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The effect of superoxide dismutase mimetic and catalase on the quality of postthawed goat semen

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ABSTRACT

Manganese(III) meso-tetrakis(N-ethylpyridinium-2-yl)porphyrin chloride (MnTE) is a cell-permeable superoxide dismutase mimetic agent which can convert superoxide to hydrogen peroxide (H_2O_2). Supplementation of MnTE to a commercial semen extender can protect sperm from superoxide but not H_2O_2. Therefore, we proposed that addition of catalase (0.0, 200, or 400 IU/mL) in combination with MnTE (0.1 μM) may further improve the cryopreservation efficiency of goat semen in commercially optimized freezing media such as Andromed. Therefore, ejaculates were obtained from three adult bucks twice a week during the breeding season and diluted with Andromed supplemented with or without MnTE and catalase and were frozen in liquid nitrogen. Sperm parameters and reactive oxygen species contents were evaluated 2 hours after dilution (before freezing) and after freezing/thawing. The results revealed that all the treatments significantly ($P \leq 0.05$) improved sperm motility, viability, and membrane integrity after freezing and reduced reactive oxygen species content compared with the control group, but maximum improvement was obtained in MnTE + 400 IU/mL catalase. In addition, supplementation with these antioxidants significantly ($P \leq 0.05$) increases the cleavage rate after IVF. In conclusion, the results of present study suggest that addition of antioxidant MnTE or catalase to commercial optimized media, such as Andromed, improves total motility, membrane integrity, and viability of goat semen samples after thawing. But the degree of improvement for these parameters significantly ($P \leq 0.05$) higher when MnTE and catalase were simultaneously added to the cryopreservation media.

1. Introduction

Despite advances in semen cryopreservation, a variety of injuries occur at cellular and molecular levels during semen cryopreservation which may impair sperm function and fertilization potential [1,2]. Major drawbacks of semen cryopreservation are reduction of sperm viability, motility, plasma membrane integrity, and impaired function of the survived cell population [3]. These phenomena are accounted by osmotic stress, cold shock, intracellular ice crystal, and oxidative stress induced by reactive oxygen species (ROS) [4]. Among the aforementioned factors, excessive production of ROS plays a central role in induction of cryoinjury. Reactive oxygen species acts as a double-edged sword in many physiological phenomena. Basal

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levels of ROS are required for normal sperm motility, capacitation, and acrosome reaction, whereas excess ROS lead to various forms ofcellular damages, including impairment of membrane, DNA damage, and apoptosis [5,6]. Somatic cells have acquired a delicate balance between ROS generation and ROS scavengers but unlike most somatic cells, sperm as one of the smallest cells of the body has little cytoplasm. This makes sperm susceptible to ROS injuries which can affect sperm motility [7], viability [8], DNA integrity, and energy metabolism [9]. Moreover, ROS can also be produced by exogenous sources such as semen handling during cryopreservation process, exposure to light, oxygen, and shearing forces generated by centrifugation. Therefore, supplementation of semen diluents with antioxidants has been advised, and a wide variety of antioxidants have been used for semen cryopreservation in bovine [10], water buffalo [11], stallion [12], ram [13] and goat [14]. Antioxidants used during semen cryopreservation can be divided into two main categories: cell-permeable and impermeable antioxidants. In addition, antioxidants can be categorized as enzymatic, enzyme-mimetic, and nonenzymatic. Recent research has suggested that cell-permeable enzyme-mimetic antioxidants are considered as valuable compounds for reducing intracellular ROS levels [15]. In this regard, we and other researchers have shown that addition of cell-permeable enzyme-mimetic antioxidants to commercially optimized cryopreservation media with antioxidant activity can further improve the quality of these media [13,14]. Manganese(III) meso-tetrakis(N-ethylpyridinium-2-yl) porphyrin chloride (MnTE) is a manganese porphyrin compound with a superoxide dismutase (SOD) mimetic activity which has been shown to successfully rescue animal models for some oxidative stress–related diseases [16]. We previously proposed that 0.1 μM of MnTE can improve sperm membrane and acrosomal integrity. Furthermore, we showed that *in vitro* rate of blastocyst formation after freeze-thawing of goat semen supplemented with MnTE was higher compared with that of its corresponding control [14].

