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Effect of tris–extender supplemented with various concentrations of strawberry (*Fragaria* spp.) on bull semen preservability

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ABSTRACT

Objective: To evaluate effect of tris-extender supplemented with various concentrations of strawberry (*Fragaria* spp.) on bull semen preservability. **Methods:** Pooled bull semen were extended with tris-citrate-fructose egg yolk diluent (control, 0% strawberry) and various concentrations of tris strawberry (TSB) (1%-6%) to achieve 60 million motile spermatozoa per milliliter. Extended semen were subjected to semen freezing protocol. Semen assessment including motility, alive%, abnormality%, intact sperm membrane (hypo-osmotic swelling test) and conception rate were carried out for both chilled and frozen semen. **Results:** Results showed that sperm motility after chilling was enhanced in groups treated with various concentrations of TSB from 1% to 5% and exhibited higher significance ($P < 0.0001$) at 6-day post-chilling. In frozen semen, 3%, 4%, 5% and 6% concentrations gave the best significance ($P < 0.0001$) on sperm motility in comparison with the control. Concentration 1% revealed the highest significance ($P < 0.0001$) on alive% as compared to the control. Hypo-osmotic swelling test was maintained as the control. Concentration 3% gave the lowest significance ($P < 0.0001$) considering abnormality%. The conception rate upon using frozen semen in insemination showed higher conception rate in concentrations of 5% and 6% in cattle. **Conclusions:** It is concluded that 1%-5% concentrations of TSB ameliorate bull semen characteristics after chilling, and 3%-6% concentrations of TSB improve bull semen characteristics after freezing. Higher conception rate exists in 5% and 6% concentration of TSB.

1. Introduction

Spermatozoa are produced by spermatogenesis process. Nowadays, semen freezing is important for maintaining the supergenetic constitutions of males, and for the use of frozen semen in artificial insemination as well as *in vitro* fertilization (IVF)[1]. Artificial insemination with frozen semen is of great importance in breeding and selection programs implicated in increasing productivity of farm animals.

The spermatozoal potency of preserved semen is affected by many factors including storage temperature, cooling regime, chemical ingredients of the diluent, cryoprotectant concentration, oxygen free radicals level, seminal plasma constituents and sanitary measures[2]. Cryopreservation of spermatozoa induces reduced semen quality, thus so many efforts are being done to improve sperm viability post-freezing. Variations in the extender and protocol used for freezing

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process differ according to species. These variations are due to their specific species and seminal plasma composition specialities[3]. Experimental trials still try to obtain the best extender for each species using different cryoprotectants. Cryoprotectants are included in the semen extender to minimize the oxidative cryo-injury resulting from sperm cooling, freezing and thawing of sperm cells and consequently enhancing semen quality and fertilizing capacity[4-6].

A new generation of semen extenders based on the presence of natural products is the target of our study to minimize the risk of contamination and improve the potential of cryopreservation.

Fruits containing natural antioxidants are more acceptable than synthetic antioxidants. Strawberry fruit is rich in natural antioxidants including phytochemicals mainly anthocyanins, flavonoids, phenolic compounds and ellagic acid, which have strong antioxidant activity[7]. Strawberry juice protects living cells from oxidative agents due to its strong antioxidant capacity[8]. The strawberry fruits contain adequate amounts of potassium, vitamin C and E, folic acid, carotenoids as well as phenolic compounds, so it has strong antioxidant capacity[9]. Natural extracts and infusions from fruits are added to semen extenders for preserving sperm cells in cattle due to its strong protective property[10].

2. Materials and methods

2.1. Fruit juice preparation

Fresh mature strawberry (*Fragaria* spp.) fruits (SB) were purchased from local market. They were well cleaned and cut to be squeezed in a blender machine with filter mesh. Stock solution of the SB juice (10%) in tris-citric acid-egg yolk-fructose was prepared. Then, the 10% juice was added to tris-strawberry (TSB) at concentrations of 1%-6%.

2.2. Semen processing

A basic control extender tris-citric acid-egg yolk-fructose (TCYF) was prepared according to Foote[11]. Semen samples were diluted in TCYF (control, 0% SB) and in the former concentrations of TSB (1%-6%) to ensure 60 million motile spermatozoa per milliliter. Extended semen was exposed to freezing program.

2.3. Semen quality assessment

Semen evaluation was carried out after thawing of bull spermatozoa. Also, sperm motility of raw semen was assessed for 2 h post-cooling and post-chilling daily up to 10 d. Frozen straws were thawed at 37 °C for 30 s. The measured parameters were motility%, alive%, abnormality% and membrane integrity using hypo-osmotic

swelling test (HOST%).

