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# Effect of tris egg yolk citrate (TEYC) supplemented with tomato juice on spermatozoa extracted from epididymis and preserved at refrigerated temperature of non-descriptive breed of bucks

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**Abstract**
The objectives of the study were to determine the effects of tomato juice as additive on sperms characteristics retrieved from epididymis of non-descriptive breed of slaughtered bucks during *in vitro* storage at refrigerator temperature. Eight pair of testes from slaughtered buck was collected and spermatozoa were retrieved from the epididymis at 37 ºC after adding TEYC dilutor and spermatozoa were fixed at the concentrations of $300x10^6$/ml. They were divided into five groups; control (T1) and treatments T2, T3, T4 and T5 using diluted spermatozoa with 4, 6, 8 and 10 ml of tomato juice per 100 ml of diluent, respectively. Individual motility, live spermatozoa, normal spermatozoa, HOST reacted spermatozoa and motility degeneration rate were observed *in vitro* storage of refrigerated temperature at 0, 12, 24, 36 and 48 h. No significant ($p>0.05$) improvement was observed by adding diluent into collection tubes either supplemented before and after refrigerated temperature. The study also revealed a highly significant ($p< 0.01$) correlation between the various spermatozoa evaluation parameters. In conclusion, addition of diluent with different treatment groups (4%, 6%, 8% and 10% added tomato juice) the Spermatozoal sample if used within 12 h in 4%, 6% and 8% tomato juice added treatment groups, the motility remained up to 50% that could be utilized for fertility purpose and the correlation coefficient of all the parameters indicated the retrieved spermatozoa was potential for fertility.

**Keywords:** Correlation coefficient, fertility, *in vitro*, spermatozoa, tomato juice

## Introduction
India is at number one in milk production. However, productivity of India is still very low as compared to developed countries. Among the total milk and meat production the goat contributes 4% of the total milk and 16% of the total meat production in India (Anonymous 2012) [5].

Epididymis spermatozoa lacks seminal plasma containing the secretion of accessary glands. Seminal plasma has many advantages over being there, its molecules interacts with sperm surface and function during the transit through the male and female genital tracts, it has an effect on post-testicular maturation process such as capacitation and the interaction of gametes. Besides, seminal plasma also exerts specific effects on the susceptibility of sperm to cold shock (Fickel *et al*., 2007) [14].

Optimization of the management includes the need to development of efficient semen storage methods. Current methods of semen storage are only effective for shorter periods of time and need to be improvised it. Improvements in the methods of liquid storage of spermatozoa are limited due to lack of basic knowledge about the biochemical mechanisms regulating spermatozoa functions *in vivo* and *in vitro*. Lipids are known to have a major impact on the structure and function of spermatozoa both *in vivo* and *in vitro* (Giovannucci *et al*., 1995) [16].

Tomato contains various compounds (e.g. carotenoid, vitamin C and flavonoids) that are responsible for the antioxidant properties. Tomatoes contain an array of phytochemicals, although, major attention has been focused on lycopene, the main carotenoid in tomato products possesses the greatest quenching ability of singlet oxygen among the various carotenoid (Di-Mascio *et al*., 1989) [11], and is effective in protecting blood lymphocytes from

Nitric Oxide ($NO_2$) radical damage (Bohm *et al*., 1995) [8]. Lycopene, the red colored, natural pigment synthesized by plants and microorganisms (Agarwal and Rao, 2000) [1]. Antioxidants nutrients, including lycopene and other carotenoids, neutralize the adverse effects of free radicals. Hence, the balance between free radicals and antioxidants is important for maintaining healthy body system (Fawzi *et al*., 2000) [13]. Lycopene is one of the most potent antioxidant (Di-Mascio *et al*., 1989 and Miller *et al*., 1996) [11, 19] with having a singlet-oxygen-quenching ability ten times higher than that of α-tocopherol (Di-Mascio *et al*., 1989) [11]. Dietary inclusion with α-tocopherol, a major lipid-soluble antioxidant presents in cell membranes, has been demonstrated to reduce the susceptibility to lipid peroxidation, improved the semen characteristics and fertility (Geva *et al*., 1996) [15]. Previous findings, may be led to expect that the use of lycopene rich sources may exert positive influence on semen quality characteristics. Thus, the present work was carried out to study the effect of tomato juice, as a lycopene-rich source as additive with tris egg yolk citrate extenders at different levels on slaughtered buck spermatozoa retrieved from epididymis, stored at refrigerated temperature.

