Effect of time interval between the semen collection and its dilution on seminal attributes during semen cryopreservation in goats

ABSTRACT

A study was conducted to evaluate the best suitable time to dilute the fresh ejaculated semen for cryopreservation in goats. 10 healthy bucks of Sirohi breed maintained at goat frozen semen station were selected for the experiment. The semen was collected from each buck in morning hours using artificial vagina. Just after collection, the semen was divided into three groups (I, II and III) and diluted as per the experimental design viz. 0, 5 and 10 minutes post collection with tris egg yolk semen extender and subject to freezing. The frozen semen was evaluated after 7 days of storage in LN_2 cylinder. On evaluation a significantly ($P<0.05$) higher values for percent viable sperm and mitochondria activity were observed in group II compared to I & III group. Significantly($P<0.05$) higher values for percent viable ROS+ ve sperm and acrosome disrupted acrosome were observed in group I followed by Group III and II. The path velocity and kinematic characters exhibited significantly ($P<0.05$) higher values in group II compared to other group during experiment. The interpretation of the result suggests that both early and late dilution of neat semen result in poor post thaw semen quality. The neat semen should be diluted at 5 minutes of collection to achieve best post thaw semen quality in goats.

Keywords: Cryopreservation, Dilution, Semen, Goat, Seminal attributes

UNDER PEER REVIEW

INTRODUCTION

Cryopreservation has been considered as a stressful process for spermatozoa. The process involves subjecting the sperm cell to variable range of temperature within a short period of 5 hours. The spermatozoa are naturally maintained at 37°C throughout their journey from spermatogenesis to fertilization in the female reproductive tract. Use of assisted reproductive techniques like Artificial Insemination (AI), involves the collection of semen through use of artificial means (artificial vagina or electroejaculation). The collected semen is later diluted, equilibrated and frozen for its storage at -196°C. During insemination, semen is again subjected to thawing temperature of 37°C for retrieval of metabolic processes. Cryopreservation of semen represents a useful tool in the management of reproduction in goat production[1,2]. However, during cryopreservation spermatozoa are exposed to physical and chemical stress that results in adverse changes in membrane lipid composition, sperm

motility, viability and acrosome status. All these changes reduce the fertilizing ability of goat spermatozoa after cryopreservation[3]. In goats, the supplementation of semen extender with several antioxidants, such as quercetin, vitamin C and resveratrol, improved post-thawing sperm motility, viability, membrane and DNA integrity[4,5,6]. Supplementation of minerals in the animal diet to improve health[7,8] and reproductive performance, as well as supplementation of antioxidant[9] and membrane protectors in the semen extender are the areas where extensive research is being conducted. But meagre literarure is available to understand the effect of different processes involved in semen cryopreservation and their influence on post thaw semen quality particulary in goats. Cryopreservation in goat is different from that of cattle and buffalo. Secretion of bulbourethral enzyme in the goat semen has tendency to interact with egg yolk or skimmed milk in semen extender make it more liable to membrane damage[10]. The degree of reaction between sperm-semen extender and its relation with the metabolic retrieval in goat need to be studied to minimize the losses during semen freezing in goats. So, the present study was carried out to evaluate the effect of time interval between the semen collection and its dilution on seminal attributes during semen cryopreservation in goat.

MATERIALS AND METHODS

The present study was conducted at Goat semen frozen station, College of Veterinary Sciences and Animal Husbandry, DUVASU, Mathura. During the experiment, 10 healthy Sirohi bucks of similar age and weight (1.5-2 years, 38.0 ± 2.0kg) used as semen donor at semen station were selected. The semen was collected in the collection arena in the semen collection center near the laboratory. The semen from individual buck was collected in morning hours using the artificial vagina maintained at 38°C. Just after the collection neat semen was immediately transferred to the laboratory. The semen was then divided into 3 equal groups and subjected to treatment as per the experimental design. In Group I (0 min) semen was instantly mixed with the formulated dilutor, in group II (5 min) the collected

