



Effect of *Moringa oleifera* Leaves Extract on Quality of Preserved Boar Semen

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ABSTRACT

Background: Moringa leaf extract (MLE) is one of the natural ingredients that can improve the quality of preserved semen by preventing sperm lipid oxidation and increase the sperm storage time. The aim of the present study is to evaluate the effect of *Moringa oleifera* leaves extract on boar semen quality during short-term preservation at liquid state.

Methods: Four mature healthy Large White Yorkshire boars of about 1.5-2 years age group having good reproductive health was selected for the study. Ten ejaculates were collected from each boar. Each ejaculate was divided into 4 aliquots (equal volume and sperm concentration) and *Moringa oleifera* leaves extract (MLE) @ 0 µg/ml (control), 100 µg/ml, 200 µg/ml and 300 µg/ml w/v was mixed in each aliquot after extension of semen samples with beltsville thaw solution (BTS) extender. The semen was evaluated for initial motility, percent live sperm, sperm abnormalities, sperm membrane integrity, acrosomal integrity and level of lipid peroxidation. Ten gilts/sows were artificially inseminated in each group with the liquid preserved semen to record the conception rates and litter size.

Result: The percent sperm motility, live sperm, plasma membrane integrity and acrosomal integrity of boar spermatozoa were decreased significantly ($P < 0.01$) while preserving the semen sample by using BTS extenders along with 100 µg/ml, 200 µg/ml and 300 µg/ml *Moringa oleifera* leaves extract at 18°C for 48 hours. The mean percent of motile sperm, live sperm, intact plasma membrane, intact acrosome among the groups i.e., 100 µg/ml, 200 µg/ml and 300 µg/ml *Moringa oleifera* leaves extract were increased significantly ($P < 0.01$) as compared to control at 24 and 48 hours of preservation. The mean percentage of sperm abnormalities and lipid peroxidation among the groups i.e., 100 µg/ml, 200 µg/ml and 300 µg/ml *Moringa oleifera* leaves extract were increased significantly ($P < 0.05$) at 0, 24 and 48 ($P < 0.01$) at hours of preservation as compared to control group. The supplementation of *Moringa oleifera* leaves extract in BTS extender at concentration of 100 µg/ml, 200 µg/ml and 300 µg/ml significantly improved the Large White Yorkshire boar's semen quality during short term preservation at 18°C as compared to control. The conception rate and average litter size at birth among the groups were increased significantly ($P < 0.01$) as compared to control (C). The significantly ($P < 0.01$) higher conception rate and litter size at birth was recorded in T3 group i.e. 300 µg/ml *Moringa oleifera* leaves extract supplemented semen. In conclusion, the concentration of 300 µg/ml *Moringa oleifera* leaves extract was found to be better among other concentrations.

Key words: Boar semen, BTS extender, *Moringa oleifera* leaves extract, Preservation, Quality.

INTRODUCTION

Pig farming is an integral component of the traditional agricultural system in India's North Eastern region (Talukdar *et al.*, 2019). Farmers in this region have a lot of opportunities to grow in this sector because the majority of the people in this area aren't vegetarians. Pork is a significant part of the diet in the Northeast and achieving self-sufficiency in pork production is a critical step toward improving food security (Talukdar *et al.*, 2022). The people of Mizoram consume about 14 kg (average) of meat annually, against the national per capita consumption of meat of 7.5 kg (Statistical Handbook Mizoram, 2010). Indigenous pigs make up the majority of the pig population in the region and significant crossbreeding is needed to improve it.

Artificial insemination (AI) is the most frequently used reproduction technique in modern pig farming because it has several advantages. A rapid gain in genetic improvement is one of them. The intense use of AI has increased the interest to develop proper conditions to store semen better for longer periods of time without affecting its fertility (Bondan *et al.*, 2016). However, quality of semen stored for longer periods deteriorate gradually, probably

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related to the oxidative stress formed during its storage (Vongpralub *et al.*, 2016).

