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Artificial insemination in Cape Verdean Goats with Cooled Semen stored for 24 or 48 Hours

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ABSTRACT

The fertilizing capacity of goat semen stored at 5oC was evaluated in Cape Verdean goats. The semen was collected from three Canarian bucks and was diluted in Tris-egg yolk 2.5% extender to yield a final concentration of 150x106 mobile spermatozoa per 0.25 mL straw. The straws were inserted into a container and were maintained at 5oC for 24 (T24) or 48 hours (T48). A total of 133 goats were randomly assigned to receive one type of cooled semen. Estrus was synchronized with intravaginal sponges containing 60 mg of MAP for six days, plus 37.5 µg of d-cloprostenol and 200 IU of eCG, both of which were injected 24 h before sponge removal. Motility, strength, and HOST-reacted spermatozoa were recorded after cooling, according to treatment. Timed artificial insemination was performed at approximately 37 hours after sponge removal. Seminal motility (58.8±11.1 and 51.3±2.5%) and strength (2.9±0.5 and 2.8±0.3) did not differ (P>0.05) between the T24 and T48 samples. Swelled spermatozoa were superior (P<0.001) at T24 (63.5±19.7%) compared to T48 (31.2±22.1%). The pregnancy rates were similar (P>0.05) at T24 (26.5%) and T48 (21.5%). Goat semen maintains acceptable quality after cooling at 5ºC for 24 and 48 hours and can be used in artificial insemination programs in Cape Verde goats. Certain adjustments, including the time of artificial insemination relative to sponge removal, must be made to achieve better pregnancy rates.

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INTRODUCTION

The Republic of Cape Verde has a dry, subtropical climate, characterized by a short rainy season (3 months) and a dry season that lasts approximately 9 months. This long period of drought impairs livestock rearing. Also, the technologies currently employed in this sector do not meet the demand for food in the country. It therefore has become necessary to define sustainable alternatives to improve the performance of the agricultural sector and to increase the food supply for Cape Verde's people. One possible alternative is sustainable intensification of the traditional farming practices that already exist in the country, such as the dairy goat rearing.

The combination of animal breeding programs with reproductive biotechnologies can increase the productivity of herds. Artificial insemination (AI) can be inserted into this context, because it is one of the most important biotechnologies of reproduction, and it is widely used for the genetic improvement of livestock (Nunes *et al*., 2002). AI, associated with livestock control and the identification of genetically superior individuals, should be considered for a gradual increase in the production of herds.

The maximum storage period for goat semen is an important point to be defined. The success of goat semen cooled for 12 to 24 h at 5ºC was reported in Brazilian dairy goats (Siquiera *et al*., 2009). If it is possible to establish that the storage of semen for 48 h at 5ºC does not reduce its fertilizing capacity, it would be possible to collect semen from bucks on the Island of Fogo in the morning and then perform the inseminations on the Island of Santo Antão, which constitutes the greatest distance to be covered within the Cape Verdean archipelago.

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The objective of this study was to determine whether semen cooled for 48 hours at 5°C has the same fertility potential as semen cooled for 24 hours at 5° C in artificial inseminated Cape Verdean goats.

MATERIALS AND METHODS

The goats used in this experiment belonged to 32 different properties, but all with very similar rearing systems. The production system included goats maintained in raised conditions during the day, when forage was abundant, or housed in pens of rocky ground during the night and fed two to three times per day with native plants, green or dry (depending on the local availability), and small amounts of corn once per day.

Three males of the Canary breed, sexually mature and previously approved by andrological exam, were used as semen donors. These breeders were kept in individual pens with a concrete solarium and a covered area, receiving native plants, dry or green (depending on the local availability). They also received concentrated feed, corn, and mineral salt.

A total of 133 Cape Verdean goats of different categories (nulliparous, lactating and dry goats) were randomly assigned into two groups to be artificially inseminated with semen cooled at 5ºC for 24 h (T24) or 48 hours (T48), using the Embrapa transcervical artificial insemination technique (Fonseca *et al*., 2011). Synchronous estrus was induced as previously reported (Fonseca *et al*., 2005a), consisting of six-day treatment with an implant containing 60 mg of medroxyprogesterone acetate (MAP; Progespon®, Syntex S.A., Indústria Bioquímica e Farmacêutica, Buenos Aires, Argentina) plus an intramuscular injection of 200 IU of eCG (Novormon® 5.000, Syntex S.A., Indústria Bioquímica e Farmacêutica, Buenos Aires, Argentina) and 37.5 mg of d-cloprostenol (Prolise®, ARSA S.R.L., Buenos Aires, Argentina) 24 hours before sponge removal.

