The Haryana Veterinarian (June, 2024) **63(1),** 65-68 ISSN 0033-4359 (P) **Research Article**

MORINGA OLEIFERA LEAVES EXTRACT ENHANCES FRESH AND CRYOPRESERVED SEMINAL ATTRIBUTE OF JAMNAPARI BUCK

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ABSTRACT

The study was conducted to assess the impact of ethanolic extract of *Moringa oleifera* at different concentrations (2.0 and 5.0%) on the seminal parameters before and after cryopreservation of extended Jamnapari buck semen. A total of twelve ejaculates were collected twice a week from two Jamnapari bucks. Each ejaculate was diluted in a TFYG (Tris-citric-acid-fructose-egg-yolk-glycerol) extender keeping 80 million/mL and divided into three equal aliquots, which were supplemented with ethanolic extract of *Moringa oleifera* (0.0, 2.0 and 5.0%; Treatment C, T1 and T2, respectively), filled in 0.25 mL straws, cooled to and equilibrated for 4 hours at 4° C and then frozen in LN2 vapour. Frozen straws were thawed at 37° C for 30 seconds in a water bath for the post-thaw evaluation. Sperm motility, percent live sperm, sperm abnormalities, plasma membrane integrity (HOST) and enzyme leakage (AST ALT, ACP and AKP) were evaluated at both pre-freeze (on dilution) and post-thaw stages. The results indicated that the addition of ethanolic extract of *Moringa oleifera*, at both 2.0 % and 5.0% concentrations significantly ($P<0.05$) improved sperm motility, sperm viability, and HOS-positive spermatozoa. Further, the sperm abnormalities and enzyme leakage were significantly ($P<0.05$) lower in *Moringa oleifera* - treated groups than in control group. The results were the best with 5% ethanolic extract of *Moringa oleifera* supplementation. In conclusion, the study showed that *Moringa oleifera* at a concentration of 5% exhibited superior protection of sperm structures and functions as compared to 2.0% *Moringa oleifera* and the control group.

Keywords: Cryopreservation, Jamnapari, *Moringa oleifera*, Semen, TFYG

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Extender used for goat semen containing egg yolk is detrimental to spermatozoa due to presence of enzyme phospholipase A in seminal plasma secreted from bulbo-urethral gland. Furthermore, the phospholipase A hydrolyses the lecithin of the egg yolk to fatty acids and lysolecithin which are spermicidal and also cause coagulation of the storage medium (Roy 1957; Iritani and Nishikawa 1963), however no latest research available which suggest spermicidal effect of caprine seminal plasma at 10% egg yolk. Cryopreservation induces biochemical, functional, and ultra-structural alterations in spermatozoa (Tamburrino *et al.*, 2023). These alterations encompass the enhancement of permeability in the plasma and acrosome membranes of the spermatozoa, resulting in the disruption of their morphology, motility, and chromatin structure (Gandini *et al.*, 2006). ROS generation is the primary factor responsible for the decline in goat sperm quality during cryopreservation, as it leads to damage to the mitochondrial membranes of spermatozoa (Bansal *et al.*, 2011). Oxidative stress leads to the production of reactive oxygen species (ROS) and/or an imbalance of antioxidant defense mechanisms. This results inmembrane lipids peroxidation and sperm injury (Mahanta *et al.*, 2012). Moreover, the elevated level of polyunsaturated fatty acids in the plasma membranes of ruminant spermatozoa makes them more prone to lipid peroxidation (Bucak *et al.*, 2009). Spermatozoa possess an inherent enzymatic antioxidant defense system (Andrabi, 2009). Nevertheless, the antioxidants of seminal plasma are unable to cope up with reactive oxygen species (ROS) associated injuries (Neild *et al.*, 2005). In order to mitigate ROS associated injuries, natural antioxidants can be used as an additive (Bilodeau *et al.*, 2001). Indeed, addition of antioxidants in semen improves the seminal attributes and fertility of cattle (Iqbal *et al.*, 2016; Javed *et al.*, 2019) and caprine semen (Atessahin *et al.*, 2008). *Moringa oleifera* contains a lot of antioxidants, especially in the leaves (Das *et al.*, 2012). Ascorbic acid, flavonoids (quercetin, kaempferol, potassium, vitamin E, selenium, vitamin C, magnesium and proanthocyanidin), polyphenolics, and carotenes are found in *Moringa oleifera* leaves (Jayawardana *et al.*, 2015). These compounds lower the free radicals production and reduce the risk of sperm cell death (Wang *et al.*, 2017). Carrera-Chavez (2020) observed that a methanolic extract of *Moringa oleifera* seed improved post-thaw semen motility and antioxidant capacity in rams. To date, no literature available regarding antioxidative properties of *Moringa oleifera* leaves extract in TFYGdiluter on the post-thaw seminal attributes of Jamnapari bucks.

