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Preservability of Buffalo semen using tris-extender enriched with different concentrations of Strawberry [*Fragaria spp.*]Juice

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Abstract: As fruit, strawberry (SB) has been proved to contain natural antioxidants which are more acceptable than synthetic ones. This study aimed to find out the effect of strawberry juice in different concentrations, when added to basic tris extender (TCYF), on preserved buffalo semen. Semen was collected from 5 mature buffalo bulls; once weekly/ 5 weeks. Semen samples were diluted in TCYF as control (0 % SB) and in the different concentrations from the 10 % stock SB juice (1 to 6%). Diluted semen was processed and stored in liquid nitrogen (LN_2). Sperm motility of the chilled buffalo semen was evaluated 2 hours after cooling and chilling up to 10 days. Frozen straws were thawed at 37°C/ 60s. The motility, alive, abnormality and membrane integrity (HOST) percentages were assessed. Moreover, conception rate data was attained from 145 artificially inseminated buffalo cows. The results revealed the maintenance of sperm motility, at SB enrichment concentrations 2 and 3%, up to the third day of chilling. Frozen semen explored improvement in sperm motility post-thawing and higher conception rate at concentrations 1 and 2%.In conclusion, addition of strawberry juice (1-3%) to basic tris extender has its beneficial effect as a natural diluent for improving semen characteristics and conception rate in buffalo.

Introduction

Semen is a male high valued product. Its preservation either by chilling or freezing to be used in artificial insemination is of great importance[1]. Sperm cryopreservation is of a great demand for conserving the super genetic constitution of males[2]. Cryoprotectants are included in the semen extender to minimize the physical and chemical stresses resulting from cooling, freezing and thawing of sperm cells and consequently improving viability and subsequent fertilizing capacity [3, 4, 5]. A new generation of semen extenders free from animal origins 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 an important source of natural antioxidants and phytochemicals mainly anthocyanins, flavonoids, phenolic compounds and ellagic acid which have strong antioxidant activity[6]. Fresh juice of strawberry detoxify living cells from oxidative agents due to its high antioxidant capacity[7]. The strawberry fruit is rich in potassium, vit. C, E, folic acid, carotenoids as well as phenolic compounds, so it has strong antioxidant capacity[8]. Natural extracts and infusions from fruits in extenders for preserving animal semen have strong protective property in preserving cattle and caprine semen[9]. According to the former information, the present study aimed to investigate the effect of the addition of different concentrations from SB juice to the basic tris extender in

maintenance of chilled and frozen buffalo semen characteristics for long preservation time to be used in an artificial insemination program.

Material and Methods

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 TCYF was prepared. Then, the stock was added to tris extender (TSB) to prepare concentrations of 1 to 6%.

Semen processing: A basic control extender (Tris-citric acid-egg yolk-fructose, TCYF) was prepared according to Foote [10]. Semen samples were diluted in TCYF (control, 0% SB) and in the former concentrations of TSB (1-6%) 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 minutes and were then dipped in LN_2.

Semen quality assessment: The assessment was undertaken on after freeze-thawing of diluted buffalo bull spermatozoa. Also, sperm motility was evaluated for raw semen, 2 hours after cooling and chilled semen daily up to 10 days. Frozen straws were thawed at 37°C/ 60 s. The parameters studied were subjective semen characteristics (motility%, alive%, abnormality% and membrane integrity (hypo-osmotic swelling test, HOST %).

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 [11].

Live and abnormal spermatozoa (%): This was evaluated using eosin-nigrosin stained smear as described by [12]. Two hundred spermatozoa were assessed.

Sperm membrane integrity (%): Sperm membrane integrity was assessed using the HOST [13]. Two hundred spermatozoa were assessed and the percentage of spermatozoa with curled tails (swollen/ intact plasma membrane) was calculated.

Statistical analysis: Statistical analysis data were analyzed by using the SAS [14] computerized program v. 9.2 to calculate the analysis of variance (ANOVA) [15] for the different parameters between control and additives replications. A significant difference between means was calculated using Duncan multiple range test at $P<0.05$.

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

$$CR = \frac{no.of\ conceived\ cows}{total\ no.of\ inseminated\ cows} \times 100$$

Results

In chilled semen (Table 1), the concentrations of 2 - 3 % TSB extender exhibited significant ($P<0.0001$) maintenance of sperm motility (40.00 ± 5.77 and 41.67 ± 1.67%, respectively) at the third day of chilling when compared to the control (0% SB) chilled diluted semen (13.33 ± 3.33%).