Semen was collected using an artificial vagina on days 1 and 2 before insemination, with the intention of providing doses cooled for 48 and 24 hours, respectively. The bucks were alternately assigned to the cooling treatments. After collection, the semen was kept in a water bath at 37°C until the macroscopic (volume, color, and odor) and microscopic (motility, strength, and concentration) assessments were performed. The volume was measured in a 15 mL conical tube, graduated at 0.1 mL. Motility (0-100%) and strength (0-5) were evaluated subjectively by means of a phase contrast microscope. The sperm concentration was determined using a Neubauer chamber, filled with a solution of sperm and distilled water at a ratio of 1:400.

Immediately after being classified as suitable to be processed, with at least 70% motility and a minimum strength of 3, the ejaculate was diluted in Tris-egg yolk at 2.5% extender, adapted from Evans and Maxwell (1987), until a final concentration of 600 million viable sperm per milliliter, packaged into 0.25 mL straws (150 x 10^6 per straw). The straws were inserted into a container (Botutainer®; Botutech-Botucatu, Botucatu Brazil) adapted for cooling and transporting the semen. This adaptation consisted of housing a set of conical tubes of 15 and 50 mL, one inside the other, and the space between the tubes was filled with 35 mL of alcohol at 70° GL. The set of tubes was then placed inside the container. The central compartment of this equipment was also filled with 100 mL of alcohol at 70° GL. The alcohol was used to improve the thermal diffusion.

A thermometer (200-45 GULterm®, Gulton of Brazil, São Paulo, Brazil) was coupled to the Botutainer®, which allowed for monitoring of the temperature inside it. When the temperature reached 10°C, one of the two ice packs that came with the Botutainer® was replaced with another previously frozen pack, to maintain the temperature inside the container between 5 and 10°C. After the cooling period the samples were again assessed for motility and strength with a phase contrast microscope. The hypoosmotic swelling test was also performed, according to the methodology proposed by Fonseca *et al*. (2005b), in a 125 mOsm/L fructose/citrate solution.

For each female, a single timed insemination was performed, using the Embrapa artificial insemination technique (Fonseca *et al*., 2011) 37 h after sponge removal. Data were recorded at insemination: the body condition score (BCS), ranging from 1 for skinny animals to 5 for obese; depth of semen deposition (DSD), ranging from 0 to 5, with 0 indicating vaginal deposition, 1 to 4 indicating the number of cervical rings transposed and 5 indicating intrauterine insemination; and the temperature inside the Botutainer® at the withdrawal of the insemination dose. The kidding rate was recorded for both treatments, as well as the correlation between the interval from sponge removal to artificial insemination and the kidding rate.

The means of sperm motility and strength and the kidding rates of the two different periods of cooling (24 or 48 hours) were evaluated by the Kruskal-Wallis test. Data regarding the time interval between sponge removal and artificial insemination and the depth of semen deposition were subjected to one-way analysis of variance (SAEG System; Ribeiro Júnior, 2001). Friedman's two-way analysis of variance by ranks was applied to evaluate differences in HOST-reacted spermatozoa from two periods of cooling (BioEstat 2.0; Ayres *et al*., 2000). All of the tests were performed at 5% significance.

RESULTS AND DISCUSSIONS

Fresh seminal parameters did not differ ($P>0.05$) among the bucks (Table 1).

Table 1: Parameters (mean ± SD) of fresh semen collected by artificial vagina from Canarian bucks.

Male	Volume (mL)	Motility (%)	Strength (0-5)	Concentration (billions/mL)
Buck 1	0.9 ± 0.2	81.0 ± 4.1	3.7 ± 0.4	2.8 ± 0.3
Buck 2	0.9 ± 0.1	76.0 ± 5.4	3.5 ± 0.3	2.7 ± 0.5
Buck 3	1.0 ± 0.3	80.4 ± 4.1	3.6 ± 0.3	2.8 ± 0.6

With the adaptations made in the Botutainer®, the cooling curve was 0.08°C/minute for both treatments (Fig. 1). This rate of temperature fall was slightly lower than that recommended by Machado and Simplício (1995), who argued that for goat semen, the rate of cooling must remain between 0.25 and 0.35°C per minute, until the temperature of 5°C is reached. However, the use of relatively slow cooling rates, less than 0.33° C/minute, allows for homogeneous and suitable dehydration, thereby minimizing injuries to the cell membrane and preventing the induction of premature capacitation and acrosome reaction (Watson, 2000). Also, Bispo (2005) established that the curve at 0.5°C and 0.03°C per minute showed satisfactory results for goat semen cooled at 5°C and diluted in egg-yolk citrate–based media. It was observed that the curve inside the Botutainer® was very close to the curve recommended by Bispo (2005), which allowed for the use of this equipment for this procedure. The equipment also efficiently maintained its internal temperature at between 5° C and 10 ° C (Fig. 1).

