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The effect of cryopreservation on goat semen characteristics related to sperm freezability

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ABSTRACT

Seminal quality parameters were used to evaluate the effect of freeze–thawing procedure on goat sperm characteristics, and to relate possible changes in sperm parameters to cryopreservation success. Semen samples ($n = 110$) were frozen with TRIS and milk-based extenders and thawed. Sperm quality parameters (motility, morphology and acrosome) were compared between fresh and frozen–thawed samples. Sperm freezability was judged by classifying the semen samples as "suitable" or "not suitable" according to the sperm quality parameters assessed before and after thawing. Fertility data was obtained after cervical insemination with frozen semen doses. The ejaculates were grouped into two categories according to their fertility results.

In experiment 1, significant differences were found between semen extenders ($P < 0.001$), bucks ($P < 0.05$) and ejaculates within the same male ($P < 0.05$) in terms of sperm quality. There was no seasonal effect ($P > 0.05$) on the majority of the sperm parameters assessed after thawing. Moreover, significant differences ($P < 0.001$) in semen parameters assessed in fresh semen and frozen–thawed samples were found between groups. The effect of the freeze–thawing procedure on sperm quality parameters was also different ($P < 0.05$) between extenders within the same group. The number of sperm quality parameters that had changed after cryopreservation was lower in "suitable" semen samples before and after thawing. In experiment 2, no differences ($P > 0.05$) in semen parameters assessed in fresh semen and frozen–thawed samples were found between groups. The effect of freezing and thawing on sperm quality parameters were different ($P < 0.05$) between extenders within the same group. Only mean beat cross frequency (BCF) values were significantly higher ($P < 0.05$) in TRIS diluted samples that led to successful pregnancies after artificial insemination. In conclusion, CASA-derived motility parameters, together with traditional semen assessment methods, give valuable information on sperm quality before and after freezing. Therefore, the identification of ejaculates as "good" or "bad" based on fresh and post-thaw semen parameters studied in the present experiment were good indicators of goat semen freezability, although the fertilizing capacity of frozen–thawed goat spermatozoa are not revealed by this quality study.

1. Introduction

The cryopreserved goat semen can be almost indefinitely stored in liquid nitrogen, it facilitates the supply of genetic material, as well as building up gene banks to encourage endangered breeds or valuable individuals (Watson and Holt, 2001), particularly associated with

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artificial insemination (AI) (Leboeuf et al., 2000). This is especially important in the case of Florida male goat, Spanish autochthonous breed under special protection because it is in danger of extinction. However, the biggest obstacle to the exploitation of frozen semen is that the freeze–thawing process of goat sperm generally leads to a decrease in the percentage of motile and viable sperm cells after thawing as a result of damage to membrane integrity and ultrastructure (Watson, 2000).

The evaluation of sperm motility, morphology and acrosomal status is an essential criterion in the evaluation of the quality of a semen sample prior to its use for AI (Salamon and Maxwell, 2000). In this sense, it has been widely demonstrated that cryopreservation leads to a decrease in sperm motility measured objectively by computer-aided methods in the goat (Dorado et al., 2007, 2009) and other animal species (Thurston et al., 2001; Martinez-Pastor et al., 2005). In the case of sperm morphology, it has been demonstrated that a decrease in the number of morphologically normal sperm in ejaculates leads to reduced fertility (Chandler et al., 1988; Gravance et al., 1998). Therefore, the lower fertility of the cryopreserved semen samples may well be a result of a decrease in the number of normal sperm in these samples (Gravance et al., 1997). On the other hand, the presence of an acrosomal cap is important in the fertilization process and has been also highly related with fertility of frozen semen (Lindsay et al., 2005).

The most common methods to evaluate sperm motility is the microscopic observation of semen samples. This subjective estimation has a reduced fertility predictive value because of the high variability observed between individuals and laboratories (Holt et al., 1994; Verstegen et al., 2002). A more objective and precise assessment of sperm motility can be achieved with Computer-Assisted Sperm Analysis (CASA), in which each sperm head trajectory is reconstructed and its kinetics derived in different species (Verstegen et al., 2002; Mortimer and Maxwell, 2004).

