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Cryopreservation of bull sperm: Effects of extender supplemented with different cryoprotectants and antioxidants on sperm motility, antioxidant capacity and fertility results

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ABSTRACT

The objectives of this study were to compare glycerol (G) and ethylene glycol (EG) at different concentrations and trehalose (T) or cysteine (C; with/without) in Tris extender for cryopreservation of bull semen. Twenty-four ejaculates obtained from three bulls were included in the study. Each ejaculate was divided into four equal aliquots and diluted using both of the Tris extenders with G (5% or 7%) or EG (3% or 5%). After that, each extenders were divided into three equal aliquots and diluted using both of the 5 mM C or 25 mM T, and control (without additives) was cooled to 4 °C and frozen in 0.25 ml French straws. The addition of 3% and 5% EG without antioxidants resulted in the least Computer-Assisted Sperm motility Analysis (CASA) motility as compared with the other groups. Treatment with 25 mM T in 3% EG beneficially effected acrosome morphology as compared with the other groups. Also, treatment with 3% EG with 25 mM T and 5% EG resulted in a greater rate of total abnormalities. Treatment with 3% G yielded a slightly greater percentage of membrane integrity by Hypo-Osmotic Swelling Test (HOST) assessment than that of the other groups. Treatment with 3% EG with 5 mM C resulted in the greatest concentration of malondialdehyde (MDA). The glutathione peroxidase (GPx) antioxidant activity was increased in the C-treatment groups when compared to the other groups. Treatment with 5% EG and 5 mM C resulted in less chromatin damage and detrimental impacts on tail moment. Treatment with 5% EG led to greater non-return rates of inseminated cows. However, this result was not considered to be statistically important.

1. Introduction

Artificial insemination (AI) is the first generation biotechnological advancement that has made a profound contribution to the genetic improvement, particularly in dairy bulls through which a single ejaculate from males

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is used for insemination of multiple females. This impact could not have been possible without long standing freezing of bull semen. Freezing–thawing processes lead to the generation of reactive oxygen species (ROS) that impair sperm motility, membrane integrity, and fertilizing potential (Hu et al., 2010). The success of cryopreservation depends not only on preserving the motility of the spermatozoa but also on maintaining their metabolic function (Watson, 2000). The major factor affecting the results of insemination with frozen–thawed semen is the addition of cryoprotectants and spermatozoal damage due to the formation of internal ice crystals due to the increase in solute concentration in the extension media or interaction of both physical factors (Aboagla and Trade, 2004; El-Harairy et al., 2011). The cryoprotectants are added to extenders to protect the sperm from damage during freezing process (Singh et al., 1995). The amount and type of cryoprotectants in semen diluent influence these events and their effects on the sperm cells during freezing (El-Harairy et al., 2011). Glycerol (G) is the most widely used cryoprotectant for bull sperm because it reduces the mechanical damage to spermatozoa during the freezing process (De Leeuw et al., 1995). Specifically, when used in greater than typical concentrations, G can cause great osmotic damage to spermatozoa because G passes through the sperm membrane much slower than other cryoprotectants (Garner, 1991; Guthrie et al., 2002). Other researchers have surmised through previous studies that a low molecular weight cryoprotectant, such as EG, may cause less damage to spermatozoa than when G is used because its low molecular weight allows it to cross the plasma membrane more easily (Moore et al., 2006). In recent years, G and EG have been used as cryoprotectant in bulls (Tasdemir et al., 2013) and goats (Tuncer et al., 2013). Cryopreservation reduces the functional and structural integrity of bull spermatozoa, and is associated with reactive oxygen species (ROS) production. Oxidative stress during freezing of mammalian semen can cause functional and structural damage to spermatozoa involving ROS-mediated pathways (Baumber et al., 2005). Although bull semen has a natural defense system against the ROS, it is considered insufficient in protecting spermatozoa under cryopreservation mediated stress (Nichi et al., 2006). Therefore, the addition to semen extenders of suitable antioxidants is suggested to reduce oxidative damage during freeze–thawing of bull spermatozoa (Ansari et al., 2011).

