Research Article

Evaluation of Adding Prickly Pear Extracts to the Diluted Ram's Semen at Preservation

Hasanain Jihad Neamah

Middle Technical University – Al-Kut Technical Institute, Iraq.

Abstract | It is known that ram's sperms are sensitive to oxidative stress during cooling storage. The purpose of this study was the evaluation of adding an aqueous prickly pear extract (PPE) in ram semen on sperm parameters during cooling storage. Three levels of PPE were added to the diluted ram semen (PPE1=5mg +Tris extender, PPE2=10 mg +Tris extender, and PPE3=30 mg +Tris extender), and compared with control group (Tris extender without addition of PPE). Motility of sperm, hypo osmotic swelling test (HOS-Test), and dead sperm percentage were assessed after 2 and 48 hour at preservation. The PPE2 group exhibited the significantly ($p \leq 0.05$) enhanced sperm motility at 2 and 48 hour of cooling. A non-significant ($p > 0.05$) increase in HOS-test percentage for all groups after 2 hours of cooling was observed. The PPE1 and PPE3 groups recorded the lowest percentage of HOS-test after 48 hours, however, groups PPE2 and the control recorded the significant ($p \leq 0.05$) excess in this characteristic. The sperm dead percentage were reduced by adding 10 mg of prickly pear extract (PPE2) in comparison with other groups at 2 hours post-cooling. The aqueous prickly pear extract has the potential to be used as an additive in ram semen to improve sperm motility, plasma membrane integrity, and dead sperm percentage at cooling preservation.

Keywords | Prickly pear extract, Dead sperm, HOS-Test, Ram semen, Sperm motility.

Received | December 17, 2022; **Accepted** | January 25, 2023; **Published** | March 25, 2023
***Correspondence** | Hasanain Jihad Neamah, Middle Technical University – Al-Kut Technical Institute, Iraq; **Email:** Hassanain.alkenani@mtu.edu.iq
Citation | Neamah HJ (2023). Evaluation of adding prickly pear extracts to the diluted ram's semen at preservation. J. Anim. Health Prod. 11(1): 94-98.
DOI | http://dx.doi.org/10.17582/journal.jahp/2023/11.1.94.98
ISSN | 2308-2801

INTRODUCTION

The sperm are sensitive to osmotic pressure, acidosis, loss of energy, denaturation of proteins, and reactive"oxygen species (ROS) (Haris et al., 2020). The ROS is important factor of sperm cells damage (Bernard and Krause, 2007). The accumulation of ROS leads to oxidative stress and cell death. To avert oxidative damage, the sperm cells have a natural protection regulation (antioxidants); these antioxidants are found normally in all biological environments (Halliwell and Gutteridge, 1999). The mitochondria dysfunction lead to increase ROS level so the enzymatic and non-enzymatic processes in cells fail to counteract the oxidation (Castellini et al., 2021). The ROS effects increase when its' production is high than the antioxidant activity (Pinto et al., 2020). To decrease the preservation effect on sperm cells, the extenders which are added to semen contain preventive components that enable the sperm to survive a long time (Bustani and Baiee, 2021).

The sperm cells have high concentrations of polyunsaturated fatty acids which"increase peroxidation of lipids in cells by increasing free radicals. This process leads to loss of membrane integrity, making sperm less able for fertilization (Van Tran et al., 2017). The studies showed a significant effect on semen quality by adding different types of antioxidant such as adding zinc oxide (Neamah et al., 2022), the *Opuntia ficus-indica* extracts (Banana et al., 2021), and astaxanthin and taurine (Neamah and Houbi, 2020). Kuti, (2004) referred that the cactus pear has effective compounds such as flavonoids, dihydroflavonol, and flavones, in addition, cactus contains natural antioxi-

dants including carotenoids, polyphenolic compounds, vitamins, chelators, and enzymes (Fernández-López et al., 2010). Hence it was hypothesized that addition of extract of prickly pear (fruit of cactus) may safeguard the spermatozoa due to presence of rich antioxidants. The study object was to investigate the prickly pear extract's effect on ram semen characteristics at post cooling phase.

