Animal Reproduction Science 118 (2010) 231–235

Contents lists available at ScienceDirect

Animal Reproduction Science

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

The fertility of ram sperm held for 24 h at 5 °C prior to cryopreservation☆

Phillip H. Purdy[a,*], Eva Mocé[b], Robert Stobart[c], William J. Murdoch[c], Gary E. Moss[c], Brent Larson[c], Shawn Ramsey[d], James K. Graham[e], Harvey D. Blackburn[a]

[a] USDA-ARS-NCGRP, National Animal Germplasm Program, 1111 S. Mason St., Fort Collins, CO 80521-4500, USA
[b] Instituto Valenciano de Investigaciones Agrarias-Centro de Tecnología Animal (IVIA-CITA), Polígono La Esperanza, n° 100, 12400-Segorbe (Castellón), Spain
[c] Department of Animal Science, University of Wyoming, Laramie, WY 82071-3684, USA
[d] Department of Animal Science, Texas A&M University, College Station, TX 77843-2471, USA
[e] Department of Biomedical Sciences, Colorado State University, Fort Collins, CO 80523, USA

ARTICLE INFO

Article history:
Received 5 August 2008
Received in revised form 18 May 2009
Accepted 18 June 2009
Available online 26 June 2009

Keywords:
Fertility
Holding time
Ram
Sperm
Artificial insemination

ABSTRACT

Diluted ram sperm can be held for 24 h at 5 °C prior to cryopreservation without impacting cryosurvival rates, however, the effects this storage has on subsequent fertility are unknown. These studies were conducted to evaluate the fertility of semen held for 24 h (to mimic shipping semen to a cryopreservation center), prior to freezing. Semen from Suffolk rams ($n = 3$ in experiment 1 and $n = 6$ in experiment 2) with initial motility of greater than 70%, were diluted to 200×10^6 sperm/mL, in one step, with a Tris–egg yolk–glycerol diluent. In experiment 1, diluted samples were cooled to 5 °C over 2 h, and then divided. Sperm in one fraction were loaded into 0.5 mL straws, frozen (T0) and stored in liquid nitrogen until thawing. Sperm in the second fraction were held at 5 °C for 24 h (T24) before being frozen. In experiment 2 ejaculates were collected and divided into two fractions. Sperm in one fraction were treated with cholesterol-loaded cyclodextrin (CLC) and sperm in the other served as control. Both fractions were diluted, cooled, and cryopreserved as described in experiment 1. Stage of the estrous cycle was synchronized in ewes ($n = 196$) using controlled internal drug releasing devices (CIDR) for 12 d and at CIDR removal each ewe was administered PMSG (500 IU in experiment 1 and 350 IU in experiment 2) immediately before insemination. Ewes were stratified by age and randomly assigned to one of the semen treatments; experiment 1: Fresh (F), T0, or T24; experiment 2: F, T24, or CLC, and inseminated laparoscopically 56 h after CIDR removal. Differences in fertility were detected between experiments, but not for treatments within experiments. Differences in fertility were also observed due to ewe age, with the 3-year-old ewes having the greatest fertility (50.7%) and 6-year-old ewes having the least fertility (9.6%; $P < 0.05$). Differences in the prolificacy rates due to semen treatment were also observed but differences due to ewe age were not detected. Therefore, sperm can be held at 5 °C for 24 h prior to cryopreservation without altering sperm fertility.

Published by Elsevier B.V.

☆ Mention of a trade name or proprietary product does not constitute a guaranty or warranty by the USDA and does not imply approval to the exclusion of other products that may be suitable. USDA, Agricultural Research Service, Northern Plains Area, is an equal opportunity/affirmative action employer. All agency services are available without discrimination.

* Corresponding author. Tel.: +1 970 495 3258; fax: +1 970 221 1427.
E-mail address: phil.purdy@ars.usda.gov (P.H. Purdy).

0378-4320/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.anireprosci.2009.06.014

1. Introduction

The sheep industry has not been able to utilize many of the assisted reproductive technologies (ART) in general and AI in particular, as other livestock industries, due to inefficiencies in collecting, freezing and inseminating frozen ram semen. Furthermore, few commercial studs routinely collect and freeze ram semen, and there is still a need to optimize cryopreservation and breeding protocols for ram semen. The USDA National Animal Germplasm Program (NAGP) is charged with developing a national repository of animal germplasm and tissue to provide genetic security and facilitate genetic understanding of livestock species (Blackburn, 2004). This task is complicated by the lack of ART infrastructure for the U.S. sheep industry and by the general inability to efficiently cryopreserve ram semen.

