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Kinematic Response of Buck Sperm to Low-density Lipoproteins in Fresh Diluted, Short Term and Long Term Stored Semen

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ABSTRACT

The present study was designed to evaluate kinematic response of sperm cell to low-density lipoproteins (LDL) in fresh diluted, short-term (4°C) or long-term (-196°C) stored semen. Four healthy bucks of similar age and weight were selected as semen donor. The semen was collected twice a week using artificial vagina. The semen after initial evaluation was pooled and divided into three aliquots, each diluted with TRIS based extender containing 8% LDL to reach final concentration of 200 million sperm/ml. The first aliquot was evaluated after 15 to 20 minutes of its storage at 37 °C, second after it storage at 4°C for 48 hours and third was cryopreserved and evaluated after seven days of storage. Percent live sperm, sperm responsive to hypo osmotic swelling test and those exhibiting rapid progression were significantly ($P \leq 0.01$) higher in fresh diluted followed by short and long term sored semen. A significant ($P \leq 0.01$) decrease in the kinematic characters (average path velocity (VAP, µm/sec), straight line velocity (VSL, µm/sec), Linearty (Lin%), Straightness (Str %), Wobble (WOB%), beat cross frequency (BCF %) and maximum amplitude-lateral head displacement (ALH, µm) was observed in short term followed by long term store semen as compared to fresh diluted semen. Low-density lipoprotein was able to maintain the curvilinear velocity (VCL, µm/sec) of sperm subjected to 4°C during short term storage. In conclusion, decrease in temperature during semen storage alter the sperm path and its velocities, but LDL has a protective effect on sperm flagellar assembly and mitochondrial energy production system that sustained the sperm capacity to travel total distance per unit time upto 4°C during short term storage.

Keywords: Buck, CASA, kinematic character, semen storage, sperm

Semen management for its storage and utilization requires extenders. Extender provide environment necessary for the survival of sperms in artificial medium. It open up opportunity to prepare multiple dose from single ejaculate. Semen is inseminated as fresh diluted, stored at 4 °C (short term) or cryopreserved at -196 °C (long term) (Mara *et al.,* 2007). But unlike other farm animals, dilution of goat semen with extender containing egg yolk or skimmed milk results in loss of sperm viability, motility and plasma membrane integrity affecting its quality (Gangwar *et al.,* 2016). The seminal proteins in plasma (SBUIII, Phospholipase A_2) react with egg yolk or skimmed milk to form lethal compounds that damage sperm cells (Leboef *et al.,* 2000; Iritani and Nishikawa, 1963). Further, egg yolk has also been reported to influence the mitochondrial respiratory chain affecting the energy production system and is also a source of bacterial contamination (Cooter *et al.,* 2005). Use of lower egg yolk level in extender has been proposed as an alternate to overcome the interactive losses during its storage (Bispo *et al.,* 2011). But the process decreases the cryoprotective capacity of extender and increased sperm susceptibility to cold shock and cryoinjuries (Watson, 2000). To overcome these looses, egg yolk is now being replaced with low density lipoproteins (LDL) - a major constituent responsible for cryoprotective effect of egg yolk. LDL that is thought to reduce the lethal interactive looses and maintains flagellar apparatus and mitochondria electron transport chain (Chaveiro *et al.,* 2006). It has been reported that 8% LDL in extender has better capacity to maintain sperm characters in diluted buck semen utilized

for insemination (Anand and Yadav, 2016). So, the study was conducted to evaluate kinematic response of sperm to low density lipoproteins in fresh diluted and short term or long term stored semen.

MATERIALS AND METHODS

Experimental procedure

Prior to the start of experiment low-density lipoproteins (LDL) was separated from egg yolk as per the method described by Moussa *et al.* (2002). Four healthy Barbari bucks of similar age and weight were selected as semen donor. A total of 48 (12 from each buck) ejaculates were collected. After initial evaluation, samples collected from four bucks were pooled to reduce individual variation. The sample was divided into 3 equals aliquot and diluted with extender containing 8% LDL to reach final concentration of 200 x 10^6 sperm/ ml. The first aliquot was evaluated as fresh diluted between 15 minutes to 20 minutes of dilution, second after 48 hours of storage at 4°C and third was cryopreserved and evaluated after 7 days of storage in liquid nitrogen. Percent live spermatozoa in different treatment groups were analyzed using eosin nigrosin staining technique as described by Hancock (1952). Hypo osmotic swelling test was conducted to evaluate the plasma membrane integrity of spermatozoa (Jeyendal *et al.,* 1984).