MATERIALS AND METHODS

This study was conducted at Al-Kut Technical Institute, Microbiology Laboratory. The semen was collected from four Awassi rams (3-3.5 years old). The semen was collected from the rams three times a week, and then the fresh semen was divided into four equal parts. One was served as control (only Tris extender (Sigma chemical Co., Germany) without addition of prickly pear extract (PPE)), and other three were treatments: PPE1 treatment (5 mg PPE and Tris extender), PPE2 (10 mg PPE and Tris extender), and PPE3 (30 mg PPE and Tris extender). All groups were stored in cool temperature (5 °C). The sperm motility, hypo-osmotic swelling test (to know the integrity of the sperm's plasma membrane), and dead sperm percentage were estimated at 2, and 48 hours post-cooling.

The PPE was prepared depending on a method that described by Sakai et al. (1986). In the beginning, the cladodes of the prickly pear were dried at 60 °C, and then grinded to small particles. The powder was stored at -18 °C. A 100 grams of prickly pear powder was placed in 1000 ml volumetric flask with a distilled water, the final solution placed in the magnetic stirrer overnight, and then the filtrations were completed by using Whatman's filter paper (no 1). It was centrifuged (30 rpm/ minutes), and the supernatant was placed in the oven at 40 °C, and then the dried powder was stored at 5 °C until used.

The sperm motility was evaluated according to Walton, (1933) depending on microscope assessment. The HOS-test was carried out depending on method described by Jeyendran et al. (1984) with some modifications by adding 180 µl warm hypo-osmotic solution (150 mOsm / L) to 20 µl diluted semen, the solution kept at 37 °C (water bath) for 30 min. Two drops of the solution were put on a warm slide and covered by a coverslip, and the observations were done with a 40x magnification to observe the coiling sperm tail and sperm head swollen. The results of HOS-test were calculated based on a percentage of the intact of plasma membrane.

The dead sperm percentages were estimated according to Evans and Maxwell, (1987) with some modifications. The staining solutions were prepared by adding eosin Y (1.67 g) (HIMIDIA, India) and nigrosin (10 g) (HIMIDIA, India) to distilled water (100 ml). The staining procedure was done by adding the staining solution (eosin Y and nigrosin solution) (30µl) to the equal volume of diluted semen. Then the smear was made on a clean glass slide to observe under a light microscope (Hancock, 1951).

The data were estimated using SPSS software (SPSS/version 26). The values are listed as mean and standard error. The statistical value between all treatments was counted by a one-way ANOVA test. The statistical model was:

"Yij = µ + Ai + eij"

Where:

Yij = Observations,

µ = means,

Ai = the treatments effects,

eij = Error term.

For the comparison among the different periods (2 and 48h) within each group the following model was used:

"Yij = µ + Pi + eij"

where

Pi= Periods effect.

RESULTS

A significant (p≤0.05) increase in sperm motility was noticed by adding 10 and 5 mg prickly pear extract (PPE2 and PPE1 groups) respectively after two hours of cooling storage (Table 1). Further, a non-significant variation among the PPE3 group (30 mg PPE) and the control group was observed. The results revealed significant (p≤ 0.05) impact on sperm cells motility after 48 hours post-cooling, where the PPE2 group recorded the highest percentage of sperm motility compared with other groups. The groups PPE1 and the control showed increasing in sperm motility than the PPE3 group (Table 1). While a significant (p≤ 0.05) variation was observed between 2 and 48 hours post-cooling periods for all groups.

