RESEARCH ARTICLE

Effect of Tomato Juice in Tris-Yolk-Citrate Extender on Refrigeration Preservation of Cauda Epididymal Spermatozoa of Buck

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Abstract

The study was carried out on the preservation of epididymal spermatozoa of buck at refrigerated temperature without and with tomato juice as a supplement in Tris egg yolk citrate extender. The eight pairs of testicles including epididymis (total 16) from slaughtered bucks were collected within 2–4 hours of their slaughter. Sperms collected from cauda epididymis were preserved at refrigerated temperature up to 48 hours in tris egg yolk citrate extender at 300 million sperm/mL with different concentration of tomato juice (0%, 4%, 6%, 8%, and 10%) and the physical characteristics of spermatozoa were assessed to know the effect of tomato juice (Tj). The mean dead, abnormal and HOST non-reacted spermatozoa increased significantly (p <0.05) at every 12-hour intervals of preservation in the dilutor without and with different concentration of tomato juice. Tomato juice exerted an adverse effect on physical characteristics of sperm during refrigeration preservation. All the three sperm traits studied however revealed significant (p <0.01) positive interrelationships with correlations of 0.31 to 0.72.

Keywords: Correlation, Epididymal spermatozoa, Refrigerated temperature, Slaughtered bucks, Tomato juice.

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Introduction

Obtaining caudal epididymal sperms is an important technique in the propagation and conservation of male germplasm with high genetic merit after a serious injury or from dead animals (Dong *et al*., 2008), and endangered species (Santiago-Moreno *et al.,* 2006) and pets (Leibo and Songsasen, 2002). Tomato contains different compounds (*e.g*. carotenoids, vitamin C and flavonoids), that may be responsible for the antioxidant properties. Although tomato contains an array of phytochemicals, most of the attention has been focused on lycopene, the main carotenoid in tomato products, which possesses the greatest quenching ability of singlet oxygen among the various carotenoids (Di-Mascio *et al*., 1989). Therefore, the objective was to study the effect of tomato juice supplementation in semen extender on goat cauda epididymal sperm when preserved at refrigeration temperature.

Materials and methods

The eight pairs of testicles including epididymis (total 16 testes) were collected from the Government approved slaughterhouse, in a sterile plastic bag with utmost care in air-tight sterile cryobox (5°C) and brought to the laboratory within 2–4 hours of the slaughter of animals. Spermatozoa were retrieved separately from the right and left cauda epididymis at room temperature by making several small incisions over cauda epididymis with a BP blade and adding 5 ml prewarmed 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 to make specific concentration (300x10^6/mL) of the spermatozoa. The retrieved cauda epididymal spermatozoa extended with TEYC dilutor were tested in five groups by adding different concentrations of tomato juice (0% as control T1 group and 4%, 6%, 8% and 10% as treatment T2, T3, T4 and T5 groups, respectively) for their effect on *in vitro* storage of sperms up to 48 hours at refrigeration temperature.

For extraction of juice at least two ripened and healthy tomato fruits (Variety: GN Tom. 2) were washed with running tap water, wiped with 70% alcohol, cut, mashed with a spoon in a dish and the juice extracted was collected into centrifuge tubes and centrifuged at 3000 rpm for 15 minutes. The supernatant was re-centrifuged and the second supernatant was collected into a sterile glass bottle as per Bayemi *et al*. (2015) for use in the extender.

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The extended cauda epididymal spermatozoa in TEYC with different concentrations of tomato juice were examined for sperm parameters, *viz*., dead sperm count (%), morphologically abnormal sperm (%) and HOST non-reacted sperms (%) at 12 h, 24 h, 36 h and 48 h of storage. The data thus obtained were statistically analyzed by using 2 factors Factorial Randomized Block Design (RBD) on aliquots of spermatozoa obtained from 8 pairs of epididymis as replicates with different concentrations of additive and different time intervals of storage. The data were arcsine transformed before analysis for ANOVA and the significance among different means of various treatments and time intervals were compared by using the critical difference (CD) test at 5% level of significance. Further, the correlation coefficient among spermatozoal parameters studied was carried out by MS Excel office.

