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The effect of Eggplant peel extract addition (*Solanummelongena*) on Farahani ram sperm after oxidative stress (freeze-thawing)

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Abstract

The aim of this study was to evaluate the effect of Eggplant peel extract on post-thawed ram sperm quality in a Tris-based extender. Semen was collected by artificial insemination vagina. Samples were pooled to prevent individual effects. Then ram semen samples were obtained, extended with Tris-based extender and supplemented with 0%, 2%, 4%, 6%, and 8% Eggplant peel extract. Later, samples were frozen by liquid nitrogen in the straw (0.25 ml). After thawing, sperm motility, viability (Nigrosine–eosin staining), membrane integrity with Hypo osmotic (Host) and morphology abnormality (Hancock test) were evaluated. Results showed that the value of 2% (62.4) of the Eggplant extract peel had a significant effect on sperm motility and membrane integrity after thawing. Also, the 2% and 4% of Eggplant peel extract groups had the most significant motility as compared to the control group and, the least motility was regarded as the most concentration of 8% of the Eggplant peel extract as compared to the control group. Thus, adding 2% and 4% of the Eggplant peel extract to Tris-based extender preserved Farahani ram sperm after thawing.

Keywords: Eggplant peel, extract, Semen

Introduction

Sperm preservation is a crucial factor for the success of fertility in livestock (Ros-Santaella and Pintus, 2021). Ruminant sperm is vulnerable to free radicals caused by oxidative stress. The membranes of ruminant sperm cells contain large amounts of unsaturated fatty acids, which make these cells sensitive to the attack of free radicals. Freezing and thawing of sperms is an effective method to curb the production of free radicals in spermatozoa. Sperm motility has been reported to be about 60% 63% (Hiemstra et al, 2005; Saha et al, 2022) after thawing in cattle, ram and poultry. Various plant products contain antioxidant compounds such as tannins, flavonoids, curcumanoids, coumarins, lignans, terpenoids and phenolics (Jeong et al, 2004). Several studies have shown that during the freeze-thaw process of sperm, the use of natural antioxidants had positive effects on sperm parameters (Zanganeh et al, 2013; Khodaei-Motlagh et al, 2014; DaghighKia et al, 2016). Eggplant has phenolic compounds as free radical scavengers (Cao et al, 1996). Research

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shows that Eggplant contains about 10 types of superoxide coatings (SOS) (Hanson et al, 2006). Therefore the use of Eggplant extract in rats increased sperm concentration in the epididymis of the treated groups due to the antioxidant and protective potential of Eggplant extract against free radicals (Tiwari et al, 2009). The antioxidant properties of Eggplant extract show its activity in the face of elevated oxidative stress, for boosting sperm production by the secretion of more testosterone (Saalu et al, 2007).

Eggplant peel contains large amounts of antioxidants such as nasunin. This purple pigment belongs to the family of flavonoids and has strong antioxidant properties (Babu and Venkatesh, 2009). In the same line, this study was designed to investigate the effect of Eggplant peel extract on qualitative parameters of Farahani ram sperm after freezing.

Materials and Methods

Location, Animals and Semen collection

This research was carried out on the farm of Arak University with geographical characteristics of 341864760 (latitude), 496426590 (longitude) and 168722 (altitude) at breeding season (Autumn).

In this experiment, five fertile rams of Farahani breed (live weight 60.5 kg ±0.4 kg, and 3.5 years old) were used. Semen was obtained twice a week by an artificial vagina (42-43 °C). Then, rams were fed with one of three iso-nitrogenous and iso-energetic diets. An oestrus ewe was used to stimulate rams for optimal jumping and ejaculation. After, the semen samples were immediately transferred to the laboratory. Semen was evaluated and samples with volume >0.75 mL, > 80% progressive motility and > 3 × 10^9 sperm/ mL (concentration) were selected for examination. Finality, semen samples were pooled to eliminate individual differences between rams.

Preparation of Eggplant peel extract

For the preparation of the Eggplant peel extract, collected Eggplant peel was dried at room temperature for 15 days. In brief, dried plants of Eggplant peel (50 g) were powdered, soaked in 400 mL of 70% ethanol for 24 hours and the mixture was filtered by Whatman (No.1 paper). Soxhlet system was used for the extraction of Eggplant peel extract. Ethanol was evaporated from the extract on a rotary evaporator at 50 °C. The extract was then maintained at 4 °Cuntilused (Shahmohammadi et al, 2014).

