Theriogenology 85 (2016) 1097–1105

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Effect of colloid (Androcoll-Bear, Percoll, and PureSperm) selection on the freezability of brown bear (*Ursus arctos*) sperm

M. Álvarez-Rodríguez [a,*], M. Álvarez [b,c], L. Anel-López [d], E. López-Urueña [b,c], P. Manrique [b,c], S. Borragán [e], J.M. Morrell [f], P. de Paz [c,g], L. Anel [b,c]

[a] Department of Clinical and Experimental Medicine (IKE), Linköping University, Linköping, Sweden
[b] Animal Reproduction and Obstetrics, University of León, León, Spain
[c] ITRA-ULE, INDEGSAL, University of León, León, Spain
[d] SaBio IREC (CSIC-UCLM-JCCM), Albacete, Spain
[e] Cabárceno Park, Cantabria, Spain
[f] Swedish University of Agricultural Sciences (SLU), Clinical Sciences, Uppsala, Sweden
[g] Molecular Biology (Cell Biology), University of León, León, Spain

ARTICLE INFO

Article history:
Received 13 April 2015
Received in revised form 17 November 2015
Accepted 24 November 2015

Keywords:
Brown bear sperm
Androcoll
PureSperm
Percoll
Freezability

ABSTRACT

The development of a species-specific conservation protocol that involves artificial insemination with frozen semen needs to validate an effective methodology for freezing semen. Colloid centrifugation has been suggested and widely applied as an effective tool for selecting animal spermatozoa for artificial breeding. The objective of the present study was to compare different methods of centrifugation, single layer using Androcoll-Bear and Percoll and double layer using PureSperm 100 (in two different discontinuous gradients 40%–80% and 45%–90%), for the selection of fresh brown bear sperm samples. In the before freezing group, all selected samples showed a higher progressive motility and viability (except Percoll for motility 43.0 ± 5.3 [$P < 0.05$]); all colloids except PureSperm 45/90% rendered samples with fewer damaged acrosomes. In the after thawing group, all tested centrifugation colloids showed a good capacity to decrease the number of damaged acrosomes. Furthermore, PureSperm treatment (45/90%) resulted in an increase in apoptotic-like changes not only immediately after thawing but also after the incubation test, leading us to suggest that this gradient could induce some kind of deleterious effects on the sperm samples. On the other hand, PureSperm treatment (40/80%) yielded a quality preservation capacity similar to Androcoll-Bear in number of damaged acrosomes, different relative to the control (control, 5.3 ± 0.6; PureSperm 80, 2.0 ± 0.3; Androcoll, 2.1 ± 0.9 [$P < 0.05$]) but a decrease in the number of viable spermatozoa recovered after thawing relative to the control (control, 21.2 ± 3.1; PureSperm 80, 13.7 ± 2.7 [$P < 0.05$]). In conclusion, Androcoll-Bear constitutes a useful tool for handling of brown bear ejaculates owing to its simple handling and procedure with a reliable sperm selection and freezability. This colloid yielded an improvement in several sperm parameters in brown bear frozen-thawed semen; the selected spermatozoa of fresh samples with this colloid showed a better resistance to freezing compared with the control sample not only for motility but also for viability.

* Corresponding author. Tel.: +46 10 10 32 616; fax: +46 10 10 33 4789.
E-mail address: manualvro@gmail.com (M. Álvarez-Rodríguez).

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http://dx.doi.org/10.1016/j.theriogenology.2015.11.021

1. Introduction

The Cantabrian brown bear (*Ursus arctos*) is the last indigenous brown bear population in the Iberian Peninsula and is considered to be at risk of extinction (Real Decreto 439/1990, regulation of the National Catalog of Endangered Species). The development of a Genome Resource Bank for this species has been proposed as a useful adjunct to other conservation methods as it would maximize the reproductive potential of males by overcoming the restrictions of time and space [1].

The development of a species-specific conservation protocol that includes artificial insemination with frozen semen needs to validate an effective methodology for freezing semen [2,3]. Banking frozen semen does not aid a conservation effort if the after thawing semen quality is too poor to achieve fertilization.

In brown bears, recent studies have mainly focused on the optimization of extender composition, including egg yolk [4–6], several additives [7,8], glycerol addition mode [5,9,10], and the glycerol concentration, and freezing rate used [11]. Together, these studies have resulted in improved methods for semen storage *in vitro* in these species.

