

Original Research Article

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Post-Thaw Evaluation of Cryopreserved Boer Crossbred Buck Semen Extended in TEYG (Universal) Extender and its Fertility Rate

S. Manivannan*, M. Selvaraju, R. Ezakial Napolean and P.S.L. Sesh

Department of Veterinary Gynaecology and Obstetrics, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

*Corresponding author

ABSTRACT

Keywords

Post-Thaw evaluation, TEYG, Fertility rate.

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Two Boer crossbred bucks aged between 4 1/2 and 5 years which are maintained at Frozen Semen Bank, Department of Veterinary Gynaecology and Obstetrics, Veterinary College and Research Institute, Namakkal were utilized for this study. The semen was collected from two adult Boer crossbred bucks. The collected ejaculates were immediately transferred to semen processing laboratory and kept at 34°C water bath. The semen collected from the two bucks were pooled in to a sterile glass tubes and immediately subjected to further processing and cryopreservation and finally stored in liquid nitrogen container. These semen straws were subjected evaluation by CASA at various storage periods. The sperm motility parameters decreased slowly as the duration increases. A total of 10 Tellicherry does were selected and all the does were inseminated with frozen thawed buck semen prepared from TRIS universal diluent and the pregnancy was confirmed by ultrasonography at 45 days of gestation. The fertility rate was 70 per cent using semen diluted with TEYG universal diluent. The survival rate and fertility rate was higher in cryopreserved Boer crossbred buck semen diluted with TEYG extender.

Introduction

The Boer breed of goat is well known for its good meat production ability and high prolificacy and butits adaptability in a tropical and sub-tropical region is difficult (Ramsay *et al.*, 1987). Hence the development of Boer crossbred is essential to utilize its germplasm in order to maximize the profit to the goat farmers. Artificial insemination (AI) has become an inevitable tool in the breeding management of most of the domestic animals. It has been successfully implemented in breeding of bovines but is still lacking in goats for its efficiency. The commercial use of cryopreserved semen has not been popular in caprine reproduction. Tris- egg- yolk based

extenders have been widely used for freezing of buck semen (Moussa *et al.*, 2002).

Sperm motility, in general and characteristics of sperm motion in particular could be some of the indicators of the quality of spermatozoa. Commonly, by evaluating the proportion of progressive motile percent at different stages, the quality of semen is monitored. However, evaluation of characteristics of sperm motion may provide valuable information on why certain samples despite containing good proportion of progressive motile spermatozoa pre freezing poorly freezable. Conventional methods of

measurement of sperm kinematic characters is cumbersome, time consuming and subjective. To get over these difficulties Computer assisted semen analysis (CASA) is the equipment of choice to provide precise and accurate information on sperm motion characteristics. Therefore, a study was undertaken to analyze the changes in motility characteristics of Boer cross spermatozoa during after cryopreservation by Computer Assisted Semen Analysis (CASA) technique and its fertility rate.

In the last decades, oestrus synchronization together with AI is extensively applied in the reproductive management of goat to propagate the superior germplasm (Amle *et al.*, 2017). In recent years, real-time ultrasonography has been used more frequently for pregnancy diagnosis in small ruminants (Hesselink and Taverene, 2017). At this juncture, a study was conducted to assess the post thaw sperm kinematic character by CASA and their efficacy on fertility rate of Boer crossbred buck semen.

Materials and Methods

Two Boer crossbred bucks aged between 4 1/2 and 5 years which are maintained at Frozen Semen Bank, Department of Veterinary Gynaecology and Obstetrics, Veterinary College and Research Institute, Namakkal were utilized for this study. The semen was collected from two adult Boer crossbred bucks. The semen was collected twice in a week and each time two ejaculates per buck was collected. The semen donor was allowed to mount on the dummy. Two false mounts were given before semen collection during every time. On third mount, the penis was directed in to AV and the semen was collected in a collection cup. The collected ejaculates were immediately transferred to semen processing laboratory and kept at 34°C water bath. The semen collected from the two

bucks were pooled in to a sterile glass tubes and immediately subjected to analysis and immediately subjected to further processing and cryopreservation and finally stored in liquid nitrogen container. This semen straws were subjected evaluation by CASA at various storage period.