Extender preparation and cryopreservation

The Tris-based extender was composed of Tris, 3.634 g (hydroxymethyl-aminoethane, Merck 64271, Germany), fructose, 0.5g (Germany) (v/v), glycerol (5%) (w/v), citric acid, 1.99g (Merck Germany) (w/v), egg yolk (15%) (w/v), and penicillin (100,000 units internationally) in 100 ml distilled water used to reach the desired volume (Evans and Maxwell, 1987). Experimental treatments included five extenders supplemented with different levels (0, 2, 4, 6 and 8%) of Eggplant peel extract. The diluted semen was gradually cooled to 4 °C for two hours and was subsequently aspirated into 0.25 mL French straws (IMV, L'Aigle, France) (4 ×10^8 spermatozoa/ml), sealed with polyvinyl alcohol powder and balanced at 4 °C for one hour. Then, the straws were exposed to liquid nitrogen (LN) vapor (4-5 cm above the LN), for 10 min, plunged into the LN and stored until thawed and then used for the assessment of sperms. The thawing point was 37 ° C for duration of 45 seconds, and the frozen samples were capped in a water bath for 30 min for the process evaluation design and dissemination of the results.

Semen evaluation

Motility

The sperm parameters were evaluated at 0 and 1 h after thawing using computer-assisted semen analysis (CASA- using the Hamilton-Thorne motility analyzer). The variables analyzed included total motility (TM) and progressive motility (PM). At least 200 spermatozoa were analyzed for each evaluation (Bucak et al, 2010).

Membrane integrity

Sperm membrane integrity was evaluated using the hypo-osmotic swelling test (HOST). Initially, 30 µL of thawed diluted semen was added to 300 µL of a 100mOsM hypo-osmotic solution (4.9 g sodium citrate+ 9 g fructose per liter of double-distilled water) in microtubes and incubated at 37 °C for 30 min. Following incubation, 10 µL of the mixture was placed on a warmed glass slide (37 °C) under a cover glass and two hundred sperms were counted (CKX41; Olympus, Tokyo, Japan) at ×400 magnification and then the percentage of sperms with swollen curved and tails was recorded (Jeyendran et al, 1992 and Garcia-Artiga, 1994).

Sperm abnormality

Hancock solution (62.5 mL formalin (37%), 150 mL sodium saline solution, 150 mL buffer solution, and 500 mL bi-distilled water) were applied to assess spermatozoa with abnormal morphology of semen samples (Rasul et al, 2001) Three drops of each semen were added to eppendorf tubes containing one mL of Hancock solution. A drop of spermatozoa was placed on a slide and evaluated for sperm abnormalities under microscope at 40× magnification.

Sperm viability

Sperm viability (live/dead, %) was examined using eosin-nigrosin (nigrosin 10 g, eosin-Y 1.67 g, and sodium citrate 2.9 g, dissolved in 100 mL distilled water) staining method. The sperm suspension smears were prepared by mixing 10 µL of sperm sample with 20 µL of eosin-nigrosin stain on a warm slide and immediately spreading the suspension (< 30s). After air drying, viable and nonviable sperms were assessed by counting 200 spermatozoa at the magnification of 400× using phase-contrast microscopy (CKX41; Olympus, Tokyo, Japan) (Evans et al, 1987).

Statistical analysis

All the data in this experiment were analyzed in a complete randomized design. All treatments were replicated five times. The normality of all the variables was tested with PROC UNIVARIATE on the residuals obtained from PROC MIXED using different times and its interaction with fixed effects. Repeated-measures ANOVA were performed on all the variables in PROC MIXED. The data were analyzed by MIXED procedure of SAS version SAS version 9.1 (SAS, 2004). $P < 0.05$ was considered the significance level. The POLYANOVA model (linear, quadratic, and cubic) was employed for data due to having more comprehensive and informative results. The results were expressed in least squares means ± standard error of the mean.

Results

The effects of Eggplant peel extract on total and progressive motility parameters of frozen–thawed ram semen are presented in Table 1. Samples cryopreserved in 2 and 4% Eggplant peel extract had higher percent total (62.4% and 58.4%) and progressive (53.4%and 49.2%) motility compared to the other groups ($P<0.01$).

Table 1 shows the results of different concentrations of Eggplant peel extract supplementation on membrane integrity, viability and abnormality parameters of frozen-thawed ram spermatozoa. The number of viable sperm increased ($P<0.01$) in the extender containing 2% Eggplant peel extract (57.6%) compared to 6% and 8% extract groups (38.8% and 37.4%, respectively). The highest amount of spermatozoa plasma membrane integrity

(60.4%) was observed in 2% Eggplant peel extract treatments (P<0.01).