Cryopreservation is reported to compromise the fertility of goat spermatozoa based on pregnancy rates from AI (Gacitua and Arav, 2005; Purdy, 2006). There is limited research on motility analysis of goat sperm by the CASA system. Moreover, no attempts have yet been made to associate the motility of goat semen with *in vivo* fertility after AI. The objective of this study was to analyse the effect of the freeze–thawing procedure on goat sperm motility and whether CASA measures can be used as an indicator, together with traditional semen assessment methods, of the sperm freezability and the potential fertility of the cryopreserved sperm.

2. Materials and methods

2.1. Animals and semen collection

Semen was obtained from two mature Florida bucks (2-years-old), located in the south of the Iberian Peninsula (37°53′N–4°46′W). Semen was collected from each animal twice a week, in different and non-consecutive days, using an artificial vagina and estrous females as mounts for the bucks. Ejaculates were equally distributed among males and seasons over a 1-year period.

2.2. Freezing and thawing protocol

After collection, semen samples were diluted with a isothermal glycerol free TRIS-citric acid extender (Biladyl A, Minitüb, Tiefenbach, Germany) at a ratio of 1:9 (semen to Biladyl A, v:v) and centrifuged at $1125 \times g$ for 20 min. The seminal plasma was discarded and the semen pellet was suspended to a final concentration of 250×10^6 rapid progressive spermatozoa/mL (assessed by CASA system) with two different commercial extenders: TRIS-based extender (Triladyl, Minitüb, Tiefenbach, Germany) and skim milk-based extender (Gent, Minitüb, Tiefenbach, Germany) containing both filtered egg yolk (20%, v:v). The diluted sperm suspensions were equilibrated at 5 °C for 5 h and loaded into 0.5 mL straws. They were then frozen in nitrogen vapour for 20 min and transferred to a liquid nitrogen container for storage at −196 °C until used. Thawing was carried out at 24 h after cryopreservation by immersing in a circulating water bath at 39 °C for 30 s.

2.3. Semen evaluation

The volume of each ejaculate was measured in a graduated tube shortly after collection and sperm concentration was determined by manual counting with a Neubauer hemacytometer (Brand, Wertheim, Germany) within 10–20 min after collection.

Aliquots of fresh semen (100 μl) were diluted in DPBS (Dulbecco's phosphate buffered saline, Sigma–Aldrich, Steinheim, Germany) at a sperm concentration of approximately 40×10^6 spermatozoa/mL and used to assess sperm motion. In the in vitro study, an aliquot of diluted semen was incubated at 37 °C for 5 min, before motility evaluation using the computerized system Sperm Class Analyzer (SCA, Microptic SL, Barcelona, Spain). For evaluation, a 10 μl drop of the sample was placed on a pre-warmed Makler chamber (Sefi Medical Instruments Ltd., Haifa, Israel) and a minimum of 200 sperm were counted under a phase contrast microscope (Olympus BH-2, Tokyo, Japan) at 37 °C and 100× magnification. At least 2 drops from each semen sample were evaluated and 3 microscopic fields were filmed for each of the sub-samples.

The semen variables studied were: total motile spermatozoa (MS, %) and rapid progressive motile spermatozoa (RPMS, %); curvilinear velocity (CLV, μm/s); straight line velocity (SLV, μm/s); average path velocity (APV, μm/s); amplitude of lateral head displacement (LHD, μm) and beat cross frequency (BCF, Hz). The main software settings were: frame rates: 25 frames/s, number of frames: 16 frames/object, velocity limit for slow sperm: 30 μm/s, velocity limit for medium sperm: 60 μm/s, minimal linearity (straightness) for progressive fast sperm: 90%, frames min (amplitude of lateral head displacement): 7, and filter for particles: 20%.

The sperm suspension was also used to assess sperm morphology and acrosome integrity. The percentage of spermatozoa with abnormal morphology and intact membrane-acrosome was estimated on Spermac-stained smears (Minitüb, Tiefenbach, Germany). Counts of 200 cells per slide were evaluated using a 100× oil immersion objec-