Almost all cells contain substances and enzymes that neutralize the toxic effects of various reactive oxygen species (ROS), but the amount of antioxidants in the sperm is lower

than that of other cells and is more vulnerable to oxidative stress (Sreegith *et al.*, 2005). Plasma membrane of mammalian sperm, fish and birds is full of unsaturated fatty acids and phospholipids. High amounts of unsaturated fatty acids with multiple bonds cause sperm to become very sensitive to lipid peroxidation, which is positively correlated with male infertility (Surai *et al.*, 1998). Oxidation of fatty acids leads to the production of reactive oxygen species (ROS). These radicals are necessary in normal conditions for some activities and sperm physiological processes (Talukdar *et al.*, 2016) but excessive production of ROS in the sperm can reduce membrane fluidity, DNA fracture, damage to proteins and ultimately reduced sperm motility and fertility (Peris *et al.*, 2007). Protection of the balance between the production of ROS and the antioxidant system is essential to maintain the survival of the sperm, which is balanced by the storage and freezing of the sperm (Breque *et al.*, 2003). In order to prevent increased sperm lipid oxidation and increase the sperm storage time in the laboratory, the use of natural antioxidants in the semen diluent environment seems necessary.

Moringa leaf extract (MLE) is one of the natural ingredients that can improve the quality of preserved semen. Active compounds found in *Moringa oleifera* leaves are polyphenols, β -carotene, vitamins A, C and E, protein, calcium, amino acids and several natural anti-oxidant agents thus; it is widely used in the developing country (Luqman *et al.* 2012). In addition, it possesses many phytochemicals, including crude fibres, reducing sugars, resins, alkaloids, flavonoids, organic acids, sterols, tannins, saponins, proteins, polyphenols and other antioxidants (Mishra *et al.*, 2011) such as kaempferol, quercetin, ellagic acid and apigenin glucoside (Mousa *et al.*, 2019). According to Yameogo *et al.* (2011), Moringa leaves contain higher iron than other vegetables, 17.2 mg/g. Moringa Leaf Extract (*Moringa oleifera* LAM) contains antioxidants, including vitamin C 17.3 mg and vitamin E 113 g/100 g, which protect the body against the damaging effects of free radicals by neutralizing them before they can cause cell damage and disease (Kurniasih 2013). By lowering the free radical concentration in sperm cytoplasm, *Moringa oleifera* leaves extract (MOLE) increases the male reproductive potentials (D'Cruz and Mathur, 2005). MOLE acts as an antioxidant (El-Nagar, 2017) or antimicrobial agent (Hammad *et al.*, 2019) which improves sperm quality. The present research work has been carried out with the objective to evaluate the effect of *Moringa oleifera* leaves extract on boar semen quality during short-term preservation at liquid state.

MATERIALS AND METHODS

The study was conducted on 4 numbers of Large White Yorkshire (LWY) boars at the age of 1.5-2 years, maintained at Livestock Farm Complex, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram. All the animals in the study had a routine examination to make sure their general and

reproductive health was good. The boars were kept separately in clean, hygienic boar enclosures with enough food and water for them to drink. The semen was collected from each boar by gloved hand technique (Shipley, 1999) using dummy as mount for 2 times in a week (Talukdar *et al.*, 2023). Semen collection was carried out in the morning time. The semen sample selected for further processing has a mass activity minimum of 3+ and a sperm motility percentage of 70 or higher. A total of 40 ejaculates, 10 from each boar were taken for the study.

