Bölüm 3

Oxidative and Nitrosative Challenges in Male and Female Gametes

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Abstract

Reactive nitrogen species (RNS), primarily nitric oxide (NO), play a dual role in reproductive physiology, acting as essential signaling molecules at physiological levels while exerting cytotoxic effects when dysregulated. In males, NO modulates critical processes such as capacitation, acrosomal reaction, and sperm motility via cGMP-mediated pathways, and supports spermatogenesis through regulation of the blood-testis barrier and steroidogenesis. However, elevated NO levels lead to the formation of peroxynitrite ($ONOO^-$), a potent oxidant that induces lipid peroxidation, DNA fragmentation, protein nitration, and apoptosis in spermatozoa—hallmarks of oxidative stress-related infertility. In females, NO contributes to folliculogenesis, ovulation, and oocyte maturation by regulating angiogenesis, cumulus expansion, and meiotic competence through redox-sensitive transcription factors and EGF-like signaling. Nonetheless, excessive RNS disrupts mitochondrial function and DNA repair in oocytes, accelerating cellular aging and compromising developmental competence. Clinical and experimental data underscore the correlation between elevated peroxynitrite levels and impaired sperm parameters, increased DNA fragmentation, and diminished antioxidant capacity in infertile men. These findings position RNS as both physiological regulators and pathological disruptors of fertility, suggesting that redox homeostasis is critical for reproductive success. Targeted antioxidant strategies aimed at neutralizing excess RNS may offer novel therapeutic avenues for managing infertility. Understanding the precise molecular mechanisms of nitrosative stress in gametogenesis can inform both diagnostics and interventions in reproductive medicine.

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1. INTRODUCTION

Oxidative stress, an imbalance between reactive oxygen/nitrogen species and the antioxidant defenses, undermines gamete integrity in both sexes (Jacobs and Bennett, 2025). In spermatozoa, elevated reactive species initiate lipid peroxidation in the plasma membrane, which damages polyunsaturated fatty acids and disrupts membrane fluidity and integrity (Alahamar, 2019; Aitken et al., 2019). This peroxidative damage impairs motility, capacitation, and the ability to fuse with the oocyte (Hussain et al., 2023). Moreover, oxidative stress can fragment sperm DNA and oxidize nucleotides, reducing fertilization potential (Aitken et al., 2019, Jacobs and Bennett, 2025). In oocytes, excessive reactive species induce lipid peroxidation in membranes and organelles, leading to mitochondrial dysfunction, apoptosis, and loss of developmental competence (Kalaivani et al., 2022). Lipid peroxidation products like malondialdehyde further propagate radical chain reactions, exacerbating cellular damage in female gametes (Kalaivani et al., 2022). Overall, oxidative stress and resultant lipid peroxidation degrade the functional and genetic quality of both sperm and oocytes, thereby reducing fertility potential (Aitken et al., 2019, Jacobs and Bennett, 2025).

1.1. Male Fertility

Numerous studies have investigated the impact of modifying specific technical parameters such as cryoprotectant concentrations, including supplementary additives, and cooling and warming rates on cryopreservation outcomes (Holt, 2000). The mechanisms underlying cryodamage to spermatozoa are considered multifactorial, encompassing cold shock, osmotic stress, intracellular ice crystal formation, oxidative stress, and various combinations. Incorporating additives with antioxidative properties has been reported to mitigate the detrimental effects of reactive oxygen species (ROS) and cold-induced injury. (Amidi et al., 2016). Semen cryopreservation remains a subject of considerable scientific and commercial interest, driven by the expanding demand for meat and the strategic use of artificial insemination (AI) to manage reproduction and regulate crossings for interbreeding. Nonetheless, the widespread implementation of AI using frozen-thawed semen is constrained by suboptimal fertility outcomes, primarily attributed to structural and functional impairments incurred by spermatozoa during the cryopreservation cycle (Salamon and Maxwell, 1995). Mammalian spermatozoa are characterized by a high content of polyunsaturated fatty acids, rendering them highly vulnerable to lipid peroxidation, particularly following cryopreservation. This oxidative stress compromises membrane integrity, disrupts cellular functionality, and leads

