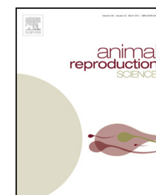
Animal Reproduction Science 138 (2013) 213–219

Contents lists available at SciVerse ScienceDirect

Animal Reproduction Science

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



Function of ram spermatozoa frozen in diluents supplemented with casein and vegetable oils

I. Del Valle[a,*], A. Souter[b], W.M.C. Maxwell[b], T. Muiño-Blanco[a], J.A. Cebrián-Pérez[a]

[a] *Departamento de Bioquímica y Biología Molecular y Celular, Facultad de Veterinaria, Instituto Universitario de Investigación en Ciencias Ambientales de Aragón (IUCA), Zaragoza, Spain*
[b] *Faculty of Veterinary Science, University of Sydney, Sydney, Australia*

ARTICLE INFO

Article history:
Received 15 October 2012
Received in revised form 21 February 2013
Accepted 27 February 2013
Available online 14 March 2013

Keywords:
Ram sperm
FITC-PNA
Palm oil
Coconut oil
Casein
Cryopreservation

ABSTRACT

The aim of this study was to assess biologically safer components as alternatives to egg yolk for the frozen storage of ram semen using casein, coconut or palm oil in either Salamon's diluent (S) or a swim-up medium (SU). Ejaculates were frozen as pellets and sperm motility (subjectively) and acrosome integrity (FITC-PNA/PI) by flow cytometry were assessed at 0, 3 and 6 h after thawing and incubation at 37 °C. Three experiments were done: different concentrations of palm oil (5%, 10% and 20%); casein added as emulsifier and protective agent; and differences between egg yolk, coconut and palm oil in S and SU. 20% of oil added to SU accounted for a lesser percentage ($P < 0.05$) of motile cells compared to rest while no differences were found between different oil levels on viable cells. When casein was added to diluents containing 5% of palm oil, no differences were found between palm or casein ($P > 0.05$). No differences were found when S and SU were compared neither as groups nor between S alone and containing coconut or palm oil; however, SU alone yielded less motility than SU 5% coconut. However, in both groups, S and SU, egg yolk accounted for the greatest values in both bases. These results indicate that none of biologically safer media components (casein, palm or coconut oil) used in this study maintained the function of ram spermatozoa after freeze-thawing better than S-containing egg yolk. The application of vegetable oils as substitutes for egg yolk in diluents for the cryopreservation of ram spermatozoa requires further research.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Despite the widespread commercial application of artificial insemination with frozen semen in sheep (Salamon and Maxwell, 2000), fertility after cervical insemination of ewes with frozen–thawed semen remains variable and problematic. Differences in fertility between rams and among ejaculates within a ram might be attributed to differences in seminal plasma components among individual males that compromise both viability and fertility (O'Meara et al., 2005). Furthermore, lambing results with frozen–thawed ram semen similar to those with fresh semen have only rarely been obtained after cervical insemination (Salamon and Maxwell, 2000).

There have been few improvements in diluent components for freezing ram semen since the routine incorporation of egg yolk or milk with glycerol as agents to protect the spermatozoa during cooling and freezing, respectively. The two diluents most widely used for prefreezing dilution of ram semen are based on Tris, fructose, egg yolk and glycerol (Visser and Salamon, 1973) or lactose,

* Corresponding author. Current address: Department of Human Metabolism, Academic Unit of Reproductive & Developmental Medicine, Level 4, The Jessop Wing, Tree Root Walk, Sheffield S10 2SF, UK. Tel.: +44 01142261072.
E-mail addresses: i.delvalle@sheffield.ac.uk, mejizebra@yahoo.es (I. Del Valle).

0378-4320/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.anireprosci.2013.02.022

milk and egg yolk (Blackshaw, 1960). Two of these additives, egg yolk and milk, are of animal origin, and concerns have been raised in recent years about their potential risk as vehicles for disease-causing pathogens (Marco-Jimenez et al., 2004). Furthermore, frozen–thawed sperm quality may also be influenced by individual quality differences inherent in egg yolk due to the numbers of days after laying and the storage period (Fukui et al., 2008) that makes it difficult to analyze the beneficial effects of a particular extender. Such concerns have developed alongside a recession in the sheep industry in Spain (MAPA, 2006). These factors have contributed to a need to develop new extenders for freezing ram semen that avoid the use of animal products, which must be inexpensive and achieve at least the same post-thaw sperm quality as the standard diluents.

