Animal Reproduction Science 139 (2013) 38–44

Contents lists available at SciVerse ScienceDirect

Animal Reproduction Science

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

Comparison of cryoprotective effects of iodixanol, trehalose and cysteamine on ram semen

Ümüt Cirit[a,*], Haydar Bağış[b], Kamber Demir[d], Cansu Agca[c], Serhat Pabuccuoğlu[d], Ömer Varışlı[e], Charlotte Clifford-Rathert[f], Yuksel Agca[c,**]

[a] Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Dicle University, 21280, Diyarbakir, Turkey
[b] Adiyaman University, Faculty of Medicine, Department of Medical Genetics, Adiyaman, Turkey
[c] Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, 1600 East Rollins Street, Columbia, MO 65211, United States
[d] Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Istanbul University, 34320, Avcilar, Istanbul, Turkey
[e] Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Harran University, Sanliurfa, Turkey
[f] Lincoln University of Missouri, Cooperative Extension and Research, Jefferson City, MO 65101, United States

ARTICLE INFO

Article history:
Received 29 May 2012
Received in revised form 18 March 2013
Accepted 21 March 2013
Available online 30 March 2013

Keywords:
Ram
Electro-ejaculated semen
Iodixanol
Trehalose
Cysteamine

ABSTRACT

This study was conducted to improve cryosurvival of electroejaculated (EE) ram semen in the presence of iodixanol (OptiPrep™), trehalose or cysteamine. A tris-based extender was used to prepare 12 extenders containing OptiPrep™ (Op), trehalose (Tr) or cysteamine (Cy) alone, or different combinations of these compounds. Extenders were designated as follows: Tris (control), Op1.25 (1.25% Op, v/v), Op2.5 (2.5% Op, v/v), Op5 (5% Op, v/v), Tr50 (50 mM Tr), Tr100 (100 mM Tr), Cy (5 mM Cy), OpTr (2.5% Op and 100 mM Tr), OpCy (2.5% Op and 5 mM Cy), TrCy (100 mM Tr and 5 mM Cy), OpTrCy1 (2.5% Op, 100 mM Tr and 5 mM Cy) and OpTrCy2 (1.25% Op, 50 mM Tr and 2.5 mM Cy).

A two-step dilution was used and glycerol was added at 5 °C in the second step. Diluted samples were equilibrated for 1 h, loaded in 0.25 mL straws and frozen in a programmable freezing machine. Supplementation of 5% OptiPrep™ significantly protected post-thaw progressive motility, membrane integrity, acrosomal integrity and morphological damages. Trehalose supplementation protected membrane integrity of ram sperm; however, it did not help post-thaw motility and morphology. Supplementation of 5 mM cysteamine had detrimental effect on cryosurvival of EE ram semen. These results demonstrate that the supplementation of iodixanol increases the cryosurvival of EE ram semen in a dose-dependent manner.

1. Introduction

Commercial semen production centers play important role on extensive use of AI. However, semen collection from large numbers of untrained rams results in complications in sperm bank establishments (Marco-Jiménez et al., 2005). Many rams refuse to serve a ewe that is not in estrus even after considerable training and nearly 50% of the rams refuse to serve a "dummy" ewe (Terrill, 1940). Thus, electroejaculation could be a useful alternative, which eliminates the need for ram training (Foote, 2002). However, electroejaculation is known to alter semen characteristics (Quinn and White, 1966; Lightfoot, 1968; Marco-Jiménez et al., 2005), diminishes semen quality

* Corresponding author. Tel.: +90 412 248 80 20 8679; fax: +90 412 248 80 21.
** Corresponding author. Tel.: +1 573 884 0311; fax: +1 573 884 7521.
E-mail addresses: ucirit@dicle.edu.tr, ucirit@istanbul.edu.tr (Ü. Cirit), agcay@missouri.edu (Y. Agca).

0378-4320/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.anireprosci.2013.03.010

(Mattner and Voglmayr, 1962) and decreases pregnancy rate (Hulet et al., 1964). On the other hand, spermatozoa collected with AV were more resistant to cold shock compared to spermatozoa ejaculated electrically (Quinn et al., 1968).

We hypothesize that if a newly developed semen diluent be successful for freezing of electroejaculated (EE) ram semen, the diluent will likely have a greater probability of being successful for freezing of semen collected by use of an artificial vagina (AV). Therefore, semen was collected by ES in the present study.

Recently, it has been demonstrated that iodixanol helps maintaining membrane integrity and functionality during the cryopreservation process when added to a freezing extender and contributes to preserving sperm motility after the freeze-thaw cycle in bull semen (Saragusty et al., 2009).

The exact mechanism by which iodixanol protect spermatozoa during cryopreservation is not clearly understood. However, Saragusty et al. (2009) suggest that iodixanol appears to protect sperm membranes and preserve their motility throughout the processes of freezing and thawing, at least in part, by two possible mechanisms. They have demonstrated that its addition elevates the glass transition temperature, an effect that may become significant at lesser temperatures when the iodixanol concentration in the unfrozen fraction increases by the removal of water from the solution. They have also showed that iodixanol alters ice crystal formation in a non-colligative way, resulting in a more sperm-friendly environment (Saragusty et al., 2009).

