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Chilled and post-thawed semen characteristics of buffalo semen diluted in tris extender enriched with date palm pollen grains (TPG)

Reda I. El-Sheshtawy*, Walid S. El-Nattat, Samy I.A. Shalaby, Mohamed I. Shahba, Islam E. Al-Se'dawy

Animal Reproduction and AI Department, Veterinary Division, National Research Center, Dokki, Giza, Egypt

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ABSTRACT

Objective: The benefits of dates palm and their pollen grains in the human nourishment system have their consideration and importance in medical and nutritional point of view. The present study aimed to investigate a new perspective for the use of date palm pollen grains (DPPG) aqueous extract or infusion in preservation of chilled and frozen buffalo semen.

Methods: Pooled buffalo semen were diluted by Tris-Citrate-Fructose egg yolk (TCFY) diluent (considered as control) and diverse concentrations of clarified soaked pollen grains in Tris-Citrate-Fructose (TCF) diluent in concentrations of 50, 100, 150, 200 and 250 mg DPPG/5 mL TCF. Five concentrations were kept without egg yolk (TPG) while other five concentrations contained 20% egg yolk (TPGY). Each of the two dilutions was enumerated according to the concentration of DPPG. Extended semen is then chilled (for 7 d) and cryopreserved. Motility, alive sperm and intact sperm membrane (HOST) % were recorded as characteristic sperm parameters.

Results: After 7 d of chilling, significant ($P < 0.0001$) high percent of motile sperms was recorded for TPG 50, 100, 150, 200 and 250 (66.67, 65.00, 61.67, 61.67 and 63.33, respectively). After freeze thawing, TPGY 150 and 250 showed the significant ($P < 0.0001$) high percent of motile sperms (43.75 and 45.00, respectively) while TPGY 100, 200, 250 and TPG 50, 150 revealed the high results concerning HOST (80.61, 83.99, 80.61, 88.33 and 83.33, respectively) whereas the highest alive sperm percentage was recorded with TPGY 150 (96.30) compared to the control.

Conclusions: The aqueous cold infusion or extract of the DPPG, added to the TCF extender with or without the addition of egg yolk, proved its good preserving and maintaining capacity of chilled and thawed buffalo bull sperms which was expressed mainly by the sperm motility.

1. Introduction

The use of natural extract and infusions from fruits, vegetables and their seeds in extenders for preserving animal semen was introduced for their protective properties in preserving cattle

and caprine sperms [1]. This innovative technique has resolved some problems in semen cryopreservation especially the bacterial contamination of extended semen, the presence of phytohormones in these natural products that protect the spermatozoa against the phospholipase A enzyme in the ejaculate [2]. Coconut water [3,4] and tomato juice [5,6] had given good results in semen preservation.

Palm dates pollen grains extract is also one of the potent fruits that has been investigated for its reproductive impact in rabbit bucks [7]. Gu *et al.* [8], Al-Farasi *et al.* [9] and Mansouri *et al.* [10] had examined the potent antioxidant activity of the aqueous extract of dates. This activity was attributed to the wide range of phenolic compounds in dates including p-coumaric, ferulic and sinapic acids, flavonoids and procyanidins.

*Corresponding author: Reda I. El-Sheshtawy, Animal Reproduction and AI Dept., Veterinary Research Division, 13 National Research Centre, Dokki 12622, Giza, Egypt.

Tel: +202 33371635

Fax: +202 37601877

E-mail: rielsheshtawy@gmail.com

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Admission of date palm pollen grains (DPPG) suspension into the nourishment system of male increased sperm counts and their motility, consequently it improves fertility through normalization of serum testosterone [11]. Date palm pollen is used as a traditional medicine for male fertility by improving sperm count, motility, morphology and DNA quality with increase in weight of testis and epididymis. These effects are due to the increase in plasma testosterone levels as DPPG is rich in flavonoids [12].

Hence, the present study aimed to investigate the potency of different concentrations of cold aqueous infusion or extract of the DPPG, added to the Tris-citrate-fructose extender with addition of egg yolk (TPGY) or without egg yolk (TPG), in preserving and maintaining good chilled and after thawing fertile sperms.

2. Materials and methods

2.1. Preparation of different semen extenders

Tris Base Extender: Tris-citric acid-fructose without egg yolk (TCF) or with egg yolk (TCFY) diluents, were prepared according to Foote *et al.* [13]. TCFY was used as control extender.

