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Curcumin has protective and antioxidant properties on bull spermatozoa subjected to induced oxidative stress

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ABSTRACT

Over the past decades, there has been an emphasis on assessment of the use of natural compounds in the prevention or repair of oxidative injury to spermatozoa. Curcumin (CUR) is a natural phenol with powerful antioxidant properties. The aim of the present study was to examine if CUR could reverse reactive oxygen species (ROS)-mediated alterations to the motility, viability and intracellular antioxidant profile of bull spermatozoa subjected to a prooxidant (*i.e.*, ferrous ascorbate – FeAA). Spermatozoa were washed from recently collected semen samples, suspended in 2.9% sodium citrate and subjected to CUR treatment (5, 10, 25 and 50 μmol/L) in the presence or absence of FeAA (150 μmol/L $FeSO_4$ and 750 μmol/L ascorbic acid) during a 6 h *in vitro* culture. Spermatozoa motility characteristics were assessed using the SpermVision computer-aided spermatozoa analysis (CASA) system. Cell viability was examined with the metabolic activity (MTT) assay, ROS generation was quantified using luminometry and the nitroblue-tetrazolium (NBT) test was used to quantify the intracellular superoxide formation. Cell lysates were prepared at the end of the culture to assess the intracellular activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) as well as the concentrations of glutathione (GSH) and malondialdehyde (MDA). Treatment with FeAA led to a reduced spermatozoa motility ($P < 0.001$), viability ($P < 0.001$) and decreased the antioxidant characteristics of the samples ($P < 0.001$) but increased the ROS generation ($P < 0.001$), superoxide production ($P < 0.001$) and lipid peroxidation ($P < 0.001$). The CUR treatment led to a preservation of spermatozoa motion ($P < 0.001$), mitochondrial activity ($P < 0.001$) and antioxidant characteristics ($P < 0.05$ with SOD and GSH; $P < 0.01$ with CAT and GPx), revealing the concentration range of 25–50 μmol/L CUR to be the most effective for sustaining spermatozoa viability. Data from the present study suggest that CUR exhibits significant protective and ROS-scavenging characteristics which may prevent oxidative insults to spermatozoa and thus preserve the functional activity of male gametes.

1. Introduction

Oxidative stress (OS) resulting from an imbalance between reactive oxygen species (ROS) generation and

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the available antioxidant defense mechanisms has been repeatedly associated with male reproductive dysfunction (Sikka, 1996; Hendin et al., 1999; Pasqualotto et al., 2000; Garrido et al., 2004). Spermatozoa are highly susceptible to oxidative insults as plasma membranes of these cells are rich in polyunsaturated fatty acids – the primary site of action for lipid peroxidation (LPO) (Aitken et al., 1989), while the cytoplasm is restricted to the midpiece and contains very few antioxidant systems to provide a proper protection against ROS-mediated cellular damage (Bromfield, 2014; O'Flaherty, 2014). Seminal OS may contribute to mitochondrial dysfunction (Baumber et al., 2000), DNA fragmentation (Morte et al., 2008), and oxidative breakdown of lipids and proteins (Aitken et al., 1989; Mammoto et al., 1996; Baumber et al., 2000), which are in turn associated with spermatozoa cell motility loss. A decreased capacity for sperm-oocyte fusion (Mammoto et al., 1996; Guthrie and Welch, 2012; Ahmad et al., 2015), poor fertilization rates and alterations during embryogenesis are other abnormalities that can result from oxidation events (Lewis and Aitken, 2005; Simões et al., 2013).

Numerous recent studies have shown that *in vitro* administration of hydrophilic or lipophilic antioxidant supplements in human or veterinarian andrology practices has positive effects on the structural integrity or functional activity of male gametes (Yun et al., 2013; Petruska et al., 2014). A variety of antioxidants either scavenge ROS directly or inhibit ROS toxicity in semen of a variety of mammalian or avian species (Bréque et al., 2003; Peña et al., 2003; Zeitoun and Al-Damegh, 2015). Much attention has been devoted, particularly on use of herbal medicines or the derivatives, in the prevention and/or treatment of complications related to ROS overproduction in male reproductive cells and tissues.