The HOS-test percentages were reported in Table (2). There were non-significant (p>0.05) effects of adding several concentrations of PPE as comparison with the control group at 2 hours post-cooling. The results in the table (2) noticed no significant (p>0.05) variation between PPE2 and the control group at 48 hours post-cooling, whilst, on the other hand, groups PPE1 and PPE3 recorded (p<0.05) the lowest percentages of HOS-Test as compared to other groups. The results in Table (2) refer to a significance (p≤0.05) variation between 2 and 48 hours periods for all groups.

The dead sperm percentage results were shown in Table (3). After two hours of cooling, the dead sperm decreased significantly (p≤0.05) in group 2 (10 mg PPE) as compared to all other groups, while non-significant variations

Table 1: The impact of adding prickly pear extract on the sperms motility percentage of ram semen preserved for different cooling periods. Mean ± Standard error

Significance	After 48 h	After 2 h	treatments
*	50.00 ± 1.65$^{b\,B}$	85.00 ± 0.55^{abcA}	Control
*	51.25 ± 1.25$^{b\,B}$	86.00 ± 0.6$^{ab\,A}$	PPE 1
*	60.00 ± 0.28$^{a\,B}$	88.00 ± 0.4$^{a\,A}$	PPE 2
*	44.00 ± 1.25$^{c\,B}$	81.00 ± 0.35$^{c\,A}$	PPE 3
	*	*	Significance

The different small letters in the same column refer to significant variations within groups, and capital letters within each row indicate comparison among periods within each group. PPE 1: 5 mg prickly pear extract; PPE 2: 10 mg prickly pear extract; PPE 3: 30 mg prickly pear extract.*: p≤0.05.

Table 2: Effect of adding prickly pear extract on hypo-osmotic swelling test of ram semen preserved for different cooling periods . Mean ± Standard error

Significance	After 48 h	After 2 h	treatments
*	42.00 ± 1.00$^{a\,B}$	76.00±1.2$^{a\,A}$	Control
*	37.00 ± 3.00$^{b\,B}$	75.14 ±1.4$^{a\,A}$	PPE 1
*	42.30 ± 1.00$^{a\,B}$	76.00±0.9$^{a\,A}$	PPE 2
*	35.50 ± 0.50$^{b\,B}$	75.03±1.00$^{a\,A}$	PPE 3
*	*	N.S	Significance

The different small letters within same column refer to significant variations within groups, and capital letters within each row indicate comparison among periods within each group. PPE 1: 5 mg prickly pear extract; PPE 2: 10 mg prickly pear extract; PPE 3: 30 mg prickly pear extract.*: p≤0.05. N.S.: non-significant.

Table 3: Effect of adding prickly pear extract on dead sperm percentage of ram semen preserved for different cooling periods. Mean ± Standard error

Significance	After 48 h	After 2 h	treatments
*	55.00 ± 0.3$^{b\,B}$	17.00 ± 1.03$^{a\,A}$	Control
*	55.02 ± 0.3$^{b\,B}$	17.00 ± 1.08$^{a\,A}$	PPE 1
*	75.11± 0.2$^{a\,B}$	14.00 ± 0.47$^{b\,A}$	PPE 2
*	74.23 ± 0.2$^{a\,B}$	18.00 ± 0.47$^{a\,A}$	PPE 3
	*	*	Significance

The different small letters in the same column refer to significant variations within groups, and capital letters within each row indicate comparison among periods within each group. PPE 1: 5 mg prickly pear extract; PPE 2: 10 mg prickly pear extract; PPE 3: 30 mg prickly pear extract.*: p≤0.05.

among the control, PPE1, and PPE3 was observed at the same time.

There was a reduction ($p \leq 0.05$) in the dead sperm percentage in control and PPE1 as compared to other both groups (Table 3). There was a significant ($p \leq 0.05$) increase in dead sperm percentage in each group at 48 hours cooling as compared to 2 hours.

