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Some Semen Characterestics, Preservation and Post-Thawing Motility of Buffalo Bull Semen Using Pomegranate Supplement.

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ABSTRACT

The aim of the present investigation was to evaluate the impact of pomegranate juice of various concentrations on some semen characteristics in chilled and post thawed of extended bull semen. Semen samples were diluted in Tris-citric acid-egg yolk-fructose extender and made into 6 aliquots, the 1st served as control while pomegranate juice was added at the concentrations of 10, 20, 30, 40 and 50% to the other five aliquots. The diluted samples were kept in glass tubes and cooled from 37°C to 5°C in a cold cabinet, and maintained at 5°C for 8 days. Sperm motility (SM%) in chilled and post-thawing sperm parameters, including individual motility%, alive sperm%, membrane integrity%, and total sperm abnormality% were assessed. The results elaborated that the addition of 10% pomegranate juice in the chilled extended buffalo bull semen improved SM% while the other concentrations failed to preserve along 8 days. Furthermore, supplementation of extender with 10% pomegranate significantly ($P<0.002$ at least) increases post-thaw motility when compared to the control group. In conclusion, the addition of pomegranate juice at concentration of 10% to semen diluent induced remarkable physiological effects on bull semen quality during preservation and improved its frozen-thawed quality.

Keywords: buffalo bull, preservation, motility, post thawing, pomegranate.

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INTRODUCTION

One of the crucial elements in semen preservation and freezability is the chemical composition of the extender and the type of the cryoprotectant used (1). Pomegranate juice is beneficial fruit of medicinal impact (2, 3). They have strong antioxidant capacity due to their high contents of polyphenols and their contents of fructose, glucose, citric acid and malic acid (4, 5). The high inclusion of pomegranate juice with polyphenols enables its potential to scavenge free radicals (6). Navindra *et al* (7) recorded that pomegranate juice is rich in vitamins A, C and E which act as antioxidant.

Türk *et al* (8) and Mansour *et al* (9) found that pomegranate juice improves sperm quality. Moreover, Forest *et al* (10) recorded its great influence in treatment of erectile dysfunction in male patients. Semen diluents enriched with pomegranate juice exhibited increased protective effect against lipid peroxidation during storage of roosters' semen (11).

Consequently, the current study was designed to investigate the physiological effect of pomegranate juice on the quality of buffalo bull semen-preserved in both liquid and frozen conditions.

MATERIALS AND METHODS

This investigation was done at the semen freezing Center, Ministry of Agriculture, Abbasia, Egypt.

Experimental Animals:

Three mature buffalo bulls were used. Each bull was fed balanced concentrate and barseem hay ad libidium. Water was offered to those animals ad libidium all the day using manual water through system.

Experimental Materials:

Pomegranate was bought from the local Egyptian market then its juice was prepared according to Türk *et al* (8). The other chemical reagents used for the preparation of extender were purchased from Deisenhofen, Germany.

Semen collection:

Semen was collected once weekly by means of an artificial vagina. After collecting the semen, the ejaculates were transferred to the laboratory within 30 seconds and kept in a water bath for 10 min at 37°C for preliminary evaluation of sperm motility and live percentages by means of conventional methods.

Semen extenders:

1. TRIS base extender: Tris-citric acid-fructose egg yolk (TCFY) diluent, prepared according to Foote *et al*. (12), was used as control extender.
2. TRIS-Pomegranate diluent: pomegranate juice was filtrated and was added to TRIS in concentrations 0.5/4.5 ml (10%), 1/4 ml (20%), 1.5/3.5 ml (30%), 2/3 ml (40%), 2.5/2.5 ml (50%) to obtain a final volume 5 ml in each tube.

Semen Processing and Experimental design:

Only ejaculates of >70% initial motility and 60 million motile sperm cells /mL were used in the following experiments after being pooled to avoid the bull influence:

Experiment I: This experiment was designed to explore the influence of pomegranate juice on the preservation of chilled bull semen in TCFY extender. Pomegranate juice was added to the extended semen at the rate of 0.0, 10%, 20%, 30%, 40% and 50%. After dilution, the extended semen was incubated at 5°C to be examined at days 1, 2, 3, 7 and 8 post semen collection, for sperm motility% (SM%).

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Experiment II: This experiment was established to investigate influence of pomegranate juice (0.0, 10%, 20%, 30%, 40% and 50%) on the post-thawing motility of bull semen extended in TCFY. The diluted semen was cooled slowly, loaded into 0.25 ml straws at 5°Cand and equilibrated for 4 h. The straws were placed horizontally on freezing racks at a height of 4.0 cm above the level of liquid nitrogen, for 10 minutes . The straws were then immersed in liquid nitrogen. After few weeks, frozen bull semen was thawed in a water bath at 37°C for 30 seconds.

Semen characteristics including SM%, alive sperm (AS%), sperm abnormality (SA%) and membrane integrity [hypo osmotic swelling test (HOST) %] were studied as follows:

Motility.

A drop of semen was placed on a pre-warmed (37 °C) glass slide and pre-warmed cover slip**.** Visual motility was assessed microscopically (x 400) at 37 °C with closed circuit television (13).

Live and abnormal spermatozoa (%)

This was evaluated using eosin-Nigrosin stained smear as described by Sidhu and Guraya (14). Two hundred spermatozoa were assessed.

Sperm membrane integrity

Sperm membrane integrity was assessed using HOST (15). Two hundred spermatozoa were assessed and the percentage of spermatozoa with curled tails (swollen/ intact plasma membrane) was calculated.

