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Short Communication

EFFECT OF HONEY SUPPLEMENTATION ON CAUDA EPIDIDYMAL BUCK SPERMATOZOA PRESERVED AT REFRIGERATED TEMPERATURE

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SUMMARY

The aim of this study was to evaluate the effects of honey supplementation in Tris egg yolk citrate extender on plasma membrane functional integrity of cauda epididymal buck spermatozoa at refrigerated temperature. Ten pairs (n=20 testis) of testicles from ten matured non-descript buck irrespective of breed presented for slaughter at government approved slaughter house were collected. Prepared 10 percent honey solution was used in experiment groups. The cauda epididymal spermatozoa were diluted with Tris egg yolk citrate extender and five equal aliquots were made i.e. control and T1 to T4. In control group, no honey was added in Tris egg yolk citrate extender, while T1, T2, T3 and T4 were supplemented with 1, 2, 3 and 4 per cent Tris Honey, respectively and preserved at refrigerated temperature (4-5 ºC). The plasma membrane functional integrity of cauda epididymal spermatozoa was measured by hypo-osmotic swelling test (HOST) at 0, 12, 24, 36 and 48 hours. Significantly higher percentage of HOST reacted spermatozoa, was found in T1 and T2 as compared to control, T3 and T4 at 48 hours after post freezing.

Keywords: Buck, Cauda epididymal spermatozoa, Honey, HOST

Goat semen can be preserved temporarily at room temperature, 24-48 hours at refrigerated temperature (Ferdinand *et al.*, 2012) or for long term at ultra-low temperature (Beltran *et al.*, 2013). However, only limited period of storage at ambient temperature as well as poor quality on post-cryopreservation (Yimer *et al.*, 2014) encourage the workers to preserve the semen at refrigerated temperature which is cheaper and more feasible than cryopreservation of semen at ultra-low temperature. Moreover, by preserving semen at refrigerated temperature, one can minimize the additional stress which occurs during cryopreservation of spermatozoa. Egg yolk is being routinely used in most of semen cryopreservation protocols in various species (Priyadharsini *et al.*, 2011). Honey is known to contain high amount of a variety of simple sugars (Fuller, 2004) which might serve both as a source of nutrition and non-penetrating cryoprotectant to sperm cells during cryopreservation.

A functional membrane is requisite for the fertilizing ability of spermatozoa, as it plays an integral role in sperm capacitation, acrosome reaction, and binding of the spermatozoon to the egg surface (Ramu and Jeyendran, 2013). The hypo-osmotic swelling test (HOST) evaluates the functional integrity of the sperm's plasma membrane and also serves as a useful indicator of fertility potential of sperm (Ramu and Jeyendran, 2013). The present study was designed to evaluate the effect of honey supplementation in tris egg yolk citrate extender on plasma membrane integrity of paired cauda epididymal buck spermatozoa at refrigerated temperature.

Sample collection and processing: Ten pairs of testicles were collected immediately after slaughter under strict hygienic conditions from apparently healthy, matured, non-descript buck irrespective of breed at government approved slaughter house and transferred to the laboratory in ice packs as early as possible. Testes were washed and cleaned with R.O. water. Fascia, blood vessels and sheath of testes were removed with the help of BP blade and thumb forceps. Care was taken to prevent the damage to the epididymis. Spermatozoa were retrieved separately from the right and left cauda epididymis at room temperature by the incision method. Several small incisions were made on the cauda of epididymis enable spermatozoa swim out in to five ml prewarmed (37 ºC) Tris egg yolk citrate (TEYC) diluter in a petri dish. Cauda epididymal sperm samples extended with TEYC diluter to make a final volume of 20 ml.

Preparation of honey extender: The product of Navsari Agricultural University, Navsari "Nauroji" Honey was used for the experiment. Honey solution was prepared by adding 1 ml honey to 9 ml distilled water (v:v) to obtain a honey solution of 10 % concentration and centrifuged the solution at 3000 rpm for 5 minute. After centrifugation, the supernatant was collected in sterile vial and stored at refrigerator temperature (4-5 ºC).

Experimental groups: To compare the effect of honey as additive on refrigerated cauda epididymal sperm parameters, 20 ml of diluted cauda epididymal sperm samples from individual epididymis were divided into five aliquots and supplemented with 0/5 ml (control), 0.5/4.5 ml (T1-TH 1%), 1.0/4.0 ml (T2- TH 2%), 1.5/3.5 ml (T3-TH 3%) and 2.0/3.0 ml (T4- TH 4%) of TEYC/honey extender (v/v) to obtain a final volume of 5 ml in each tube

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and preserved at refrigerated temperature (4-5 ºC) up to 48 h and evaluated at every 12 h interval.

Evaluation of Plasma Membrane Functional Integrity: To evaluate the plasma membrane integrity of spermatozoa, HOST was determined by mixing 0.1 ml of diluted semen with 1.0 ml of hypo-osmatic solution. The tube containing the mixture was incubated at 37 ºC for 30 minutes. A drop of semen from the mixture was placed on a clean dry glass slide and covered with cover slip. The sperms characterized by varying degrees of coiling or swelling of tail were considered to have an intact plasma membrane (HOS positive sperm) and the sperms without tail curling were considered to have damaged membrane (HOS negative sperm). Total 200 spermatozoa were counted in phase contrast microscope under 10X and percentage of spermatozoa exhibiting tail curling was calculated.