Cysteine (C) is a non-essential amino acid, scavenger of free radicals and precursor molecule of glutathione and enhances intracellular glutathione production both *in vivo* and *in vitro* (Malo et al., 2010; Tuncer et al., 2010) and to prevent hydrogen peroxide-mediated loss of sperm motility in bulls (Bilodeau et al., 2001). Additionally mammalian cells can only utilize C, which has been shown to penetrate the cell membrane easily (Sarıözkan et al., 2009a,b). In recent years, C has been used as an antioxidant in the cryopreservation of boar (Taylor et al., 2009), bull (Tuncer et al., 2010) and dog sperm (Michael et al., 2007). Sugar maintains the osmotic pressure of the diluents by inducing cell dehydration and less ice crystal formation into the spermatozoa (Purdy, 2006).

Sugar is utilized by spermatozoa as an energy source through glycolysis and mitochondrial oxidative phosphorylation to support sperm motility and movement (Naing et al., 2010). Many researchers have studied the effect of sugar supplementation in semen extender on the quality of cryopreserved spermatozoa. Trehalose (T) is a nonreducing disaccharide which is able to protect the integrity of cells against a variety of environmental stresses such as dehydration, heat, cold and oxidation (Chen and Haddad, 2004). Furthermore, T had been extensively used to improve sperm quality variables in semen cryopreservation and its protective effects improved the freezing capacity of goat spermatozoa due to increase in membrane fluidity resulting from the depression of membrane transition temperature, allowing the sperm membrane to tolerate low-temperature effects (Tasdemir et al., 2013; Tuncer et al., 2013). The extender containing T improved antioxidant action and reduced the oxidative stress induced by cryopreservation (Aisen et al., 2005; Hu et al., 2010).

The objectives of the present study were to compare G and EG at different concentrations as cryo-protectants and T or C (with/without) as antioxidants in Tris extender for cryopreservation of bull semen and to compare microscopic sperm variables (motility, acrosome and total abnormalities, HOS test, sperm motion characteristics), concentrations of malondialdehyde (MDA) and glutathione peroxidase (GPx) and DNA integrity of frozen–thawed semen. Conception rates of the cows artificially inseminated with frozen–thawed semen were also assessed.

2. Materials and methods

2.1. Animals, semen collection and semen processing

Three Holstein bulls (3 or 4 years of age) were housed at the Lalahan Livestock Central Research Institute (Ankara, Turkey), and maintained under uniform feeding and housing conditions. A total number of 24 ejaculates were collected from the bulls by using of an artificial vagina twice a week, according to AI standard procedures. The volume of ejaculates was measured in a conical tube graduated at 0.1 ml intervals and sperm concentration was determined by means of an Accucell photometer (IMV, L'Aigle, France). Sperm motility was estimated using phase-contrast microscopy (200×). The ejaculates containing spermatozoa with >80% forward progressive motility and concentrations greater than 1.0×10^9 spermatozoa/ml were used in the study. Immediately after collection, the ejaculates were immersed in a warm water bath at 35 °C until their assessment. A Tris-based (T) extender (189.5 mM of Tris, 63.2 mM of citric acid, 55.5 mM of fructose, 20% (v/v) egg yolk, and 1000 ml of distilled water at a pH of 6.8) was used as the base for the experimental extenders. Each ejaculate was divided into four equal aliquots and diluted using both of the T extenders with G (5% or 7%) or EG (3% or 5%). Subsequently, each of the extenders was divided into three equal aliquots and diluted using both of 5 mM C or 25 mM T, and control (without additives) to a final concentration of approximately 60×10^6 spermatozoa per ml (15×10^6 total spermatozoa in each 0.25 ml straw). Diluted semen samples were cooled to 4 °C in 4 h. Subsequently semen was