Small Ruminant Research 113 (2013) 383–389

Contents lists available at SciVerse ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Effects of different doses of trehalose supplementation in egg yolk extender in frozen–thawed Angora buck semen

Pürhan Barbaros Tuncer[a,*], Umut Taşdemir[b], Serhat Büyükleblebici[c], Taner Özgürtaş[d], Erdem Coşkun[e], Halil Erol[a], Fevzi Nuri Aydın[d], İsmail Safa Gürcan[f]

[a] Livestock Central Research Institute, Lalahan, Ankara, Turkey
[b] General Director of Food and Control, Ankara, Turkey
[c] Aksaray Technical Sciences Vocational School, Aksaray, Turkey
[d] Biochemistry and Clinical Biochemistry Laboratory, Gulhane Military Medical Academy, Ankara, Turkey
[e] Department of Toxicology, Faculty of Pharmacy, Gazi University, Ankara, Turkey
[f] Department of Biostatistics, Faculty of Veterinary, Ankara University, Ankara, Turkey

ARTICLE INFO

Article history:
Received 6 August 2012
Received in revised form 8 March 2013
Accepted 25 April 2013
Available online 23 May 2013

Keywords:
Angora buck
CASA parameters
COMET test
Cryopreservation
Oxidative stress
Trehalose

ABSTRACT

Few studies have been done on the effects of trehalose supplementation in the cryopreservation of Angora buck semen. The objective of present study was to investigate the effects of the addition of trehalose at different doses in semen extenders, on in vitro semen quality parameters, anti-oxidant enzymes activities and DNA damage after the freeze–thaw process in Angora buck semen. Semen samples from 5 mature Angora bucks (3 and 4 years of age) were used in this study. The bucks, belonging to the Livestock Central Research Institute were maintained under uniform breeding condition. A total number of 40 ejaculates were collected twice a week from the bucks using an artificial vagina, during the breeding season and the semen pooled to minimize individual variation. Each pooled ejaculate was split into 7 equal aliquots and diluted (37 °C) with base extenders supplemented with the trehalose (12.5, 25, 50, 75, 100 and 150 mM), and a base extender with no additives (control). Diluted samples were aspirated into 0.25 ml French straws, and equilibrated at 5 °C for 4 h and then were frozen at a digital freezing machine. The freezing extender supplemented with 50 mM trehalose led to the greatest percentages of CASA motility (53.6 ± 4.69), in comparison to the other groups after the freeze–thawing process ($P < 0.05$). The addition of different doses of trehalose did not provide any significant effect on the percentages of post-thaw sperm motion characteristics (VAP, VSL and LIN), compared to the control ($P > 0.05$). The freezing extender with 150 mM trehalose group led to the highest percentages of acrosome abnormalities ($P < 0.05$) and 50 mM trehalose group had the lowest percentages of total abnormalities ($P < 0.001$), in comparison to the others. There were no significance differences in the DNA integrity among treatment groups ($P > 0.05$). The different doses of trehalose did not show any effectiveness on the maintenance GPx, LPO, GSH, CAT and total antioxidant activities, when compared to the control ($P > 0.05$). Therefore, the additions of 50 mM and 75 mM doses of trehalose will be useful in increasing post thaw motility on Angora buck semen.

1. Introduction

The cryopreservation of mammalian sperm is a complex process that involves many factors in order to produce satisfactory results (Purdy, 2006). Cooling and freeze–thawing

* Corresponding author at: Ministry of Food, Agriculture and Livestock, Livestock Central Research Institute, Lalahan, Ankara, Turkey.
Tel.: +90 532 350 39 46.
E-mail address: barbarostuncerp@hotmail.com (P.B. Tuncer).

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http://dx.doi.org/10.1016/j.smallrumres.2013.04.012

produce physical, chemical and oxidative stress on the sperm membrane, which result in reduced sperm viability and fertilizing ability (Evans and Maxwell, 1987). Cold shock in sperm is also generally associated with oxidative stress and the generation of reactive oxygen species (ROS), by dead sperm and atmospheric or molecular oxygen in the environment. Oxidative stress is a cellular condition generally characterized by an imbalance between the production of ROS and the scavenging capacity of the antioxidants. When the production of ROS exceeds the available antioxidant defence system, significant oxidative damage occurs to the sperm organelles through the damage of lipids, proteins and DNA (Gomez et al., 1996; Bucak and Uysal, 2008; Bucak et al., 2009).