One way to quickly improve the quality of cryopreserved ram semen would be to collect the semen where the ram resides and ship the semen to a central processing center with the expertise in cryopreserving the semen, in much the same way cooled stallion semen is currently used (Backman et al., 2004). This system would permit semen from a ram to be cryopreserved without the owner having to transport the ram, house the ram in a collection facility, or have to have the equipment for or expertise in cryopreservation. Diluted ram semen can be held at 5 °C for up to 48 h prior to cryopreservation without detrimental effects on sperm physiology (Purdy, 2006). In addition, new cryopreservation technologies, such as treating ram sperm with cholesterol-loaded cyclodextrins (CLC) show promise in increasing sperm cryosurvival rates (Morrier and Bailey, 2003; Morrier et al., 2004; Mocé et al., 2009). These studies were conducted to determine the fertilizing potential of ram sperm cryopreserved 24 h after collection in conjunction with CLC treatment.

2. Materials and methods

2.1. Semen collection and handling

Adult rams and ewes were housed at the University of Wyoming (Laramie, WY, USA). Animals were fed a diet providing 100% of their nutritional needs, and provided water *ad libitum*. All protocols for working with the sheep were approved by the University of Wyoming Institutional Animal Care and Use Committee.

Single ejaculates from sexually mature Suffolk rams were collected using electro-ejaculation (Evans and Maxwell, 1987) during the month of October and ewes were inseminated the following month. After collection, the percentage of motile sperm in each ejaculate was determined using bright field microscopy (Nasco Advanced Laboratory Microscope, Nasco Farm and Ranch Supplies, Fort Atkinson, WI, USA) at 400× magnification and 23 °C to ensure that each sample had a minimum of 70% motile sperm. The volume and sperm concentration (Spermacue, IMV Corp., Minneapolis, MN, USA) were determined and ejaculates were diluted in one step (in 50 mL centrifuge tubes) to 200×10^6 sperm/mL with Tris–egg yolk–glycerol medium (300 mM Tris, 28 mM glucose, 95 mM citric acid, 5% (v/v) glycerol, 15% egg yolk, 1 mg/mL streptomycin sulfate and 0.06 mg/mL benzylpenicillin; Sanchez-Partida et al., 1998) at 37 °C.

2.2. Experiment 1

Diluted semen samples were cooled to 5 °C over 2 h and split into two aliquots. One aliquot from each ejaculate was immediately (designated T0) loaded into 0.5 mL CBS straws (IMV Corp., Minneapolis, MN, USA) and cryopreserved as described by (Sanchez-Partida et al., 1998). The second aliquot was held for 24 h (T24) at 5 °C, before being packaged into straws and cryopreserved.

Sperm were frozen in liquid nitrogen vapor using a Mini Digitcool UJ400 programmable freezer (IMV Corp., Minneapolis, MN, USA). The sperm were cooled from 5 °C to −5 °C at 4 °C/min; from −5 °C to −110 °C at 25 °C/min; and from −110 °C to −140 °C at 35 °C/min. After reaching −140 °C the straws were plunged into liquid nitrogen for storage.

Frozen straws were thawed in 37 °C water for 30 s and were analyzed for the percentage of motile sperm, using a computer assisted semen analysis system (IVOS Version 10.9, Hamilton Thorne Bioscience, Beverly, MA, USA) with a 10× phase contrast objective and the following settings: 50 frames acquired, frame rate of 60 Hz, minimum contrast of 60, minimum cell size of 5 pixels, VAP (path velocity) cutoff of 20 μm, progressive minimum VAP cutoff of 50 μm/s, VSL (progressive velocity) cutoff of 30 μm/s, static head size of 0.24–3.66, and magnification of 1.89. For each sample, an aliquot was diluted 1:3 (v/v; sample to diluent) with Tris-buffered saline (Purdy and Graham, 2004) and 5 μL was placed onto a Standard Count Analysis Chamber (Spectrum Technologies, Healdsburg, CA, USA). A minimum of 500 sperm and five fields were analyzed to estimate the percentage of motile sperm from each treatment.

2.3. Experiment 2

In experiment 2, conducted the following year, semen samples from six rams were collected and split prior to diluting the sperm. One aliquot was diluted with the Tris–egg yolk–glycerol medium to 200×10^6 sperm/mL. The second aliquot was treated with cholesterol-loaded methyl-β cyclodextrin (2 mg/120×10^6 sperm or CLC (3.33 mg/200×10^6 sperm) for 15 min at 23 °C and then diluted in the same manner as the first aliquot (Mocé and Graham, 2006). Both were then cooled to 5 °C over 2 h and held at 5 °C for 24 h before being packaged into straws and cryopreserved as described above.

Straws were thawed and motility analyses conducted, as described previously. In addition, sperm plasma membrane integrity (PMI) was determined in this experiment using flow cytometry, as described by Garner et al. (1994). Briefly, the sperm were stained with SYBR-14 (to detect cells from debris) and propidium iodide (to detect cells lacking intact plasma membranes (Garner et al., 1994)). A minimum of 10,000 sperm were analyzed from each sample.

Download English Version:

https://daneshyari.com/en/article/2073758

Download Persian Version:

https://daneshyari.com/article/2073758

Daneshyari.com