Increase in the level of Eggplant peel extract resulted in a clear decline in sperm normality (P<0.01) and the highest amount of abnormality (17.2%) was observed in 8% Eggplant peel extract treatments (P<0.01).

The POLYANOVA model evaluation of the data in the current study showed that adding 2% Eggplant peel extract enhance, sperm quality; however, adding incremental levels of Eggplant peel extract linearly reduced sperm quality in the current experiment.

Table 1. The effect of different concentrations of Eggplant (*Solanummelongena*) peel extract on post-thaw sperm motility, viability, membrane integrity, progressive and morphology of frozen-thawed Farahani ram spermatozoa

Treatment (%)	Post-thawing				
	Motility	Viability	Membrane integrity	Progressive	Sperm abnormality
0	57.0±1.58[a]	52.8±6.72[a]	44.8±3.03[b]	49.0±3.16[a]	29.4±1.67[a]
2	62.4±5.72[a]	57.6±2.30[a]	60.4±3.20[a]	53.4±4.82[a]	24.0±2.12[b]
4	58.4±4.27[a]	42.2±1.92[b]	47.0±3.08[b]	49.2±5.31[a]	23.4±1.51[b]
6	40.6±14.79[b]	38.6±8.14[b]	36.6±7.89[c]	29.8±15.05[b]	19.2±3.56[c]
8	30.2±7.59[b]	37.4±1.67[b]	31.8±1.78[c]	19.2±5.11[c]	17.2±3.34[c]
Statistical comparisons					
Linear	0.01	0.02	0.01	0.01	0.01
Quadratidc	0.19	0.45	0.23	0.08	0.12
Qubic	0.65	0.78	0.10	0.14	0.38

[a,b,c] Different letters indicate mean values at each time of storage within post-thawing analysis (P < 0.05).

Discussion

This study was carried out for the first time to evaluate the effect of different concentration of Eggplant peel extract in semen extender containing Tris on post-thawed ram sperm quality. Freezing improved lipid peroxidation due to the curbing of the production of ROS which adversely influences the intercellular organelles (Maxwell and Watson 1996). Therefore, supplementation of extender may mitigate these damaging effects and provide a suitable protection for sperm. The percentage of motility for the groups that used the doses of 2 and 4% Eggplant peel extract in this study was significantly higher than that the control groups. Sperm survival and motility decreased significantly in the post-thawed process. The quality of frozen sperm can be improved by adding antioxidants (Hsieh et al, 2000). A little amount of reactive oxygen species (ROS) is produced by sperm under physiological conditions as required for sperm capacity and acrosomal reaction, but large amounts of ROS are negatively related to sperm motility and number (Agarwal et al, 2006). In our study, the concentrations of 0% (control), 2% and 4% of Eggplant peel extract preserved sperm motility (57%, 62.4% and 58.4%, respectively) post-thawing. The antioxidant effects of Eggplant peel are not limited to its phenolic and anthocyanin compounds, and due to its vitamin C content, it is also important in reproduction (EunJu et al, 2011; Kadivec et al, 2015). This finding is in agreement with previous studies, which suggested the maintenance of motility during cryopreservation using antioxidants. In earlier studies, it was shown that Solanum melon fruits extract has an effect on the mitochondria found in the body of the spermatozoon where energy is synthesized in the form of adenosine triphosphate, which eventually increases sperm motility (Adelakun et al, 2020).

Our results are in line with the findings of Modaresi and Khodadadi, (2014) who found higher production sperm when the Aloe vera extract (contains vitamins C, A

and B) was added to treatments. Also, the use of thistle extract (like Eggplant) in rams increased sperm viability (Kistanova, 2005). Antioxidants in Eggplant peel extract (such as Nasonin) probably affected sperm progressive and improved membrane health and survival in 2% and 4% Eggplant peel extract compared to groups 6 and 8%.

Stresses due to the freeze-thaw process can compromise the health of plasma and acrosomal membranes, motility, fertility and the number of live sperm (Leboeuf et al, 2000). Freezing protection leads to changes in the DNA and cytoskeleton of sperm, which reduces its motility. Eggplant extract has an effect on sperm mitochondria (Duke, 1997). Ascorbic acid in the Eggplant extract is the first antioxidant defense line of plasma sperm and inhibits the oxidation of lipoproteins (Pursel and Graham, 1967).