## Materials and Methods

**Collection of testicles:** A total 16 mature testicles (8 pair) were collected during the study period from the Government approved slaughter house, in a sterile plastic bag with utmost care in air-tight sterile cryobox (5 ºC) and brought to the laboratory within 2-4 h after slaughtering the goat.

**Preparation of tomato juice (Tj) extract:** 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 was extracted on the same day of collection of testes. The tomato juice was prepared by standard procedure reported by Bayemi *et al*. (2015) [6].

**Retrieval of Epididymal spermatozoa:** Spermatozoa were retrieved separately from the right and left cauda epididymis at laboratory room temperature by making several small incisions over cauda epididymis with a BP blade and added 5 ml pre-warmed Tris Egg Yolk Citrate (TEYC) dilutor i.e. at 37ºC to swim out the spermatozoa in a petri dish and the prepared samples had $\geq$70% individual sperm motility were selected for further analysis and extended with TEYC dilutor in order to make specific concentration of the spermatozoa.

**Sperm concentration ($x10^6$/ml):** The sperm concentration was calculated briefly as follows: 0.1 ml of spermatozoa plus dilutor was added in 9.9 ml of 2% eosin solution in first test tube. After gentle mixing, 1.0 ml solution from first test tube was taken out and poured in 9.0 ml of 2% eosin solution in second test tube. A cover slip was fixed on the haemocytometer slide and the slide was charged with the help of capillary pipette using diluted semen from second test tube and allowed it to spread under the cover slip. After waiting for 5 minutes to allow the sperms to settle, the slide was placed under microscope and number of sperms (N) present in the four corner chambers and one middle chamber (16 X 5 = 80 small chambers) were counted. Finally, the sperm concentration per ml of spermatozoa plus dilutor was estimated using following formula:

Cauda epididymal sperm concentration/ml = N x 50 x $10^6$.

The sperm concentration (300 $x10^6$) per ml was fixed by adding further dilutor to Cauda epididymis retrieved sperm concentration (ml).

$$\text{Dilutor required per ml} = \frac{\text{Cauda epididymal sperm concentration per ml}}{\text{Required sperm concentration per ml}}$$

**Experimental groups:** Moreover, the retrieved cauda epididymal spermatozoa extended with Tris Egg Yolk Citrate dilutor were tested in five groups by adding different concentrations (0% as control T1 group and 4%, 6%, 8% and 10% as treatment T2, T3, T4 and T5 groups, respectively) of tomato juice as additive and observed their effect on *in vitro* storage at refrigerated temperature up to 48 h.

**Evaluation of cauda epididymal spermatozoa extended with dilutor:** The cauda epididymal spermatozoa extended with dilutor was examined for spermatozoa evaluation parameters *viz.* individual sperm motility (%), live sperm count (%), morphological normal sperm (%), HOST reacted sperms (%) (Kumar *et al*., 2018) [17] and motility degeneration rate (%) (Campos *et al*., 2004) [9] after adding different concentrations of tomato juice, at 37 ºC temperature in a water bath and initial evolution carried out immediate considered as (0 h). Further, the sperm samples were preserved at refrigerated temperature up to 48 h and evaluated for above parameters at 12 h, 24 h, 36 h and 48 h intervals.