2.3.1. Sperm motility

Sperm motility was performed microscopically with closed circuit television system[12].

2.3.2. Live and abnormal spermatozoa (%)

This was assessed using eosin-Nigrosin stained smear[13].

2.3.3. Sperm membrane integrity (%)

The integrity of sperm membrane was performed using the HOST[14].

2.3.4. Conception rate

Conception rate no. of buffalo cows ($n=145$) were inseminated with TSB extended buffalo bull semen. Another no. of cows was inseminated with bull semen diluted with TCYF (control group). Pregnancy was confirmed by rectal palpation 2 months later after insemination. The inseminated cows were used via the cooperation in Beni-Suef Governorate. Conception rate was calculated according to the equation:

$$\text{Conception rate} = \frac{\text{Number of conceived cows}}{\text{Total number of inseminated cows}} \times 100$$

2.4. Statistical analysis

Data were analyzed by means of the SPSS (2005)[15] computerized program v. 14.0 to calculate the analysis of variance (ANOVA) [16] for the different parameters between control and additives replications. Significant differences between means were calculated using Duncan multiple range test at $P<0.05$.

3. Results

In chilled semen, concentrations of strawberry from 1% to 5% in tris-extender exhibited higher sperm motility% ($P<0.0001$) at 6 days post-chilling (60.00 ± 5.77 , 53.33 ± 3.33 , 58.33 ± 1.67 , 68.33 ± 4.41 and 53.33 ± 3.33 , respectively) (Table 1).

Sperm motility% of frozen semen in concentrations 3%, 4%, 5% and 6% were significantly different (40.00 ± 0.00 , 40.00 ± 0.00 , 43.33 ± 3.33 and $43.33 \pm 3.33\%$, respectively) with control (31.67 ± 1.67) ($P<0.0001$). Sperm alive% of concentration 1% was significantly different in comparison with the control (85.33 ± 0.33 and 80.33 ± 0.33 , respectively) ($P<0.0001$). HOST% was maintained as the control. Sperm abnormality% of concentration 3% gave the lowest value (20.33 ± 0.33) (Table 2).

The conception rate upon using frozen semen in insemination showed higher conception rate in concentrations of 5% and 6% TSB in cattle (Table 3).

Table 1

Sperm motility% of chilled cattle semen using different concentrations of TSB extender.

Treatment	2 h	Days					
		1	2	3	4	5	6
Control	88.33 ± 1.67 ^a	75.00 ± 5.00 ^a	73.33 ± 3.33 ^{cd}	63.33 ± 8.82 ^b	56.67 ± 6.67 ^c	53.33 ± 8.82 ^b	41.67 ± 1.67 ^c
1%	88.33 ± 1.67 ^a	80.00 ± 5.77 ^a	83.33 ± 1.67 ^{ab}	81.67 ± 1.67 ^a	73.33 ± 7.26 ^{ab}	73.33 ± 1.67 ^a	60.00 ± 5.77 ^{ab}
2%	88.33 ± 1.67 ^a	81.67 ± 1.67 ^a	68.33 ± 1.67 ^d	61.67 ± 1.67 ^b	60.00 ± 2.89 ^{bc}	55.00 ± 5.00 ^b	53.33 ± 3.33 ^b
3%	90.00 ± 0.00 ^a	91.67 ± 1.67 ^a	78.33 ± 1.67 ^{bc}	76.67 ± 1.67 ^a	73.33 ± 1.67 ^{ab}	70.00 ± 2.89 ^a	58.33 ± 1.67 ^{ab}
4%	85.00 ± 0.00 ^a	83.33 ± 1.67 ^a	83.33 ± 1.67 ^{ab}	81.67 ± 1.67 ^a	78.33 ± 1.67 ^{ab}	76.67 ± 3.33 ^a	68.33 ± 4.41 ^a
5%	86.67 ± 1.67 ^a	86.67 ± 3.33 ^a	85.00 ± 2.89 ^a	83.33 ± 3.33 ^a	81.67 ± 1.67 ^a	76.67 ± 4.41 ^a	53.33 ± 3.33 ^a
6%	88.33 ± 1.67 ^a	81.67 ± 4.41 ^a	48.33 ± 1.67 ^e	46.67 ± 3.33 ^c	41.67 ± 6.01 ^d	38.33 ± 4.41 ^c	23.33 ± 3.33 ^d
f-cal	1.27	1.98	35.17	12.07	9.48	9.21	16.53
P	0.333	0.136 9	0.000 1	0.000 1	0.000 3	0.000 3	0.000 1

Data were expressed as mean ± SE. Different superscript letters within column (a, b, ...etc) are significantly differed according to Duncan multiple range test ($P < 0.05$).