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MATERIALS AND METHODS

Preparation of *Moringa oleifera* leaves extract

The mature, healthy and fresh *Moringa oleifera* leaves were harvested from Experimental Farm of College of Horticulture, ANDUAT Kumarganj, Ayodhya U.P. Fifty grams of dried *Moringa oleifera* leaves powder was extracted using a modified technique proposed by Khalaf *et al.* (2008).

Animals

Twelve semen ejaculates were collected from two Jamnapari buck, (2-3 years) reared at Deep Frozen Semen Laboratory, College of Veterinary Science & Animal Husbandry, ANDUAT Kumarganj, Ayodhya of Uttar Pradesh under standard management conditions.

Semen Collection, Processing and Freezing

Buck ejaculates were harvested twice in a week using AV and transfer to laboratory for analysis of mass motility (10X) and sperm concentration (Accucell photometer, IMV, France).

Selected ejaculates with visual motility above 75 % were diluted using a Tris-citric-acid-fructose-egg-yolk-glycerol (TFYG) extender. Diluted semen was splited into three equal aliquots having different concentrations of 0%, 2%, and 5% of ethanolic extract of *Moringa oleifera* control (C) and treatment groups (T_1 and T_2), respectively, to make final sperm concentration of 80 million per ml. Diluted semen were automatically filled into 0.25 mL French straws (ISEVO, IMV, France) and equilibrated in a cold cabinet (Minitube, Germany) at 4° C for 4 hours. Straws were arranged horizontally and suspended in LN_2 vapour inside a foam box at 4 cm height above liquid nitrogen for 20 min, then dipped in liquid nitrogen (Najafi *et al.*, 2014).

Evaluation of semen's quality

Seminal attributes (sperm motility, viability and morphology) at post-dilution and post-thaw stage (Hafez and Hafez, 2000). The assessment of sperm plasma membrane integrity was carried out using a HOS test (Amorim *et al.*, 2009). The seminal plasma was separated from freshly diluted and frozen-thawed samples through centrifugation at 3000 rpm for duration of 20 minutes. The levels of enzyme leakage in seminal plasma (AST, ALT, AKP, and ACP) were measured using commercially available kits on the BEACON B Auto200 analyzer.

Statistical analysis

The values were presented as means ± SE for all variables. Statistical analysis was done with one-way ANOVA using Graph Pad Prism 5 software.

