Journal of Animal Research: v.6 n.3, p. 493-497. June 2016

DOI: 10.5958/2277-940X.2016.00051.6

Kinematic Response of Barbari and Sirohi Buck Spermatozoa to High Egg Yolk Concentration in Semen Extender

M. Anand*, S. Yadav and J. Kumar

[1]*Department of Veterinary Physiology, College of Veterinary Sciences and Animal Husbandry, Pt. Deen Dayal Upadhaya Pashuchikitisha Vigyan Vishwa Vidyalay Evam Go Anusandhan Sansthan, Mathura, Uttar Pradesh, INDIA*

*Corresponding author: M Anand; Email:drmukulanandvet@gmail.com

Received: 10 March, 2016 **Accepted**: 25 April, 2016

ABSTRACT

The experiment was designed to evaluate kinematic characteristic exhibited by spermatozoa in diluted Barbari and Sirohi buck semen using computer assisted semen analysis. Four normal healthy adult bucks (2 Barbari and 2 Sirohi) reared at the experimental goat sheds of department were selected for semen collection. Semen was collected twice a week using artificial vagina. A total of 8ejaculates (sixteen ejaculates from each breed) were collected from each buck. After initial semen evaluation, ejaculates from similar breed were pooled. The pooled semen sample of two breeds were dilutedwith TRIS based extender containing 20 per cent egg yolkto final concentration of 100×10^6 spermatozoa per ml. Motility patterns and path velocities of spermatozoa were evaluated using computer assisted semen analyser (CASA). It was observed that proportion of spermatozoa exhibiting rapid progression were highest in both the breeds, following by slow progression and non-progression.A significantly ($p<0.01$) higher values of non-progressive spermatozoa were observed in Barbari buck semen. Different path velocities *viz.* VCL (µm/sec), VAP (µm/sec), VSL (µm/sec), Lin (%), Str(%), WOB(%), BCF(hz), ALH(µm) and DNC(μm^2/sec) exhibited by spermatozoa were recorded during the experiment. The VCL (µm/sec), ALH(µm) and DNC(μm^2/sec) were significantly($p<0.01$, $p<0.05$) in Barbari buck semen while a significantly($p<0.01$, $p<0.05$) higher values of VSL,Lin (%),Str (%), WOB(%)and BCF (hz) were observed for Sirohibuck.The result indicated that Barbari buck spermatozoa covered more distance per unit time in the vertical direction (Y-axis) and short X axial movements (horizontal movement), while the spermatozoa of Sirohi Buck moved at slower velocity with more distance covered in horizontal direction (X axis) and lesser vertical displacementresulting in lower values of VCL and higher values of VAP and VSL as compared to Barbari buck. So, it can be concluded that a variable response is exhibited by Barbari and Sirohi buck spermatozoato egg yolk concentration, indicating a breed specific variation in motion characteristic and path velocities of spermatozoa.

Keywords: Barbari buck, cryopreservation, kinematic response, spermatozoa, Sirohibuck

A major step for successful conception is timely transport of spermatozoa to the actual site of fertilization. Spermatozoa are ejaculated or inseminated in the lower reproductive tract of female and later travel to site of fertilization. Motility a phenomenon exhibited by live spermatozoa is largely responsible for their transport. It is a coordinated process that utilizes the energy generated by mitochondria for its progression in female reproductive tract (Piomboni *et al.* 2012). Out of all theparameters that are being utilized for semen evaluation, motility is one of the most complex phenomena exhibited by spermatozoa (Kozdrowski *et al.* 2007). The semen after ejaculation comes in contact with various stressors *viz.* temperature variation, sunlight etc. that directly or indirectly affects the sperm morphology and physiology influencing motion kinematics of spermatozoa (Fuerst-Waltl *et al.* 2006). Furthermore,there exist a lethal interaction between the egg yolk in semen extender and seminal plasma in goats that influence the quality of semen after dilution (Leboef *et al.* 2000). The interaction affects plasma membrane, acrosomal integrity and diminishes motility leading to poor postthaw semen quality.So, various protocols have been proposed for semen cryopreservation in goat that include spermatozoa washing (Islam *et al.* 2006), use of lower egg yolk concentration in extender and semen cryopreservation with use ofhigh egg yolk concentration

and low spermatozoa count. Gunjan *et al.* (2014) reported better results with 20% egg yolk in semen extender diluted to final concentration of 100 million spermatozoa per ml for Barbari buck. Ranjan *et al.* (2015) reported use of 10 per centegg yolk in semen extender tofinal concentration of 100 million spermatozoa per ml in Jamunapari bucks while Bispo *et al.* (2011) recommended the use of 3 per cent egg yolk in semen extender with dilution to final concentration of 400 million spermatozoa per ml. Hence, no specific protocol has been established till date. Further, the literature is lacking for the motility patterns and path velocities exhibits by spermatozoa after dilution in indigenous breeds of goat.