The motility patterns and kinematic characteristic of spermatozoa were evaluated through computer assisted semen analyzer using sperm counting chamber, negative phase contrast and 10 x objectives. Settings of the CASA system (Biovis CASA 2000, Version 4.6, India) designed using algorithm based on size, shape, detection of sperm head were as follow: Frames/s - 60, number of frames acquired - 61, max velocity (for tracking): (um/s) - 150 motility min, curvilinear velocity (VCL): (um/s) - >25 motility min, average path velocity: (um/s) - >10 motility min, straight-line velocity: (um/s) - >1 min, track length (% of frames) - 51, aspect - 0-99,999, area - 2-20, axis (major) - 4-20, axis (minor) - 2-10, compact- ness - 0-50, perimeter ratio - 0-99,999, minimum cell size on major axis - 20, minimum cell size on min axis - 10 magnification - ×10 phase, calibration × (pixels/unit) - 1.905 µ, Y (pixels/unit) - 1.905 µ, size of image - 1280 × 960 pixels. Semen was diluted and adjusted to a concentration of about 50 × 10^6 spermatozoa per ml for computer aided motility analysis. A 10 µl of diluted semen sample was loaded in metallic sperm counting chamber with surface graticule of 100 x 0.01 mm^2 (sperm processor, Welcomenagar, India) and a range of 3-6 fields were acquired for motility analysis.

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Science (SPSS® Version 20.0 for Windows®, SPSS Inc., Chicago, USA). The means were compared using Analysis of Variance, Duncan's multiple range test and presented as mean ± standard error (SE).

RESULTS

The effect of low-density lipoproteins on viability, progressive motility and membrane integrity in fresh dilute, short term and long term stored semen has been presented in Fig. 1.

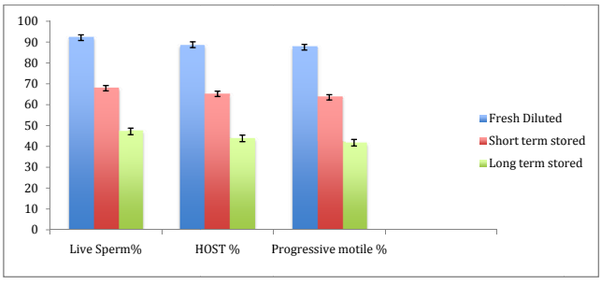

Fig. 1: Mean (± SE) values of physical seminal attributes recorded in fresh dilutes, short-term and long-term stored semen

Significantly ($P \leq 0.01$) higher value was observed in fresh diluted semen, which decreased subsequently after short term and long term storage. The kinematic character exhibited by sperm in fluid medium is the cumulative effect of sperm physiological processes and its morphological integrity. Different path velocities and motion kinematics of sperm cells evaluated using computer-assisted semen analyzer (CASA) has been presented in Fig. 2 and Table 1. A significantly ($P \leq 0.01$) higher value for sperm with rapid progression was observed in fresh diluted semen while the values for sperm with slow progression and those exhibiting non-progression were significantly ($P \leq 0.01$) higher in the post thaw. No significant difference was observed in the curvilinear velocity (VCL) exhibited

Table 1: Mean (± SE) values of different path velocities exhibited by sperm in fresh dilutes, short-term and long-term stored semen

Parameter Stage	(VCL) µm / sec	(VAP) µm / sec	(VSL) µm / sec	(LIN) %	(STR) %	(WOB) %	(BCF) hz	(ALH) µm
Fresh Diluted	152.25^{A} ±2.19	82.125^{A} ±1.63	77.50^{A} ±1.69	51.21^{A} ±1.16	92.90^{A} ±0.35	54.39^{A} ±1.08	33.21^{A} ±0.75	4.03^{A} ±0.24
Short-term stored	148.75^{A} ±2.36	70.00^{B} ±1.06	58.87^{B} ±1.82	39.39^{B} ±0.74	81.58^{B} ±2.27	47.29^{B} ±0.57	24.98^{B} ±0.73	4.90^{B} ±0.13
Long-term stored	90.25^{B} ±4.32	39.37^{C} ±2.63	31.12^{C} ±2.94	31.80^{C} ±2.52	68.38^{C} ±4.14	42.66^{C} ±1.62	17.48^{C} ±1.38	3.18^{C} ±0.20

Different superscripts (A,B) with in columns differ significantly

Mean values marked with the capital letter show difference at ($P \leq 0.01$)

Curvilinear velocity (VCL), Average path velocity (VAP), Straight line velocity (VSL), Linearty (Lin%), Straightness (Str %), Wobble (WOB%), Beat

Cross Frequency (BCF %) and maximum Amplitude-Lateral Head displacement (ALH))

by sperm cell in fresh diluted and short-term stored semen that significantly ($P \leq 0.01$) decrease in long term stored semen. The values for different kinematic characters (average path velocity (VAP), straight line velocity (VSL), Linearty (Lin%), Straightness (Str %), Wobble (WOB%), beat cross frequency (BCF %) and maximum amplitude-lateral head displacement (ALH) were significantly higher in fresh diluted semen, which significantly ($P \leq 0.01$) decreased during short term followed by long term storage.