RESULTS AND DISCUSSION

The impacts of ethanolic extract of *Moringa oleifera* on the seminal parameters of frozen buck semen are depicted in Table 1. Semen samples cryopreserved with

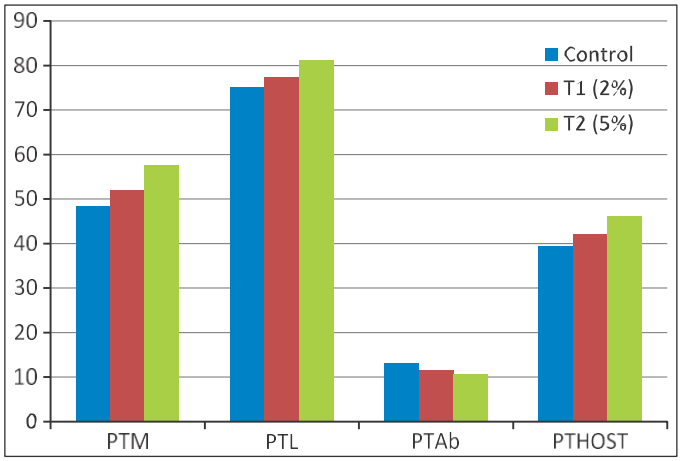

Fig. 1. Image showing post-thawed motility, livability and abnormality of control and ethanolic extract of Moringa oleifera (T1 and T2) treated group (P<0.05)

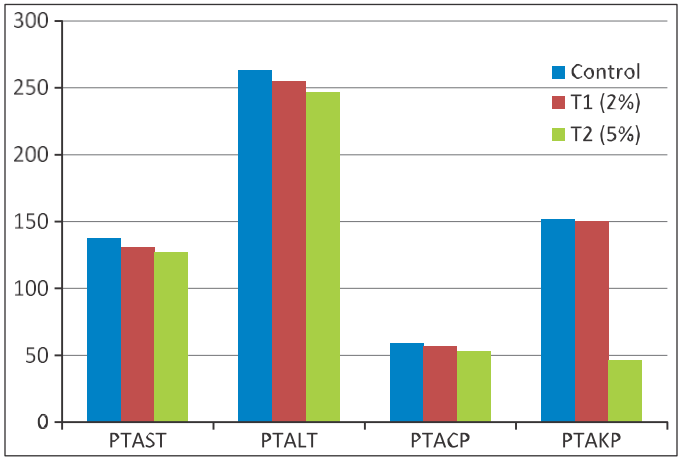

Fig. 2. Image showing post-thawed seminal plasma enzyme leakage (AST, ALT, ACP and AKP) of control and ethanolic extract of Moringa oleifera (T1 and T2) treated group (P<0.05)

5.0% ethanolic extract of *Moringa oleifera* in TFYG extender had significantly (P<0.05) higher percent post-diluted and post-thaw sperm motility, viability and morphological normal sperm followed by 2.0% ethanolic extract of *Moringa oleifera* as compared to the control extender (Fig. 1). The mean post-thaw motility recorded was in line with the findings of Hammad *et al.* (2019). Conversely lower (Mina & Husam, 2020) and higher (Wahjuningsih *et al.*, 2019) values were reported in previous studies. Our findings on sperm viability were in tune with Saurabh (2021), and lower than the findings of Mina and Husam (2020), but higher than those reported by Doidar *et al.* (2018).

Cryopreservation causes membrane dysfunction and excessive cell dehydration, leading to osmotic shock (Neild *et al.*, 2005). The damage is scribed to intracellular and extracellular ice crystals formation along with an increase in solute quantity (Mazur, 1984). However, cryopreservation can lead to alterations in sperm mitochondrial membrane fluidity, potentially leading to release of reactive oxygen species and changes in sperm membrane potential (Said *et al.*, 2010). ROS causes ATP depletion, which in turns

Table 1. Effect of ethanolic extract of Moringa oleifera (2.0% & 5.0%) supplementation in Tris extender on sperm quality parameters and enzyme leakage during cryopreservation of Jamnapari bucks semen