Table 1: Sperm motility of Chilled buffalo semen using different concentrations of TSB.

periods / treatment	2 hours	Days		
		1	2	3
Control	$90.00^{a} \pm 0.00$	$63.33^{a} \pm 3.33$	$33.33^{b} \pm 3.33$	$13.33^{c} \pm 3.33$
TSB-1%	$88.33^{a} \pm 1.67$	$73.33^{a} \pm 3.33$	$43.33^{a} \pm 3.33$	$23.33^{b} \pm 3.33$
TSB-2%	$86.67^{ab} \pm 1.67$	$73.33^{a} \pm 3.33$	$45.00^{a} \pm 2.89$	$40.00^{a} \pm 5.77$
TSB-3%	$78.33^{c} \pm 3.33$	$66.67^{a} \pm 3.33$	$45.00^{a} \pm 2.89$	$41.67^{a} \pm 1.67$
TSB-4%	$81.67^{bc} \pm 1.67$	$71.67^{a} \pm 1.67$	$46.67^{a} \pm 3.33$	$26.67^{b} \pm 3.33$
TSB-5%	$88.33^{a} \pm 1.67$	$46.67^{b} \pm 6.67$	$16.67^{c} \pm 3.33$	$6.67^{cd} \pm 3.33$
TSB-6%	$85.00^{ab} \pm 0.00$	$30.00^{c} \pm 2.89$	$0.00^{d} \pm 0.00$	$0.00^{d} \pm 0.00$
f-cal	5.46	18.92	36.98	22.13
Sig.	0.0042	0.0001	0.0001	0.0001

Different superscripts (a, b…etc.) indicate significant difference between means using Duncan multiple range test ($P<0.05$).

Frozen semen (Table 2) explored significant ($P<0.0035$) improvement in sperm motility post-thawing at concentrations of 1 and 2% (46.67 ± 6.01 and 51.67 ± 1.67%, respectively). The lowest significant ($P<0.015$) sperm abnormalities were observed in concentration 5% (24.33 ± 0.67%). Sperm membrane integrity (HOST) % was maintained in all concentrations used as compared to the control.

Table 2: Sperm parameters of post-thawed buffalo semen using different concentrations of TSB.

periods / treatment	Motility	Alive	HOST	Abnormality
Control	$30.00^{bcd} \pm 5.77$	$77.67^{a} \pm 2.67$	$76.00^{ab} \pm 1.00$	$29.67^{ab} \pm 3.18$
TSB-1%	$46.67^{a} \pm 6.01$	$69.00^{a} \pm 1.00$	$76.33^{ab} \pm 1.33$	$31.67^{a} \pm 1.67$
TSB-2%	$51.67^{a} \pm 1.67$	$70.67^{a} \pm 0.67$	$80.67^{a} \pm 0.67$	$32.00^{a} \pm 1.15$
TSB-3%	$38.33^{abc} \pm 6.01$	$71.67^{a} \pm 1.67$	$81.67^{a} \pm 1.67$	$31.67^{a} \pm 0.33$
TSB-4%	$43.33^{ab} \pm 3.33$	$78.00^{a} \pm 4.16$	$80.33^{a} \pm 0.88$	$30.67^{ab} \pm 0.67$
TSB-5%	$23.33^{d} \pm 3.33$	$74.67^{a} \pm 4.67$	$72.33^{b} \pm 2.33$	$24.33^{c} \pm 0.67$
TSB-6%	$26.67^{cd} \pm 3.33$	$71.67^{a} \pm 1.67$	$76.33^{ab} \pm 4.10$	$26.00^{bc} \pm 1.00$
f-cal	5.71	1.62	2.69	4.02
Sig.	0.0035	0.2146	0.0596	0.0150

Different superscripts (a, b…etc.) indicate significant difference between means using Duncan multiple range test ($P<0.05$).

Table 3: Effect of addition of SB to TCYF on a field conception rate test.

Treatment	Conception rate%
Control (TCFY)	25
TSB-1%	42.86
TSB-2%	66.67
TSB-3%	36.84
TSB-4%	33.33
TSB-5%	37.21
TSB-6%	25

Conception rate (Table 3) upon using frozen semen in insemination of buffalo cows showed higher conception rate in concentrations 1 and 2% (42.86 and 66.67%, respectively).

Discussion

The results of the present study in chilled semen revealed significant (P<0.0001) maintenance of sperm motility at concentrations 2 and 3% up to the third day of chilling. This indicates that it could be used in field insemination up to the third day of chilling. Frozen semen explored improvement in sperm motility post-thawing at concentrations 1 and 2%. The conception rate upon using frozen semen in insemination showed higher conception rate in concentrations 1 and 2%. The higher conception rate at these concentrations coincided with the higher sperm motility at these concentrations as sperm motility is the main criterion in semen evaluation [16].

The improved results for semen preservability by incorporation of SB juice in tris extender, as a cryoprotectant, is mainly due to its strong antioxidant properties. This strong antioxidant capacity is due to its high contents of vitamins, flavonoids and phenolic compounds as these components are strong antioxidants [17,18]. SB is rich in anthocyanins, the main poly-phenolic compounds, which have strong antioxidant property as radical scavenger and alleviating oxidative stress and cellular damage [19, 20]. 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 their abilities to scavenge free radicals that cause cellular damage [21].

In conclusion, the enrichment of basic tris extender with 1-3% SB juice has its beneficial effect in cryopreservation of buffalo semen as a natural diluent.

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