone production is acutely reduced in a number of conditions associated with ROS production and oxidative stress in the testis. Examples are cryptorchidism (Chaki et al, 2005), aging (Zirkin and Chen, 2000), and IR injury (Turner et al, 2005). It is also true that steroidogenesis itself produces ROS, largely from mitochondrial respiration and the catalytic reactions of the steroidogenic cytochrome P450 enzymes (Peltola et al, 1996; Hales, 2002; Hanukoglu, 2006). The ROS produced by spermatogenesis, if unchecked by

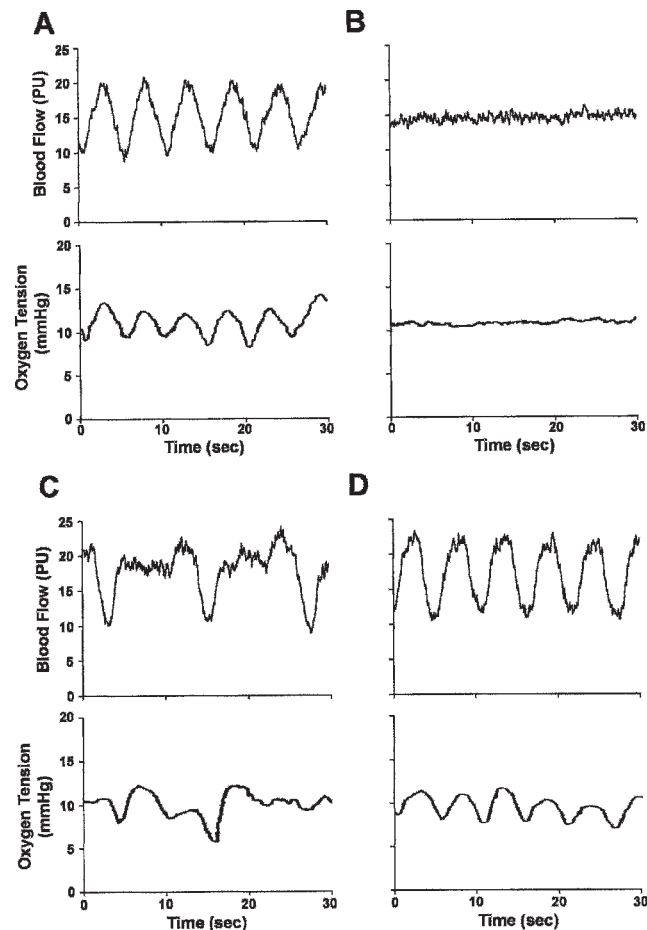


Figure 1. Testicular microvascular blood flow and simultaneously measured interstitial oxygen tensions in (A) control rat testis, (B) rat testis 1 hour after repair of 1 hour ischemia induced by testicular torsion, (C) rat testis 24 hours after repair of 1 hour torsion, and (D) rat testis 7 days after repair of 1 hour torsion. Normal vasomotion does not return until 7 days after torsion repair. PU indicates arbitrary perfusion unit. (Reprinted with permission from Lysiak et al, 2000a.)

intracellular antioxidants, can also damage mitochondrial membranes and contribute to the inhibition of subsequent steroid production (Luo et al, 2006). In the average male, the oxidative damage from steroidogenesis may be more of a chronic than an acute factor and has been hypothesized to be important in the declining testosterone production seen in the aging testis (Chen and Zirkin, 1999; Luo et al, 2006).

Increased NO from a variety of stresses also decreases testosterone secretion (Del Punta et al, 1996; Kostic et al, 1998). This might come in part from the formation of peroxynitrites, but in other tissues, both NO and ischemia increase the transcription factor hypoxia inducible factor (HIF)-1 α (Brune and Zhou, 2003; Zhou et al, 2003). Interestingly, preliminary results from our lab have localized HIF-1 α to Leydig cells in the testicular interstitium (Figures 3A and 3B). We are

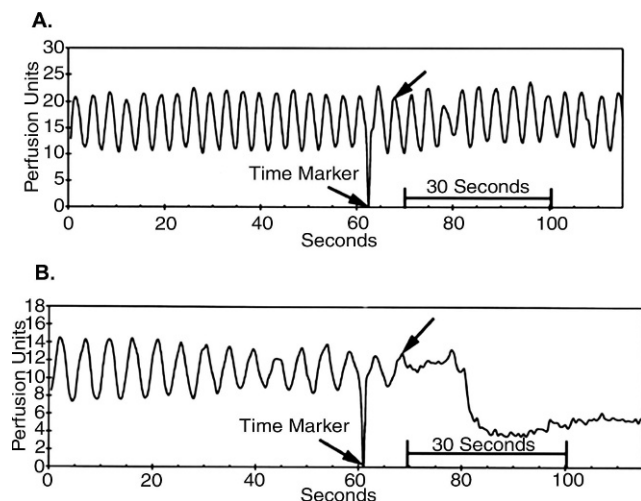


Figure 2. Vasomotion of the testicular microvasculature when infused in vivo (arrows) via the testicular artery with (A) vehicle only or (B) 20 μ L 5 mM SIN-1 (NO donor). SIN-1 eliminated vasomotion, illustrating a potential effect of up-regulated NO on testicular microvascular blood flow (Turner and Wilson, unpublished data).