The *Moringa oleifera* plant was identified at Dept. of Forestry, Mizoram University, Aizawl, Mizoram, India with accession Number: 1628. For preparation of the *Moringa oleifera* leaves (MLE) extract, the fresh *Moringa oleifera* leaves was collected and washed with distilled water, dried in shaded area at room temperature then grinded and sieved. The ethanolic extract of *Moringa oleifera* leaves was prepared as per the method described by Dina *et al.* (2021). The powder was suspended in 70% ethyl alcohol in a conical flask. The rotary shaker set at 200 rpm for 48 hrs at 30°C and conical flask was kept on it, after that the extract was filtered through a whatman filter paper no.1 and the solvent was evaporated using a rotary evaporator at 40°C for 24 hrs. Then the extract was collected in labelled vials and stored at 4°C for further use.

Each ejaculate of boar was mixed @1:2 ratio with BTS extender and divided into four aliquots, each of which was diluted with 0 μ g/ml (without MLE, control), 100 μ g/ml (T1), 200 μ g/ml (T2) and 300 μ g/ml (T3) *Moringa oleifera* leaves extract and preserved at liquid state at 18°C in a BOD incubator. The preserved semen samples were evaluated at 0 hour i.e. immediately after dilution, 24 and 48 hours. The stored semen samples were tested for percent progressive motile sperm, live sperm count, sperm abnormalities by Eosin-Nigrosin staining method (Blom, 1950), plasma membrane integrity estimated by Hyper Osmotic Swelling Test (Jeyendran *et al.*, 1984), acrosomal integrity estimated by Giemsa staining method (Watson, 1975). The lipid peroxidation assay was carried out by using commercially available kit (Cat. no. 705002, Cayman Chemical, Ann Arbor, MI).

In each group, 10 gilts/sows were artificially inseminated with liquid-preserved semen having total sperm concentration of 2.5×10^9 with dose rate of 90 ml semen after 24 and 48 hours from the beginning of oestrus. The pregnancy was diagnosed after 30th day of AI by using ultrasonography. The conception rate and litter size at birth were calculated and reported.

The data collected from the study were subjected to statistical analysis using a suitable formula as per Snedecor and Cochran (1994) for meaningful and accurate comparison and interpretation.

RESULTS AND DISCUSSION

Percent sperm initial motility, live sperm, Plasma membrane integrity, intact acrosome, normal sperm, LPO

level of Large White Yorkshire boar semen after supplementation of *Moringa oleifera* leaves extract in different experimental groups were depicted in (Table 1). The conception rate and litter size at birth of inseminated gilt/sows by using *Moringa oleifera* leaves extract supplemented semen samples in different experimental groups were depicted in (Table 2 and 3).

The mean percent motile sperm among the groups i.e., 100 µg/ml (T1), 200 µg/ml (T2) and 300 µg/ml (T3) *Moringa oleifera* leaves extract were increased significantly ($P<0.01$) as compared to control (C) at 24 and 48 hours of preservation except at 0 hour i.e., immediately after dilution in all the groups. The mean percent of motile sperm in different hours of preservation were decreased significantly

Table 1: Per cent sperm initial motility, live sperm, plasma membrane integrity, intact acrosome, normal sperm, LPO level of large white yorkshire boar semen after supplementation of *Moringa oleifera* leaves extract in different experimental groups (Mean±SE).