Tris-Pollen Grain Extender (TPG): Pollen grain extract was prepared after well grinding of DPPG in a mortar to acquire a fine powder grade and sieved. 50, 100, 150, 200 and 250 mg of this powder was soaked in 2×5 test tubes each containing 5 mL of TCF diluents. All tubes were put in cooling incubator (adjusted at 10 °C) for 5 d with daily vortex and finally centrifuged to get the supernatant TPG extender. Five tubes were kept without egg yolk addition and enumerated TPG 50, TPG 100, TPG 150, TPG 200 and TPG 250, while the other five tubes had received 20% egg yolk and enumerated TPGY 50, TPGY 100, TPGY 150, TPGY 200 and TPGY 250 later after soaking and centrifugation.

2.2. Semen collection and initial evaluation

Three mature cattle bulls maintained at Artificial Insemination Center, General Organization for Veterinary Services Ministry of Agriculture, Abbasia, Egypt, were used as semen donors. Ejaculates were collected using a bovine adapted artificial vagina at weekly intervals for 3 weeks. Semen samples were initially evaluated for subjective sperm motility and sperm concentration. Ejaculates fulfilling minimum standard of sperm motility (70%) and sperm morphology 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 the water bath before dilution.

2.3. Semen processing

Semen samples were diluted with TCFY extender (control) and other aliquots of pooled semen samples were diluted with the different concentrations of TPG and TPGY extenders in order to provide a concentration of 60 million sperm/mL. Extended semen was slowly cooled to 5 °C and equilibrated for 2 h. Then, they were packed into 0.25 mL polyvinyl French straws. After equilibrium periods, the straws were horizontally placed on a rack and pre-frozen in a liquid nitrogen vapor 4 cm above liquid nitrogen surface for 10 min and were finally dipped in liquid nitrogen. A fraction of extended semen from control

and the different concentrations of TPG TPGY was chilled for 7 d and inspected for sperm motility every 24 h.

2.4. Assessment of semen quality parameters

The assessment was undertaken on cooled and after thawing of bull spermatozoa. Also, sperm motility was evaluated for raw semen, 2 h after cooling and chilled semen daily up to 7 d. Frozen straws were thawed at 37 °C/1 min. The parameters studied were subjective semen characteristics (motility, alive and hypoosmotic swelling test (HOST) %) [14].

2.5. Statistical analysis

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

3. Results

3.1. Effect of different concentrations of TCF enriched with DPPG on sperm motility of cooled and chilled buffalo bull semen

Sperm motility was significantly higher with all concentrations of DPPG used after 2 h of cooling if compared to control. Although, after 7 d of chilling, the TPG 50, 100, 150, 200 and 250 revealed a significantly ($P < 0.0001$) higher percent of motile sperms compared to the control and the other TPGY concentrations (Table 1).

3.2. Effect of different concentrations of TCF enriched with DPPG on post thawing semen characteristics

TPGY 250 [(45.00 ± 2.04)%], 150 [(43.75 ± 2.39)%] and 200 [(38.75 ± 5.15)%] showed that its post-thawing motility was apparently higher than the control TCFY (37.5 ± 4.79).

Table 1

Effect of Pollen grain addition to Tris diluent on the motility of buffalo semen after cooling and chilling.

Treatment	Motility % 2 h after cooling at 5 °C	Motility % 7 d after cooling at 10 °C
Control	78.75 ± 5.54 ^B	41.67 ± 1.67 ^B
50 mg without egg	83.75 ± 1.25 ^{AB}	66.67 ± 1.67 ^A
100 mg without egg	83.75 ± 2.39 ^{AB}	65.00 ± 2.89 ^A
150 mg without egg	91.25 ± 1.25 ^A	61.67 ± 1.67 ^A
200 mg without egg	83.75 ± 2.39 ^{AB}	61.67 ± 1.67 ^A
250 mg without egg	90.00 ± 2.04 ^A	63.33 ± 1.67 ^A
50 mg with egg	87.50 ± 2.50 ^A	8.33 ± 1.67 ^C
100 mg with egg	85.00 ± 2.04 ^{AB}	3.33 ± 1.67 ^D
150 mg with egg	91.25 ± 1.25 ^A	0.00 ± 0.00 ^D
200 mg with egg	90.00 ± 0.00 ^A	0.00 ± 0.00 ^D
250 mg with egg	91.25 ± 1.25 ^A	0.00 ± 0.00 ^D
F-value	3.07	377.98
P	<0.007	<0.0001

Dissimilar superscript (A, B, C and D) are significantly different within column via multiple range Duncan test at $P < 0.05$.

Table 2

Effect of Pollen grain addition to Tris diluent on the post-thawing buffalo semen characters.

