RESEARCH ARTICLE

Assessment of Pomegranate Juice as Antioxidant in Extender on Cauda Epididymis Spermatozoal Quality of Buck at Refrigerated Temperature

Amarjeet*, CT Khasatiya, LM Chaudhary

Abstract

The present investigation was carried out to study the refrigeration preservation of the cauda epididymal retrieved spermatozoa of buck in Tris egg yolk citrate (TEYC) dilutor containing pomegranate juice as antioxidant additive. The retrieved cauda epididymal spermatozoa extended in TEYC dilutor were studied in five groups by adding different concentration of pomegranate juice as additive (0% as control T1 group and 5%, 10%, 15% and 20% as treatment T2, T3, T4 and T5 groups, respectively) and storing at refrigerated temperature up to 48 hr. The results showed that the control extender had the least dead, abnormal and HOS non-reacted sperm percent among all treatments tested and that with increasing the pomegranate juice concentration in dilutor, the percentage of the dead, abnormal and HOST non-reacted spermatozoa increased significantly. The same trend was observed at all 12 hourly storage intervals indicating its detrimental effect on epididymal sperms of bucks at refrigeration temperature. The dead, abnormal, and HOST non-reacted sperm were significantly and positively interrelated with each other (r = 0.53–0.83). It was concluded that the inclusion of pomegranate juice in TEYC dilutor did not show any beneficial/antioxidant effect on epididymal sperms of buck in fresh or refrigerated semen and in fact all the levels of pomegranate juice (5% to 20%) were detrimental to cauda epididymal spermatozoa of buck.

Keywords: Buck, Cauda epididymis, Pomegranate juice, Refrigerated temperature, Spermatozoa

Ind J of Vet Sci and Biotech (2019): 10.21887/ijvsbt.15.2.7

Introduction

Goat is an animal of Asian origin and distributed all over the world and is the primary source of livelihood of people below the poverty line. As per the 19th Livestock Census of India, the total goat in the country was 135.17 million numbers in 2012. The epididymal sperm collected from various domesticated and wild animals at different time intervals after post-mortem showed a substantial loss in the sperm characteristics after preservation at 4–5°C (Dong *et al.,* 2008), which may be due to the action of the reactive oxygen species (ROS) formed *via* lipid peroxidation of the membrane lipids of spermatozoa (Perumal *et al.,* 2011). Flavonoids and other phenolic compounds, as well as vitamins A, C, and E in pomegranate, have antioxidant activities (Cao *et al*., 1998; and Seeram *et al.,* 2008). The pomegranate juice was shown to improve the sperm quality of rats (Mansour *et al.,* 2013). Semen diluents, enriched with pomegranate juice, exhibited an increased protective effect against lipid peroxidation during the storage of roosters' semen (Al-Daraji, 2015). In the view of above fact and meager literature available on the effect of pomegranate juice on the preservability of cauda epididymal sperms, the present study was conducted on goat epididymal sperm quality and preservability in Tris-yolk-citrate extender without and with varying levels of pomegranate juice.

Materials and methods

The present investigation was conducted at Navsari, Gujarat, which is located geographically on Arabian coastline at 20°57' to 20°95' North latitude and 72°56' to 72°93' East longitude at an elevation of 9 m above the mean sea level. A total 16 mature testicles (8 pair) of bucks were collected during a period of six months (September 2018 to February 2019) from the Government approved slaughterhouse, in a sterile plastic bag with utmost care and were carried in air-tight sterile cryo box (5°C) to the laboratory within 2-4 hours of slaughter of bucks. The spermatozoa were retrieved separately from the right and left cauda epididymis by making several small incisions over cauda epididymis with a BP blade and adding 5 ml pre-warmed Tris egg yolk citrate (TEYC) dilutor at 37°C to swim out the spermatozoa in a petri dish. The prepared samples with ≥70% individual sperm motility were selected for further study and extended with TEYC dilutor

Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science & AH, NAU, Navsari-396 450, Gujarat, India

Corresponding Author: Amarjeet, Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science & AH, NAU, Navsari-396 450, Gujarat, India, e-mail: aamarkargwal@gmail.com

How to cite this article: Amarjeet, Khasatiya, C.T. and Chaudhary L.M. (2019). Assessment of Pomegranate Juice as Antioxidant in Extender on Cauda Epididymis Spermatozoal Quality of Buck at Refrigerated Temperature. Ind J Vet Sci and Biotech, 15(2): 26-29.

Source of support: Nil

Conflict of interest: All authors declare no conflict of interest.