Stages	Additive concentration (%)	Progressive motility (%)	Sperm livability (%)	Sperm abnormality (%)	HOS reactive sperm (%)	AST (µmole/L)	ALT (µmole/L)	ACP KAU/ 100 ml	AKP KAU/ 100 ml
Post-diluted	Control (C)	78.67±0.8^{a}	86.33±0.6^{a}	6.83±0.60^{b}	49.00±1.0^{a}	60.33±2.7^{c}	119.3±3.20^{c}	27.17±1.8^{c}	43.17±2.6^{c}
	2.0% (T_1)	79.67±0.8^{b}	87.17±0.70^{b}	6.33±0.67^{ab}	52.17±1.28^{b}	59.17±2.55^{b}	115.2±2.69^{b}	26.00±1.7^{b}	42.33±2.36^{bc}
	5.0% (T_2)	81.00±0.8^{c}	88.67±0.6^{c}	5.67±0.56^{a}	55.67±1.2^{c}	56.50±2.4^{a}	110.7±2.5^{a}	24.00±1.7^{a}	39.83±2.2^{a}
Post- thaw	Control (C)	48.33±0.67^{A}	75.00±0.97^{A}	13.17±1.4^{B}	39.33±0.42^{A}	137.2±4.73^{C}	263.5±4.49^{C}	59.17±1.35^{C}	151.80±5.10^{C}
	2.0% (T_1)	52.00±0.37^{B}	77.33±1.17^{B}	11.67±1.0^{A}	42.17±0.79^{B}	131.00±4.1^{B}	255.20±3.89^{B}	56.33±0.88^{B}	149.70±5.08^{B}
	5.0% (T_2)	57.67±0.84^{C}	81.17±0.95^{C}	10.67±0.9^{A}	46.17±0.87^{C}	127.30±3.9^{A}	246.70±3.70^{A}	53.00±0.93^{A}	145.80±4.90^{A}

AST-ALT = Asparate and Alanine amino-transferases, ACP-AKP = Alkaline and acid Phosphatases, Mean±SE values bearing different superscript within the stage (a, b, c / A, B, C) in a column differ significantly (P<0.05).

result in insufficient phosphorylation of the axoneme and lipid peroxidation. These effects ultimately decrease both motility and survival (El-Sherbiny *et al.*, 2022). *Moringa oleifera* leaves possess potent antioxidant properties by virtue of its ascorbic acid, carotenes, flavonoids and polyphenol content (Jayawardana *et al.*, 2015). These compounds have been shown to decrease the presence of free radicals and mitigate the possibility of cellular death (Wang *et al.*, 2017). Moreover, Carrera-Chavez (2020) observed that a 0.5 mg/mL concentration of *M. oleifera* seed extract effectively maintained the integrity of the sperm membrane.

The average percent HOS-reactive sperm cells were significantly (P<0.05) higher in extender containing 5.0% ethanolic extract of *Moringa oleifera* at both post-diluted and post-thaw stage followed by 2.0% ethanolic extract of *Moringa oleifera* than those of control extender (P<0.05, Table 1). Our findings are in tuned with Doidar *et al.* (2018), but were lower (El-Seadawy *et al.*, 2022) and higher (Kumar, 2022) values were also observed. Plasma membrane functionality and integrity are vital sperm parameters to consider when evaluating sperm quality because an intact plasma membrane is required for sperm cell survival (Makarevich *et al.*, 2010). Moreover, it plays essential roles in sperm metabolism, capacitation, acrosome reaction, eventually sperm-oocyte fusion (Forouzanfar *et al.*, 2013).

The significantly (P<0.05) lower values of AST, ALT, ACP and AKP activities in plasma of post-thawed semen were recorded in extender containing 5.0% as compared to 2.0% ethanolic extract of *Moringa oleifera* and the control extender (P<0.01, Table 1 & Fig. 2). Elevated enzyme activity in the extracellular medium indicates cold shock associated damage of spermatozoa membrane (Singh *et al.*, 1992). The assessment of enzymes also indicates the effectiveness of the extender in preserving the membrane integrity of spermatozoa during freezing and thawing (Prasad *et al.*, 2000). The addition of an ethanolic extract of *Moringa oleifera* leaves to the extender may have enhanced protection of the sperm membrane, leading to the preservation of structural integrity and cell membrane permeability, ultimately resulting in reduced enzyme leakage. These findings align with the results reported by Srivastava and Kumar (2014). *Moringa oleifera* leaves are used for sperm preservation because they contain significant amounts of protein, calcium, potassium, magnesium, vitamin E, selenium, vitamin C, fat, and phenolic components (Ayodele *et al.*, 2014). Moreover, *Moringaoleifera* leaves contain a flavonoid compound that enhances antioxidant activity and prevent lipid peroxidation in sperm (Moyo *et al.*, 2012).