UNDER PEER REVIEW

semen was kept at 37°C for 5 minutes and then mixed with dilutor while in group III (10 min) the neat semen maintained at 37°C was mixed after 10 minutes post collection. Just after dilution, semen was immediately equilibrated for 4 hours at 4°C and later subjected to freezing using a semen programmable freezer, which sequentially reduces the temperature from 4°C to -140°C within 7min with use of Liquid Nitrogen vapours The frozen semen straws (0.25 ml, French mini) were stored for 7 days in liquid nitrogen (-196ºC). The stored semen was evaluated after 7 days for various seminal parameters (viability, mitochondrial activity, percent viable sperm affected by reactive oxygen species (ROS) and percent viable sperm with acrosomal damage) using flow cytometer (Single blue laser bench top Easycyte, IMV, France) and computer assisted semen analyzer (IVOS 2, IMV, France). Data generated was statistically analysed using SPSS software (SPSS for Windows, V 19.0; SPSS, Inc., Chicago, IL, USA)[11]. Data are presented as mean ± SE. The comparison among treatment means was made by Duncan's multiple range test[12] with significance level of $P<0.05$.

RESULTS

The study was conducted to evaluate the effect of time dependent change in the post thaw semen quality during dilution of fresh ejaculated semen with extender for semen cryopreservation. The values were recorded for the viability, mitochondrial activity, percent viable sperm affected by ROS and percent viable sperm with acrosomal damage evaluated through flow cytometry are presented in Figure 1. The values for the viability and mitochondrial activity were significantly ($P<0.05$) higher in the group II compared to group I and group III during the experiment. The different path velocities and kinematic characters that includes, curvilinear velocity (VCL, µm/sec) (average path velocity (VAP, µm/sec), straight line velocity (VSL, µm/sec), Linearity (Lin%), Straightness (Str %), Wobble (WOB%), beat cross frequency (BCF %) and maximum amplitude-lateral head displacement

UNDER PEER REVIEW

(ALH, µm) exhibited by spermatozoa in different groups during the experiment have been presented in Table 1. A significantly ($P<0.05$) higher values of path velocity VCL, VAP, VSL, Lin, Str, and kinematic characters have been exhibited by sperm in group II followed by group III and group I.

DISCUSSION

The values for the % viable ROS +ve sperm and those with disrupted acrosome were significantly ($P<0.05$) higher in Group I. ROS production is the characteristic of metabolically active spermatozoa under stress. Any disturbance in the electron transport chain in mitochondrial energy production system leads to increased production of ROS. The plasma membrane integrity that determines the sperm capacity to withstand cryodamage greatly affects the ROS generation in post thaw semen. ROS mainly act upon the plasma membrane. Acrosome membrane with inherent capacity to undergo capacitation under the influence of ROS is most liable for damages during increased ROS production[13]. The cryocapacitation and acrosomal cap damage are cellular exocytic-like events that influence the fertilizing ability of sperm, a fundamental prerequisite for successful conception after insemination[14]. During the experiment, higher values of ROS and acrosomal damage in group I indicates the higher sperm susceptibility to membrane damage due disturbed metabolic function leading to metabolic stress, delayed energy production and death of spermatozoa. Semen is maintained in quiescent state in the male reproductive tract. Spermatozoa become metabolically active and motile as it is released from male reproductive tract. Significantly ($P<0.05$) lower values for seminal attribute in group I indicates that sudden change in the sperm environment due to dilution is detrimental for sperm. Dilution of semen prior to attainment of complete metabolism may cause death of spermatozoa. Also, too much delay in semen dilution deprives the active spermatozoa from energy source in seminal plasma. Absence of external energy source (semen extender) during delayed dilution induced

UNDER PEER REVIEW

metabolic stress to spermatozoa leading to ROS generation resulting in efflux of cholesterol and phospholipids from plasma membrane making them liable to cryo-injuries and sperm death[15] leading to significantly (P<0.05) lower values in group III compared to group II. Significantly (P<0.05) lowers values of mitochondrial activity in the group I may be attributed to inability of spermatozoa to regain its metabolic activity immediately after ejaculation and instant dilution leading to cell death.

The different path velocities and kinematic characters exhibited by spermatozoa in different groups during the experiment have been presented in Table 1. A significantly (P<0.05) higher values of path velocity and kinematic characters that includes curvilinear velocity (VCL, µm/sec) (average path velocity (VAP, µm/sec), straight line velocity (VSL, µm/sec), Linearity (Lin%), Straightness (Str %), Wobble (WOB%), beat cross frequency (BCF %) and maximum amplitude-lateral head displacement (ALH, µm) have been exhibited by spermatozoa in group II followed by group III and group I. The motility is the result of energy transfer and its utilization by the spermatozoa tail[16]. Energy production through mitochondria and its utilization by sperm cell both depend upon the plasma membrane integrity that maintain the sperm internal environment. During the experiment the significantly (P<0.05) higher values of ROS+ve sperm indicative of increased ROS production might have disrupted the inter environment of spermatozoa through efflux of membrane proteins, phospholipids and cholesterol changing the selective permeable mature of membrane that disturbed the energy production and utilization mechanism leading to significantly lower values of path velocity and kinematic character during the experiment.