DISCUSSION

Adding prickly pear extract in the semen improved sperm motility at cooling. This finding is supported by the already published literature, where Allai et al. (2017) reported the significant enhancement of ram semen characteristics during storage at 5 °C by adding prickly pear essential oil and yolk- skim milk-based extenders. In this study, the sperm cells motility increased by adding 10 mg of prickly pear extract at cooling for 2 and 48 h, while it was significantly reduced while used 30mg PPE. These results supported with findings of Allai et al. (2016), who reported that the high concentrations of prickly pear may decrease sperm motility.

Other plant-based antioxidants were also been reported in the literature to improve sperm characteristics. The addition of 45 and 30 ppm of *Capparis spinosa* improved sperm cells motility, viability, and reduction of DNA"fragmentation in diluted human semen at 37 °C for 24 hours (Khojasteh et al., 2021). Another study proved that adding 2 to 10 mg/L *Rhodiola rosea* extract improved HOS-Test of diluted boar semen post-cryopreservation (Zhao et al., 2009).

In our study, there was a non-significant variation of HOS-Test after 2 hours of cooling between all groups, and there were non-significant differences showed between adding 10 mg prickly pear extract and the control group after 48 hours, while the negative effect on the sperm cells was recorded by adding 5 and 30 mg PPE. Similar to these observations, Hu et al. (2009) reported that adding 0.5 mg of *Gynostemma pentaphyllum* extract to the diluted boar semen enhanced motility, acrosome and plasma membrane integrity, mitochondrial activity, and sodium oxide dismutase (SOD) post-cryopreservation, while adding 1 mg decrease several parameters.

Adding *Diospyros kaki* at the rate of 1 and 6% to the bull's semen improved sperm motility after 11 days of cooling, and cactus extract with 2, 4, and 6% enhanced the conception rate in cows (El-Sheshtawy and El-Nattat, 2017). Other study showed that adding 0.5 – 1.5 µg / mL of *Albizia harveyi* extracts to the bulls semen lead to reduction of malondialdehyde (MAD) and increased total antioxidant capacity post-cryopreservation (Sobeh et al., 2017)."The positive differences were found in goats' sperm cells motility, viability, and plasma membrane integrity by adding 2, 4, and 6% of *Salvia Rosmarinus* extract, moreover, 2%

decreased the MDA level (Zanganeh et al., 2013). "The positive results in roosters' semen were obtained after 48 hours of cooling at 4 °C by adding 4% and 6% of *Salvia Rosmarinus* extract, while, 8% concentration had harmful effects on sperm cells parameters compared with the control group"(Touazi et al., 2018)."The addition of 0.5 – 5 mg/mL of *Moringa oleifera* to the ram semen enhanced several sperm parameters post-cryopreservation. Thus, an extract concentration had a protective effect on the motility and antioxidant activity of the semen samples"(Carrera-Chávez et al., 2020).

CONCLUSION

The cactus extracts are an important antioxidants that are added to diluted sperm at cooling preservation. In this study, the adding cactus extract (prickly pear extract) at the rate of 2 mg improved individual sperm motility, enhanced plasma membrane integrity and decreased the dead sperm cell percentages. Whereas, adding high concentration of prickly pear extract reduced sperm quality parameters during preservation comparison with the control group.

ACKNOWLEDGMENTS

The author thanks the workers in the microbiology laboratory at the Kut Technical Institute for assisting during the experiments.

CONFLICT OF INTEREST

The authors report no conflict of benefit.

NOVELTY STATEMENT

The manuscript objective is the evaluation of adding aqueous prickly pear extract to the ram semen parameters after 0 and 24 hours of cooling at 5 °C. The prickly pear can be used as a semen additive with a specific concentration and a good source of antioxidants without any harmful effect on sperm viability.

AUTHORS CONTRIBUTION

H. J. Neamah conceived and planned the experiments, and sample preparation, performed the analytic calculations, the final version of the manuscript, and wrote the manuscript.

