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**ARTICLE**

# Zinc protects sperm from being damaged by reactive oxygen species in assisted reproduction techniques

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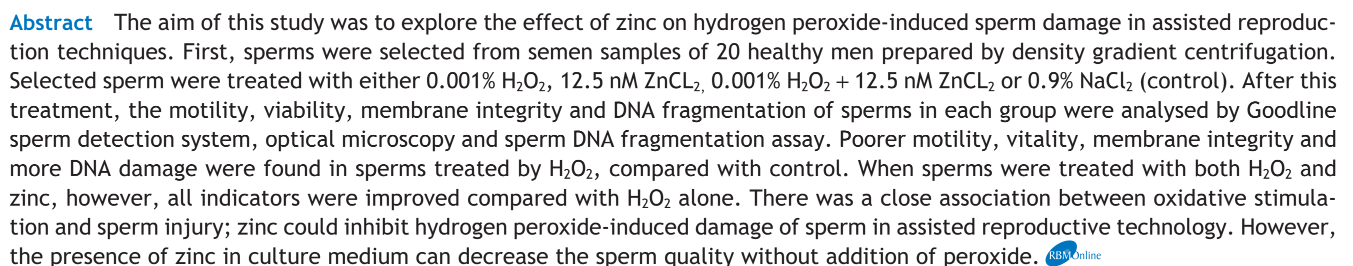

**Abstract** The aim of this study was to explore the effect of zinc on hydrogen peroxide-induced sperm damage in assisted reproduction techniques. First, sperms were selected from semen samples of 20 healthy men prepared by density gradient centrifugation. Selected sperm were treated with either 0.001% $H_2O_2$, 12.5 nM $ZnCL_2$, 0.001% $H_2O_2$ + 12.5 nM $ZnCL_2$ or 0.9% $NaCl_2$ (control). After this treatment, the motility, viability, membrane integrity and DNA fragmentation of sperms in each group were analysed by Goodline sperm detection system, optical microscopy and sperm DNA fragmentation assay. Poorer motility, vitality, membrane integrity and more DNA damage were found in sperms treated by $H_2O_2$, compared with control. When sperms were treated with both $H_2O_2$ and zinc, however, all indicators were improved compared with $H_2O_2$ alone. There was a close association between oxidative stimulation and sperm injury; zinc could inhibit hydrogen peroxide-induced damage of sperm in assisted reproductive technology. However, the presence of zinc in culture medium can decrease the sperm quality without addition of peroxide.

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**KEYWORDS:** assisted reproduction, DNA damage, oxidative stress, reactive oxygen species, sperm

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## Introduction

Infertility is a public health problem for many couples of childbearing age. Growing evidence indicates that oxidative stress plays a fundamental role in the cause of male infertility by negatively affecting sperm quality and function, which can be a primary cause of male infertility (Benedetti et al., 2012). Seminal oxidative stress results from an imbalance between reactive oxygen species (ROS) production and ROS scavenging by seminal antioxidants (Agarwal et al., 2014). Thse are are toxic substances that are produced by cells in the normal metabolism. High levels of semen ROS can cause sperm dysfunction, sperm DNA damage and reduced male reproductive potential. Oxidative stress occurs commonly in spermatozoa through lipid peroxidation because the sperm membrane contains high amounts of polyunsaturated fatty acids. The concept of improving fertility potential of infertile patients with oxidative stress by certain antioxidants has gained considerable attention in assisted reproductive technology and infertility practice (Chow, 1991; Donnelly et al., 1999).

Zinc is a micronutrient required for the action of more than 200 metallo-enzymes. It plays a vital role in male fertility. Deficiency of zinc can impair spermatogenesis and decrease serum testosterone levels (Wong et al., 2002). Studies have shown that it plays a central role in normal testicular growth, spermatogenesis, and sperm physiology (Elgazar et al., 2005); It conserves genomic integrity in the sperm and stabilizes connection of sperm head to tail (Tuerk and Fazel, 2009). Deficiency of zinc is associated with hypogonadism and insufficient growth of secondary sex characteristics in human beings (Sandstorm and Sandberg, 1992). Low seminal zinc levels were coupled with a decrease in fertilizing ability of sperm (Pandy et al., 1983) and decreased the synthesis of testosterone (Ebisch et al., 2007). Also, it helps in stabilizing polymeric macromolecules, such as RNA, DNA and protein. Earlier studies have shown that zinc present in the prostatic secretion provides stability to human sperm chromatin (Bjorndahl and Kvist, 2011; Suzuki et al., 1995).

