

## A Study on the Effects of Chilling Period on Sperm Quality of Sannan and Jamunapari Goats

### Short Communication

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### ABSTRACT

The objective of the present study was to examine the effects of chilling period on semen quality of Sannan and Jamunapari. Semen of goat breeds were collected with artificial vagina and chilled at 5-9 °C. Volume, colour and pH were determined on fresh semen. After dilution, microscopic examination was performed for the progressive motility and sperm viability. Diluted semen of both breed was compared by microscopic examination from day 0 to 3 during chilling. Hemocytometer was loaded with 10 µL of semen to evaluate the sperm count. Eosin stain (1%) was used to assess the viable sperm. Although viability and sperm velocity of both goat breed decreased ( $P < 0.05$ ) in a time dependent manner during chilling, the viability between the Sannan and Jamunapari did not differ significantly. However there was a significant difference for means of sperm velocity ( $P < 0.05$ ). Progressive motility and viability decreased gradually during chilling at 5-9 °C. In order to obtain higher successful rate of insemination, chilled goat semen should be supplied to the farmers within a day.

**KEY WORDS** chilling period, goat semen, velocity, viability.

### INTRODUCTION

Artificial insemination (AI) with fresh, chilled or frozen-thawed semen is a basic tool in goat breeding, allowing the diffusion of caprine semen with high genetic value (Leboeuf *et al.* 2000; Hidalgo *et al.* 2007). Nowadays, some chilling methods have been yielding expressive results in conserving semen for several days (Iguer Ouada, 2001), allowing semen exchange between countries. Semen quality and its relationship to fertility are of major concern in animal production. Abeygunawardena *et al.* (2001) stated that the proportion of calving from AI was negligible in Eastern Province (EP) and Northern Province (NP) when compared to other regions of Sri Lanka. In Jaffna peninsula, AI center

at Thirunelvely has been involving in AI service since 1957. At this center, fresh goat semen is diluted with semen extenders to prepare diluted semen which is stored in the refrigerator before supplying to the farmers. Even though the successful rate of insemination of goat semen is low in Jaffna Peninsula than in the other regions of Sri Lanka, there are no studies regarding the doe factors, semen factors, inseminators and successful rate of the AI service of goat semen in Jaffna. As goats play a vital role in the economy of the poor and marginal farmers, successful insemination rate of goat semen have to be increased to obtain the higher pregnancy rate. Thus, the objective of the present study was to examine the effects of chilling period (at 5-9 °C) for 3 days on the viability and velocity of goat semen.

## MATERIALS AND METHODS

### Collection of goat semen

Fresh semen of Sannan (5 years old) and Jamunapari (nearly 7 years old) was collected by using artificial vagina twice a week for 6 months from AI centre at Thirunelveli. It was then stored in the refrigerator at 5-9 °C for further evaluation.

Totally twelve semen samples were collected from both breed. Prior to semen collection, the goats were sexually aroused, by visual, olfactory and auditory stimuli. Three goats for both breed were used for the present study.

### Gross examination of semen

General examination including volume, colour, viscosity and pH was performed before dilution with 2.9% (wt/v) sodium citrate buffer solution. Volume and pH were measured with graduated cylinder and pH paper (sigma) respectively. Colour and viscosity were assessed by visual observation.

### Microscopic examination of semen

After dilution (1:100) with sodium citrate buffer solution, microscopic examination was performed for the progressive motility. Diluted semen of both breed was compared for the progressive motility using hemocytometer (Improved Neubauer) and stop watch from day 0 to day 3 during chilling. The number of small boxes on which sperm moved was counted.

Hemocytometer was loaded with 10 µL of semen (1:100 dilutions) by using micropipette to evaluate the progressive motility and sperm viability. Eosin stain was used to assess the viable sperms. Determination of viability was conducted as triplicate for each sample and the whole set of calculations were repeated for more than 18 samples for both goat breeds.

### Statistical analysis

Statistical analysis was performed by using Prism 5.04. Ordinary two-way ANOVA with Bonferroni post-test for multiple comparisons was performed to analyze sperm viability and velocity of both breeds.

## RESULTS AND DISCUSSION

The semen colour of both breeds was creamy white, their odour was similar to the fresh milk and the average pH was 7. Although sperm viability (Table 1) and velocity (Table 2) of both breeds decreased ( $P<0.05$ ) in a time dependent manner during chilling at 5-9 °C, the viability of Sannan and Jamunapari semen did not differ by breed. However there was a significant difference between means of sperm

velocity ( $P<0.05$ ). Percentage of dead sperms increased from day 0 to day 3. Udeh and Oghenesode (2011) stated that the drastic decline in sperm motility and viability could be attributed to gradual depletion of nutrients such as potassium, sodium and plasma protein during chilling.

**Table 1** Comparison of the sperm viability of Sannan and Jamunapari during chilling

Storage time (hrs)	Goat breeds (Mean±SE)	
	Sannan (%) (n=18)	Jamunapari (µm/s) (n=18)
0 h	93.02±0.69 <sup>ax</sup>	91.13±0.26 <sup>ax</sup>
24 h	86.18±0.62 <sup>bx</sup>	87.74±0.22 <sup>bx</sup>
48 h	80.96±0.75 <sup>cx</sup>	81.91±0.22 <sup>cx</sup>
72 h	76.64±0.53 <sup>dx</sup>	77.57±0.24 <sup>dx</sup>

<sup>x, y</sup>: the means within the same row with different letter, are significantly different ( $P<0.05$ ).

<sup>a, b, c, d</sup>: the means within the same column with different letter, are significantly different ( $P<0.05$ ).

SE: standard error.

**Table 2** Comparison of the sperm velocity of Sannan and Jamunapari during chilling

Storage time (hrs)	Goat breeds (Mean±SE)	
	Sannan (%) (n=18)	Jamunapari (µm/s) (n=18)
0 h	22.46±1.10 <sup>ax</sup>	19.06±0.79 <sup>ay</sup>
24 h	16.87±0.83 <sup>bx</sup>	14.21±1.18 <sup>by</sup>
48 h	11.19±0.88 <sup>cx</sup>	8.923±0.88 <sup>cy</sup>
72 h	4.99±0.36 <sup>dx</sup>	4.347±0.75 <sup>dy</sup>

<sup>x, y</sup>: the means within the same row with different letter, are significantly different ( $P<0.05$ ).

<sup>a, b, c, d</sup>: the means within the same column with different letter, are significantly different ( $P<0.05$ ).

SE: standard error.

In our present study sodium citrate buffer solution was used as semen diluents for maintaining osmotic balance. Sodium Citrate-based extender was found better than Milk-based extender for sperm motility (Schindler and Amir, 1961; Lopez *et al.* 1999) and however, some researchers reported that the higher lambing rate was obtained from semen diluted with Sodium Citrate compared to the Milk-based extenders (Salamon and Robinson, 1962). In general, a buffering solution for goat sperm media should have a pKa of 7.0 (pH range of 6.0-8.0). In the freezing of goat semen, the quality of sperm motility is important in determining the post-thawing survivability of the spermatozoa in terms of progressive motility. Further, the velocity measurements of sperm motility are useful in predicting the fertility of semen (Holt and North, 1984; Budsworth *et al.* 1988; Aitken, 1990; Sarma *et al.* 1996; Tardiff *et al.* 1997; Kirk *et al.* 2005).

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