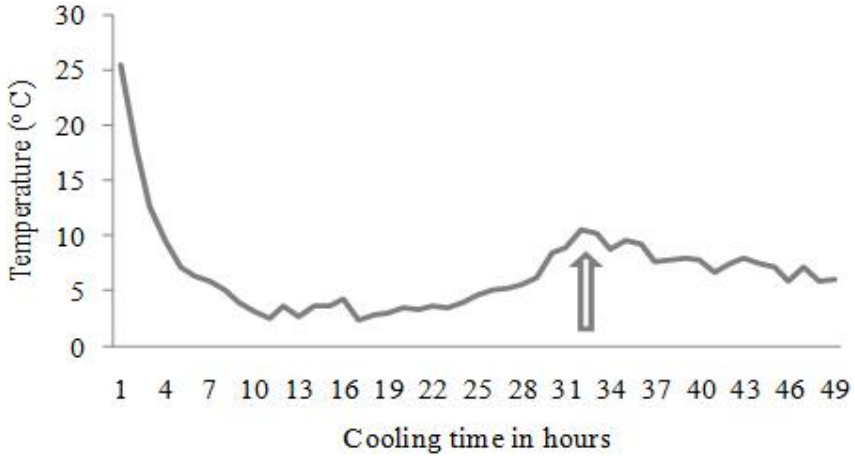

Fig. 1: Curve of temperature inside the Botutainer®; the arrow indicates the moment of replacement of the recyclable ice.

Sperm motility and strength at the time of collection and after 24 or 48 hours of cooling at 5°C were recorded (Table 2). Siqueira *et al*. (2009), using similar conditions for semen preservation, reported similar motility of 66% and 62% and strength of 3.4 and 3.2 for semen cooled for 12 and 24 h, respectively. Similarly, in the present study, there was no difference (P>0.05) between the maximum (48 hours) and minimum (24 hours) storage periods, in agreement with Roca *et al*. (1997), who did not find a difference (P>0.05) for sperm viability in goat semen stored at 5°C for 24 or 48 hours and diluted in Tris-egg yolk 2% medium. Salvador *et al*. (2006) also reported similar motility (60%) for goat semen stored at 5°C for 24 or 48 hours (in milk-based extender). In contrast, Islam *et al*. (2006) and Viana *et al*. (2006), using a methodology similar to that of the present study, observed the motility of goat semen cooled at 5°C for 24 (56%) or 48 h (48 and 40%). These findings imply that the container used in the present study efficiently maintained the temperature indicated for goat semen.

Table 2: Parameters of Canarian goat semen before and after cooling for 24 or 48 hours at 5°C.

Seminal parameters	Cooling period		
	Fresh	24 hours	48 hours
Ejaculations	22	10	12
Motility (%)	79.7 ± 4.7^{a}	58.8 ± 11.1^{b}	51.3 ± 2.5^{b}
Strength (1 to 5)	3.5 ± 0.3^{a}	2.9 ± 0.5^{b}	2.8 ± 0.3^{b}
Swelled spermatozoa (%)	-	63.5 ± 19.7^{A}	31.2 ± 22.1^{B}

^{a,b}Means with different superscripts within rows differed (Kruskal-Wallis, P<0.05). ^{A,B}Means with different superscripts within rows differed (Friedman, P<0.001).

In addition to motility and strength testing, the hypoosmotic swelling test was also performed, to analyze spermatozoa changes during the preservation period. The HOST test, adapted for goats (Fonseca *et al*., 2005b), revealed that the goat semen lost (P<0.001) membrane integrity from 24 to 48 h of storage. This finding was also verified by Roca *et al*. (1997), Islam *et al*. (2006), Viana *et al*. (2006) and Silva (2010), who worked with

goat semen cooled at 5ºC. This test should be considered in conjunction with motility and strength testing when choosing the best time for storage, especially in association with pregnancy rate. The changing of the ice packs in the 48 h containers when they reached 10ºC should also be considered as a possible cause of membrane damage in the present study.