unaware of any direct studies of the relationship between HIF-1 and Leydig cell testosterone secretion, but the location of HIF-1 α in those cells is suggestive that the two may be intertwined. Additional preliminary evidence has shown that HIF-1 α protein is expressed in the mouse testis under normoxic conditions (Figure 3C), which has made the transcription factor a target of interest in our lab.

Under normoxic conditions in other tissues, HIF-1 α is rapidly degraded (Semenza, 1999) and is not detected on Western blots (Figure 3C). Under hypoxic conditions the protein is stabilized, localizes to the nucleus, and binds to its partner, HIF-1 β , forming the HIF-1 dimer. HIF-1 binds to the hypoxia response element(s) in the promoter region of target genes, which leads to increases in those genes' expression (Williams and Benjamin, 2000; Powell et al, 2002).

The constitutive presence of HIF-1 α in the testis (Figure 3) is interesting because the "normoxic" testis has long been reported to be relatively hypoxic (Setchell, 1978; Lysiak et al, 2000a). Those 2 facts, together, suggest that HIF-1 may play a role in normal Leydig cell function. Although much remains to be clarified, preliminary findings (Turner and Lysiak, unpublished data) suggest that constitutive HIF-1 may provide an initial protective mechanism against NO effects on Leydig cell testosterone production, but very large increases in NO are associated with oxidative stress, which may override the effects of HIF-1 and inhibit testosterone production. The degree to which this is true for oxidative stress, generally, or the specific conditions of the testis remains to be investigated.

Germ Cell Responses to Oxidative Stress

Apoptosis results from the activation of an intracellular program that leads to cell death without the induction of an inflammatory response (Thompson, 1995). GCA is a significant process even in conventional spermatogenesis (Matsui, 1998), but it is clear that the process is up-regulated in a number of the stress conditions already mentioned, such as toxin exposure, cryptorchidism, and testicular torsion (Brinkworth et al, 1995; Turner et al, 1997; Yin et al, 1998). With the IR injury caused by testicular torsion of sufficient duration, for example, the seminiferous epithelium undergoes a catastrophic induction of GCA (Bozlu et al, 2003; Rodriguez et al, 2006) and that induction coincides with the increase in testicular oxidative stress (Turner et al, 1997). Although the details of apoptosis induction have not been elaborated in all causes of oxidative stress, in the case of testicular IR it is caused by a cytokine-induced stress-kinase stimulation of E-selectin expression in the testicular vascular endothelium, which leads to testicular neutrophil recruitment and an increase in intratesticular ROS. ROS, in turn, cause peroxidative damage to cell membranes and the initiation of GCA (Turner et al, 1997; Lysiak et al, 2001, 2003).

Any severe induction of GCA increases the requirement that Sertoli cells engulf large numbers of dying germ cells. This may overwhelm usual Sertoli cell processes and initiate a switch-on of cytokine expression involving nuclear factor kappa B (Lysiak et al, 2005) or cytokines like IL-1 and IL-6 (Cudicini et al, 1997). How Sertoli cells handle the demand for increased engulfment and phagocytosis of germ cells when faced with a large increase in GCA remains an unexplored facet of testicular oxidative stress.

There are numerous molecular pathways to apoptosis, depending on proximate causes and the specific tissue involved. There is a prominent role for the so-called mitochondrial pathway to GCA after IR injury to the testis in both rats (Lysiak et al, 2000b) and mice (Lysiak et al, 2007). The primary effect of oxidative stress is on the mitochondrial membrane, where associations between proapoptotic and antiapoptotic members of the Bcl-2 family (eg, Bax and Bcl-X_L or Bcl-2 and Bcl_w, respectively) are altered (Adams and Cory, 1998; Hengartner, 2000) allowing the release of cytochrome c and the eventual activation of a caspase cascade, which ultimately results in the fragmentation of a cell's DNA (Wyllie, 1980; Green, 1998). Consistent with this pathway, Bax is the predominant proapoptotic molecule in the rat testis, where it exhibits increased expression after IR-induced oxidative stress (Lysiak et al, 2001). Not all testicular stresses activate the mitochondrial pathway as primary oxidative stress does. For example,