Superoxide dismutase is one of the enzymatic antioxidants, which converts superoxide anion (O_2^-) into hydrogen peroxide (H_2O_2) and oxygen (O_2), whereas catalase can convert this H_2O_2 into oxygen and water [17,18]. Therefore, supplementation with MnTE is expected to increase H_2O_2, which is a relatively stable cell-permeable molecule with high oxidant potential. In other words, the association of SOD mimetic (MnTE) and peroxide scavengers (catalase) may promote better sperm freezing performance. Therefore, the aim of this study was to address whether addition of catalase in combination with MnTE or alone into commercially optimized freezing media could further improve the efficiency of cryopreservation of goat semen.

2. Materials and methods

2.1. Chemicals

All chemicals used in this study were purchased from Sigma Chemical CO. (St. Louis, MO, USA) unless stated otherwise. MnTE was a gift from Dr Ines Batinić-Haberle (Department of Radiation Oncology, Duke University Medical School, Durham, NC 27710, USA). Andromed as a semen extender was obtained from Minitube, Germany.

2.2. Semen collection, processing, and freezing–thawing

Semen collection and processing procedures were carried out according to Forouzanfar et al. [14]. In brief, ejaculates were obtained by artificial vagina from three adult Bakhtiari bucks aged 2 to 3 years, twice a week during the breeding season (September/October 2013) in the animal farm of Reproductive Biotechnology Research Center at Royan Institute (Isfahan, Iran latitude 32°39′N). Samples from each male were kept separated and transported at 35 °C to the laboratory for microscopic assessment within 30 minutes. Only ejaculates showing higher than 70% motile and 80% morphologically normal sperm and more than 1 mL in volume were used for experiments. To minimize individual differences, semen samples from the three bucks in each replicate were pooled. A total of six ejaculates from each goat were processed. For freezing, all the treatments were repeated six times with the pooled semen samples. Andromed was used as the base freezing extender. Each pooled semen sample was divided into six equal aliquots and diluted (1:20 [v:v]) with the extender containing no antioxidant (control group), 0.1 μM of MnTE (Mn), 200 IU/mL catalase (CAT 200), 400 IU/mL catalase (CAT 400), 0.1 μM of MnTE plus 200 IU/mL catalase (Mn + CAT 200), and 0.1 μM of MnTE plus 400 IU/mL catalase (Mn + CAT 400). Concentrations of MnTE and catalase were based on our previous report [14] and Roca et al. [19], respectively. The diluted semen was cooled to 4 °C over a period of 2 hours and drawn into 0.5-mL French straws (Biovet, L'Agile, France), heat sealed and stored at 4 °C for 1 hour for more equilibration. The straws were exposed to liquid nitrogen (LN_2) vapor for 12 minutes, plunged into LN_2, and stored in LN_2 until thawed and used for evaluation of sperm parameters and IVF. Thawing was carried out by plunging sealed straws in a 37 °C water bath for 30 seconds. The thawed samples were individually evaluated by a single trained individual.

2.3. Semen evaluation after thawing

2.3.1. Measurement of sperm motility

For assessment of sperm motility after thawing, four or five straws from one treatment in each group were thawed and pooled. The samples were diluted with a fertilization medium (Tyrode's albumin lactate pyruvate medium, Fert-TALP) to a final concentration of 1×10^6 spermatozoa/mL. The percentages of rapid progressive (class A), sperm that swim fast in a straight line; slow progressive (class B), sperm that move forward but tend to travel in a curved motion; and nonprogressive (class C), sperm that do not move forward despite that they move their tails, as well as total motility which refers to the population of sperm that display any type of movement, were assessed according to the software setting of the computer-assisted sperm analysis system (VideoTest Sperm software 2.1; 1990–2004, VideoTest Ltd, Russia)