**Statistical analysis:** The data generated were statistically analyzed by using Randomized Block Design (RBD) on aliquots of spermatozoa obtained from different pairs of testicles with different additive concentrations at different time intervals by carrying out arcsine transformation of data. As samples from separate pairs of goat testicles considered replication and observation were recorded with different concentrations of additive at different time intervals further pooling was carried out by split plot ANOVA and the significance among different means of various treatments and time intervals as well as different pairs of testicles generated were compared by using Critical Difference (CD) test at 5% level of significance. Further, the correlation coefficient among spermatozoa evaluation parameters was carried out by MS Excel office.

## Results and Discussion

Effect of tomato juice with tris egg yolk citrate extender on mean motility, live, normal, HOST reacted and motility degeneration rate percentage of spermatozoa fluctuated with (4%, 6%, 8% and 10%) additive concentrations at 0 h as well as at 12 h, 24 h, 36 h and 48 h intervals (Tab.1, 2, 3, 4 and 5), respectively.

The mean motility, live, normal and HOST reacted percentage of spermatozoa decreased, while the mean motility degeneration rate of spermatozoa increased in tandem with increased storage periods (Tab.1, 2, 3, 4 and 5). These results were in agreement with Al-Daraji (2014) [4], who found that sperm quality and fertility in chicken semen were generally decreased when semen was stored for 72 h in *vitro*; Daramola and Adekunle (2017) [10] reported the mean motility and membrane integrity of West African Dwarf bucks spermatozoa decreased during storage and contrast to the

present finding significant decreased motility degeneration rate observed with increased time intervals reported by Aguiar *et al*. (2013) [2] in non-defined breed of goats.

The mean motility percentage and live percentage of spermatozoa were observed significantly higher ($p< 0.05$) in control group as compared to other treatment groups (Tab.1 & 2). However, treatment T2 group (4%, tomato juice) had least decreased mean motility and live percent as compared to other treatment groups (6%, 8% and 10% of tomato juice). The results were found contrary to the finding reported by Rosato *et al*. (2012) [20] and Akalin *et al*. (2016) [3] when used lycopene extenders in rabbit and ram ejaculated semen, respectively as well as reported by Al-Daraji (2014) [4] tomato juice used extender in chicken ejaculated semen.

The mean normal percentage of spermatozoa and HOST reacted spermatozoa were observed at par ($p>0.05$) between control T1 group and treatment T2 (4%, tomato juice) added group (Table 3 & 4). These results were also found contrary to the finding reported by Rosato *et al*. (2012) [20], they used lycopene with Tris-citrate-glucose extenders in rabbit ejaculated semen as well as tomato juice with Extender ($AD_2E$) in chicken ejaculated semen reported by Al-Daraji (2014) [4].

The mean motility degeneration rate of epididymal retrieved spermatozoa form testicles of non-descriptive breed of goats was observed non-significantly ($p>0.05$) higher between treatment T2 group (4%, tomato juice) and control (T1) group and increased with increased concentration of tomato juice when preserved at refrigerated temperature (Table 5). As compared to the findings Aguiar *et al*. (2013) [2] reported significantly decreased motility degeneration rate when time intervals increased in non-defined breed of goats.

The various spermatozoa evaluation parameters studied were found to be decreased as the concentration of additive (tomato juice) increased, the reason behind might be due to increase the concentration of citrate present in the tomato juice.

Moreover, the present research work was carried out with 300 x $10^6$/ml concentration of spermatozoa and different concentration of spermatozoa might be affected sperm motility percentage at various concentrations of additives. The sperm motility was found decreased with increased concentration and length of storage in 5 °C reported by Wahjuningsih *et al*. (2012) [22].

The study further envisaged a significant ($p< 0.01$) positive correlations between the motility percentage and spermatozoa evaluation parameters (Table 6). Similarly, in some earlier studies, correlation had been established between sperm motility, live percentage, normal percentage and HOST reacted spermatozoa (Zubair *et al*., 2013) [23] in bull and also between sperm motility with live percentage (Bohlooli *et al*., 2012) [7] in ram and (El-Tarabany *et al*., 2015) [12] in rabbit as well as sperm motility with live percentage and HOST reacted spermatozoa reported by Sharma *et al*. (2012) [21] in cattle crossbred bulls (Jersey x local hill cattle).