CONCLUSION

So, it can be concluded that both the early and delayed dilution had detrimental effect on post thaw semen quality of buck semen. Fresh ejaculated semen should de diluted after 5 minutes of collection for its use in semen cryopreservation.

UNDER PEER REVIEW

SIGNIFICANCE STATEMENT

Artificial insemination in goats is a novel technology recently adapted in field conditions. And the process of Cryopreservation is considered to be a stressful process for spermatozoa. The process involves subjecting the sperm cell to variable range of temperature within a short period of 5 hours, that leads to disruption of plasma membrane leading to production of ROS This study evaluated the effect of time dependent change in the post thaw semen quality during dilution of fresh ejaculated semen with extender for semen cryopreservation. It was concluded that both the early and delayed dilution had detrimental effect on post thaw semen quality of buck semen. It is hence recommended that the fresh ejaculated semen should de diluted after 5 minutes of collection for its use in semen cryopreservation.

Data Availability: All relevant data are within the paper and its supporting information files.

References:

1. Purdy PH. A review on goat sperm cryopreservation. Small Ruminant Research 2006;63(3):215–25.

2. Leboeuf B, Restall B, Salamon S. Production and storage ofgoat semen for artificial insemination. Animal Reproduction Science. 2000;62(1–3):113–41

3. Barbas JP, Mascarenhas RD. Cryopreservation of domesticanimal sperm cells. Cell Tissue Bank. 2009;10(1):49–62.

4. Daramola JO, Adekunle EO. Cryosurvival of goat spermatozoa in Tris-egg yolk extender supplemented with vitamin C. Arch. Zootec. 2015;64:261–268. doi: 10.21071/az.v64i247.402.

5. Lv C, Larbi A, Wu G, Hong Q, Quan G. Improving the quality of cryopreserved goat semen with a commercial bull extender supplemented with resveratrol. Animal Reproduction Science. 2019;208:106127. doi: 10.1016/j.anireprosci.2019.106127.

UNDER PEER REVIEW

6. Seifi-Jamadi A, Ahmad E, Ansari M, Kohram H. Antioxidant effect of quercetin in an extender containing DMA or glycerol on freezing capacity of goat semen. Cryobiology. 2017;75:15–20. doi: 10.1016/j.cryobiol.2017.03.002.

7. Keshri A, Roy D, Kumar V, Kumar M, Kushwaha R, Vaswani S. Impact of different source chromium sources on physiological response, blood biochemicals and endocrine status of heat stress in dairy calves. Biological Rhythm Research. 2022; 53(1):58-69.

8. Singh A, Kumar M, Kumar V, Roy D, Kushwaha R,Vaswani S. Effect of Nickle supplementation on antioxidant status, immune characteristics and energy and lipid metabolism in growing cattle. Biological Trace element research, 2019;190(1):65-75.

9. Mittal P, Anand M, Madan AK, Yadav S, Kumar J. Antioxidative capacity of vitamin E, vitamin C and their combination in Bhadawari bull semen. Veterinary World. 2014; 7(12:): 1127-1131.

10. Roy A. Egg yolk coagulating enzyme in the semen and Cowper's gland of the goat. Nature. 1957; 179:318-319.

11. SPSS. 2008. Statistical Packages for Social Sciences, Version 21.0, SPSS Inc., Illinois, USA.

12. Duncan DB. Multiple range and multiple “F” tests. Biometrics. 1955; 11:1-42.

13. Alahmar, AT. 2019. Role of Oxidative Stress in Male Infertility: An Updated Review. Journal of Human Reproduction Science, 2019; 12(1):4-18.

14 Lindsay G, Evans G, Maxwell WMC. Flow cytometric evaluation of sperm parameters in relation to fertility potential. Theriogenology. 2005; 63:445–457.