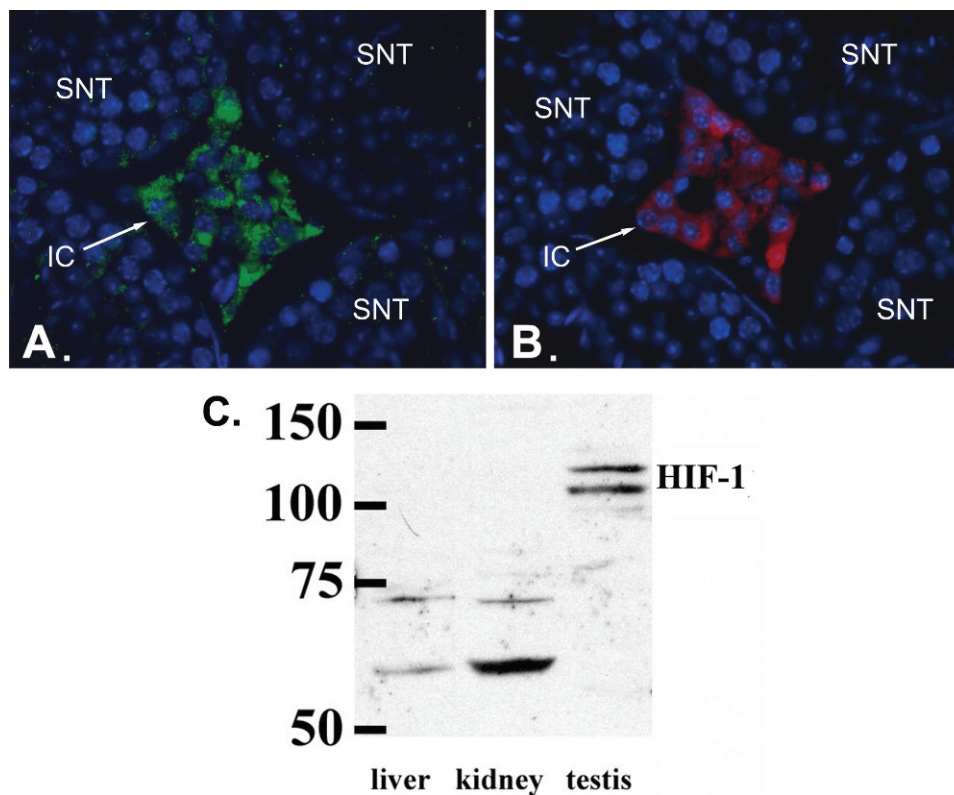


Figure 3. Immunofluorescent detection of hypoxia inducible factor (HIF)-1 α (A) and the Leydig cell marker 3 β -hydroxysteroid dehydrogenase (B), suggesting colocalization of the 2 proteins in interstitial cells (IC) nested between seminiferous tubules (SNT). Western blot analysis of proteins from normoxic mouse liver, kidney, and testis (C) illustrates the constitutive presence of HIF-1 α in testis but not in liver and kidney.

Boekelheide and colleagues (Boekelheide et al, 1998; Lee et al, 1999) have shown that certain organic toxicants induce GCA through a pathway involving Fas ligand (FasL) and Fas, members of the TNF superfamily of ligands and receptors (Nagata, 1997). FasL is secreted by Sertoli cells, and its receptor, Fas, is on the germ cell membrane. Fas-FasL binding initiates the intracellular “death domain” pathway, which, like the mitochondrial pathway, eventually leads to DNA degradation via the caspase cascade. In theory, this Sertoli cell-induced GCA can be selective to particular germ cells, especially for the GCA that occurs during conventional spermatogenesis.

Conclusions

Approximately 15% of couples attempting to conceive are clinically infertile, and male-factor infertility is involved in fully one-half of those cases (Sigman and Jarow, 2007). Conditions like varicocele, cryptorchidism, testicular torsion, or endocrinopathy, all of which are associated with testicular oxidative stress, are strongly associated with testicular dysfunction; in fact, approximately 45% of male infertility patients present with at least 1 of these indications (Sigman and Jarow,

2007). Further, approximately 25% of male infertility patients have abnormal semen analyses in the absence of any recognized cause (Sigman and Jarow, 2007). The proportion of these men experiencing occult testicular oxidative stress is unknown, but such stress could come from unappreciated factors in a patient’s history such as excessive alcohol consumption, drug use (including steroids), unsuspected toxicant exposure, or even excessive exercise. Thus, from both known and unknown conditions, testicular oxidative stress likely plays a larger than appreciated role in male infertility. Such a conclusion suggests that the development of new, more efficacious antioxidant therapies may be important for the treatment of hypospermatogenesis.

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