In the present study, the highest concentration of ascorbic acid (8% Eggplant peel extract) significantly reduced total and progressive motility. It can be inferred that the excessive addition of ascorbic acid, like other antioxidants, to the diluent alters the properties of the plasma membrane, allowing lipoperoxidation to affect the membrane fluidity and the production of free radicals (Naijian et al, 2013). Adding high levels of the extract reduces sperm function by inhibiting the activity of enzymes involved in oxidation and reduction and upsetting the balance between antioxidant capacity and the production of free radicals (Roca et al, 2004).

The integrity of sperm membrane was significantly preserved using 2% of Eggplant peel extract (Table 1). Increased free radicals in semen caused lipid peroxidation and damage to the sperm membrane and its acrosome (Hidiroglou and Knipfel, 1984).

The use of Eggplant extract significantly increased the number of rat sperm (Tiwari et al, 2009). Sperm abnormalities were reported to be significant in this study. Free radicals in semen alter sperm motility and morphology (Agarwal et al, 2006).

The freeze-thaw process causes changes in the morphology of the sperm, which in turn cause damage to the acrosome membrane and the mitochondria of the sperm. This causes only a small percentage of sperm to have a healthy membrane and normal mitochondrial activity after cryopreservation, resulting in fewer sperm counts after cryopreservation (Holt and North, 1994). Addition of aqueous extract of rosemary containing polyphenols to goat semen extender improved the parameters of motility, viability, plasma membrane integrity and reduced the number of morphological abnormalities of sperm. Furthermore, it protected goat sperms from free radical damage (Zanganeh et al, 2013).

Many plant extracts contain biochemical compounds such as flavonoids and phenolic compounds that have antioxidant properties and prevent cellular damage caused by free radicals (Sefidkon and Jamzad, 2005). The thawing process can damage the cytoplasm of the cell, the cytoplasmic membrane, and the structure of the DNA, leading to impaired motility, sperm survival, and infertility (Leboeuf et al, 2000). Oxidative stress can be reduced by antioxidants containing phytonutrients such as flavonoids, anthocyanins and phenolic compounds (Babu and Venkatesh, 2009).

The findings of this study showed that the adding of Eggplant peel extract did not change compared to the control treatment in Farahani ram semen. Adding incremental levels of 6 and 8% Eggplant peel extract was shown to have negative effects on sperm quality characters.

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Conflict of interest

The authors declared no conflict of interest.

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تأثیر افزودن عصاره پوست بادمجان (Solanummelongena) بر اسپرم قوچ فراهانی پس از تنش اکسیداتیو (انجماد- ذوب)

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چکیده

هدف از این مطالعه بررسی اثر عصاره پوست بادمجان بر کیفیت اسپرم قوچ پس از ذوب در رقیق‌کننده بر پایه تریس بود. مایع منی توسط واژن مصنوعی جمع‌آوری شد. نمونه‌ها برای جلوگیری از اثرات فردی باهم مخلوط شدند. سپس نمونه‌های مایع منی قوچ با بر پایه تریس رقیق شد و با عصاره پوست بادمجان ۰، ۲، ۴، ۶ و ۸ درصد تکمیل شد. سپس نمونه‌ها توسط نیتروژن مایع در پایوت (۰/۲۵ میلی‌لیتر) منجمد شدند. پس از ذوب، تحرک اسپرم، زنده ماندن (رنگ‌آمیزی نیگروزین-ائوزین)، یکپارچگی غشاء با محلول هیپواسموتیک (Host) و ناهنجاری مورفولوژی (تست هانکوک) مورد بررسی قرار گرفت. نتایج نشان داد که مقدار ۲ درصد (۶۲/۴) پوست عصاره بادمجان تأثیر معنی‌داری بر تحرک اسپرم و یکپارچگی غشاء پس از ذوب داشت. همچنین گروه‌های ۲ و ۴ درصد عصاره پوست بادمجان دارای بیشترین تحرک نسبت به گروه کنترل و کمترین تحرک در غلظت ۸ درصد عصاره پوست بادمجان در مقایسه با گروه شاهد بود. بنابراین، افزودن ۲ و ۴ درصد عصاره پوست بادمجان به رقیق‌کننده بر پایه تریس سبب حفظ اسپرم قوچ فراهانی پس از ذوب شد.

کلمات کلیدی: پوست بادمجان، عصاره، مایع منی

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