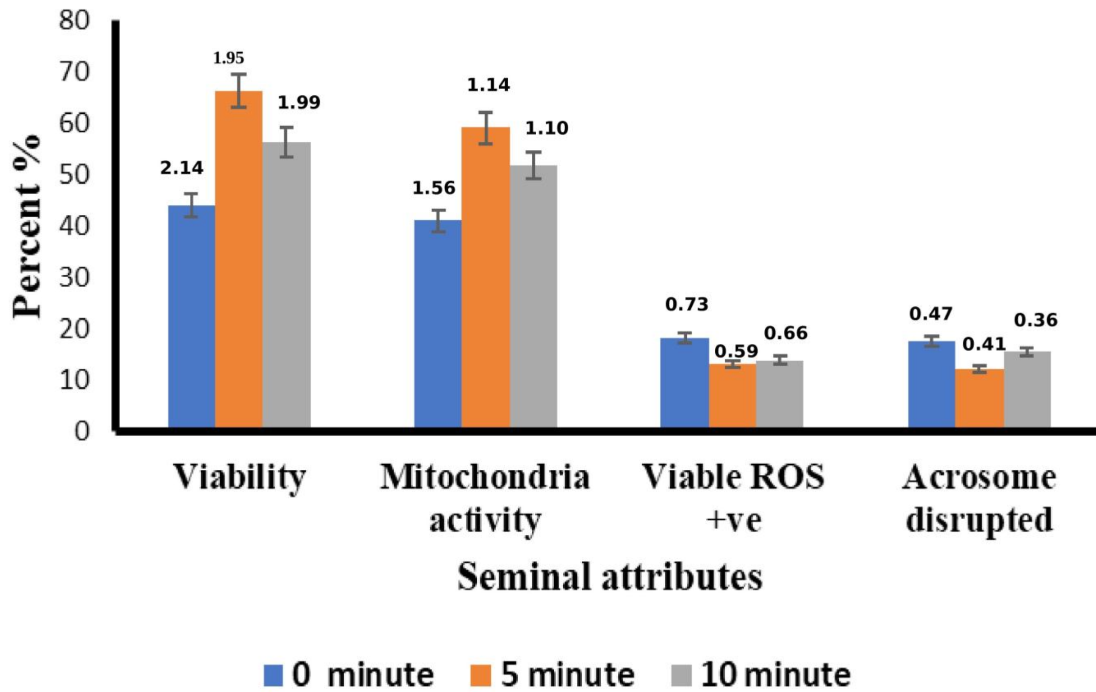
15. Anand M, Baghel G, and Yadav S.. Effect of egg yolk concentration and washing on sperm quality following cryopreservation in Barbari buck semen, Journal of Applied Animal Research, 2017;45:1, 560-565.

UNDER PEER REVIEW

16. Anand M, Yadav S. Assessment of motion characters of frozen-thawed Sirohi goat semen using computer assisted semen analyzer, Veterinary World. 2016; 9(2): 203-206.

UNDER PE

Fig. 1- Seminal parameters exhibited by frozen-thawed spermatozoa subjected to different thawing procedure



UNDER PEER

Table 1. Effect of time interval between the semen collection and its dilution on motility, path velocity and kinematic characters during semen cryopreservation in goats

Time / Parameter	0 minute (Immediately after collection)	5 minutes (Intermediate dilution time)	10 minutes (Delayed dilution time)
% Total motile	43.00±2.54[a]	59.86±1.84[c]	50.86±2.21[b]
%Rapid progressive	28.86±2.84[a]	39.14±1.05[b]	35.00±1.35[ab]
VCL (µm / sec)	75.71±11.49 [a]	109.69±2.74[b]	91.29±3.28[ab]
VAP (µm / sec)	44.00±3.65[a]	66.71±9.23[b]	55.57±15.14[a]
VSL (µm / sec)	37.57±3.61[a]	56.14±2.29[b]	47.71±2.68[ab]
LINEARITY (%)	21.57±1.96[a]	30.43±1.13[b]	26.29±1.06[ab]
STRAIGHT (%)	35.86±2.49[a]	49.00±1.46[b]	42.23±1.67[ab]
BCF (hz)	15.43±1.34[a]	22.57±0.78[b]	19.43±0.94[b]
Max ALH (µm)	3.14±0.26[a]	4.711±0.18[b]	3.71±0.28[a]

Mean values with different superscript differ significantly ($P<0.05$)