Generally sperm motility is expressed in terms of mass and progressive individual motility. Analysis of motility through visual perception lacks precision. Also, thedifferent pattern exhibited by spermatozoa responsible for successful timely transport and fertilization cannot be evaluated. So,taking into account the importance of motility patterns exhibited by spermatozoa, the kinematics of spermatozoa are now studied using computer assisted semen analysis (CASA). It provides objective and reproducible data on a number of sperm motion parameters and enhance the value of motility assessment to fertility prognosis resulting in high correlations among several CASA motility parameters and the *in vivo* fertility of spermatozoa (Mortimer, 2000; Foote, 2003; Chang *et al.* 2004). The higher egg yolkconcentration in semen extender gives better protection and improves the cryosurvival rate in most of the domestic species. The study was conducted to evaluate the kinematic characteristics and path velocities exhibited by spermatozoa in response to high egg yolk concentration at low spermatozoa count in Barbari (small size) and Sirohi (large size) goat.

MATERIALS AND METHODS

Experimental procedure

Four normal, healthy adult bucks (two Barbari and two Sirohi) reared at the experimental goat sheds of department were selected for semen collection. Semen was collected twice a week using artificial vagina. Immediately after collection, semen was evaluated and samples with more 85% of live spermatozoa were selected for further study. A total of 32 samples (eight from each buck) were collected. The ejaculates of similar breed were pooled separately to minimize the individual variation. The pooled samples of both thebreeds were diluted with TRIS-glycerol egg yolk (20%) extendertofinal concentration of $100x10^6$ spermatozoa per ml. The sample were analysed for per cent live (Hancock, 1952) and different motility patterns exhibited by spermatozoa using computer assisted semen analysis (CASA with software Biovis Motility plus V4.59 serial # 7337). The setting of the instrument for the assessment of motility characteristicsof goat spermatozoa were; frames per second -60. Number of frames acquired-61, Max Velocity (for tracking)(µm/sec)->10, motility min.VCL(µm/se)- >1.Min track length (% of frames) – 51, Aspect -0 to 50, perimeter ratio-0 to 99999. Area – 2to 20, axis (major) – 4 to 20. Axis (minor) -2 to 10, compactness -0 to 50, perimeter ratio -0 to 99999. Minimum cell size on major axis -20. Minimum cell size on minor axis-10 magnification -10X phase calibration X (pixels/unit) –1.9050 micron.Y (pixels/unit)- 1.905 micron. Size of image –1280x960 pixels. A 10 microliter (10µl) of dilution semen sample was loaded in metallic sperm counting chamber and a range of 2-4 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 paired t test and presented as mean ± standard error (SE).

RESULT

The different motility patterns exhibited by spermatozoa in Barbari and Sirohi bucks have been presented in figure 1. A non-significantly higher values of rapid progressive and slow progressive spermatozoa was observed in Sirohi compared to Barbari bucks while a significant (p<0.01) higher value for non-progressive motile sperm was observed in Barbari as compared to Sirohi buck.

Different path velocities exhibited by spermatozoa in diluted semen of Barbari and Sirohi buck has been presented in table-1. A significant difference was observed for path velocities recorded in both the breeds of goat. The curvilinear velocity (VCL, µm/sec) was significantly (p<0.01) higher in Barbari as compared to Sirohi buck.

Average path velocity (VAP, µm/sec) was non-significantly (p>0.01 or 0.05) higher in Sirohi as compared to Barbari buck spermatozoa. Significantly (p<0.05) lower value of straight-line velocity (VSL, µm/sec) was observed in Barbari as compared to Sirohi buck. A significantly (p<0.01) higher value of Lin (%), WOB (%) and BCF (hz) were observed in Sirohi buck spermatozoa. Str (%) was significantly (p<0.05) higher for Sirohi buck spermatozoa. Amplitude lateral displacement (ALH, µm) was significantly (p<0.01) higher in Barbari buck semen compared to Sirohi buck semen. A significantly (p<0.05) higher value of DANCE (DNC, µm^2/sec) was observed for Barbari buck spermatozoa.

DISCUSSION

The different motility patterns exhibited by spermatozoa in semen are indicative of its quality. It has been observed that rapid moving spermatozoa seem to have a high probability of crossing the cervical barrier during artificial insemination (AI) after cervical deposition especially in small ruminants (Sundararamand and Edwin, 2008).

Table 1: Kinematic response of Barbari and Sirohi buck spermatozoa to high egg yolk concentration in semen extender

Parameters	Units	Barbari buck	Sirohi buck
Live percent	%	91.00± 1.68	93.00± 1.22
Total motile spermatozoa	%	88.32±1.55	90.10±1.149
Curvilinear velocity (VCL)	µm / sec	134.75^{b}± 3.96	117.50^{a}±3.66
Average path velocity (VAP)	µm / sec	64.75±1.11	66.75±1.70
Straight line velocity (VSL)	µm / sec	60.00^{A}±0.91	63.50^{B}±1.66
Linearity (Lin)	%	44.63^{a}±1.41	54.10^{b}±0.78
Straightness (STR)	%	90.40^{A}±0.82	93.53^{B}± 0.64
Wobble (WOB)	%	48.72^{a}± 1.21	57.15^{b}± 0.6602
Beat cross frequency (BCF)	hz	25.78^{a}±0.54	29.38^{b}±0.65
Amplitude-lateralhead displacement (ALH)	µm	5.20^{b}±0.18	3.63^{a}±0.06
DANCE (DNC)	µm²/sec	575.40^{B}± 45.66	355.68^{A}±19.51

Mean with lowercase letter (a,b) show difference at (p<0.01) and capital letter (A,B) at (p<0.05) between column.