Submitted: 16/07/2019 **Accepted:** 31/07/2019 **Published:** 25/11/2019

in order to make specific concentrations (300x10^6/ml) of the spermatozoa. These retrieved cauda epididymal spermatozoa extended with TEYC dilutor were studied for quality traits by adding different concentration of pomegranate juice (0% as control T1 group and 5%, 10%, 15% and 20% as treatment T2, T3, T4 & T5 groups, respectively) during *in-vitro* storage at 5°C temperature up to 48-hour.

The fresh ripened pomegranate fruit of average size was purchased from the commercial market. It was washed and peeled with sterilized hand on the same day of collection of testes. The fully red seeds were separated from the fruit in a clean dish and squeezed the seed by keeping into a sterile porous cloth and then filtered with Whatman filter paper. The filtered juice was centrifuged at 4000 rpm for 15 minutes, and the clear watery juice was collected in a sterile glass bottle and autoclaved. The preserved semen samples were evaluated for dead sperm count, morphologically abnormal sperm, and HOST (hypo-osmotic swelling test) non-reacted sperms (Campos *et al*., 2004) at 12-hour, 24-hour, 36-hour and 48-hour intervals of storage.

Data were statistically analyzed using 2 Factors Factorial RBD for ANOVA using goats as eight replicates, and treatment, storage time, and their interaction as main effects. Before the analysis, data were arcsine transformed, and the significance among different means was compared by using critical difference (CD) test at 5% level of significance. The correlation coefficients among spermatozoal parameters were carried out by MS Excel office.

Results and discussion

The result on quality parameters of semen assessed at different time intervals of preservation at refrigerated temperature with or without adding pomegranate juice in Tris-yolk-citrate extender is presented in Tables 1-3.

Table 1: Mean percentage of dead spermatozoa in TEYC extender with different concentration of pomegranate juice at different time intervals of refrigeration following retrieval from epididymes of buck (n = 8)

	Refrigeration storage interval (H)					
Juice Treatment (T)	*0 h*	*12 h*	*24 h*	*36 h*	*48 h*	*Overall*
T1 (Control)	17.86 (10.44)	19.61 (12.19)	21.96 (14.81)	26.20 (20.38)	28.79 (23.94)	22.88^{c} (16.35)
T2 (5%)	19.33 (11.81)	21.35 (14.00)	23.46 (16.56)	27.64 (22.25)	29.78 (25.38)	24.31^{bc} (18.00)
T3 (10%)	20.23 (13.13)	23.13 (16.31)	25.22 (19.06)	29.21 (24.75)	31.87 (28.88)	25.93^{b} (20.43)
T4 (15%)	23.44 (16.88)	26.67 (21.06)	28.71 (24.06)	32.67 (30.13)	35.19 (34.13)	29.33^{a} (25.25)
T5 (20%)	24.23 (17.94)	26.81 (21.44)	29.15 (24.81)	33.83 (32.06)	36.23 (35.88)	30.05^{a} (26.43)
Overall Mean	21.02_{z} (14.04)	23.51_{y} (17.00)	25.70_{x} (19.86)	29.91_{w} (25.91)	32.37_{v} (29.64)	26.50 (21.29)
SEm	T - 0.71 H - 0.33 TxH -0.75	–	CD	T - 2.06 H - 0.93 TxH - NS	–	CV%-7.99

Analysis carried out using Arcsine transformation and figures in the parenthesis are original means.

Table 2: Mean percentage of abnormal spermatozoa in TEYC extender with different concentration of pomegranate juice at different time intervals of refrigeration following retrieval from epididymes of buck (n = 8)

	Refrigeration storage interval (H)					
Juice Treatment (T)	*0 h*	*12 h*	*24 h*	*36 h*	*48 h*	*Overall*
T1 (Control)	31.13 (27.44)	33.81 (31.50)	37.47 (37.38)	43.17 (46.94)	45.75 (51.31)	38.27^{d} (38.91)
T2 (5%)	32.42 (29.38)	35.26 (33.81)	39.24 (40.25)	44.49 (49.19)	46.74 (53.00)	39.63^{c} (41.13)
T3 (10%)	34.73 (33.00)	37.39 (37.25)	40.11 (41.56)	40.93 (43.13)	45.40 (50.75)	39.71^{c} (41.14)
T4 (15%)	38.19 (38.56)	41.01 (43.25)	44.18 (48.63)	48.71 (56.38)	52.04 (62.00)	44.82^{b} (49.76)
T5 (20%)	39.61 (40.94)	42.52 (45.81)	46.00 (51.75)	50.85 (60.00)	53.59 (64.56)	46.52^{a} (52.61)
Overall Mean	35.22_{y} (33.86)	38.00_{x} (38.33)	41.40_{w} (43.91)	45.63_{v} (51.12)	48.70_{v} (56.30)	41.72 (44.61)
SEm	T - 0.36 H - 0.36 TxH -0.81		CD	T - 1.06 H - 1.02 TxH - NS		CV% -5.51

Analysis carried out using Arcsine transformation and figures in the parenthesis are original means.