The data recorded at the time of AI showed homogeneity between treatments (Table 3).

Table 3: Data recorded (mean ± SD) at the time of artificial insemination (AI) in estrus-induced Cape Verdean goats, with cooled semen at 5ºC for 24 or 48 hours

	Cooled semen		
Variable	24 hours	48 hours	Total
Body condition score (1 to 5)	2.7 ± 0.4	2.5 ± 0.3	2.6 ± 0.4
Internal container temperature (ºC)	7.7 ± 2.2	6.3 ± 1.4	7.0 ± 2.0
Interval from sponge removal to AI (hours)	36.7 ± 1.0	37.2 ± 2.7	37 ± 2.1
The depth of semen deposition (0 to 5)	3.3 ± 1.6	3.5 ± 1.5	3.4 ± 1.6

A positive correlation (r=0.27; P<0.01) was noted between the interval from sponge removal to AI and the depth of semen deposition. This finding could imply that the goats were inseminated in an inadequate time, possibly at estrus onset, when cervical dilation was not satisfactory to provide transposition of the cervical rings. Thus, it is expected that inseminations performed at intervals greater than those used in this study (37 h) would make it easier to pass through the cervical rings with the inseminating gun, which could improve fertility rates, because these rates are directly affected by the depth of semen deposition (Traldi, 2006, Fonseca *et al*. 2010; Maia, 2010).

It was also found that a longer interval from sponge removal to AI, in addition to facilitating the deposition of semen deeper into the female reproductive tract, was also positively correlated with kidding rate (r=0.29; P<0.001). Based on this finding, it can be concluded that 37 hours after sponge removal is too soon to perform artificial insemination with cooled semen when using the hormonal protocol suggested here. Some studies (Lebouef, 2000; Machado and Simplício, 2001; Barbas *et al*., 2006) have recommended that timed AI in goats must occur, on average, 44 hours after the removal of progestin implants. Fonseca *et al*. (2011) suggested that AI must occur at between 48 and 55 hours after the removal of the implants, according to the semen used (fresh/cooled/frozen-thawed).

The kidding rate did not differ (P > 0.05) between animals from T24 (26.5; 18/68) and T48 (21.5; 14/65). The 24.1% (32/133) kidding rate reported in the present study is inferior to the 64% (Eppleston *et al*., 1994), 74% (Roca *et al*., 1997) and 71 % (Mara *et al*., 2007) pregnancy rates reported in earlier artificial insemination programs using goat semen cooled at 5°C and induced estrus. It is known that increases in conception rates have been achieved (in goats) when insemination was performed in the final third of estrus (Smith, 1986, quoted by Siqueira *et al*., 2009) and that the correct identification of estrus had a positive impact on artificial insemination programs (Nuti, 2007). It is important to highlight that in protocols for estrus and ovulation synchronization using progestin implants, insemination must be performed 42 to 60 hours after the removal of the implants (Machado and Simplicio, 2001; Traldi, 2007; Fonseca *et al*, 2010). In the present study, as a function of local Cape Verdean conditions, it was not possible to follow this recommendation, and the inseminations had to be anticipated (37.0 ± 2.1 hours; Table 3). This anticipation might have negatively affected the kidding rates.

Also, it is known that a low body condition score (<2.5) negatively affects follicular growth and plasma progesterone concentrations (Viñoles *et al*. 1999), interfering with the results of hormonal treatments. The animals in this experiment were inseminated at a body condition score of 2.6 ± 0.4, very close to the limit stipulated by Viñoles *et al*. (1999). This fact might have affected the responses of the animals to the hormonal treatment and, consequently, the kidding rate.

Conclusion:

In conclusion, the results of this study showed that goat semen maintains acceptable quality after cooling at 5^0C for 24 and 48 hours, and it can be used in artificial insemination programs in Cape Verdean goats. Some adjustments, including the time of artificial insemination relative to sponge removal, must be made to achieve better pregnancy rates. However, there have been no studies or reports about the reproductive rates of Cape Verdean goats. This lack is significant when evaluating the data, making it difficult to classify the results as satisfactory or unsatisfactory, because there are no specific parameters for comparison. However, the conclusion is that the methodology used was efficient in spreading genetic material in the archipelago of Cape Verde in a restricted and orientated form, which is a factor that should be considered in the context of local breed preservation and programmed breeding.

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