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] (CUR) is a major bioactive chemical component of turmeric powder, found in the herbal remedy and dietary spice turmeric. This vibrant yellow spice is derived from the rhizome of the plant *Curcuma longa* Linn., and is obtained by crushing the plant roots into powder form. The CUR is the phytochemical that gives a yellow color to turmeric and is now recognized as being responsible for most of its therapeutic effects (Aggarwal et al., 2007).

CUR is a potent scavenger of an array of ROS including superoxide and hydroxyl radicals (Reddy and Lokesh, 1994) as well as nitrogen dioxide (Unnikrishnan and Rao, 1995). This molecule is an effective LPO inhibitor (Sreejayan and Rao, 1994).

Inconsistent data are, however, available with respect to the effects of CUR on male fertility. Several *in vivo* (Salahshoor et al., 2012) as well as *in vitro* reports (Bucak et al., 2012; Soleimanzadeh and Saberivand, 2013) provide evidence for the involvement of CUR in energy-promoting and protective events in the testicular tissue, spermatogenesis and spermatozoa physiology. Furthermore, CUR reverses the reproductive toxicity caused by a variety of endogenous (Wei et al., 2009) or exogenous factors (El-Wakf et al., 2011; Dev et al., 2013). Other studies, however, implicate a negative involvement of CUR in pathways related to human and murine spermatogenesis (Naz, 2011; Xia et al., 2012) suggesting a cautious and thorough investigation of potentially toxic and contraceptive effects of CUR.

Previous studies (Bansal and Bilaspuri, 2008a; Mojica-Villegas et al., 2014; Tvrdá et al., 2015a) have emphasized that ferrous ascorbate may serve as a highly suitable ROS promoter for mammalian spermatozoa when these cells are separated from the primary antioxidant protection of the seminal plasma. Based on this inconsistent evidence, there is need to further examine the effects of CUR on spermatozoa. The present study was designed to explore the *in vitro* impact of CUR on bull spermatozoa exposed to oxidative stress induced by ferrous ascorbate.

2. Material and methods

2.1. Semen collection and experimental design

Ejaculates ($n = 50$) were obtained from five adult Holstein Friesian breeding bulls (Slovak Biological Services, Nitra, Slovak Republic) that were on a regular collection schedule using an artificial vagina. Each sample had to meet the quality criteria given for the corresponding breed. Institutional and national guidelines for the care and use of animals were followed, and all experimental procedures were approved by the State Veterinary and Food Institute of Slovak Republic (no. 3398/11-221/3) and Ethics Committee.

The *in vitro* treatment followed the protocol designed by Bansal and Bilaspuri (2008a,b, 2009). Each semen sample was centrifuged (800*g*) at 25 °C for 5 min, seminal plasma was removed, the pellet was washed twice with 2.9% sodium citrate dissolved in distilled water (SC; pH 7.4; Centralchem, Bratislava, Slovak Republic), re-suspended in 2.9% SC using a ratio of 1:20 (for cell lysis) or 1:40 (for immediate experimental assessments) and divided into ten equal groups. To one group (Control 1; SC Control) 2.9% SC was added, and with another group (Control 2; FeAA Control) there was supplementation with an ROS inducer, *i.e.*, ferrous ascorbate (FeAA) comprising 150 μmol/L $FeSO_4$ (ferrous sulfate; $FeSO_4 \cdot 7H_2O$; Sigma-Aldrich, St. Louis, MO, USA) and 750 μmol/L ascorbic acid (Centralchem), diluted in 2.9% SC. The remaining eight (experimental) groups were supplemented with 5, 10, 25 or 50 μmol/L CUR (Sigma-Aldrich) in the presence or absence of FeAA. Specific CUR concentrations were selected based on results obtained from a previous CUR standardization study with bull semen (Tvrdá et al., 2015b). All suspensions were incubated at 37 °C.

After culture periods of 0, 2 and 6 h, spermatozoa motility variables, mitochondrial activity, ROS generation and intracellular superoxide production were assessed for each group. Moreover with the group where there was culturing for 6 h, centrifugation occurred at 800*g* at 25 °C for 10 min, the media were removed and the resulting pellet was sonicated at 28 kHz for 30 s on ice using RIPA buffer (Sigma-Aldrich) with protease inhibitor cocktail suitable for mammalian cell and tissue extracts (Sigma-Aldrich). Subsequently the samples were centrifuged at 11,828*g* at 4 °C for 15 min to purify the lysates from the residual cell