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Comparison of different methods of goat sperm selection and capacitation for optimization of assisted reproductive technologies

Carolina Cerqueira Sarmento Olivares[a], Jeferson Ferreira da Fonseca[b], Luiz Sérgio de Almeida Camargo[c], Joanna Maria Gonçalves de Souza-Fabjan[a,*], André Luís Rios Rodrigues[a], Felipe Zandonadi Brandão[a]

[a] Faculty of Veterinary Medicine, Fluminense Federal University, Av. Vital Brasil Filho, 64, CEP 24230-340 Niterói, RJ, Brazil
[b] Embrapa Goats and Sheep, Estrada Sobral/Groaíras, km 04, CP 145, CEP 62010-970 Sobral, CE, Brazil
[c] Embrapa Dairy Cattle Research Center, Rua Eugênio do Nascimento, 610, CEP 36038-330, Juiz de Fora, MG, Brazil

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ABSTRACT

This study evaluated different methods of frozen–thawed sperm selection and capacitation in goats for further use in reproductive biotechnologies. In Experiment 1, semen was processed by the following techniques: mini-Percoll, swim-up, or washing by centrifugation. In Experiment 2, mini-Percoll selected-sperm was subjected to capacitation induction by incubation with: 50 μg/mL heparin, 10 μM, 50 μM, or 100 μM of sodium nitroprusside (SNP). Motility, vigor, acrosome, and plasma membrane (PM) integrity were evaluated after thawing and after each treatment of sperm selection or capacitation. In Experiment 1, washing by centrifugation presented greater (43%; $P<0.05$) spermatozoa recovery rate than the other treatments. The swim-up technique showed the lowest ($P<0.05$) progressive motility (41%). Spermatozoa presenting both intact PM ($P=0.0002$) and acrosome ($P=0.0004$) showed an interaction effect between the buck and swim-up technique. In Experiment 2, the addition of 100 μM SNP resulted in greater ($P<0.05$) motility and vigor (38%; 4.7), respectively, than did heparin (28%; 4.3). An enhancement ($P<0.05$) in vigor was obtained after all treatments in comparison with the evaluation after thawing (3.3). In conclusion, mini-Percoll was better than swim-up for preparing frozen–thawed goat sperm, whereas washing by centrifugation technique presented similar rates to mini-Percoll and could also be used. The use of 100 μM SNP resulted in better motility and vigor than heparin treatment.

1. Introduction

The *in vitro* embryo production (IVP) has the advantage of selecting and using both male and female gametes, allowing the birth of several kids from a single high genetic merit female. Many studies have been carried out in an attempt to determine the most suitable conditions for *in vitro* maturation, fertilization and development in order to maximize goat embryo production rate and quality (Souza-Fabjan et al., 2014). Among the different factors that may affect IVP results, probably the main ones are oocyte (Souza-Fabjan et al., 2014) and spermatozoa quality (Hansen, 2006). However, studies on goat spermatozoa preparation have received little

* Corresponding author. Tel.: +55 21 2629 2595; fax: +55 21 2629 2433.
E-mail address: joannavet@gmail.com (J.M.G. de Souza-Fabjan).

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attention in IVP systems (Palomo et al., 1999; Rho et al., 2001).

The introduction of *in vitro* fertilization (IVF) during the 1980s led to the development of a wide range of different sperm separation methods. Initially, the sperm preparation started with simple washing of spermatozoa and then evolved to separation techniques based on principles like migration, or density gradient centrifugation. The ideal sperm separation technique should (i) be quick, easy and cost-effective, (ii) isolate as much motile spermatozoa as possible, (iii) not cause sperm damage or nonphysiological alterations of the separated sperm cells, (iv) eliminate dead spermatozoa and other cells, including leukocytes and bacteria, (v) eliminate toxic or bioactive substances like decapacitation factors or reactive oxygen species (ROS), and (vi) allow processing larger volumes of ejaculates (Henkel and Schill, 2003).

In 1990, a method of sperm preparation consisting of a reduced volume of a discontinuous Percoll gradient (mini-Percoll) was proposed (Ord et al., 1990). The two-layer Percoll gradient showed a higher sperm recovery than the mini-Percoll method, but the latter resulted in a higher percentage of motility and a higher sperm survival rate at 24 h (Kunathikom et al., 1998). However, the number of studies on the effect of swim-up and Percoll methods on goat spermatozoa is very limited in comparison with human or bovine, and the present authors are not aware of any published study comparing the goat spermatozoa obtained by swim-up or mini-Percoll separation on goats.