To our knowledge, the use of iodixanol for cryopreservation of ram semen has not been reported. One of the aims in the present study was to investigate the effects of adding varying concentrations of iodixanol to the freezing extender on spermatological traits before and after freezing.

Addition of trehalose to semen extenders containing glycerol increases cryosurvival of ram semen collected by artificial vagina (Aisen et al., 2002; Matsuoka et al., 2006; Berlinguer et al., 2007; Bucak et al., 2007; Tonieto et al., 2010; Jafaroghli et al., 2011; Quan et al., 2012). However, trehalose solely could not protect spermatozoa against the adverse effects of freezing in extenders which do not contain glycerol (Soylu et al., 2007; Nur et al., 2010) or extenders containing less (4.5%) egg yolk (Valente et al., 2010). Furthermore there have been very few reports that investigated the effects of trehalose addition to semen extenders which contain glycerol on EE ram semen (Yamashiro et al., 2011; Quan et al., 2012), and the optimum concentration of trehalose has not been established.

Cysteamine enhanced post-thaw motility by elevating the antioxidant capacity of ram (Bucak et al., 2007) and goat (Bucak et al., 2009) semen collected by AV. To our knowledge, the use of cysteamine and/or the combination of trehalose and cysteamine in the cryopreservation of EE ram semen has not been reported.

Although several research groups have developed different extenders and protocols for freezing ram semen, overall fertility results were not comparable to those obtained with fresh semen and natural mating (Nur et al., 2010). Thus, development of better freezing extenders is crucial for enhancing pregnancies following AI in sheep (Aisen et al., 2000). The main aim of this study was to develop new extender compositions for freezing of electroejaculated ram semen.

2. Materials and methods

2.1. Extender preparation

All chemicals were purchased from Sigma Chemical Co. (Saint Louis, MO) unless otherwise noted. OptiPrep™ (60% w/v iodixanol in water; Axis-Shield PoC AS, Oslo, Norway) was used as a source of iodixanol. A tris-based extender (tris 27.1 g/L, citric acid 14 g/L, fructose 10 g/L, egg yolk 15% (v/v), pH 6.8) was used as the base extender. The base extender without glycerol were divided into 12 equal parts and 12 different freezing extenders were prepared by the addition of OptiPrep™ (60% iodixanol in water), trehalose or cysteamine, or different combinations of these substances. Extenders were designated as follows: Tris (control), Op1.25 (1.25% OptiPrep™, v/v), Op2.5 (2.5% OptiPrep™, v/v), Op5 (5% OptiPrep™, v/v), Tr50 (50 mM trehalose), Tr100 (100 mM trehalose), Cy (5 mM cysteamine), OpTr (2.5% OptiPrep™ and 100 mM trehalose), OpCy (2.5% OptiPrep™ and 5 mM cysteamine), TrCy (100 mM trehalose and 5 mM cysteamine), OpTrCy1 (2.5% OptiPrep™, 100 mM trehalose and 5 mM cysteamine) and OpTrCy2 (1.25% OptiPrep™, 50 mM trehalose and 2.5 mM cysteamine). Each of these 12 extenders was divided into two parts and described as Fraction A and B. Then 10% glycerol (v/v) was added into each of the Fractions B, so final glycerol concentration was 5%. Average osmolarity values of Tris (control), Op1.25, Op2.5, Op5, Tr50, Tr100, Cy, OpTr, OpCy, TrCy, OpTrCy1 and OpTrCy2 extenders were 291, 313, 312, 319, 365, 426, 318, 431, 325, 436, 437 and 360 mOsm/kg for Fractions A (the parts not contain glycerol) and were 1713, 1753, 1759, 1768, 1802, 1883, 1725, 1886, 1761, 1890, 1912, 1692 mOsm/kg for Fractions B (the parts with glycerol), respectively.

2.2. Collection and dilution of the semen

Two to five years old Dorset rams ($n = 4$) were housed at the Lincoln University sheep research farm and used during the studies. Semen collection and freezing parts of the study lasted approximately two weeks. To collect semen, rams were adequately restrained physically, the prepuce was cleaned to remove debris and a lubricated probe was inserted into the rectum (Nur et al., 2010). An electroejaculator (Lane manufacturing Inc., Denver, CO, USA) was used implementing a stimulation interval of 5 s. Semen samples were obtained from all rams following a maximum of 3 or 4 electrical stimuli. Ejaculated semen was collected in pre-warmed 50 mL plastic conical tubes during out of season (May and June). The ejaculate was kept in an insulated Styrofoam box containing warm heat pads (30 °C) and transported to the laboratory within 50 min of collection. To eliminate individual differences, semen samples of 4 rams were pooled. Only ejaculates of good