Another important factor to take into account when developing methods for improving sperm quality is that damage caused by the semen processing procedures themselves may result in important deleterious changes at the end of the process. Thus, for the ejaculates of some species, centrifugation of semen before cryopreservation is necessary to reach an appropriate cellular concentration (dog [12], stallion [13,14], and brown bear [2,6]) or even to remove seminal plasma (goat [15]) and clean urine-contaminated samples (horse [16] and brown bear [17]). Despite previous studies assessing the deleterious effects of centrifugation, this procedure does not appear to be harmful for brown bear spermatozoa [18,19]. Regarding seminal plasma, some studies have shown beneficial effects of its addition during semen processing and cryopreservation (deer [20] and ram [21]), whereas other authors have reported deleterious effects (dog [12], goat [22], ram [23], stallion [13,14], and bull [24]).

Colloid centrifugation has been widely used as an effective tool for selecting animal spermatozoa for artificial breeding [25]. Density gradient centrifugation is a widely used technique not only for separating motile from nonmotile sperm, but also for removing contaminating agents, obtaining a final sperm suspension free of seminal plasma, leukocytes, microbial contamination, and other debris [26].

First, the PureSperm100 (Nidacon, Gothenburg, Sweden) density gradient centrifugation technique is designed to select viable and morphologically intact human spermatozoa for assisted reproductive technologies [27]. This two-layer gradient has been used previously with effective results in the selection of brown bear sperm samples [17,28].

Second, Percoll has been used for the selection of sperm in many different species, enabling a comparison between different gradients (e.g., human [29,30], bovine [31], and marmoset [32]) in the vast majority of these studies.

Finally, Androcoll-Bear represents a new method for selecting the best spermatozoa that has been developed at the Veterinary Faculty of the Swedish University for Agricultural Sciences, using single-layer centrifugation (SLC) through species-specific colloids. Single-layer centrifugation–selected sperm samples have good motility, normal morphology, intact plasma membranes, and good chromatin integrity (reviewed by Morrell and Rodriguez-Martinez [33]).

Thus, the objectives of this study were to compare three different centrifugation products and protocols Androcoll-Bear, Percoll, and PureSperm100 (in two different discontinuous gradients 40/80% and 45/90%) for the selection of fresh sperm, and the freezability of the samples selected analyzing the effect on quality of both immediately after thawing and after a thermal stress test consisting of incubation at 37 °C up to 2 hours.

2. Materials and methods

2.1. Reagents and animal regulation

All products were obtained from Sigma (Madrid, Spain) except Equex STM Paste (Minitüb, Tiefenbach, Germany) and PureSperm100 (Nidacon, Gothenburg, Sweden). Androcoll-Bear was provided by Prof. Morrell (Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden).

2.2. Animals and sample collection

Semen samples from eight sexually mature male brown bears (between 7 and 20 years) were obtained by electroejaculation during the breeding season (late April to early July). Animals were housed in a semi-free regimen in Cabárceno Park (Cantabria, Spain; 43° 21′ N, 3° 50′ W; altitude, 142 m) and fed with a diet based on chicken meat, bread, and fruit. Animal manipulations were performed in accordance with Spanish Animal Protection Regulation RD1201/2005, which conforms to European Union Regulation 2003/65. All experiments were performed after obtaining approval from the Ethical Committee for Experimentation with Animals of León University, Spain (February 3, 2010).

The males were immobilized by teleanesthesia, using 750 mg of zolazepam HCl plus tiletamine HCl (Zoletil100; Virbac, Carros, France) and 6 mg of medetomidine (10 mg/mL, Zalopine; Orion Pharma Animal Health, Finland). After immobilization, the males were weighed, and pulse, oxygen saturation, and breathing were monitored. Before electroejaculation, the preputial area was shaved and washed with physiological saline serum (sodium chloride 0.9% wt/vol intravenous infusion BP; Ecoflac plus container sizes, 500 mL; B. Braun Medical Inc.), and the rectum was emptied of feces. The bladder was catheterized during semen collection to prevent urine contamination. Electroejaculation was carried out with a PT Electronics electroejaculator (PT Electronics, Boring, OR). The transrectal probe was 320-mm long with a diameter of 26 mm. Electric stimuli were given until ejaculation (10 V and 250 mA on average).