**Table 1:** Motility percentage of epididymal spermatozoa (n=8) at different concentrations of additive and time intervals as well as overall motility percentage of spermatozoa retrieved from different pairs of goat testicles

| Time intervals (H) / Treatment (T) | 0 h | 12 h | 24 h | 36 h | 48 h | Average (T) Mean |
|---|---|---|---|---|---|---|
| T1 (Control) | 60.66 (75.94) | 54.68 (66.56) | 48.77 (56.56) | 42.63 (45.94) | 37.68 (37.50) | $48.88^{a}$ (56.50) |
| T2 (4%) | 57.84 (71.56) | 50.79 (60.00) | 43.90 (48.13) | 39.13 (40.00) | 35.17 (33.44) | $45.36^{b}$ (50.63) |
| T3 (6%) | 56.62 (69.69) | 49.33 (57.50) | 41.91 (44.69) | 37.32 (36.88) | 32.88 (29.69) | $43.61^{c}$ (47.69) |
| T4 (8%) | 58.02 (71.88) | 50.23 (59.06) | 42.65 (45.94) | 37.34 (36.88) | 33.53 (30.63) | $44.35^{bc}$ (48.88) |
| T5 (10%) | 53.40 (64.38) | 46.06 (51.88) | 40.29 (41.88) | 35.27 (33.44) | 30.57 (25.94) | $41.12^{d}$ (43.50) |
| Average (H) Mean | $57.31_{v}$ (70.69) | $50.22_{w}$ (59.00) | $43.50_{x}$ (47.44) | $38.34_{y}$ (38.63) | $33.96_{z}$ (31.44) | 44.67 (49.44) |
| -- | -- -- | -- | -- | -- | SEm | CD | C.V.% |
| -- | -- -- | -- | -- | T | 0.55 | 1.60 | 5.89 |
| -- | -- -- | -- | -- | H | 0.42 | 1.16 | -- |
| -- | -- -- | -- | -- | TH | 0.93 | NS | -- |

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

Average means bearing different superscripts among various treatments (T) and average means bearing different subscripts between time intervals (H) differ significantly by C.D. test ($p<0.05$)

**Table 2:** Live percentage of Epididymal spermatozoa (n=8) at different concentrations of additive and time intervals as well as overall live percentage of spermatozoa retrieved from different pairs of goat testicles

| Time intervals (H) / Treatment (T) | 0 h | 12 h | 24 h | 36 h | 48 h | Average (T) Mean |
|---|---|---|---|---|---|---|
| T1 (Control) | 77.32 (94.88) | 72.34 (90.44) | 68.36 (86.00) | 63.90 (80.13) | 60.95 (75.63) | $68.57^{a}$ (85.41) |
| T2 (4%) | 73.76 (91.38) | 69.52 (87.25) | 67.22 (84.63) | 63.58 (79.88) | 60.58 (75.56) | $66.93^{b}$ (83.74) |
| T3 (6%) | 73.22 (91.50) | 70.89 (89.06) | 65.25 (82.06) | 62.36 (78.13) | 59.55 (73.94) | $66.26^{bc}$ (82.94) |
| T4 (8%) | 73.54 (91.44) | 70.86 (88.69) | 66.69 (83.69) | 63.36 (79.50) | 61.32 (76.69) | $67.15^{b}$ (84.00) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| T5 (10%) | 72.30 (90.44) | 68.95 (86.81) | 63.62 (79.75) | 62.06 (77.56) | 59.22 (73.38) | 65.23$^{c}$ (81.59) |
| Average (H) Mean | 74.03$_{v}$ (91.93) | 70.51$_{w}$ (88.45) | 66.23$_{x}$ (83.23) | 63.05$_{y}$ (79.04) | 60.32$_{z}$ (75.04) | 66.83 (83.54) |
| -- | -- | -- | -- | -- | -- | SEm | CD | C.V.% |
| -- | -- | -- | -- | -- | T | 0.45 | 1.31 | 2.75 |
| -- | -- | -- | -- | -- | H | 0.29 | 0.81 | -- |
| -- | -- | -- | -- | -- | TH | 0.65 | NS | -- |