to diminished motility and fertilization capacity, thereby adversely affecting the efficacy of artificial insemination (Bucak et al., 2008). The freeze–thaw process imposes cold shock on spermatozoa; a condition intricately associated with oxidative stress driven by the overproduction of free radicals (Uysal and Bucak, 2008). Free radicals are highly reactive molecular species characterized by one or more unpaired electrons. In seeking to stabilize themselves, they readily interact with adjacent stable molecules, abstracting electrons and converting the target into a new radical. This chain reaction initiates various oxidative events that may culminate in significant cellular damage. From a physiological perspective, free radicals play regulatory roles in key reproductive processes, including maturation, capacitation, hyperactivation, the acrosome reaction (AR) of sperm cell, and fusion of sperm and oocyte. Conversely, under pathological conditions, excessive free radical activity contributes to lipid peroxidation (LPO), DNA fragmentation, and the initiation of apoptotic pathways (Kothari et al., 2010).

Reactive nitrogen species (RNS), a subclass of reactive oxygen species (ROS), play a pivotal role in male reproductive physiology by participating in cell signaling and maintaining sperm function when present at physiological levels (Doshi et al. 2012, Kothari et al. 2010). Among RNS, nitric oxide (NO) is particularly important in regulating sperm capacitation, acrosomal reaction, and motility via cyclic guanosine monophosphate (cGMP)-mediated signaling (Beckman et al., 1990). NO facilitates sperm hyperactivation, necessary for oocyte penetration, by modulating calcium influx and protein phosphorylation cascades (de Lamirande and Gagnon, 1995). Additionally, NO contributes to the regulation of the blood-testis barrier, a critical structure for spermatogenesis and germ cell maturation, through its interaction with tight junction proteins (Lee and Cheng, 2004). It also influences steroidogenesis by modulating Leydig cell function, affecting testosterone biosynthesis (Rosselli, 1998). However, excessive NO concentrations ($>1\ \mu M$) generate toxic compounds such as peroxynitrite, leading to oxidative damage of lipids, proteins, and DNA within spermatozoa (Pryor and Squadrito, 1995). This nitrosative stress compromises sperm motility, morphology, and viability, thus impairing fertility outcomes (Nobunaga et al., 1996). Furthermore, NO-induced DNA fragmentation in sperm correlates with poor IVF success rates and increased miscarriage risk (Amiri et al., 2007). Hence, while NO is indispensable for male fertility at low concentrations, its dysregulation poses a significant threat to reproductive health (Agarwal, 2008).

1.2. Female Fertility

Reactive nitrogen species, particularly NO, act as pivotal signaling mediators in the regulation of ovarian physiology, modulating processes such as folliculogenesis and ovulation (Bezdíček et al., 2025). Nitric oxide is primarily synthesized in ovarian tissues through different isoforms of nitric oxide synthase (NOS), contributing to vascular remodeling and angiogenesis in developing follicles (Bezdíček et al., 2025). At physiological levels, NO modulates redox-sensitive pathways by activating transcription factors such as Nuclear Factor kappa B (NF-κB), which further enhance Hypoxia-inducable factor 1-alpha (HIF-1α) expression, linking oxygen sensing to angiogenic gene expression (Bonello et al., 2007). This redox activation supports the expression of vascular endothelial growth factor (VEGF), facilitating adequate blood supply to maturing follicles (Bonello et al., 2007). In ovulatory follicles, NO interacts with EGF-like growth factors such as amphiregulin and epiregulin inducing cumulus expansion and enhancing oocyte meiotic competence (Park et al. 2004). Experimental findings in murine models have demonstrated that appropriate NO concentrations facilitate both nuclear and cytoplasmic maturation of oocytes, whereas suboptimal NO availability impairs developmental potential (Bu et al., 2003). Additionally, nitrosative stress induced by excess NO or peroxynitrite has been shown to accelerate the process of oocyte aging by disrupting mitochondrial activity, cytoskeletal integrity, and DNA repair mechanisms (Tatone et al., 2006).