Egg yolk is the main animal-derived component of the Tris-based diluent which protects spermatozoa from cold shock damage during cooling (Watson, 1981; Holt et al., 1992). The main constituent of egg yolk responsible for this protective effect is the low density lipoprotein fraction (Watson, 1976; Bergeron and Manjunath, 2006). To minimize the risk of animal disease transmission, egg yolk has been replaced with vegetable lipid sources such as lecithin (Aires et al., 2003). The use of a soybean lecithin-containing diluents for bull semen cryopreservation resulted in greater insemination success compared with the egg yolk-containing extender (Aires et al., 2003). Studies with coconut water as cryo-protector for ram semen gave appropriate results of sperm quality (Gutierrez et al., 2006). The major protein of milk, casein, is a component of milk extenders that it could act as an emulsifier (Dickinson, 1989). Casein micelles can protect stallion, goat, ram, and bull sperm during storage at low temperature (Martin, 1966; Batellier et al., 1997; Leboeuf et al., 2003) and bull sperm during freezing in a medium with glycerol (Choong and Wales, 1963). The protective action of milk on sperm storage at low temperature is analogous to the protective action of egg yolk, and it has been suggested that it may involve a BSP protein-casein micelle (protein–protein) interaction (Bergeron et al., 2007).

Based on these observations, the hypothesis was investigated that vegetable lipids and casein could serve as alternatives to egg yolk for the frozen storage of ram semen. The effect was examined of casein and two oils, coconut oil, derived from the kernel of the coconut (*Cocos nucifera*), and palm oil derived from the fleshy mesocarp of the fruit of the oil palm (*Elaeis guineensis*), which have been widely used in the nutrition industry and have similar qualitative fatty acid composition (FAO, 1996). Moreover, because ice crystals are one of the main causes of cryo-damage to spermatozoa, some of the water in the freezing media was replaced with these oils to interfere with regular ice crystal formation, as oil in water emulsion promotes irregular ice crystal formation (Andreeva et al., 2008).

2. Materials and methods

2.1. Reagents and media

Stains for determining the acrosome status of spermatozoa using FITC/PNA and Propidium Iodide and other general chemicals were purchased from Sigma Chemical Co (St. Louis, MO). Coconut oil and palm oil were purchased from Spectrum Chemicals & Laboratory Products (Gardena, CA).

The media used in the experiments were Salamon's medium (TRIS 299.75 mM, glucose 27.75 mM, citric acid 94.73 mM, 20% egg yolk and 5% glycerol) (Salamon, 1977) and modified swim-up medium (García-López et al., 1996) containing NaCl 50 mM, KCl 10 mM, $MgSO_4$ 0.4 mM, K_2HPO_4 0.3 mM, HEPES 21 mM, glucose 2.7 mM, piruvic acid 10 mM, sodium L-lactate 18.65 mM, sucrose 200 mM and 5% glycerol. These media were used alone or with different additives such as coconut oil, palm oil, casein or egg yolk. Media containing oil were sonicated for at least 3 min (60%, 6 limit) before use. Androhep (AH; Minitüb, Tiefenbach, Landshut, Germany) with modified pH for ram (pH 7.3, with NaOH 1 M) was used for motility and acrosome integrity assessments.

2.2. Semen collection and freeze-thawing procedure

Semen was collected by artificial vagina from three Merino rams aged 2–5 years during reproductive season at the University of Sydney, Camperdown, NSW. Two ejaculates were successively collected and pooled from each male. The semen from individual rams was kept separate according to the procedure usually used in the AI centres. Semen was diluted (1:5) with different media at 30 °C immediately after collection and assessed for motility and acrosome integrity of spermatozoa, then cooled slowly to 5 °C over 1.5 h and frozen following the pellet method described by Evans and Maxwell (1987). Briefly, 250 μl of cooled semen were pipetted on a block of dry ice to form one pellet. When pellets were frozen, they were submerged into liquid nitrogen. The frozen pellets were stored in liquid nitrogen and the studies were carried out within 1 week after freezing. Pellets were thawed in dry glass tubes (37 °C) and immediately diluted (1:1) with modified AH to follow a 6 h incubation at 37 °C. Sperm quality parameters were assessed before freezing (fresh diluted sample before cooling), immediately after thawing (0 h) and following incubation for 3 and 6 h in a water bath at 37 °C.

2.3. Experimental design and trials

A series of in vitro experiments were performed to compare important functional sperm parameters immediately after thawing and at several time points after incubation at 37 °C, to mimic the temperature in the female reproductive tract. A swim-up modified medium (SU) (García-López et al., 1996) and the standard Salamon's medium for ram semen cryopreservation (S) (Salamon, 1977) were comparatively assessed.

2.3.1. Experiment 1

Effect of increasing palm oil concentration (0%, 5%, 10% and 20%) in SU related to SU as control and Salamon's with 20% egg yolk (SY) as aimed goal. All media contained 5% glycerol and their osmolality was 340 mOsm/kg (before addition of glycerol). The oil was emulsified mechanically by sonication, avoiding the need for any other emulsifier in the medium. The media was supplemented as follows:

Download English Version:

https://daneshyari.com/en/article/8405124

Download Persian Version:

https://daneshyari.com/article/8405124

Daneshyari.com