Computer Assisted Semen Analyzer (CASA)

In this study CASA was employed to study the kinematic character of Boer crossbred buck semen extended in TRIS universal extender. The CASA version used in this study was CASA: IVOS version No.14 was used to analyze sperm kinematic characteristics. The CASA settings were fixed with slight modification as described by Sundaraman *et al.*, (2008).

A two µl of neat pooled semen sample was diluted with 400 µl of Tris solution. After dilution, two µl of sample was loaded in one chamber of pre warmed Leja slide and kept in microscopic stage of CASA. Four fields were scanned automatically and sperm motility characteristics were determined with a 10X objective at 37°C. The sperm kinematic characteristics viz., motility (per cent), progressive motility (per cent), VAP (Average Path Velocity, µm/sec), VSL (Straight Line Velocity, µm/sec), VCL (Curvi Linear Velocity, µm/sec), ALH (Amplitude of Lateral Head displacement, µm), LIN (Linearity Index; $LIN = \{VSL/VCL\} \times 100$) and the sperm the concentration were measured by using CASA.

Post thaw semen analysis by CASA

Frozen straws were taken from the storage container and placed into a water bath maintained at 37 °C for 30 seconds. After wiping the straws, the laboratory seal of semen straw was cut and the semen was

poured into a sterile test tube. A 2 µl of post thaw semen sample was diluted with 200 µl of Tris / NS. After dilution, two µl of sample was loaded in one chamber of pre warmed Leja slide and placed in microscopic stage of CASA. Four fields were scanned automatically and sperm motility characteristics were determined with a 10X objective at 37°C. The motility characteristics were analyzed for frozen thawed semen as done for fresh semen samples as explained earlier.

Estrus synchronization and evaluation of fertility rate

Ten non pregnant pluriparous does were selected in villages in and around Namakkal. The selected does were subjected to estrus synchronization using intra vaginal sponge + PGF₂α + eCG protocol as explained by Baldassarre and Karatzas (2004).

The selected does were inserted with intravaginal sponges containing 0.35 mg of synthetic progesterone on day 0 and kept *insitu* for 11 days. One day before sponge withdrawal (day10) 250µg of PGF₂α and on the day of sponge removal (day11) 200 IU of equine chorionic gonadotropin were given intramuscularly. The synchronized does were inseminated at fixed time using the frozen semen straws prepared in universal extenders and the conception rate was analyzed.

Artificial insemination and pregnancy diagnosis

After 48 hours of eCG injection, the confirmation of estrus was done by examining the changes in vulva, vaginal mucous membrane, mucus discharge and by visualizing external cervical os of the does with the help of speculum along with borescope. With the help of borescope the external os was clearly visualized for the changes during estrum. All selected ten does

were artificially inseminated with previously prepared semen straws by using borescope which helped in proper deposition of semen at the mid cervix. Pregnancy diagnosis was carried out by ultrasonography after 45th day of AI. The collected was analyzed statistically. Statistical evaluations were carried out using the statistical package for social studies software (SPSS version 20).

Results and Discussion

Effect of TRIS semen extenders on sperm kinematic characteristics evaluated by CASA of post freeze stage buck semen at different storage period is depicted in Table. 1. The majority of the post-thaw sperm motility parameters such as Sperm motility, Progressive motility, Average path velocity, Straight line velocity, Curvilinear velocity, Amplitude of lateral head displacement, Beat cross frequency and Linearity index evaluated by CASA were significantly higher at 24 hours and decrease progressively as the duration storage period increases. On evaluation of effect of freezing, it was observed that, all the motility characteristics of spermatozoa were significantly influenced by freezing and thawing, which reiterates earlier findings in Boer cross and Barbari goats (Sundaraman and Edwin, 2005). All the sperm motility and velocity parameters were significantly reduced in the post-thawing semen. Changes in the osmotic pressure during semen cryopreservation and thawing critically affect the motility and survival of the spermatozoa. This may be the most important deterrent to sperm survival during cryopreservation (Jamadiet *al.*, 2017).