Semen sample with large number of rapid progressively motile spermatozoa have better chance of fertilization. The higher proportion of rapid progressively motile spermatozoa in semen of Sirohi and Barbari breeds is indicative of better semen quality with higher possibility of successful conception.Curvilinear velocity (VCL, µm/sec) is the measure of actual distance (curvilinear trajectory) travelled by spermatozoa in given time while the straight line velocity (VSL, µm/sec) defines the distance between the first and last tracked points divided by time. VCL & VSL are considered to be the most important parameters to evaluate the kinematic characters that influence the fertilizing ability of spermatozoa (Ciereszko *et al.* 1996; Rurangwa *et al.* 2002; Jobling *et al.* 2002). VAP is indicative of averaged path or distance of the sperm head trajectory divided by time. It is derived by smoothing the actual curvilinear path covered by the spermatozoa in the frames to be analysed. VAP is influenced by the curvilinear trajectory of sperm. As the sperm follow the straight path the value of VCL and VAP become closed to VSL and becomes equal when sperm moves in complete straight line. It has been reported that the progressive spermatozoa velocities (VCL, VSL and VAP) correlate better with the fertilization rates than other parameters of movement (Rurangwa *et al.* 2001).

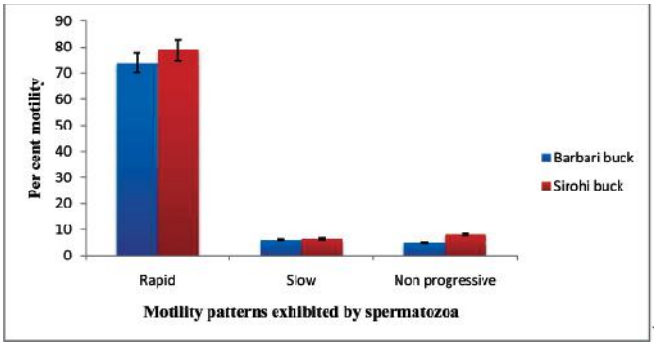

Fig. 1: Motion characteristics exhibited by spermatozoa in semen of Barbari and Sirohi buck

In the present study, Barbari buck spermatozoa exhibited higher velocity and covered greater distance in the vertical direction (Y axis) with short horizontal (X axis) movements while the spermatozoa of Sirohi buck move with slower velocity and coveredmore distance in horizontal direction (X axis) and exhibited lesser vertical

movements, leading to lower values of VCL and higher values of VAP and VSL as compared to Barbari buck indicating a better fertilizing ability. Linearity (Lin %) measures the departure of the cell track from a straight line and it is the ratio of VSL/VCL and straightness (Str %) defines the ratio of net distance moved of smoothed path distance (VSL/VCL) can be very useful indicators of curvature of the trajectory. The correlation between CASA parameters for goat semen and sperm migration in cervical homologous mucus depends on linearity that determines the sperm *in vitro* migration efficiency, where spermatozoa presenting values of Lin >50% showed better migration (Cox *et al.* 2006). The higher values of linearity and straightness in Sirohi compared to Barbari bucks is the result of variable horizontal and vertical movements. ALH corresponds to the mean width of the head oscillation as the sperm swims. Both Lin % and ALH seem to be indicators of sperm hyperactivation (Pena *et al.* 2000). Besides, the spermatozoawith higher ALH, a property that reflects the ability to penetrate mucus in the uterine cervix and to unite with the oocyte (Verstegen *et al.* 2002) have better fertilizing ability. Higher values of Lin% in Sirohi buck semen and ALH in Barbari buck semen is indicative difference in hyperactivity patternamong two breeds. BCF and WOB was found to be significantly (p<0.01) higher in Sirohi as compared to Barbari buck spermatozoa. The difference in the motion and kinematics of the spermatozoa between the two breeds may be result of breed specific variation in regard to concentration of reactive proteins (BUSgp60 and Phospholipase A2) secreted from bulbourethral gland in seminal plasma affecting membrane integrity and metabolism resulting in altered motility patterns.

CONCLUSION

It may be concluded that there exist a variation in the motion kinematics and path velocities of spermatozoa between the two species at high egg yolk concentration in extender, signifying a breed specific response of spermatozoa after dilution with extender containing egg yolk. Further studies may be conducted to study the level of these reactive proteins (BUSgp60 and phospholipase A2) in seminal plasma of different goat breedsand establish and specify breed specific egg yolk concentrations in semen extender with minimal interactive losses and better outcome.

ACKNOWLEDGEMENTS

The authors are thankful to Vice Chancellor. U.P. Pt. Deen Dayal Upadhayay Pashuchikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan. Dean, College of Veterinary Sciences and Animal Husbandry for providing funds and facilities to pursue this research work.

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