Statistical analysis:

Data were analysed using were analyzed using the SPSS (2005) computerized program 14.0 to calculate the analysis of variance (ANOVA) (16) Duncan multiple range test was used to evaluate the significant difference between means at P<0.05.

RESULTS

The present results showed that the addition of pomegranate juice at concentration of 10% to the extended bull semen, increased significantly (P<0.0001) the SM % (Table 1). Furthermore, Sperm motility of the previous concentration (35.00 ± 2.89) after 7 days of chilling was significantly (P<0.0001) higher than control and other concentrations (Table 1).

On the other hand, high concentration of pomegranate juice (more than 10%) had significantly (P<0.0001) decreased the sperm motility in comparison with control along the preservation period (8 days).

Regarding the influence of pomegranate juice on the post-thawed sperm motility, addition of pomegranate in a concentration of 10% significantly (P<0.002) improved post thawing sperm motility [Table 2].

Table 1: Sperm motility% of chilled TRIS-Pomegranate extender in buffalo bulls

	Days after cooling					F-value	P<
Treatment	1	2	3	7	8		
Control	81.67 ± 1.67^{A}	81.67 ± 1.67^{A}	63.33 ± 1.67^{A}	0.00 ± 0.00^{B}	0.00 ± 0.00	213.67	0.0001
0.5/4.5 cc	85.00 ± 0.00^{A}	83.33 ± 1.67^{A}	65.00 ± 2.89^{A}	35.00 ± 2.89^{A}	0.00 ± 0.00		
1.0/4.0 cc	41.67 ± 1.67^{B}	35.00 ± 0.00^{B}	23.33 ± 3.33^{B}	0.00 ± 0.00^{B}	0.00 ± 0.00		
1.5/3.5 cc	40.00 ± 2.89^{B}	2.00 ± 0.00^{C}	0.00 ± 0.00^{C}	0.00 ± 0.00^{B}	0.00 ± 0.00		
2.0/3.0 cc	10.00 ± 0.00^{C}	0.00 ± 0.00^{C}	0.00 ± 0.00^{C}	0.00 ± 0.00^{B}	0.00 ± 0.00		
2.5/2.5 cc	0.00 ± 0.00^{D}	0.00 ± 0.00^{C}	0.00 ± 0.00^{C}	0.00 ± 0.00^{B}	0.00 ± 0.00		
P<	0.0001	0.0001	0.0001	0.0001			

Different superscript letters (A, B, …etc) within column are significantly different at P<0.05

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Table 2: Effect of Pomegranate addition to Tris diluent on post thawing semen characteristics of buffalo bulls

Parameter	After Thawing			
	HOST%	Alive%	Abnormality%	Motility%
Control	74.00 ± 8.96^{A}	83.33 ± 4.37^{A}	18.00 ± 1.15^{B}	41.67 ± 3.07^{A}
0.5/4.5 cc	72.00 ± 1.73^{A}	91.00 ± 2.08^{A}	16.00 ± 4.36^{B}	45.00 ± 2.89^{A}
1.0/4.0 cc	74.00 ± 3.79^{A}	82.67 ± 2.91^{A}	27.00 ± 0.58^{A}	36.67 ± 2.79^{A}
1.5/3.5 cc	69.00 ± 4.62^{A}	85.00 ± 2.89^{A}	20.33 ± 1.45^{B}	41.67 ± 1.67^{A}
2.0/3.0 cc	66.33 ± 2.60^{A}	83.33 ± 0.88^{A}	17.00 ± 0.58^{B}	39.17 ± 4.17^{A}
2.5/2.5 cc	72.33 ± 7.22^{A}	85.00 ± 2.89^{A}	14.33 ± 0.33^{B}	21.67 ± 6.15^{B}
F-value	0.31	1.14	5.23	4.96
P<	0.8970	0.3904	0.0089	0.0020

Different superscript letters (A, B, …etc) within column are significantly different at P<0.05

DISCUSSION

The current investigation revealed that the addition of pomegranate juice to the extended buffalo bull semen improved the SM%, SA% post thawing and motility% in chilled semen when compared to the control results.

The present results are in compatible with Türk *et al.* (8) who recorded that epididymal sperm motility and concentration of rats received different doses of pomegranate juice were significantly higher than those of the control. On the contrary, Al-Daraji (11) reported beneficial effect when pomegranate juice is included in roosters' semen diluents and resulted in significant decline in the percent of dead and abnormal sperms before and after storage. The current findings varied slightly than those reported by El-Sheshtawy *et al* (in press) in cattle semen. Those variations were attributed to species' difference and some variations in physiological characteristics of spermatogonia.

Concerning the effect of pomegranate juice supplementation at concentration of 10% to the frozen bull semen, the current results showed that it increased significantly (P<0.002) post-thawing motility compared to other concentrations and the control.

The beneficial impact of pomegranate juice on semen preservation and freezability may be attributed to one and / or the following physiological mechanisms:

I. It contains high concentrations of antioxidants represented by flavonoids, Vit C and other phenolic compounds which were proved to be a very potent antioxidant that could inhibit lipid peroxidation in sperm membrane (17, 18, and 19).
II. Acting as energy substitutes through its high content of glucose, fructose and amino acids (mainly glutamic and aspartic acids) which become available as energy substrate (20).

In conclusion, supplementation of pomegranate juice at concentration of 10% to both chilled and frozen bull semen improve SM% and help in preserving that semen to be used in large scale. This improvement is mainly due to the strong antioxidant and antibacterial influence of pomegranate juice.

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