Analysis carried out using Arcsine transformation and figures in the parenthesis are original means.
Average means bearing different superscripts among various treatments (T) and average means bearing different subscripts between time intervals (H) differ significantly by C.D. test ($p<0.05$)

**Table 3:** Normal percentage of epididymal spermatozoa (n=8) at different concentrations of additive and time intervals as well as overall normal percentage of spermatozoa retrieved from different pairs of goat testicles

| Time intervals (H) / Treatment (T) | 0 h | 12 h | 24 h | 36 h | 48 h | Average (T) Mean |
|---|---|---|---|---|---|---|
| T1 (Control) | 62.63 (78.75) | 55.70 (68.25) | 52.72 (63.31) | 50.43 (59.44) | 46.20 (52.13) | 53.54$^{ab}$ (64.38) |
| T2 (4%) | 61.88 (77.63) | 57.54 (71.00) | 53.09 (63.94) | 50.07 (58.81) | 47.57 (54.50) | 54.03$^{a}$ (65.18) |
| T3 (6%) | 61.41 (77.06) | 56.20 (69.00) | 51.41 (61.06) | 47.50 (54.38) | 45.85 (51.50) | 52.48$^{bc}$ (62.60) |
| T4 (8%) | 60.90 (76.00) | 54.90 (66.94) | 51.28 (60.88) | 47.75 (54.81) | 44.76 (49.63) | 51.92$^{c}$ (61.65) |
| T5 (10%) | 59.36 (73.88) | 54.09 (65.56) | 51.63 (61.44) | 49.38 (57.63) | 46.06 (51.88) | 52.11$^{c}$ (62.08) |
| Average (H) Mean | 61.23$_{v}$ (76.66) | 55.69$_{w}$ (68.15) | 52.03$_{x}$ (62.13) | 49.03$_{y}$ (57.01) | 46.09$_{z}$ (51.93) | 52.81 (63.18) |
| -- | -- | -- | -- | -- | -- | SEm | CD | C.V.% |
| -- | -- | -- | -- | -- | T | 0.43 | 1.24 | 3.69 |
| -- | -- | -- | -- | -- | H | 0.31 | 0.86 | -- |
| -- | -- | -- | -- | -- | TH | 0.69 | NS | -- |

Analysis carried out using Arcsine transformation and figures in the parenthesis are original means.
Average means bearing different superscripts among various treatments (T) and average means bearing different subscripts between time intervals (H) differ significantly by C.D. test ($p<0.05$)

**Table 4:** HOST reacted percentage of epididymal spermatozoa (n=8) at different concentrations of additive and time intervals as well as overall HOST reacted percentage of spermatozoa retrieved from different pairs of goat testicle

| Time intervals(H) / Treatment (T) | 0 h | 12 h | 24 h | 36 h | 48 h | Average (T) Mean |
|---|---|---|---|---|---|---|
| T1 (Control) | 66.02(82.69) | 61.81(77.06) | 57.04 (70.13) | 52.09 (62.19) | 47.58(54.50) | 56.91$^{a}$ (69.31) |
| T2 (4%) | 64.68 (80.88) | 60.25 (75.00) | 56.35 (68.94) | 49.02 (57.00) | 46.71(53.00) | 55.40$^{ab}$ (66.96) |
| T3 (6%) | 61.57(77.06) | 57.48 (70.88) | 52.26 (62.38) | 45.49 (50.88) | 42.32(45.38) | 51.82$^{cd}$ (61.31) |
| T4 (8%) | 64.97(81.31) | 58.43 (72.25) | 53.35 (64.25) | 48.16 (55.50) | 44.80(49.69) | 53.94$^{bc}$(64.60) |
| T5 (10%) | 58.74(72.38) | 55.67 (67.75) | 52.21 (62.13) | 46.95 (53.31) | 41.07(43.25) | 50.93$^{d}$(59.76) |
| Average (H) Mean | 63.20$_{v}$(78.86) | 58.73$_{w}$ (72.59) | 54.24$_{x}$ (65.56) | 48.34$_{y}$ (55.78) | 44.49$_{z}$(49.16) | 53.80(64.39) |
| -- | -- | -- | -- | -- | -- | SEm | CD | C.V.% |
| -- | -- | -- | -- | -- | T | 0.85 | 2.47 | 4.42 |
| -- | -- | -- | -- | -- | H | 0.38 | 1.05 | -- |
| -- | -- | -- | -- | -- | TH | 0.84 | NS | -- |