CONCLUSION

This study indicated that adding 2.0% and 5.0% ethanolic extract of *Moringa oleifera* to the extender improved the quality of post-thawed Jamnapari buck semen, highest preservation efficiency was observed in 5.0% ethanolic extract of *Moringa oleifera* compared to other treatments.

REFERENCES

Allai, L., Druart, X., Ozturk, M., BenMoula, A., Nasser, B. and El Amiri, B. (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.

Amorim, E.A.M., Torres, C.A.A., Graham, J.K., Amorim, L.S. and Santos, L.V.L. (2009). The hypoosmotic swelling test in fresh rabbit spermatozoa. *Anim. Reprod. Sci.* **111(2-4)**: 338-343.

Andrabi, S.M.H. (2009). Factors affecting the quality of cryopreserved buffalo (*Bubalus bubalis*) bull spermatozoa. *Reprod. Domest. Anim.* **44(3)**: 552-569.

Atessahin, A., Bucak, M.N., Tuncer, P.B. and Kýzýl, M. (2008). Effects of anti-oxidant additives on microscopic and oxidative parameters of Angora goat semen following the freeze-thawing process. *Small Rumi. Res.* **77(1)**: 38-44.

Ayodele, A.E., Adeola, J.R. and Mayowa, A.T. (2014). Reproductive performance of rabbit does feed graded levels of *Moringa Oleifera* leaf meal-based diet. *Int. J. Sci.* pp. 49-53.

Bansal, A.K. and Bilaspuri, G.S. (2011). Impacts of oxidative stress and antioxidants on semen functions. *Vet. Med. Int.* **7**: 2-3.

Bilodeau, J.F., Blanchette, S., Gagnon, C. and Sirard, M.A. (2001). Thiols prevent H_2O_2 mediated loss of sperm motility in cryopreserved bull semen. *Theriogenol.* **56(2)**: 275-286.

Bucak, M.N., Tuncer, P.B., Sarýözkan, S. and Ulutas, P.A. (2009). Comparison of the effects of glutamine and an amino acid solution

on post-thawed ram sperm parameters, lipid peroxidation and anti-oxidant activities. *Small Rumi. Res.* **81(1)**: 13-17.

Carrera-Chavez, J.M. (2020). Effect of *Moringa oleifera* seed extract on antioxidant activity and sperm characteristics in cryopreserved ram semen. *J. Appl. Anim. Res.* **48**: 114-120.

Das, A.K., Rajkumar, V., Verma, A.K. and Swarup, D. (2012). *Moringa oleiferia* leaves extract: a natural antioxidant for retarding lipid peroxidation in cooked goat meat patties. *Int. J. Food Sci. Technol.* **47(3)**: 585-591.

Doidar, Y.A., El-Nagar, H.A., Elrefy, A. and Mousbah, A.M. (2018). Cryopreservation and quality assessment of buffalo bull (*Bubalus bubalis*) semen using new *Moringa* extender and antioxidant. *J. Anim. Poult. Prod.* **9(9)**: 375-381.

El-Seadawy, I.E., Kotp, M.S., El-Maaty, A.M.A., Fadl, A.M., El-Sherbiny, H.R. and Abdelnaby, E.A. (2022). The impact of varying doses of *Moringa* leaf methanolic extract supplementation in the cryopreservation media on sperm quality, oxidants, and antioxidant capacity of frozen-thawed ram sperm. *Trop. Anim. Health Prod.* **54(6)**: 344.