Table 2

Sperm parameters of post-thawed cattle semen using different concentrations of TSB extender.

Treatment	Motility%	Alive%	HOST%	Abnormality%
Control	31.67 ± 1.67 ^b	80.33 ± 0.33 ^b	82.00 ± 2.00 ^a	28.67 ± 1.33 ^b
1%	23.33 ± 3.33 ^c	85.33 ± 0.33 ^a	83.33 ± 1.67 ^a	31.33 ± 1.33 ^{ab}
2%	21.67 ± 1.67 ^c	79.33 ± 0.67 ^b	85.33 ± 0.33 ^a	29.67 ± 0.33 ^b
3%	40.00 ± 0.00 ^a	79.00 ± 1.00 ^b	84.33 ± 0.67 ^a	20.33 ± 0.33 ^c
4%	40.00 ± 0.00 ^a	78.33 ± 1.67 ^b	82.33 ± 2.84 ^a	30.33 ± 0.33 ^{ab}
5%	43.33 ± 3.33 ^a	71.00 ± 1.00 ^c	83.00 ± 3.00 ^a	31.33 ± 1.33 ^{ab}
6%	43.33 ± 3.33 ^a	74.00 ± 2.08 ^c	80.67 ± 3.48 ^a	33.00 ± 1.53 ^a
f-cal	15.4	15.14	0.45	15.16
P	0.000 1	0.000 1	0.830 6	0.000 1

Data were expressed as mean ± SE. Different superscript letters within column (a, b, ...etc) are significantly differed according to Duncan multiple range test ($P < 0.05$).

Table 3

Effect of addition of TSB to the TCYF on a field conception rate test in cattle.

Treatment	Conception rate (%)
Control	45.50
1%	45.83
2%	52.17
3%	40.74
4%	52.00
5%	69.23
6%	69.50

4. Discussion

Recently, scientists are interested in exploring the beneficial and the synergistic effects of the natural extracts and their multiple constituents compared to the single purified active compounds[17]. Semen cryopreservation can induce oxidative damage to spermatozoa leading to a reduction in semen quality[18], but it is important to preserve the valuable genetic constitution of our local breeds of cattle bulls.

Cryodamage induced by freezing and thawing can be minimized

by adding lipoproteins, or using the convenient cryoprotectant[19]. Semen freezing is associated with accumulation of reactive oxygen species and an alteration in the antioxidant capacity as manifested by a decrease in intracellular glutathione content that induce damage in integrity and function of spermatozoal membrane[20–22].

Seminal plasma has limited antioxidant capacity, thus the use of an extender with strong antioxidant effect is recommended to maintain the viability and subsequent fertilizing capacity of frozen spermatozoa[4]. The motility of sperm is the most important criterion used for semen assessment, both for chilled and frozen semen[23]. Concannon and Battista[24] stated that 40%-50% of sperm motility is needed for artificial insemination success. However, Linde-Forsberg and Forsberg[25] postulated that 20%-30% sperm motility is necessary for pregnancy.

In recent years, extensive researches have been conducted to investigate the effect of natural and synthetic antioxidants (herbal origins) on the viability of animal sperm during cooling and cryopreservation. Present investigation on chilled semen explored significant maintenance of sperm motility in some concentrations of strawberry up to the second, third and sixth day of chilling. This indicates that it could be used in field insemination up to these days of chilling. Frozen semen explored improvement in sperm motility post-thawing at some concentrations. The conception rate upon using frozen semen in insemination showed higher conception rate in concentrations 5% and 6%. The higher conception rates at these concentrations coincide with the higher sperm motility at these concentrations as sperm motility is the main criterion in semen evaluation[26].

Improved results in semen preservability by using strawberry juice as a cryoprotectant in semen tris-extender are mainly due to strong antioxidant properties. Strong antioxidant capacity is attributed to its high contents of vitamins, phenolic and flavonoid compounds as these components are strong antioxidants[27,28]. Canuto *et al*[29] recorded that strawberry was rich in anthocyanins that had strong antioxidant property as radical scavenger and alleviating oxidative stress and cellular damage. Main polyphenolic compounds in

strawberry responsible for antioxidation are anthocyanins[30]. The mechanisms to prevent oxidation are associated with the defense system, including antioxidant enzymes and antioxidants, which play an important role in preventing oxidative injury through elimination of the excess of oxygen free radicals that cause sperm damage[31].

Conflict of interest statement

The authors declare that they have no conflict of interest.

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