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Sperm motility assessment of epididymal sperm from post mortem goat testicles held at 5°C

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ABSTRACT

Appropriate holding conditions for post mortem testicles of goat to yield quality epididymal sperm (ES) as a source of genetic material for cryobanking and fertilization studies are lacking. In this study, the effect of storage time on the motility of ES from post mortem testicles maintained at 5°C was evaluated. In the laboratory, the cauda epididymides were excised from the testicles after 4 hr (G-1) and 24 hr (G-2) of holding time before collecting the sperm in a Tris-citrate buffered solution and evaluated using a CASA. Sperm motility profiling revealed a subpopulation of static, slow, motile and progressive ES. The proportion of static sperm in the control (16.97±6.21) and G-1 (21.53±5.60) were lower significantly than G-2 (36.13±5.05). The proportion of slow moving sperm was lower significantly than G-1 (23.31±3.57) and G-2 (25.45±3.32). The proportion of motile and progressive motile sperm decreases significantly (P<0.05) as the holding time increases at 78.46±4.64% (G-1) to 63.85±4.06 (G-2) and 45.53±8.89 (G-1) to 25.46±8.42 (G-2), respectively. The results showed that prolonged storage of post mortem testicles at 5°C could result to a reduced percentage of motile and progressively motile ES. Nevertheless, this considerable proportion of ES remained useful both for cryobanking and fertilization studies.

Key words: Epididymal sperm, Post mortem, Storage temperature, Testicles

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INTRODUCTION

There is a pressing need to preserve the germplasm of resilient breeds for future animal genetic resources cryobanking. However, the unexpected death of a candidate sperm donor may pose some problems on the application of assisted reproductive technologies for biodiversity preservation. The recovery of post mortem testicles (PMT) and cryopreservation of viable sperm from the cauda epididymides (CE) is a viable alternative in preserving male genome and maintenance of germplasm banks [5, 6, 11, 13]. Thus, optimization of conditions needed for the collection and transport of PMT to preserve ES integrity is important especially in situations wherein the site of sample collection is far from the laboratory for processing. ES viability and its fertilizing ability was reported to have been compromised when the time between death of the animal and semen collection increased while kept at ambient temperature [4]. Whereas, better ES quality could be obtained when the CE is stored at refrigeration temperature of 4°-6°C for up to 72 hr [7, 9, 10] or up to 7 days after death before use [2]. These differences reflect species variability on tolerance to cold shock of ES among mammals. In goat, few informations are available regarding the quality of ES [8, 15], especially on the motility after collection from post mortem testicles. In this study, the ES motility in relation to the time

elapsed between death of the donor animals, post mortem storage of testicles and ES recovery was evaluated.

MATERIALS AND METHODS

Medium preparation

The chemicals used were of reagent grade and were purchased from Sigma-Aldrich (USA) except for Tris-base (Promeg Corp., Madison, WI, USA). Tris-citric acid-lactose-raffinose buffer (TLB medium) was prepared a day before collection of cauda epididymides. A one liter preparation composed of 15.7 g Tris-hydroxymethyl amino methane, 8.8 g citric acid monohydrate, 14.1 g lactose, 25.4 g raffinose and gentamycin solution (50 µg/ml) using an ultra-pure water (Milli-Q, Integral 5). Before use, the medium was sterilized by filtration using a 0.2 µm syringe filter.

Testicle preparation and epididymal sperm collection

Twenty pairs of testicles from non-descript bucks (≤ 3yrs old) were collected post mortem at local abattoirs and transported to the laboratory in a Styrofoam box at ambient temperature (control) or at refrigeration temperature (5°C) for 4 hr (G-1) and for 24 hr (G-2) before processing for sperm recovery. Collection period was between April to August. In the laboratory, the CE were excised aseptically from the testicles, sliced several times with a scalpel (done without cutting blood vessels to minimized contamination) and the sperm collected, its volume recorded and the sperm concentration estimated by using haemocytometer. Sperm motility parameters were assessed using a computer assisted sperm analyzer (CASA; Hamilton Thorne IVOS II, Beverly, MA, USA). Each sample was diluted (20×10^6 cells/ml)in TLB medium and incubated for 20 min in 38°C water bath pending analysis. Then pre-warmed (38°C) chamber slide (SC20.01FA; Leja®, Nieuw-Vennep, The Netherlands) was loaded with 10 µl sample, allowed to settle for 1 min on MiniTherm© stage warmer before analysis. At least 5 fields per sample were observed and recorded. The sperm motility parameters taken were static, slow, motile and progressive motile sperm expressed in percentage.

Statistical analysis

Percentage sperm motility was analyzed using *t*-test procedure in SAS. Data were expressed as mean± standard error of mean. *P* values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

In all testicles collected, the ES was successfully recovered from the CE by slicing method. The ES mean volume was 0.5 ml with a mean sperm concentration of 2×10^9 cells/ml. Profiling of ES motility showed a subpopulation of static, slow, motile and progressive motile sperm (Table 1). The proportion of static sperm in the control (16.97±6.21) and G-1 (21.53±5.60) were significantly lower than G-2 (36.13±5.05). The proportion of slow sperm in the control (8.57±3.32) was significantly lower than both G-1 (23.31±3.57) and G-2 (25.45±3.32). The proportion of motile sperm in the control (88.03±2.21) was significantly higher than G-1 (78.46±4.64) and G-2 (63.85±4.06). Moreover, the proportion of progressive motile sperm was higher significantly in the control (68.73±3.28) than G-1 (45.53±8.89) and G-2 (25.46±8.42). Studies in ram [1], red deer and moufflon [4] showed that ES viability decreases progressively as the time between the animal's death and sperm collection increases. In this study, the results showed that prolonged storage of testicles at 5°C significantly influenced the motility of ES. Nevertheless, the considerable proportion of recoverable motile and/or progressive motile ES can still be useful for cryobanking and fertilization studies. It has been suggested that goat testicles should be transported and stored at 5°C up to a maximum of 48 hr post mortem to ensure an acceptable sperm quality for gene banking [15]. Moreover, cold storage of CE at refrigeration temperature of 4°C for up to 72 hr efficiently protected goat ES in terms of progressive motility and viability [8]. Related studies done in other mammals suggest that when valuable male animal die unexpectedly and sperm cryopreservation is not possible immediately, temporal storage of epididymides at 4°C may help preserve its genome [11, 12]. That, ES of slaughtered bulls remained fully functional for at least 60 hr at 5°C when used for artificial insemination [3].

Table 1: Effect of testicular post mortem storage on epididymal sperm motility.

Post mortem	**Profile of sperm motility (mean±SD)**			
time (hr)	static	slow	motile	progressively motile
Control	16.97±6.21[a]	8.57±3.32[a]	88.03±2.21[a]	68.73±3.28[a]
G-1	21.53±5.60[a]	23.31±3.57[b]	78.46±4.64[b]	45.53±8.89[b]
G-2	36.13±5.05[b]	25.45±3.32[b]	63.85±4.06[c]	25.46±8.42[c]

[a,b,c] Values with different superscript differ significantly (P<0.05).

CONCLUSION

The results suggest that maintaining post mortem testicles of goat at 5°C for up to 24 hr is useful in the collection of ES. The positive effect of refrigeration temperature on the sperm motility may be explained by the reduced metabolic rate of sperm cells at 5°C [14].

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Authors' contributions
ACB and HMH designed the experiments and performed the experimental works. CCD, MBO, LCO contributed ideas, gave critical feedback during data analysis and manuscript write-ups. All authors read and approved the final manuscript.

Conflict of interest
The authors declare that they have no competing interest.

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