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Hypo osmotic swelling test of caprine epididymal sperms

DILIP KUMAR SWAIN[1], PRASANT SWARNKAR[2] and SARVAJEET YADAV[3]

U.P. Pt. Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya, Mathura, Uttar Pradesh 281001 India

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Epididymis serves as the site of storage of sperms in which not only the sperms get matured but also motility and fertility are acquired. Functional and structural modifications of the sperms are the key events in the epididymis (Cooper 2007). Since the plasma membrane functional activity is crucial for the viability and fertilising ability of spermatozoa, it is very important to assess the structural and functional activity of the sperm membrane (Fonseca *et al.* 2005). The membrane integrity is evaluated by the use of a simple technique known as the HOST (Jeyendran *et al*. 1984). Literature is scanty regarding the degree of membrane intactness in different segments of the epididymis of different animals. The present study was designed in a goat model to evaluate the structural integrity of the sperm plasma membrane. The major objective of this study was to standardise the best concentration of HOST solution to characterise sperms of different segments of epididymis and secondly to evaluate the sperm membrane integrity at different segments of caprine epididymis.

Testes of buck (30) were collected immediately after slaughter of the animal from the local slaughterhouse of Mathura and were kept in chilled normal saline and transferred to the laboratory in a thermox. The laboratory processing of testes was carried out within 30 min of reaching the laboratory. Sperm was collected by flushing the epididymal segments with Tyrode-lactate.The epididymal sperm solution was collected from 30 testes and centrifuged at 3000 rpm for 30 min. The supernatant was aspirated and discarded and the pellet was resuspended in fresh Tyrode-lactate solution and the volume was made up to 1 ml. The sperm samples were transferred to the ependorff tube and kept in incubator at 37°C as sperm stock sample for evaluation.

The harvested sperms were evaluated for HOST positive spermatozoa (%) by taking 8 different hypo-osmolar solutions (50 (S1), 75(S2), 100 (S3), 125(S4), 150(S5), 175 (S6), 200 (S7) and 300 (S8)) mOsmol/L as per Fonseca *et al.* (2005). The protocol was modified according to the requirement of goat epididymal sperms. Hypo-osmotic solutions of 300 mOsm/L tri sodium citrate and fructose based solution was made as per Revell and Mrode (1994). As proposed by Correa and Zavos (1994), serial dilutions were carried out in triple distilled water to finally get hypoosmolar solution of above mentioned concentrations. The solutions were kept in eppendorf tubes at –20°C until use. Analysis and evaluation of the HOST positive sperms were carried out as per Ranjan *et al.* (2009). The statistical analysis was carried out by taking the sperms exhibiting strong coiling and swelling. Analysis of variance (ANOVA) was done by using the SPSS software version 14. The results are presented in Table 1.

Strong swelling and coiling response of different segments of epididymal sperms were considered while evaluation of the HOS response and the results are presented in Table 1. The caput epididymal sperms exhibited differential degree of swelling and coiling when treated with the hypo-osmotic solutions of different osmolar concentrations. Highest swelling and coiling response was observed at 75 mOsm/L. On the other hand the corpus and cauda epididymal sperms exhibited strong swelling and coiling at 125 mOsm/L (S3). The swelling pattern of the corpus and cauda sperms remained similar. From the results it was evident that S2 was the best concentration for the HOST for the caput sperms whereas S4 was the best for the corpus and cauda sperms.

With the progression from the caput to cauda, the degree of sperm membrane integrity got increased and hence more number of HOS positive sperms was recorded in the corpus and cauda sperms (Table 1). The cauda exhibited the highest sperm count with respect to HOST positive sperms. The hypo osmotic resistance of the cauda sperms was greater as compared to that of the corpus and caput. The exact mechanism behind the process was not understood in the study. Studies showed that the epididymal transit of the sperms causes the membrane modification by the alteration of the cell membrane composition and this alteration was mostly due to the increase in the fluidity of the sperm plasma membrane due to an increase in the cholesterol content

Present address: [1]Assistant Professor (dilip_swain @yahoo.com), [3]Professor and Head, Department of Physiology (dr.swarnkarvet@gmail.com). [2]Veterinary Officer, Aligarh sarvajeet24@hotmail.com).

Table 1. Hypo osmotic swelling and coiling response of segment specific epididymal sperms to various concentrations of hypo osmolar solutions (mean±SE)

Segment of epididymis	(S1)	(S2)	(S3)	(S4)	(S5)	(S6)	(S7)	(S8)
Caput	35.87^{c}±1.28	39.34^{d}±0.98	35.61^{c}±1.46	36.10^{c}±1.26	32.12^{b}±1.96	32.76^{b}±1.78	28.46^{a}±1.23	28.58^{a}±1.65
Corpus	36.58^{A}±1.15	37.16^{A}±1.34	36.46^{A}±1.16	41.19^{B}±1.87	32.76^{A}±1.11	32.16^{A}±1.71	32.14^{A}±1.13	35.19^{A}±1.24
Cauda	56.18^{X}±1.14	55.54^{X}±0.88	56.45^{X}±1.34	64.16^{Y}±1.37	55.62^{X}±1.66	56.46^{X}±1.18	55.36^{X}±1.73	54.18^{X}±1.15

*Values a, b, c with different superscript indicate significant difference (P<0.05) for the hypo-osmotic swollen positive sperms of caput epididymis. **Values A, B with different superscript indicate significant difference (P<0.05) for the hypo-osmotic swollen positive sperms of corpus epididymis. ***Values X, Y with different superscript indicate significant difference (P<0.05) for the hypo-osmotic swollen positive sperms of cauda epididymis.

(Cornwall 2009). In the study we did not estimate the cholesterol content but it was evident that the osmotic resistance of the sperms was rising during the transit from the caput to cauda.

Ranjan *et al.* (2009) reported 75 mOsm/L is the best concentration for the frozen thawed spermatozoa in bucks. Contrary to this finding, Fonseca *et al.* (2005) reported that 125 mOsm/L is the best concentration of hypo osmotic solution for the fresh ejaculated goat spermatozoa. The swelling response varies in different animals with respect to various concentrations of the HOS. Datta *et al.* (2009) reported 150 mOsm/L is the best concentration for the caprine cauda epididymal sperms after preservation. Matson *et al.* (2009) reported various concentrations of hypo osmolar solutions for epididymal sperms in different animals.

SUMMARY

The study was designed to find a suitable HOS solution concentration for the sperms retrieved from 3 segments of epididymis. The major aim of the study was to evaluate the pattern of osmotic resistance of the epididymal sperms to different concentrations of hypo-osmotic solutions. We reported the highest sperm coiling at 75 mOsm/L for the caput and corpus sperms whereas cauda sperms exhibited highest coiling at 125mOsm/L. The osmotic resistance of the cauda sperms was greater than that of the caput and corpus. Further studies are required to study the exact mechanism behind the sperm membrane integrity and its relationship with the epididymal transit and how the osmotic resitance of the sperms gradually increased during the epididymal transit.

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