Statistical analysis: The data pertaining to various aspects were suitably tabulated and analysed using R-3.3.2 software. The differences among the parameter means were carried out using appropriate statistical methods, viz. ANOVA, DNMRT (Duncan's New Multiple Range Test). The mean differences were considered significant at $p<0.05$ and $p<0.01$.

The HOST reacted sperm count (%) at 0 hour differed non-significantly between control (74.90±3.07), T1 (73.75±2.80), T2 (72.60±3.14), T3 (71.60±2.78) and T4 (70.40±2.64) groups (Table 1). Similarly, post-chilled HOST reacted sperm count (%) at 12, 24, 36 and 48 hours differed non-significantly between control, T1, T2, T3 and T4 groups. The corresponding pooled mean of HOST reacted sperm count (%) irrespective of treatment and control groups were reduced with increasing preservation time.

Moreover, in control group, HOST reacted sperm count (%) was non-significantly higher at 0 hour as compared to 12 hours but significantly ($p<0.01$) higher as compared to 24, 36 and 48 hours, whereas, it was non-significantly differed between 24 and 36 hours. In T1 group, HOST reacted sperm count (%) was non-significantly higher at 0 hour as compared to 12 hours but significantly ($p<0.01$) higher as compared to 24, 36 and 48 hours, however, the difference between 12, 24 and 36 hours were significant ($p<0.01$) and 36 and 48 hours was non-significant. In T2, T3 and T4 groups, HOST reacted sperm count (%) was non-significantly higher at 0 hour as compared to 12 hours but significantly ($p<0.01$) higher as compared to 24, 36 and 48 hours, however, the difference between 12 and 24, 24 and 36 hours were non-significant. Significantly ($p<0.01$) lower HOST reacted sperm count (%) was observed at 48 hours as compared to 0, 12, 24 and 36 hours in all the groups (Table 1).

EL-Sheshtawy *et al.* (2014) in bull; El-Nattat *et al.* (2016), Kandiel and Elkhawagah (2017) in buffalo reported higher HOST reacted sperm count (%) in honey additives as compared to control group in post thaw frozen semen. EL-Seadawy *et al.* (2017) reported non-significantly higher HOST reacted sperm count (%) in different concentration of propolis-ethanolic extract added groups as compared to control group at 24 hours post chilling in rabbit. Banday *et al.* (2017) found post-thaw HOST reacted sperm count (%) significantly higher ($p<0.05$) in control (60.33±1.42) than all the other Tris-based honey extender groups in ram semen.

In the present study, higher post chilled HOST reacted sperm count (%) was found in 1-2 % honey group at 48 hours. Kandiel and Elkhawagah (2017) observed

Table 1

Effect of different concentrations of Tris honey extender and storage duration on HOST reacted-spermatozoa (%)of paired cauda epididymal buck spermatozoa preserved at refrigerated temperature (Mean±S.E.)

Groups	HOST reacted- spermatozoa (%) (n=20 testicle)					Overall	F value	P value
	0 hr	12 hr	24 hr	36 hr	48 hr			
C	$74.90\pm3.07_{w}$	$69.50\pm3.07_{w}$	$63.50\pm2.85_{x}$	$59.80\pm3.08_{x}$	$52.70\pm2.91_{y}$	64.08±3.42	18.18**	0.00
T1	$73.75\pm2.80_{w}$	$68.10\pm3.02_{w}$	$62.25\pm2.87_{x}$	$58.20\pm2.86_{yz}$	$54.90\pm2.71_{z}$	63.44±3.24	17.22**	0.00
T2	$72.60\pm3.14_{w}$	$67.80\pm3.07_{wx}$	$65.05\pm3.06_{xy}$	$60.95\pm3.02_{y}$	$54.55\pm3.05_{z}$	64.19±3.33	10.59**	0.00
T3	$71.60\pm2.78_{w}$	$68.40\pm2.88_{w}$	$61.75\pm2.69_{x}$	$58.85\pm2.87_{x}$	$53.15\pm3.02_{y}$	62.75±3.22	16.46**	0.00
T4	$70.40\pm2.64_{w}$	$66.30\pm 2.72_{w}$	$61.35\pm2.88_{x}$	$56.95\pm2.77_{x}$	$51.10\pm2.73_{y}$	61.22±3.18	20.07**	0.00
Overall	72.65±2.90	68.02±2.94	62.78±2.87	58.95±2.92	53.28±2.88	—	—	—
F value	0.87	0.35	0.46	0.63	0.67	—	—	—
P value	0.48	0.85	0.77	0.64	0.62	—	—	—

$^{w-z}$Means with different subscript between a column (between time intervals) differs significantly at $p<0.05$; $p<0.01$.
** $p<0.01$; * $p<0.05$; C-Control; T1- Tris Honey 1%; T2-Tris Honey 2%; T3-Tris Honey 3%; T4-Tris Honey 4%

highest post chilled HOST reacted sperm count (%) in 0.5-1 % honey in milk based extender in buffalo semen. EL-Sheshtawy *et al.* (2014) in bull and El-Nattat et al. (2016) in buffalo observed highest post thawing HOST reacted sperm count (%) in 5 and 3% honey in Tris based extender, respectively.

CONCLUSION

Based on the results of these experiment, it was concluded that, the highest HOST reacted sperm count was found in treatment group supplemented with 1 % and 2 % honey in tris egg yolk citrate extender at 48 hours for cauda epididymal buck spermatozoa preserved at refrigerated temperature.

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