Spermatozoa must undergo capacitation and acrosome reaction to be able to fertilize, causing the release of proteolytic enzymes that may assist sperm penetration into the oocyte. Any agent that causes Ca^{2+} entry into the sperm acrosome and an increase of intracellular pH enables capacitation and later fertilization to be accomplished (Amoah and Gelaye, 1997). However, efficient systems for induction of capacitation and acrosome reaction of goat spermatozoa were still not established. It was already reported the use of heparin (Zhou et al., 2004), caffeine (Pereira et al., 2000; Lv et al., 2009), calcium ionophore (Pereira et al., 2000; Lv et al., 2009) and estrus sheep serum (Souza-Fabjan et al., 2014) as capacitating agents for goat semen. The mechanism for sperm capacitation of sodium nitroprusside (SNP) is related to the release of nitric oxide that induces the capacitation by means of intracellular mechanisms that seem to be involved with the activation of protein kinases (Rodriguez et al., 2005). Even though the efficiency of SNP was already evaluated in many species, such as bovine (Rodriguez et al., 2005) and bubaline (Boccia et al., 2007), it was never tested in caprine spermatozoa.

The aim of the present study was to enhance spermatozoa quality for assisted reproduction by comparing the effectiveness of: (i) mini-Percoll, swim-up, and washing by centrifugation procedures on the collection of high-quality frozen–thawed goat spermatozoa and (ii) heparin or SNP at different concentrations as capacitating agents.

2. Materials and methods

All the experiments were carried out at Fluminense Federal University (UFF), Niterói (Brazil, latitude 22°53′ S, longitude 43°06′ W). The procedures were approved by the local ethic committee (protocol approval: 159/11). Two experiments were performed to examine the impact of different spermatozoa preparation techniques using goat semen.

2.1. Reagents

All chemicals were from Sigma Chemical (St. Louis, MO, USA). Exceptions were Percoll gradients (90 and 45%) and buffered saline solution (DPBS; Dulbecco's Phosphate Buffered Saline) obtained from Nutricell (Campinas, Brazil) and Giemsa staining acquired from Reagen Quimibrás (Rio de Janeiro, Brazil).

2.2. Animals and spermatozoa samples

Commercial semen straws from the same batch (ejaculate) from Saanen bucks, aging 2–5 years old, sexually matured and of proved fertility were used. All straws were maintained frozen in liquid nitrogen until their use. The data presented were obtained from nine bucks (Experiment 1) or seven bucks (Experiment 2), using one straw from each buck per treatment. In both experiments, all treatments were repeated three times (triplicates). Each replicate of all treatments was performed in the same day and by the same operator.

2.3. Experiment 1

Semen straws of 0.25 mL were thawed at 38 °C for 30 s and homogenized in warmed microtubes. After thawing, each dose/straw was subjected to a different method of spermatozoa selection: mini-Percoll gradient, swim-up or spermatozoa washing by centrifugation. For all treatments, after thawing, spermatozoa were subjected to concentration, progressive motility (0–100%), and vigor analysis (0–5). After the different spermatozoa selection techniques, besides the same evaluations, spermatozoa recovery rate and acrosome and plasma membrane integrity were also observed.

2.3.1. Mini-Percoll density gradient

In view of the batch-dependence of the quality of Percoll (Avery and Greve, 1995) it is necessary to note, that during the experiments the density gradients were made from the same batch of Percoll. The gradient was formed by pipetting 400 μL of 90% Percoll solution into a 2 mL microtube and then overlaying it with 400 μL of 45% Percoll solution (Machado et al., 2009). After the preparation, the microtube was warmed to 37 °C. One straw was placed onto the top of the 45% layer and then centrifuged at 14,000 × *g* at room temperature for 5 min. After removal of supernatant, the resulting pellet was washed with 400 μL HEPES-TALP, supplemented with 3 mg/mL BSA V, 2.2 mg/mL sodium pyruvate, 50,000 IU/mL penicillin and 50 mg/mL streptomycin by centrifugation at 700 × *g* for 3 min. Finally, the pellet was resuspended in 200 μL of HEPES-TALP.

2.3.2. Swim-up

Swim-up procedure was previously described by Parrish and Foote (1987) but modified TALP was used in the present study. Frozen–thawed sperm from one straw was carefully placed in the bottom of a 15 mL tube containing 1 mL HEPES-TALP supplemented with 3 mg/mL BSA V, 2.2 mg/mL sodium pyruvate, 50,000 IU/mL penicillin and 50 mg/mL streptomycin. Spermatozoa were allowed to swim-up for 1 h, at 37 °C in a humidified 5.0% CO_2 (in air) atmosphere. After incubation, the supernatant containing the spermatozoa was harvested and transferred to another tube containing 1 mL of HEPES-TALP and centrifuged at 300 × *g* for 8 min. Finally, the pellet was resuspended in 200 μL of HEPES-TALP.

2.3.3. Spermatozoa washing by centrifugation

After thawing, semen from one straw was placed in a conic tube containing 10 mL of DPBS and washed once by centrifugation at 300 × *g* for 20 min. After discarding the supernatant the spermatozoa pellet was resuspended in 500 μL of DPBS.

2.4. Experiment 2

After thawing, two semen straws (due to the amount needed) were placed into a 2 mL microtube previously warmed and subjected to spermatozoa selection by mini-Percoll gradient method (as described in Experiment 1). After the mini-Percoll procedure, the selected spermatozoa was washed in SPERM-TALP (Parrish et al., 1988) supplemented with 6 mg/mL BSA V, 2.2 mg/mL sodium pyruvate, 50,000 IU/mL penicillin and