REFERENCES

Allai L., X. Druart, , M. Öztürk, A. BenMoula, B. Nasser, B. El Amiri (2016). Protective effects of *Opuntia ficus-indica* extract on ram sperm quality, lipid peroxidation and DNA fragmentation during liquid storage, Anim. Reprod. Sci., 175: 1-9. https://doi.org/10.1016/j.anireprosci.2016.09.013

Allai L., X. Druart, N. Louanjli, J. Contell, B. Nasser, B. El Amiri. (2017). Improvements of ram semen quality using cactus seed oil during liquid preservation in Tris egg yolk and skim milk based extenders. Small Rumin. Res., 151: 16–21. https://doi.org/10.1016/j.smallrumres.2017.02.001.

Banana H., H. J. Neamah, A. Y. Saieed (2021). Evaluation of Adding Aqueous Extract of *Opuntia Ficus Indica* Leaves To Tris Extender on the Criteria of Awassi Rams Semen Stored at Cooling. Indian J. Forensic Med. Toxicol., 15(3): 3164–3169. https://doi.org/10.37506/ijfmt.v15i3.15790

Bernard K., K. H. Krause. (2007). The NOX family of ROS-Generating NADPH oxidases: Physiology and Pathophysiology. Physiol. Rev., 87: 245-313. https://doi.org/10.1152/physrev.00044.2005.

Bustani G. S., F. H. Baiee. (2021). Semen extenders: An evaluative overview of preservative mechanisms of semen and semen extenders. Vet. World. May;14(5):1220-1233. Https://doi.org/10.14202/vetworld.2021.1220-1233

Carrera-Chávez J. M., E. E. Jiménez-Aguilar, T. P. Acosta-Pérez, J. A. Núñez-Gastélum, A. Quezada-Casasola, A. M. Escárcega-Ávila, M. F. Itza-Ortiz E. Orozco-Lucero (2020). Effect of *Moringa oleifera* seed extract on antioxidant activity and sperm characteristics in cryopreserved ram semen. J. Appl. Anim. Res., 48: 114–120. Https://doi.org/10.1111/rda.1423.

Castellini C., S. D'Andrea, G. Cordeschi, M. Totaro, A. Parisi, G. Di Emidio, C. Tatone, S. Francavilla, A. Barbonetti. (2021). Pathophysiology of Mitochondrial Dysfunction in Human Spermatozoa: Focus on Energetic Metabolism, Oxidative Stress and Apoptosis. Antioxidants (Basel), 10 (5): 695. https://doi.org/10.3390/antiox10050695

El-Sheshtawy R. I., W. S. El-Nattat (2017). Effect of Diospyros kaki enriched extender on cattle bull sperm parameters and conception rate. Asian Pac. J. Reprod., 6: 128–132.

Evans G., Maxwell W. (1987). Salamon's artificial insemination of sheep and goats. Butterworth, Sydney., p 194. Https://doi.org/10.12980/apjr.6.20170306

Fernández-López J., A. L. Almela, J. M. Obón, R.Castellar (2010). Determination of Antioxidant Constituents in Cactus Pear Fruits. Plant Foods Hum. Nutr., 65:253–259. https://doi.org/10.1007/s11130-010-0189-x

Halliwell B., J. M. C. Gutteridge. (1999). Free radicals in biology and medicine. 3rd ed. Oxford: Clarendon Press.

Hancock J. L. (1951). A staining technique for the study of temperature shock in semen. Nature (London). 169: 323-326. Https://doi.org/10.1038/167323b0

Haris F. Z., Y. S. Ondho, D. Samsudewa. (2020). Effect of vitamin E addition to frozen Simmental bull semen extender on post-thawing quality. ICALS 2019, E3S Web of Conferences 142(02002): 1-6. https://doi.org/10.1051/e3sconf/202014202002

Hu J. H., Q. W. Li, T. Zhang, Z. Jiang. L . (2009). Effect of *Gynostemma pentaphyllum* Polysaccharide on boar spermatozoa quality following freezing-thawing. Cryobiology., 59: 244–249. Https://doi.org/10.1016/j.cryobiol.2009.04.008.