1.3. Infertility

Reactive nitrogen species, such as peroxynitrite ($ONOO^-$), have emerged as critical contributors to male infertility through their damaging effects on spermatozoa structure and function (Khosravi et al., 2014). Peroxynitrite is formed by the reaction of nitric oxide (NO•) with superoxide anion (O_2^-), leading to a potent oxidant capable of inducing nitration and oxidative injury in cellular components (Beckman, 1996). In the male reproductive system, this damage is particularly detrimental, as spermatozoa have limited cytoplasmic content and weak endogenous antioxidant defenses (Agarwal et al., 2005). Khosravi et al. (2014) demonstrated that infertile men with abnormal semen parameters have significantly higher levels of seminal peroxynitrite compared to normozoospermic controls, indicating increased RNS generation (Khosravi et al., 2014). This elevation in RNS is accompanied by enhanced sperm DNA fragmentation (DF), a well-established marker of compromised sperm chromatin integrity and male infertility (Zini et al., 2001). The positive correlation between peroxynitrite

levels and DNA fragmentation percentages ($r = 0.594$, $p < 0.01$) underscores the genotoxic role of RNS (Khosravi et al., 2014). Moreover, peroxynitrite has been shown to induce lipid peroxidation, thiol group reduction, and tyrosine nitration in sperm proteins, all of which are associated with reduced sperm motility and fertilization potential (Oztezcan et al., 1999; Herrero et al., 2001). The nitration of tyrosine residues within sperm proteins leads to structural alterations that hinder key physiological processes such as capacitation and acrosome reaction (Herrero et al., 2001). Consequently, spermatozoa exposed to high peroxynitrite concentrations exhibit poor motility and abnormal morphology, which are classical hallmarks of oxidative stress-mediated infertility (Vignini et al., 2006). In conjunction with elevated RNS levels, infertile men in the study exhibited significantly lower total antioxidant capacity (TAC) in seminal plasma compared to controls (Khosravi et al., 2014). This reduction in TAC exacerbates oxidative damage by failing to neutralize excess free radicals, including both ROS and RNS (Said et al. 2003). The inverse relationship between TAC and peroxynitrite levels ($r = -0.582$, $p < 0.01$) confirms that antioxidant depletion may leave sperm vulnerable to nitrosative assault (Khosravi et al., 2014). Collectively, these findings support a mechanistic model in which increased seminal RNS, particularly peroxynitrite, contributes to male infertility by impairing sperm function and genome integrity (Agarwal and Said, 2005). Targeting RNS through antioxidant therapies may offer a promising adjunctive approach in the management of male infertility (Mahfouz et al., 2010). Understanding the oxidative basis of male reproductive failure can lead to improved diagnostic markers and therapeutic strategies aimed at preserving sperm quality and fertilization capacity (De Iuliis et al., 2009).

Reactive nitrogen species, particularly nitric oxide and its derivatives, play a critical but paradoxical role in reproductive physiology. While physiological concentrations of RNS are indispensable for processes such as sperm capacitation, oocyte maturation, and follicular angiogenesis, their excessive accumulation leads to oxidative and nitrosative stress, impairing gamete structure and function. The deleterious effects of peroxynitrite on sperm DNA integrity, motility, and membrane fluidity, as well as its role in accelerating oocyte aging, highlight the need for maintaining redox homeostasis in the reproductive tract. Understanding the molecular mechanisms underlying RNS-mediated damage offers valuable insights into the etiology of infertility and underscores the therapeutic potential of targeted antioxidant strategies. Future research focused on modulating nitrosative pathways may pave the way for improved diagnostic and interventional approaches in reproductive medicine.

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