Analysis carried out using Arcsine transformation and figures in the parenthesis are original means.
Average means bearing different superscripts among various treatments (T) and average means bearing different subscripts between time intervals (H) differ significantly by C.D. test ($p<0.05$)

**Table 5:** Motility degeneration rate of epididymal spermatozoa (n=8) at different concentrations of additive and time intervals as well as overall Motility degeneration rate of spermatozoa retrieved from different pairs of goat testicles

| Time intervals (H) / Treatment (T) | 12 h | 24 h | 36 h | 48 h | Average (T) Mean |
|---|---|---|---|---|---|
| T1 (Control) | 20.21(12.30) | 30.06(25.36) | 38.78(39.39) | 45.31(50.56) | 33.59$^{b}$(31.90) |
| T2 (4%) | 23.24(16.19) | 34.55(32.52) | 41.37(43.90) | 46.76(53.03) | 36.48$^{ab}$(36.41) |
| T3 (6%) | 24.04(17.40) | 36.66(35.87) | 43.30(47.10) | 49.28(57.34) | 38.32$^{a}$(39.43) |
| T4 (8%) | 24.59(17.68) | 36.78(36.01) | 44.08(48.47) | 49.14(57.17) | 38.65$^{a}$(39.83) |
| T5 (10%) | 25.83(19.39) | 35.88(34.58) | 43.51(47.44) | 50.27(59.07) | 38.87$^{a}$(40.12) |
| Average (H) Mean | 23.58$_{w}$(16.59) | 34.79$_{x}$(32.87) | 42.21$_{y}$(45.26) | 48.15$_{z}$(55.44) | 37.18(37.54) |
| -- | -- | -- | -- | -- | SEm | CD | C.V.% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| -- | -- | -- | -- | -- | T | 1.20 | 3.48 | 8.70 |
| -- | -- | -- | -- | -- | H | 0.51 | 1.44 | -- |
| -- | -- | -- | -- | -- | TH | 1.14 | NS | -- |

Analysis carried out using Arcsine transformation and figures in the parenthesis are original means.
Average means bearing different superscripts among various treatments (T) and average means bearing different subscripts between time intervals (H) differ significantly by C.D. test ($p<0.05$)

**Table 6:** Correlation coefficient among various spermatozoa evaluation parameters (%) of buck testicles collected from slaughter house

| Spermatozoa evaluation parameters (%) | Motility | Live | Normal | HOST Reacted |
|---|---|---|---|---|
| Motility | 1 | | | |
| Live | 0.62** | 1 | | |
| Normal | 0.41** | 0.31** | 1 | |
| HOST Reacted | 0.49** | 0.40** | 0.72** | 1 |

Correlation is significant at 1% level **$p<0.01$

## Conclusions

In conclusion, the data suggested that mean motility, live, normal and HOST reacted percentage of spermatozoa were found to be higher in control (T1) group as compared to different treatment groups (4%, 6%, 8% and 10% added tomato juice). Moreover, the spermatozoa sample if used within 12 h in 4%, 6% and 8% tomato juice added treatment groups, the motility remained up to 50% that could be utilized for fertility purpose. The correlation coefficient of all the parameters indicated the retrieved spermatozoa were potential for fertility.

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