Assisted reproduction techniques are the main method of treating infertility. The process of density gradient centrifugation for sperm preparation before treatment, freezing and recovery *in vitro* will lead to sperm oxidative damage, which affects semen parameters (De Iuliis et al., 2009; Donnelly et al., 2001a, 2001b). Human seminal plasma is a natural reservoir of antioxidants. Spermatozoa depend on scavenging systems provided by the seminal plasma, with important natural antioxidants such as vitamins C and E, superoxide dismutase, glutathione and thioredoxin that act directly as free radical scavengers (Kobayashi et al., 1991; Niki, 1991). The sperm lose the protective effect of seminal plasma in assisted reproductive technology. The concept of improving fertility potential of infertile patients with oxidative stress by certain antioxidants has gained considerable attention in assisted reproduction techniques and infertility practice (Chow, 1991; Donnelly et al., 1999). The presence of zinc in culture medium has been reported to inhibit capacitation and acrosome reaction (Andrews et al., 1994; Bilaspuri and Babbar, 2007; Liu et al., 2009; Riffo et al., 1992). Zinc, however, has been reported to protect sperm chromosomal stability *in vitro* (Blazak and Overstreet, 1982; Kotdawala et al., 2012). Therefore, the present study was undertaken to assess whether addition of zinc to the culture medium can benefit sperm and protect the human spermatozoa from oxidative damage.

## Materials and methods

### Preparation of sperm

Semen samples were collected by masturbation after the stipulated 2–7 days of abstinence from 20 men with normal semen parameters who attended the infertility clinic. The semen samples were liquefied for 15–30 min at 37°C, following which the sperm parameters were assessed in accordance with the World Health Organization guidelines (World Health Organization, 2010). Samples with high leukocyte concentrations (leukocytes $> 1 \times 10^6$ cell mL−1) were excluded. The sperm were prepared using a discontinuous PureSperm gradient (Quinn's, SAGE, USA), which consisted of two layers of PureSperm (1.5 ml): 80% and 40%. Semen sample (2 ml) was deposited on the 40% layer. The gradient was then centrifuged at 300 x g for 15 min. After centrifugation, the sperm precipitate was collected and washed twice with 2 ml of sperm washing medium (Quinn's, SAGE, USA) at 200× g for 5 min. The pellet was then resuspended in IVF medium (Scandinavian IVF, Gothenburg, Sweden) to sperm concentration of $5 \times 10^6$/ml for further study.

### Experimental design

At first, pre-experiments of gradient dose of $H_2O_2$ (0.1%, 0.01% and 0.001%) and $ZnCl_2$ (50, 25, 12.5, 6.2 nmol L-1) were conducted to decide the optimal concentration that can make a significant difference to semen motility and viability. After 5 h of incubation, significant changes were observed only at 0.001% $H_2O_2$ and 12.5 nmol/l $ZnCl_2$. Therefore, four groups of experimental samples were prepared with 0.001% $H_2O_2$, 12.5 nmol/ml $ZnCl_2$, 0.001% $H_2O_2$ and 12.5 nmol/l $ZnCl_2$, as well as the same volumes of saline (control), respectively.

### Sperm motility and viability analysis

Sperm motility and viability were determined according to 2010 WHO recommendation (World Health Organization, 2010), after 5 h and 24 h incubation of the samples in the incubator with 5% $CO_2$ at 37°C.

### Sperm hypo-osmotic swelling test

The sperm membrane integrity was assessed with hypo-osmotic swelling test according to 2010 WHO recommendation (World Health Organization, 2010), after 5 h and 24 h incubation of the samples in the incubator with 5% $CO_2$ at 37°C. In brief, the membrane integrity of spermatozoa tail could expand under hypotonic environment. Thus, following 30-min treatment with hypotonic solution, the sperm was observed under optical microscopy (×400 times) in randomly

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