El-Sherbiny, H.R., Abdelnaby, E.A., El-Shahat, K.H., Salem, N.Y., Ramadan, E.S., Yehia, S.G. and Fathi, M. (2022). Coenzyme Q10 supplementation enhances testicular volume and hemodynamics, reproductive hormones, sperm quality, and seminal antioxidant capacity in goat bucks under summer hot humid conditions. *Vet. Res. Commun.* **46(4)**: 1245-1257.

Fakurazi, S., Nanthini, U. and Hairuszah, I. (2008). Hepatoprotective and antioxidant action of *Moringa oleifera* Lam. against acetaminophen induced hepatotoxicity in rats. *Int. J. Pharmacol.* **4(4)**: 270-275.

Forouzanfar, M., Ershad, S.F., Hosseini, S.M., Hajian, M., Ostad-Hosseini, S., Abid, A. and Esfahani, M.H.N. (2013). Can permeable super oxide dismutase mimetic agents improve the quality of frozen-thawed ram semen. *Cryobiol.* **66(2)**: 126-130.

Gandini, L., Lombardo, F., Lenzi, A., Spano, M. and Dondero, F. (2006). Cryopreservation and sperm DNA integrity. *Cell Tissue Ban.* **7**: 91-98.

Hafez, B. and Hafez, E.S.E. (2000). Reproduction in Farm Animals. (7th Edn.), Awolters Klawer Company Publishers, USA, pp. 376-387.

Hammad, M.E.R., Wafa, W.M., Gabr, A.A. and Elkishky, A.Y. (2019). Different types and levels of *Moringa oleifera* leaf extract as a source of antibiotics in Friesian bull semen extender. *J. Anim. Poult. Prod.* **10(3)**: 67-71.

Iritani, A. and Nishikawa, Y. (1963). Studies on the egg-yolk coagulating factor in goat semen. III. Release of some acids accompanied by the coagulating phenomena. *Jap. J. Anim. Reprod.* **8**: 109-12.

Iqbal, S., Naz, S., Bhutta, M.F., Sufyan, A. and Awan, M.A. (2022). Antioxidant effect of *Moringa olifera* leaves extract in extender improves post thaw quality, kinematics, lipid peroxidation, total antioxidant capacity and fertility of water buffalo bull semen. *Andrologia.* **54(1)**: 14300.

Javed, M., Tunio, M.T., Abdul Rauf, H., Bhutta, M.F., Naz, S. and Iqbal, S. (2019). Addition of pomegranate juice (*Punica granatum*) in tris based extender improves post thaw quality, motion dynamics and *in vivo* fertility of Nili Ravi buffalo (*Bubalus bubalis*) bull spermatozoa. *Andrologia.* **51(8)**: 13322.

Jayawardana, B.C., Liyanage, R., Lalantha, N., Iddamalgoda, S. and Weththasinghe, P. (2015). Antioxidant and antimicrobial activity of drumstick (*Moringa oleifera*) leaves in herbal chicken sausages. *LWT-Food Sci. Technol.* **64(2)**: 1204-1208.

Khalaf, N.A., Shakya, A.K., Othman, A.A.L., Agbar, Z.E.L. and Farah, H. (2008). Antioxidant activity of some common plants. *Turk. J. Biol.* **32**: 51-55.

Kumar, R. (2022). Effect of Ginger (*Zingiber officinale*) and Ascorbic acid on cryopreservability of Buck spermatozoa. Thesis, M.V.Sc., C.V.Sc. & A.H., ANDUAT, Kumarganj-Ayodhya (UP), India.

Mahanta, R., Gogoi, A., Chaudhury, P.N.B., Roy, S., Bhattacharyya, I.K. and Sharma, P. (2012). Association of oxidative stress biomarkers and antioxidant enzymatic activity in male infertility of north-East India. *J. Obstet. Gynaecol. India.* **62**: 546-550.