Sugar maintains the osmotic pressure of the diluents by inducing cell dehydration and less ice crystal formation into the spermatozoa (Leibo and Songsasen, 2002; Purdy, 2006). Moreover, sugar has the ability to form a glass (vitrification) by depressing the membrane phase transition temperature of dry lipids. It also interacts with phospholipid membranes at low hydration and thus causes stabilization of the membranes (Aisen et al., 2002). Furthermore, 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. Glucose was suggested to be more suitable sugar than fructose, lactose or raffinose in Tris-based extender in ram sperm (Salamon and Visser, 1972). Trehalose is a nonreducing disaccharide in which the two glucose units are linked in an a,a1,1-glycosidic linkage. Trehalose 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). It had the remarkable stabilizing properties due to the formation of a nonhygroscopic glass state and protected protein and lipids membranes from degradation during the freeze–drying process. Furthermore, trehalose had been extensively used to improve sperm quality parameters in semen cryopreservation and its protective effects significantly improved the freezability 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 (Aboagla and Terada, 2003; Hu et al., 2010). The extender containing trehalose improved antioxidant action and reduced the oxidative stress provoked by cryopreservation (Aisen et al., 2005). Antioxidant treatment with trehalose significantly elevated and improved post-thawed ram sperm motility (Bucak et al., 2007). Comet assay (Single Cell Gel Electrophoresis – SCGE) is one of the most popular cytogenetic methods to detect the DNA damage in a single cell. First published in 1980s as a method using micro-gel electrophoresis of immobilized cells lysed at high salt concentrations, which had been embedded in agarose. When an electrophoretic field applied with pH conditions less than pH 10, tails were observed where the damaged DNA migrated faster than the nuclear DNA (Ostling and Johanson, 1984). High alkaline conditions (pH > 13) for DNA unwinding and electrophoresis were incorporated later (Singh et al., 1988) and allowed the detection of single and double strand breaks as well as alkali-labile sites. Thus the alkaline COMET assay can provide a comprehensive measure of DNA damage (Lewis et al., 2008).

A few studies have been done on the effects of trehalose supplementation in the cryopreservation of Angora buck semen. Thus, the objective of present study was to investigate the effects of the addition of trehalose at different doses, on in vitro semen quality, anti-oxidant enzymes activities [glutathione (GSH), lipid peroxidase (LPO), glutathione peroxidase (GPx), catalase (CAT), total antioxidant] and DNA damage after the freeze–thaw process in Angora buck semen.

2. Material and methods

2.1. Chemicals

All chemicals used in this study were obtained from Sigma–Aldrich Chemical Co. (Interlab Ltd., Ankara, Turkey).

2.2. Animals and semen collection

Semen samples from 5 Angora bucks (3 and 4 years of age), were used in this study. The bucks, belonging to the Livestock Central Research Institute (39°58′ 23.56″ N, 33°06″ 28.51″ E) were maintained under uniform breeding conditions. They were housed in a dirt lot with an in door feeding area. Bucks received a mixed ration balanced to meet minimum nutritional requirements according to NRC (National Research Council 2001) and had free access to water. A total number of 40 ejaculates were collected at the morning time (9:00 am) twice a week intervals from the bucks using an artificial vagina, during the breeding season (autumn to early winter during two months) and the semen pooled to minimize individual variation. Ejaculates which met the following criteria were evaluated: volume of 0.5–2 ml; minimum sperm concentration of 3×10^9 sperm/ml; motility of 80%. Immediately following collection, the ejaculates were placed in a water bath (35 °C), until evaluation in the laboratory. Semen assessment was performed within approximately 10 min following collection. Each group was replicated eight times. The experimental procedures were approved by the Animal Care Committee of Lalahan Livestock Central Research Institute.

2.3. Semen processing

A Tris-based extender (Tris 254 mM, citric acid 78 mM, fructose 70 mM, egg yolk 15% (v/v), glycerol 5% (v/v), pH 6.8) was used as the base extender. Each pooled ejaculate was split into 7 equal aliquots and diluted with base extenders supplemented with the trehalose (12.5, 25, 50, 75, 100 and 150 mM), and a base extender with no additives (control) for a total of 7 experimental groups to a final concentration of 200×10^6 spermatozoa/ml (single step dilution), in a 15 ml plastic centrifuge tube. Actual sperm concentrations were calculated with the aid of a haemocytometer (Smith and Mayer, 1955). Diluted samples were filled in 0.25 ml French straws, and equilibrated at 5 °C for a period of 4 h. and then were frozen at a programmed rate of −3 °C/min from +4 to −10 °C; −40 °C/min from −10 to −100 °C; −20 °C/min from −100 to −140 °C using a digital freezing machine (Digitcool 5300 ZB 250, IMV, France). After being stored for at least 24 h, straws were thawed individually (37 °C), for 30 s in a water bath for sperm evaluation. Sperm evaluation was performed on all semen samples immediately after thawing.

2.4. Semen evaluation

2.4.1. Analysis of standard semen parameters

Progressive motility as an indicator of semen quality was assessed using a phase-contrast microscope (×100 magnification), fitted with a warm stage maintained at 37 °C (Bearden and Fuquay, 2000). Sperm motility estimations were performed in 3 different microscopic fields for each semen sample and the mean of the 3 successive estimations recorded as the final motility score. Besides recording the subjective sperm motility, a computer-assisted sperm motility analysis (CASA, Version 12 IVOS,