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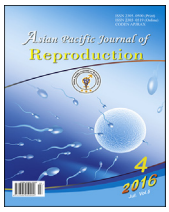

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# Effects of pomegranate juice in Tris-based extender on cattle semen quality after chilling and cryopreservation

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ABSTRACT

**Objective:** To study the effect of adding different concentrations of the pomegranate juice (PJ) to the cattle bull semen extender on post-thawing semen quality.
**Methods:** Semen was collected from five cattle-bulls at weekly intervals for 5 weeks at the Semen Freezing Center, General Organization for Vet. Services, Ministry of Agriculture. Semen samples were diluted in Tris-citric acid-egg yolk-fructose extender and divided into six aliquots, the 1st served as control while PJ was supplemented at 10%, 20%, 30%, 40% and 50% in the aliquot 2, 3, 4, 5 and 6 respectively. Diluted semen samples were subjected to cooling and cryopreservation and stored in liquid nitrogen (LN2). Sperm motility in chilled semen (over 10 d) and post-thawing sperm parameters, including individual motility, alive sperm, membrane integrity, and total sperm abnormality were assessed.
**Results:** Obtained results clearly demonstrated that the addition of 10% PJ in the chilled extended cattle semen proved to be beneficial for maintaining sperm motility percentage. On the other hand, the addition of 40% and 50% PJ failed to preserve motility all over the 10 d. Also, supplementation of extender with 10–20% PJ significantly increases the post-thaw motility and viability as compared with control group.
**Conclusions:** Supplementation of bull semen extender with 10% and 20% PJ provides good chilling and improved frozen-thawed semen quality.

## 1. Introduction

Vegetables and fruits are of the natural sources that maintain life through their contents of multiple active remedies compounds. Pomegranate is one of the beneficial fruits in medicinal treatment [1,2]. Some authors have investigated mainly the antioxidant activities of its polyphenols [3,4].

Populations in the Middle East used pomegranate (*Punica granatum*) as a herbal medicine [5]. Tezcan *et al*. showed that fructose and glucose were the major sugars and that citric and malic are the major acids [4]. The nutritive value of the pomegranate fruit has been demonstrated by Virgili and Marino [6] who explained that daily consumption of 250 mL of PJ covers about 50% of the daily requirements of vitamins A, C and E. Moreover, the fruit contained antioxidant polyphenols that present half of the fruit's antioxidant ability for counteracting the free radicals [7]. The strong antioxidant capacity of PJ was useful in fighting certain cancers [8–10]. Besides many experimental studies described PJ in improving semen quality [11,12] and erectile dysfunction in male patients [13]. El Ghazzawy *et al*. mentioned that PJ had the ability to counteract structural changes in the rat epididymis caused by plasticizer Bisphenol, which interfere with its function and contribute to infertility, *via* increasing the number of caudal epididymal sperm, decreasing sperm abnormalities and improving male fertility [14]. Administration of pomegranate extract could improve sperm characteristics and antioxidant activity in adult male Wistar rats [12] and men [15]. Al-Daraji revealed that supplementation of semen diluent with PJ significantly improved storage ability of roosters' semen and increased the protective effects against lipid peroxidation during liquid storage of roosters' semen for up to 36 h [16].

Hence, the present study was designed to investigate the effect of pomegranate juice when incorporated as a semen extender for maintaining the quality of chilled and frozen-thawed cattle bull semen.

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## 2. Materials and methods

### 2.1. Semen collection and initial evaluation

Five mature cattle-bulls reared at the Semen Freezing Center, General Organization for Vet. Services, Ministry of Agriculture, Abbasia, Egypt, were included in this study. Semen was collected from these five cattle-bulls using an artificial vagina at weekly intervals for 5 weeks. The semen samples were transferred to the adjacent lab within few seconds and initially evaluated for volume (in a graduated tube), sperm motility and live sperm percent. The neat semen samples with more than 70% motility and 80% morphologically normal spermatozoa were admitted to freezing procedure. The ejaculates were pooled in order to have sufficient semen for a replicate and to eliminate the bull effect. The semen was given a holding time for 10 min at 37 °C in a water bath before dilution.

### 2.2. Pomegranate processing

Pomegranates with intact peel were sold from the Egyptian market. They were washed, peeled and the red grains were collected in a clean dish. The grains were squeezed with gauze to obtain a clear watery juice. The juice was filtered and stored at −18 °C till used [1].