Table 3: Mean percentage of HOST non-reacted spermatozoa in TEYC extender with different concentration of pomegranate juice at different time intervals of refrigeration following retrieval from epididymes of buck (n = 8)

	Refrigeration storage interval (H)					
Juice Treatment (T)	*0 h*	*12 h*	*24 h*	*36 h*	*48 h*	*Overall*
T1 (Control)	29.48 (24.50)	31.40 (27.38)	33.31 (30.38)	39.65 (40.88)	41.44 (43.94)	35.06^{bc} (33.41)
T2 (5%)	29.69 (24.88)	31.58 (27.75)	33.44 (30.63)	37.51 (37.19)	41.06 (43.25)	34.66^{c} (32.74)
T3 (10%)	31.22 (27.06)	32.97 (29.81)	36.99 (36.50)	39.39 (40.38)	42.65 (46.00)	36.64^{b} (35.95)
T4 (15%)	34.53 (32.38)	36.17 (35.06)	37.98 (38.06)	42.16 (45.19)	46.32 (52.38)	39.43^{a} (40.61)
T5 (20%)	35.46 (33.88)	37.56 (37.38)	39.10 (39.94)	43.84 (48.06)	48.32 (55.81)	40.86^{a} (43.01)
Overall mean	32.08_{z} (28.54)	33.94_{y} (31.48)	36.16_{x} (35.10)	40.51_{w} (42.34)	43.96_{v} (48.28)	37.33 (37.15)
SEm	T - 0.53 H - 0.43 TxH -0.97		CD	T - 1.52 H - 1.21 TxH - NS		CV% -7.34

Analysis carried out using Arcsine transformation and figures in the parenthesis are original means.

Means bearing different superscripts (abc) among various treatments (T) and subscripts (xyz) between time intervals (H) differ significantly ($p < 0.05$); TxH–treatment x storage interval interaction.

The dead, abnormal and HOST non-reacted spermatozoa increased significantly ($p < 0.05$) with increasing concentration of pomegranate juice from 5% to 20% in buck epididymal sperm as compared to control dilutor, and also as the period of storage increased at refrigerated temperature, *i.e.,* observed at different time intervals with different concentration of additive (Tables 1-3). These observations on storage effect corroborated well with those of Parmar *et al*. (2012), who reported significant increase ($p < 0.05$) in dead, abnormal and HOST non-reacted sperm count at 12-hour, 24-hour, 36-hour and 48-hour of refrigeration preservation of Mehsana buck semen diluted with Tris citrate fructose yolk diluents, of course without pomegranate juice. In contrast, the rooster's ejaculated sperm extended in AD2D extender with 2–8% pomegranate juice showed a highly significant reduction in dead and abnormal sperm percentages and improved the sperm motility, viability, and acrosome integrity on dilution and during 36 hrs of refrigeration storage (Al-Daraji, 2012). Perumal and Rajkhowa (2015) also found significantly ($p < 0.05$) lower dead and abnormal spermatozoa percentage in Mithun bull semen with 8% pomegranate juice added extender compared to higher or lower levels up to 30 hr of storage at 4–6°C. These contradictory findings may be attributed to a difference in species, source of sperm and chemical composition, pH and bacterial load/contaminants of the extender as well as the quality of pomegranate juice used in different studies.

Moreover, various spermatozoal parameters (dead, abnormal and HOST non-reacted) were found to be increased in the present study as the concentration of additive (pomegranate juice) increased, the reason behind might be due to high concentration of citrate present in the pomegranate juice that perhaps altered the pH of TEYC diluents, which of course was not monitored in this study.