Treatment	Post-thawing sperm motility %	Hypo-osmotic swelling test (HOST) %	Alive sperm %
Control	37.50 ± 4.79 ^{AB}	74.38 ± 2.37 ^C	91.94 ± 1.94 ^B
50 mg without egg	25.00 ± 6.12 ^{CD}	88.33 ± 1.67 ^A	75.93 ± 0.93 ^D
100 mg without egg	22.50 ± 2.50 ^D	69.47 ± 0.53 ^D	91.31 ± 1.31 ^B
150 mg without egg	30.00 ± 0.00 ^{BCD}	83.33 ± 1.67 ^B	69.90 ± 0.10 ^E
200 mg without egg	6.25 ± 1.25 ^F	56.67 ± 1.67 ^E	90.90 ± 0.90 ^B
250 mg without egg	10.00 ± 3.54 ^{EF}	24.44 ± 0.55 ^F	60.00 ± 2.89 ^F
50 mg with egg	20.00 ± 4.08 ^{DE}	69.58 ± 0.42 ^D	91.06 ± 1.06 ^B
100 mg with egg	33.75 ± 2.39 ^{BC}	80.61 ± 0.61 ^B	90.30 ± 0.30 ^B
150 mg with egg	43.75 ± 2.39 ^A	75.64 ± 0.64 ^C	96.30 ± 1.30 ^A
200 mg with egg	38.75 ± 5.15 ^{AB}	83.99 ± 2.07 ^B	91.75 ± 1.75 ^B
250 mg with egg	45.00 ± 2.04 ^A	80.61 ± 0.61 ^B	80.54 ± 0.54 ^C
F-value	13.23	175.48	66.31
P	<0.0001	<0.0001	<0.0001

Dissimilar superscript (A–F) are significantly different within column via multiple range Duncan test at $P < 0.05$.

Concerning the intact sperm membrane test (HOST%), TPG 50 (88.33 ± 1.67) and TPG 150 (83.33 ± 1.67) and TPGY 100 (80.61 ± 0.61), 200 (83.99 ± 2.07), and 250 (80.61 ± 0.61) showed higher significant ($P < 0.0001$) difference than control (74.38 ± 2.37). Regarding the alive sperm percentage, TPGY 150 revealed higher significant ($P < 0.0001$) difference than the control TCFY (91.94 ± 1.94) and other TPG and TPGY extended semen (Table 2).

4. Discussion

Cryopreservation of bovine semen often induce an additional source for reactive oxygen (ROS) attack on sperm due to decreased activities of antioxidant enzymes and the sperm membrane become more susceptible to lipid peroxidation [16] which affect the membrane permeability [17]. Natural antioxidants exert a protective effect preserving the metabolic activity and cellular viability of cryopreserved bovine spermatozoa [18]. The results of the present study revealed good sperm motility after 7 d of chilling especially TPG 50, 100, 150, 200 and 250; so, it can be used in artificial insemination (AI) up to 7 d of chilling and pollen can be used as an alternative of egg yolk in chilled buffalo semen to be protected against cold shock. These results were slightly differed from those obtained in cattle using the same concentrations and treatments with DPPG where TPGY 150 was the best enriched extender concentration (42.50%) after 7 d of chilling [19]. Post thawing semen characteristics were superior with TPGY 150 and 250 (for sperm motility %) although in cattle bull there were no significant difference between TPG 50 (43.75%), TPGY 250 (42.50%) and the control (41.25%) [19], TPGY 100, 200, 250 and TPG 50, 150 (for intact spermatozoal membrane, HOST%) although in cattle bull there was fluctuations that agreed with these results [19] and TPGY 150 (for alive %) this disagreed with the cattle results [19]. Hence, the present results could be attributed to the interaction between the species and the antioxidant effect of DPPG through reduction of ROS level exerted by its high

content of flavonoids [11,12,20]. Flavonoids are excellent scavengers of free radicals and the number of hydroxyl group on the phenyl ring seems to enhance the antioxidant capacity of polyphenolic molecule [21,22]. On the other hand, the buffalo sperm may benefit from some components in the DPPG infusion that may interact with the outer sheath of the sperm to protect itself against cold thence it may survive in low temperature.

The antioxidant effect of DPPG is due to its high concentration of vitamin C, B (Thiamine), B₂ (Riboflavin), nicotinic acid (Niacin) and vitamin A [23,24]. DPPG improved semen preservability through reduction of bacterial growth in the semen extender that used egg yolk as anti-cold shock [25]. In conclusion, the cold aqueous extract of the DPPG, added to the Tris-citrate-fructose extender with and without the addition of egg yolk, proved its good preserving and maintaining capacity of chilled (TPG 50 to TPG 150) and after thawing (TPGY 250 and TPGY 150) fertile buffalo bull sperms which was expressed mainly by the sperm motility.

Conflict of interest statement

We declare that we have no conflict of interest.

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