### 2.3. Semen processing

A basic control extender (Tris-citric acid-egg yolk-fructose [TCYF]) was prepared according to Foote [17]. TCYF/PJ (pomegranate juice enriched extender, PJEE) [v:v] (0.5/4.5 (10%), 1.0/4.0 (20%), 1.5/3.5 (30%), 2.0/3.0 (40%) and 2.5/2.5 (50%)) were prepared and centrifuged to discard any precipitate. Semen samples were diluted in TCYF (control, 0% PJEE) and the former concentrations of PJEE to ensure 60 million motile spermatozoa/mL, cooled slowly up to 5 °C and equilibrated for 4 h. Semen was packed into 0.25 mL polyvinyl French straws (IMV, France). After equilibration periods, the straws were placed horizontally on a rack and frozen in a vapor 4 cm above liquid nitrogen ($LN_2$) for 10 min and were then dipped in liquid $LN_2$.

### 2.4. Semen quality assessment

The assessment was undertaken on after freeze-thawing of bull spermatozoa. Also, sperm motility was evaluated for raw semen, 2 h after cooling and chilled semen daily up to 10 d. Frozen straws were thawed at 37 °C/30 s. The parameters studied were subjective semen characteristics (motility %, alive %, abnormality % and membrane integrity (hypo-osmotic swelling test HOST) %).

#### 2.4.1. Subjective motility

Subjective motility was assessed using a phase-contrast microscope (100× magnification), with a warm stage maintained at 37 °C. A wet mount was made using a drop of semen placed directly on a pre-warmed slide and covered by a pre-warmed cover slip under the same temperature conditions. Sperm motility estimations were performed in three different microscopic fields for each semen sample. Visual motility was assessed microscopically with closed circuit television system [18].

#### 2.4.2. Live and abnormal spermatozoa (%)

This was evaluated using eosin-Nigrosin stained smear as described by Sidhu and Guraya [19]. Two hundred spermatozoa were assessed.

#### 2.4.3. Sperm membrane integrity

Sperm membrane integrity was assessed using the hypo-osmotic swelling test (HOST) [20]. Two hundred spermatozoa were assessed and the percentage of spermatozoa with curled tails (swollen/intact plasma membrane) was calculated.

### 2.5. Statistical analysis

Statistical analysis data were analyzed using the SPSS (2005) computerized program v. 14.0 to calculate the analysis of variance (ANOVA) [21] for the different parameters between control and additives replications. A significant difference between means was calculated using Duncan's multiple range test at $P < 0.05$.

## 3. Results

Data analysis revealed a gradual decline concerning the motility percentage of spermatozoa chilled at 5 °C for the control (0% PJEE) and the first treatment (10% PJEE) from the 1st day to the 10th day. On the contrary, the four other treatments (20–50% PJEE) abruptly declined at the 7th and 10th days. The use of 10% PJEE for extended chilled cattle semen showed the highest significant (at least $P < 0.058\,1$) motility percentage all over the 10 d. The worst results were obtained on using the 40% and 50% PJEE (Table 1).

**Table 1**
Sperm motility percentage of chilled semen in PJEE in cattle-bulls.

| Treatment | Days | | | | | *F*-value for Treat × Days | *P* < |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 7 | 10 | | |
| Control (0% PJEE) | 86.67 ± 1.67$^{A}$ | 81.67 ± 3.33$^{A}$ | 78.33 ± 1.67$^{A}$ | 45.00 ± 10.41$^{AB}$ | 23.33 ± 14.53$^{AB}$ | 3.55 | 0.000 1 |
| 10% PJEE | 88.33 ± 1.67$^{A}$ | 85.00 ± 5.00$^{A}$ | 85.00 ± 2.89$^{A}$ | 53.33 ± 8.82$^{A}$ | 33.33 ± 13.33$^{A}$ | | |
| 20% PJEE | 85.00 ± 5.00$^{AB}$ | 83.33 ± 4.41$^{A}$ | 75.00 ± 2.89$^{A}$ | 30.00 ± 7.64$^{BC}$ | 3.33 ± 3.33$^{B}$ | | |
| 30% PJEE | 78.33 ± 4.41$^{AB}$ | 78.33 ± 4.41$^{A}$ | 60.00 ± 2.89$^{B}$ | 13.33 ± 6.01$^{CD}$ | 3.33 ± 3.33$^{B}$ | | |
| 40% PJEE | 61.67 ± 13.02$^{B}$ | 50.00 ± 10.00$^{B}$ | 26.67 ± 6.67$^{C}$ | 0.00 ± 0.00$^{D}$ | 0.00 ± 0.00$^{B}$ | | |
| 50% PJEE | 18.33 ± 10.93$^{C}$ | 8.33 ± 6.01$^{C}$ | 1.67 ± 1.67$^{D}$ | 0.00 ± 0.00$^{D}$ | 0.00 ± 0.00$^{B}$ | | |
| *P* < | 0.000 2 | 0.000 1 | 0.000 1 | 0.000 4 | 0.058 1 | | |

Means with different superscripts are significantly different using Duncan's multiple range test at $P < 0.05$.