The dead sperm percentage was positively correlated ($p < 0.01$) with abnormal sperm percentage ($r = 0.53$) and HOST non-reacted sperm percentage ($r = 0.83$) in buck. The abnormal sperm percentage was positively correlated with HOST non-reacted sperm percentage ($r = 0.53$, $p < 0.01$). Similar positive correlations among these traits in fresh bull semen of different breeds have been reported by Sharma *et al*. (2012) and Zubair *et al*. (2013). Contrary to the present finding, the dead sperm percentage was found to be positively correlated with normal sperm percentage ($r = 0.12$, $p < 0.05$) in male rabbit (El-Tarabany *et al*., 2015).

Conclusions

The present study indicates that the mean percentage of dead, abnormal and HOST non-reacted buck epididymal spermatozoa were found to be lowest in control tris-yolk-citrate dilutor as compared to all pomegranate juice treatment groups (5–20%), and the values of all increased with increasing levels of additive and storage interval, suggesting its detrimental effect on cauda epididymal spermatozoa either on fresh addition or during refrigeration preservation. However, whether the cauda epididymal spermatozoa with or without additive pomegranate juice translate into better fertility or conception rates remains to be determined.

Acknowledgment

We thank Principal and Dean, veterinary college; NAU, Navsari, and their staff for kind permission, help, and co-operation for taking up this work.

References

Al-Daraji, H.J. (2012). The use of pomegranate juice for counteract lipid peroxidation that naturally occurred during liquid storage of roosters' semen. *Am. J. Pharm. Tech. Res.,* 2(4): 341-350.

Al-Daraji, H. J. (2015). The use of pomegranate juice for counteract lipid peroxidation that naturally occurred during liquid storage of roosters' semen. *Pharmacognosy Communications*, 5(1): 82-87.

Campos, A.C.N., Nunes, J.F., Monteiro, A.W.U., de Figueiredo, E.L., Pinheiro, J.H.T., Ferreira, M.A.L., and de Araujo, A.A. (2004). Viability of washed and unwashed goat sperm diluted in coconut water, cooled and storage at 4°C. *Rev. Bras. Cien. Vet.*, 11(3): 178-182.

Cao, G., Booth, S.L., Sadowski, J.A. and Prior, R.L. (1998). Increases in human plasma antioxidant capacity after consumption of controlled diets high in fruit and vegetables. *The Am. J. Clin. Nutr.,* 68: 1081-1087.

Dong, Q., Rodenberg, S.E., Huang, C. and Vandevoort, C.A. (2008). Cryopreservation of Rhesus monkey (*Macaca muitatta*) epididymal spermatozoa before and after refrigerated storage. *J. Andrology*, 29(3): 283-292.

El-Tarabany, M.S., El-Bayomi, K. and Abdelhamid, T. (2015). Semen characteristics of purebred and crossbred male rabbits. *PloS one*, 10(5): e0128435.

Mansour, S.W., Sangi, S., Harsha, S., Khaleel, M.A. and Ibrahim, A.R.N. (2013). Sensibility of male rats fertility against olive oil, Nigella sativa oil and pomegranate extract. *Asian Pacific J. Trop. Biomed.*, 3(7): 563-568.

Parmar, V.R., Suthar, B.N., Sharma, V.K., Nakhashi, H.C. and Parikh, S.S. (2012). Preservation of washed spermatozoa of Mehsana buck at refrigeration temperature. *Veterinary World*, 5(5): 294-296.

Perumal, P. and Rajkhowa, C. (2015). Effect of addition of pomegranate (*Punica granatum*) juice on the liquid storage (5°c) of Mithun (*Bos frontalis*) semen. *Indian J. Anim. Res.,* 49(4): 470-473.

Perumal, P., Selvaraju, S., Barik, A.K., Mohanty, D.N., Das, S. and Mishra, P.C. (2011). Role of reduced glutathione in improving post-thawed frozen seminal characters of poor freezable Jersey crossbred bull semen. *Indian J. Anim. Sci.*, 81(8): 807-810.

Seeram, N.P., Aviram, M., Zhang, Y., Henning, S.M., Feng, L., Dreher, M. and Heber, D. (2008). Comparison of antioxidant potency of commonly consumed polyphenol-rich beverages in the United States. *J. Agril. Food Chem.*, 56(4): 415-1422.

Sharma, M., Singh, M., Kapoor, S. and Jasial, S. (2012). Interrelationship between some routine semen evaluation parameters in Jersey X local hill crossbred bulls. *Open Vet. J.*, 2(1): 26-31.

Zubair, M., Lodhi, L.A., Ahmad, E. and Muhammad, G. (2013). Hypo-osmotic swelling test as screening for evaluation of semen of bull. *J. Ento. Zoo. Studies*, 1(6): 124-128.