Makarevich, A.V., Kubovicova, E., Sirotkin, A.V. and Pivko, J. (2010). Demonstration of the effect of epidermal growth factor on ram sperm parameters using two fluorescent assays. *Vet. Med.* **55(12)**: 581-589.

Mazur, P. (1984). Freezing of living cells: mechanisms and implications. *Am. J. Physiol.* **247**: 125-142.

Mina, S.M.J. and Husam, J.H.B. (2020). Effect of adding different level of Amla fruit extract and vitamin C to Tri-based extender on the semen properties preserved at 5º C of Awassi Ram. *Plant Archives.* **20(1)**: 1326-1330.

Moyo, B., Oyedemi, S., Masika, P.J. and Muchenje, V. (2012). Polyphenolic content and antioxidant properties of *Moringa oleifera* leaf extracts and enzymatic activity of liver from goats supplemented with *Moringa oleifera* leaves/sunflower seed cake. *Meat Sci.* **91**: 441-447.

Najafi, A., Najafi, M.H., Zanganeh, Z., Sharafi, M., Martinez Pastor, F. and Adeldust, H. (2014). Cryopreservation of ram semen in extenders containing soybean lecithin as cryoprotectant and hyaluronic acid as antioxidant. *Reprod. Domest. Anim.* **49(6)**: 934-940.

Neild, D.M., Brouwers, J.P., Colenbrander, B., Aguero, A. and Gadella, B.M. (2005). Lipid peroxide formation in relation to membrane stability of fresh and frozen thawed stallion spermatozoa. *Mol. Reprod. Dev.* **72**: 230-238.

Prasad, J.K., Satish, K., Greesh, M., Agarwal, S.K. and Uma, S. (2000). Biochemical studies pertaining to freezability of cross bred bull semen. *Indian J. Vet. Res.* **9(1)**: 37-40.

Roy, A. (1957). Egg-yolk coagulating enzyme in the semen and Cowper's gland of the goat. *Nature* (London). **179**: 318-19.

Said, T.M., Gaglani, A. and Agarwal, A. (2010). Implication of apoptosis in sperm cryoinjury. *Reprod. Biomed. Online.* **21(4)**: 56-462.

Saurabh, (2021). Effect of herbal antioxidants on cryopreservability and fertility of Murrah bull semen. Thesis, Ph.D. C.V.Sc. & A.H., ANDUAT, Kumarganj-Faizabad (UP), India.

Singh, R.S., Tomar, N.S., Sharma, K.C. and Sharma, K.B. (1992). Studies on acrosomal abnormalities of cattle and buffalo spermatozoa in relation to other semen characteristics and fertility. *Indian Vet. J.* **69(3)**: 267-268.

Srivastava, S. and Kumar, S. (2014). Incorporation of ascorbic acid, caffeine and chloroquine diphosphate in dilutor improves structural and functional status of frozen semen. *Open Access Library J.* **1(1)**: 1-12.

Tamburrino, L., Traini, G., Marcellini, A., Vignozzi, L., Baldi, E. and Marchiani, S. (2023). Cryopreservation of human spermatozoa: Functional, molecular and clinical aspects. *Int. J. Mol. Sci.* **24(5)**: 4656.

Wahjuningsih, S., Ihsan, M.N., Ciptadi, G., Isnaini, N., Rahayu, S. and Afifah, D.D. (2019). Effect of *Moringa oleifera* leaves extract on post-thawed semen quality of Senduro Goat. *In IOP Conference Series: Earth and Environmental Sci.* **247(1)**: 012055.

Wang, Y., Gao, Y., Ding, H., Liu, S., Han, X., Gui, J. and Liu, D. (2017). Subcritical ethanol extraction of flavonoids from *Moringa oleifera* leaf and evaluation of antioxidant activity. *Food Chem.* **218**: 152-158.