Jeyendran R. S., H. H. Van Der Ven, M. Perez-Pelaez, B. G. Crabo, L. J. D. Zaneveld (1984). Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics.

J. Reprod. Fertil., 70 (1): 219-228. https://doi.org/10.1530/jrf.0.0700219

Khojasteh R. M, A. Ghani, E. Ghani. (2021). *In vitro* effects of *Capparis spinosa* L. extract on human sperm function, DNA fragmentation, and oxidative stress. J. Ethnopharmacol., 269: 113702. https://doi.org/10.1016/j.jep.2020.113702

Kuti J. O. (2004). Antioxidant compounds from four Opuntia cactus pear fruit varieties. Food Chem., 85:527–533. Https://doi.org/10.1016/S0308-8146(03)00184-5

Neamah H. J., A. A. Houbi (2020). Effect of adding astaxanthin and taurine to the Tris extender on the semen quality of Awassi rams during cooling storage. Ann. Trop. Med. Pub. Health., 23(7). Https://doi.org/10.36295/ASRO.2020.23732

Neamah H. J., S. J. F. AL-Omairi, M. A. R. Al-Sarray. (2022). The effect of zinc oxide on the glutathione peroxidase and superoxide dismutase enzymes activities of ram sperm at cooling. Iran. J. Ichthyol., 9 (Special Issue 1): 462-466.

Pinto S. C. C., D. S. Almeida, M. B. R. Alves, S. A. Florez-Rodriguez, G. S. Abreu Junior, N. Britto-e-Alves, E. C. C. Celeghini, L. M. Laskoki, F. A. Souza (2020). Does supplementation of vitamin c, reduced glutathione or their association in bovine frozen semen? Arq. Bras. Med. Vet. Zootec., 72 (1): 9-17.

Sakai Y., H. Nagase, Ose, Y. Sato, T. A. Yamaada, M. Hibi, F. Yamaada (1986). Ant mutagenicity of extracts from crud drugs in Chinese medicine, Mulat. Res. 174: 1-4. Https://doi.org/10.1016/0165-7992(86)90068-0

Sobeh M., S. A. Hassan, M. A. El Raey, W. A. Khalil, M. A. E. Hassan, M. Wink. (2017). Polyphenolics from Albizia harveyi exhibit antioxidant activities and counteract oxidative damage and ultra-structural changes of cryopreserved bull semen. Molecules., 22: 1993. Https://doi.org/10.3390/molecules22111993

Touazi L., B. Aberkane, Y. Bellik, N. Moula, M. Iguer-Ouada. (2018). Effect of the essential oil of *Rosmarinus officinalis* (L.) on rooster sperm motility during 4 °C short-term storage. Vet. World., 11: 590–597. https://doi.org/10.14202/vetworld.2018.590-597

Van Tran L., B. A Malla, S. Kumar, A. K. Tyagi. (2017). Polyunsaturated Fatty Acids in Male Ruminant Reproduction - A Review. Asian-Australas J. Anim. Sci.,30(5):622-637. https://doi.org/10.5713/ajas.15.103

Walton A. (1933). Technique of artificial insemination. mp. Bur. Anim. Genet., Iiius- Edinburgh.p 56.

Zanganeh Z., Zhandi M., Zare-Shahneh A., Najafi A., Nabi M. M., Mohammadi-Sangcheshmeh A. (2013). Does rosemary aqueous extract improve buck semen cryopreservation?. Small Rumin. Res. 1; 114 (1): 120-5. Https://doi.org/10.1016/j.smallrumres.2013.05.015

Zhao H. W., Q. W. Li, G. Z. Ning, Z. S. Han, Z. L. Jiang, Y. F. Duan. (2009). Rhodiola sacra aqueous extract (RSAE) improves biochemical and sperm characteristics in cryopreserved boar semen. Theriogenology., 71, 849–857. Https://doi.org/10.1016/j.theriogenology.2008.10.006