Furthermore, membrane destabilization might occur when the sperm plasma membrane undergoes a phase transition from the liquid crystalline phase to the gel phase due to a decrease in temperature during cryopreservation (Sundaraman and Edwin, 2008).

Table.1

Effect of TRIS semen extenders on sperm kinematic characteristics evaluated by CASA of post freeze stage buck semen at different storage period								
Duration of storage period	Sperm motility (%)	Progressive motility (%)	Average path velocity (µm/s)	Straight line velocity (µm/s)	Curvilinear velocity (µm/s)	Amplitude of lateral head displacement (µm)	Beat cross frequency (Hz)	Linearity index (%)
24 hrs	59.83±1.38	39.19±1.50	70.15±3.98	58.61±3.89	145.65±5.98	6.9±0.5	21.83±0.05	40.89±0.30
48 hrs	57.31±1.68	35.69±1.50	67.45±4.08	55.61±3.19	139.55±5.38	6.6±0.5	21.03±0.05	39.56±0.5
10 th day	55.61±1.38	33.69±1.20	65.15±4.01	52.31±3.09	135.15±5.04	6.5±0.5	20.83±0.05	38.56±0.10
20 th day	53.21±1.08	30.69±1.01	63.10±4.11	51.30±4.39	133.05±4.94	6.3±0.5	20.13±0.05	38.36±0.10
30 th day	51.21±1.51	29.89±1.00	62.10±4.11	47.80±4.39	125.05±4.14	6.1±0.5	19.13±0.05	37.26±0.10

(Statistically all kinematic parameters (rows) differ significantly between different storage periods (columns))

Computer Assisted Semen Analyzer (CASA)

No	Variables	Settings
1	Frame rate(Hz)	60.00
2	Frames acquired	30.00
3	Minimum contrast	50.00
4	Minimum cell size	5.00
5	Threshold straightness	70.00
6	Medium VAP cut -off	25.00
7	Low VAP cut-off	5.00
8	Low VSL cut-off	5.00
9	Non-motile head intensity	70.00
10	Static size limit-minimum	0.52
11	Static size limit-maximum	1.99
12	Static intensity limit-minimum	0.50
13	Static intensity limit-maximum	1.25
14	Static elongation limit-minimum	17.00
15	Static elongation limit-minimum	66.00
Optic calibration		
16	Magnification	1.89
17	Camera frequency(Hz)	60.00

The irreversible changes in the sperm membrane induced by lipid phase transitions during cooling warming may possibly affect the movement characteristics of spermatozoa during semen processing for cryopreservation (Deleeuw *et al.*, 1990). In addition, frozen-thawed sperm are more vulnerable to oxidative stress due to peroxidation than sperm in freshly diluted semen (Neild *et al.*, 2005). As semen is diluted many fold in the extender it reduces the total antioxidant concentration in the medium and cells (Kumar and Das, 2005). Many sperm are killed during cryopreservation. Thus, it is likely that cryopreserved sperm cells are posed to more ROS concentration and therefore many of the surviving cells post-thaw exhibit as if they are capacitated or acrosome reacted (Bailey *et al.*, 2000). The overall effects of these events may adversely affect quality of post thawing semen.

A total of 10 Tellicherry does were selected and all the does were inseminated with frozen thawed buck semen prepared from TRIS universal diluent and the pregnancy was confirmed by ultrasonography at 45 days of gestation.

Out of 10 does 7 does were conceived (70 per cent) and the remaining does were conceived during subsequent estrus.

The effect of TRIS semen extenders on sperm kinematic characters evaluated by CASA of post freeze stage of Boer crossbred buck semen at different storage period decrease progressively as the duration storage period increases. The fertility rate was good using this frozen thawed Boer crossbred buck semen (70 per cent). TRIS extender augment fertility in does more than 50 per cent and hence, TRIS can be used as universal extender for cryopreservation with less cryo damage with maximum conception rate for Boer crossbred buck semen.

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