Results and discussion

The mean dead, abnormal and HOST non-reacted epididymal spermatozoa found at different time intervals up to 48 hours of refrigeration preservation in tris egg yolk citrate extender supplemented with different levels of tomato juice (0%, 4%, 6%, 8%, and 10%) are presented in Tables 1 to 3.

The results showed that the addition of tomato juice in TEYC dilutor, particularly with increasing concentration from 4% to 10% as compared to non-added control dilutor, was detrimental to epididymal sperm of bucks as revealed by significantly higher dead, abnormal and HOST non-reacted sperm percentage soon after dilution. The mean values of all three traits also increased gradually and significantly (p <0.01) with increasing storage intervals at 4°C. Al-Daraji (2014) found the increased percentages of dead and abnormal spermatozoa in chicken semen when stored for 72 hours *in vitro* in AD2D extender with and without tomato juice, but his results with respect to effects of tomato juice were contradictory. He found significantly reduced percentages of the dead, abnormal sperm and acrosomal abnormalities in ejaculated chicken sperm in AD2D extender added with tomato juice @ 2–8%, and the highest level was significantly beneficial in improving the sperm quality over control dilutor, in contrast, to present findings on epididymal sperm of buck in TEYC dilutor. The abnormal spermatozoa were also reported to be increased in buck semen with increased storage time up to 72 hours and up to 24 hours at refrigerated temperature without any additive by Parmar *et al*. (2012) and Daramola and Adekunle (2017), respectively. The present trend suggested that tomato juice no way protected sperm during refrigeration and that increasing juice level was, in fact, detrimental on the viability of buck epididymal sperm.

A non-significant (p >0.05) difference was observed between the control group and treatment T2 group (4%, tomato juice) in terms of mean abnormal spermatozoa and HOST non-reacted spermatozoa (Table 2 and 3). These results were contrary to the findings of Rosato *et al*. (2012) and Al-Daraji (2014), who found that the osmotic resistance (%) and abnormal spermatozoa, respectively, decreased in lycopene enriched and tomato juice treated groups than the control semen of rabbit and chicken, respectively. The reason might be due to altered pH and increased concentration of citric acid present in the tomato juice.

The variations in mean spermatozoal parameters (*viz*. dead, abnormal and HOST non-reacted spermatozoa)

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

Juice treatment (T)	*Refrigeration storage interval (H)*					
	0 h	*12 h*	*24 h*	*36 h*	*48 h*	*Overall*
T1 (Control)	12.65 (5.13)	17.63 (9.56)	21.60 (14.00)	26.06 (19.88)	29.02 (24.38)	21.39^{c} (14.59)
T2 (4%)	16.20 (8.63)	20.44 (12.75)	22.74 (15.38)	26.38 (20.13)	29.39 (24.44)	23.03^{b} (16.26)
T3 (6%)	16.74 (8.50)	19.07 (10.94)	24.72 (17.94)	27.60 (21.88)	30.41 (26.06)	23.71^{ab} (17.06)
T4 (8%)	16.43 (8.56)	19.11 (11.31)	23.27 (16.31)	26.61 (20.50)	28.65 (23.31)	22.81^{b} (16.00)
T5 (10%)	17.67 (9.56)	21.02 (13.19)	26.34 (20.25)	27.90 (22.44)	30.75 (26.63)	24.73^{a} (18.41)
Overall mean	15.94^{v} (8.08)	19.45^{w} (11.55)	23.74^{x} (16.78)	26.91^{y} (20.96)	29.64^{z} (24.96)	23.14 (16.47)
SEm	T—0.45 H—0.26 TxH—0.65		CD	T—1.31 H—0.81 TxH—NS		CV%—7.95

Analysis carried out using arcsine transformation and figures in the parenthesis are original means.
Overall means bearing different superscripts (^{abc}) among various treatments and subscripts (^{xyz}) between time intervals differ significantly (p <0.05); TxH, treatment x storage interval interaction