DISCUSSION

Sperm plasma membrane is susceptible to temperature variation (Wysokinska *et al.,* 2015). The exposure of sperm to the low or ultra low temperature stimulate the efflux of membrane constituent that make cells liable to cold shock and cryoinjuries (Medeiros *et al.,* 2002; Purdy, 2006). During the experiment a decreasing trend in values recorded for percent live sperm, sperm responsive to hypo osmotic swelling test and those exhibiting rapid progression in fresh diluted, short term and long term stored semen may be the result of increased sperm susceptibility to decrease in temperature (Fang *et al.,* 2016) and effect of lethal interactive losses that increased with exposure time during storage (Xu *et al.,* 2009). No significant difference was observed in the curvilinear velocity (VCL) exhibited by sperm cell in fresh diluted and short term stored semen indicating the beneficial role of LDL in protecting and maintaining the sperm flagellar assembly and energy production system upto 4°C. A significantly ($P \leq 0.01$) lower value observed in frozen thaw semen after long-term storage may be attributed to cryoinjuries incurred during preservation at ultralow temperature (Vera-Munoz *et al.,* 2011) that might have affected mitochondrial system and energy utilization by flagellar apparatus. Leich *et al.,* (2008) also reported that ultra low temperature result in influx of membrane constituents affecting its permeability and the energy production system that may result in reduced flagellar movements reducing capacity of sperm to cover the distance per unit time. Values recorded during the present experiment were higher than those recorded for fresh semen diluted with egg yolk based extender (Anand *et al.,* 2016). The low-density lipoproteins might have a reduced intensity of interactive losses along with better capacity to prevent efflux of membrane constituents that resulted in higher values.

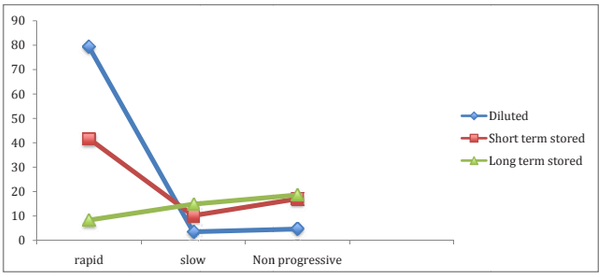

Fig. 2: Mean (± SE) values of different motility patterns exhibited by sperm in fresh dilutes, short-term and long-term stored semen

Average path velocity (VAP) indicates average path covered and is correlated with axial (X axis and Y axis)

sperm movement. The higher values of VSL indicate the sperm capacity for forward progression. VSL is supposed to have a positive correlation with travel time in female reproductive tract (King *et al.,* 2000). The VCL and VSL are considered to be the most important characteristics to evaluate the kinetic characters that influence the fertilizing ability of spermatozoa (Jobling *et al.,* 2002). VAP and VSL were significantly higher in fresh diluted semen followed by short term stored and long term stored semen indicative of reduced sperm capacity for farward progression with decrease in temperature. The sperm during its progression in female reproductive tract has to cross mucosal barriers (Doncel *et al.,* 2014). Sperm cell with higher average path velocity (VAP), straight line velocity (VSL), Linearity (Lin%) and Straightness (Str %) have better capacity to overcome reproductive barriers for better conception (Cox *et al.,* 2006). The values recorded during the experiment were higher in the diluted semen, which subsequently decreased during short-term storage and long-term storage indicating a negative effect of decreased temperature on sperm path and its velocity. Wobble (WOB%) and beat cross frequency (BCF %) indicates the oscillation of sperm trajectory about its averaged path. Spermatozoa with higher values of WOB% and BCF% are thought to posses better capacity to cross mucosal barriers in the female reproductive tract increase and probability of more sperm to reach the fertilization site (Anand *et al.,* 2016). The ALH displacement of the sperm head from its average path was higher in the fresh diluted semen followed by short and long term stored semen. Higher values for different kinematic characters exhibited by frozen thaw sperm with low density lipoproteins when compared with those recorded in Sirohi buck (Anand and Yadav, 2016) diluted with egg yolk. The reason for this difference may be reduced interactive losses and better cryoprotective capacity of extender containing low-density lipoproteins.

CONCLUSION

In conclusion, decrease in temperature during semen storage alter the sperm path and its velocities, but LDL has a protective effect on sperm flagellar assembly and mitochondrial energy production system and sustain the sperm capacity to total travel the distance per unit time upto 4°C during short term storage. The values recorded during the experiment can be a base line data to evaluate the semen diluted with extender using low-density lipoproteins. Further studies may be conducted to establish correlation between the kinematic sperm character, semen quality and fertility for better conception in goats.

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