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

Juice treatment (T)	*Refrigeration storage interval (H)*					
	0 h	*12 h*	*24 h*	*36 h*	*48 h*	*Overall*
T1 (Control)	27.33 (21.25)	34.26 (31.75)	37.24 (36.69)	39.53 (40.56)	43.76 (47.88)	36.43^{bc} (35.63)
T2 (4%)	28.09 (22.38)	32.43 (29.00)	36.87 (36.06)	39.90 (41.19)	42.40 (45.50)	35.94^{c} (34.83)
T3 (6%)	28.55 (22.94)	33.76 (31.00)	38.55 (38.94)	42.46 (45.63)	44.11 (48.50)	37.49^{ab} (37.40)
T4 (8%)	29.07 (24.00)	35.06 (33.06)	38.69 (39.13)	42.21 (45.19)	45.20 (50.38)	38.05^{a} (38.35)
T5 (10%)	30.60 (26.13)	35.87 (34.44)	38.34 (38.56)	40.58 (42.38)	43.90 (48.13)	37.86^{a} (37.93)
Overall Mean	28.73^{v} (23.34)	34.28^{w} (31.85)	37.94^{x} (37.88)	40.94^{y} (42.99)	43.88^{z} (48.08)	37.15 (36.83)
SEm	T—0.43 H—0.31 TxH—0.69		CD	T—1.24 H—0.86 TxH—NS		CV%—5.25

Analysis carried out using arcsine transformation and figures in the parenthesis are original means.
Means bearing different superscripts (^{abc}) among various treatments and subscripts (^{xyz}) between time intervals differ significantly (p <0.05); TxH, treatment x storage interval interaction

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

Juice treatment (T)	*Refrigeration storage interval (H)*					
	0 h	*12 h*	*24 h*	*36 h*	*48 h*	*Overall*
T1 (control)	23.95 (17.31)	28.16 (22.94)	32.93 (29.88)	37.88 (37.81)	42.39 (45.50)	33.06d (30.69)
T2 (4%)	25.28 (19.13)	29.72 (25.00)	33.62 (31.06)	40.94 (43.00)	42.53 (45.75)	34.42cd (32.79)
T3 (6%)	28.39 (22.94)	32.48 (29.13)	37.70 (37.63)	44.48 (49.13)	47.65 (54.63)	38.14ab (38.69)
T4 (8%)	25.00 (18.69)	31.53 (27.75)	36.61 (35.75)	41.81 (44.50)	45.16 (50.31)	36.02bc (35.40)
T5 (10%)	31.22 (27.63)	34.30 (32.25)	37.83 (38.00)	43.01 (46.69)	48.90 (56.75)	39.05a (40.26)
Overall Mean	26.77v (21.14)	31.24w (27.41)	35.74x (34.46)	41.62y (44.23)	45.33z (50.59)	36.14 (35.57)
SEm	T—0.85 H—0.39 TxH—0.86		CD	T—2.47 H—1.07 TxH—NS		CV%—6.74

Analysis carried out using arcsine transformation and figures in the parenthesis are original means. Means bearing different superscripts (^{abc}) among various treatments and subscripts (^{xyz}) between time intervals differ significantly (p <0.05); TxH, treatment x storage interval interaction

observed at different storage time and with different concentration of additive in present and other studies might be attributed to species difference, source of sperm (ejaculated or epididymal), chemical composition and nature of extender preparation used and thereby its protective properties for sperm against cold shock and ageing during storage.

In the present study, dead spermatozoa had significant (p <0.01) positive correlations with abnormal spermatozoa and HOST non-reacted spermatozoa (r = 0.31 and 0.40). Similarly, the abnormal sperm was significantly (p <0.01) and positively correlated with HOST non-reacted spermatozoa (r = 0.72). Similar correlations of membrane integrity with the viability of spermatozoa were reported by Bohlooli *et al*. (2012) and Zubair *et al*. (2013), while contrary findings of the morphological abnormalities with viability and HOST reacted spermatozoa were reported by Sharma *et al*. (2012).

Conclusion

The study suggests that the mean dead, abnormal and HOST non-reacted spermatozoa of buck epididymis were found to be lower in control tris egg yolk citrate extender as compared to that added with different concentration of tomato juice (4%, 6%, 8% and 10%) at all intervals of refrigeration storage for 48 h, suggesting detrimental effect of tomato juice at 4–10% levels used for buck epididymal sperms.

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