Parameters	Hours	C	T1	T2	T3	F value
Sperm initial motility (%)	0	82.10±0.95 ^{ab} _C	79.45±0.98 ^a _C	81.47±1.08 ^{ab} _C	84.50±0.81 ^b _C	4.653**
	24	65.17±1.35 ^a _B	65.35±1.14 ^a _B	67.12±1.04 ^a _B	71.40±0.85 ^b _B	6.745**
	48	50.05±1.68 ^a _A	50.47±1.42 ^a _A	52.75±1.24 ^a _A	57.07±1.18 ^b _A	5.285**
	F value	137.917**	145.752**	162.638**	200.753**	
Live sperm (%)	0	69.97±1.40 ^a _C	69.52±1.21 ^a _C	72.32±1.42 ^{ab} _C	74.90±1.26 ^b _C	3.460*
	24	57.17±1.34 ^a _B	56.72±1.33 ^a _B	60.85±1.16 ^{ab} _B	62.75±1.27 ^b _B	5.161**
	48	44.20±1.40 ^a _A	45.87±1.45 ^a _A	47.07±1.56 ^a _A	48.57±1.48 ^a _A	1.577 ^{NS}
	F value	86.741**	78.378**	82.229**	96.362**	
Plasma membrane integrity (%)	0	67.05±1.35 _C	68.82±1.08 _C	66.35±1.40 _C	69.27±1.20 _C	1.213 ^{NS}
	24	56.67±1.15 ^a _B	60.67±1.09 ^{ab} _B	58.30±1.11 ^a _B	63.37±1.26 ^b _B	6.306**
	48	45.20±1.45 ^{ab} _A	43.77±1.11 ^a _A	48.47±1.06 ^b _A	49.25±1.15 ^b _A	4.781**
	F value	67.526**	135.048**	48.948**	80.480**	
Intact acrosome (%)	0	82.97±1.27 _C	85.02±1.24 _C	85.57±1.30 _C	87.52±1.19 _C	2.224 ^{NS}
	24	74.62±1.24 _B	75.82±1.44 _B	75.97±1.49 _B	77.95±1.46 _B	0.947 ^{NS}
	48	65.67±1.28 _A	67.30±1.35 _A	66.32±1.57 _A	68.22±1.78 _A	0.546 ^{NS}
	F value	46.577**	43.220**	43.271**	41.370**	
Normal sperm (%)	0	89.67±0.39 _C	89.00±0.36 _C	89.62±0.32 _C	89.92±0.36 _C	12.17 ^{NS}
	24	85.95±0.52 _B	84.82±0.47 _B	86.80±0.41 _B	85.20±0.75 _B	2.455 ^{NS}
	48	79.37±0.53 ^{ab} _A	78.37±0.63 ^a _A	78.70±0.57 ^a _A	81.07±0.71 ^b _A	3.772*
	F value	114.262**	112.016**	182.223**	74.038**	
LPO (nmol)	0	0.90±0.04 _A	0.82±0.03 _A	0.80±0.03 _A	0.78±0.03 _A	1.802 ^{NS}
	24	1.02±0.02 ^b _B	0.94±0.04 ^a _A	0.91±0.03 ^a _B	0.91±0.02 ^a _B	2.904*
	48	1.18±0.02 ^b _C	1.13±0.02 ^b _B	1.02±0.02 ^a _B	0.98±0.02 ^a _B	11.667**
	F value	17.221**	16.929**	12.295**	13.459**	

* $P<0.05$; ** $P<0.01$; NS; Non-significant.

Means bearing different superscripts in lowercase in each row differed significantly.

Means bearing different subscripts in uppercase in each column differed significantly.

Table 2: Conception rate of inseminated gilt/sows by using *Moringa oleifera* leaves extract supplemented semen samples in different experimental groups.

Conception rate (%)	Control	T1	T2	T3	Chi square value
	80.0 ^a	80.0 ^a	90.0 ^b	100.0 ^c	25.23**

** $P<0.01$.

Means bearing different superscripts in a row differed significantly.

Table 3: Litter size at birth of inseminated gilt/sows by using *Moringa oleifera* leaves extract supplemented semen samples in different experimental groups (Mean±SE).

Litter size	Control	T1	T2	T3	F value
	6.70±0.35 ^a	7.05±0.36 ^{ab}	7.87±0.34 ^{ab}	8.12±0.29 ^c	3.885**

** $P<0.01$.

Means bearing different superscripts in a row differed significantly.

($P < 0.01$) at control (C), 100 µg/ml (T1), 200 µg/ml (T2) and 300 µg/ml (T3) *Moringa oleifera* leaves extract. The mean percentage of sperm motility found to be significantly ($P < 0.01$) higher in T3 group as compared to control (C), T1 and T2 at different hours of preservation. The present finding was higher than that reported by the Dina *et al.* (2021). Dina *et al.* (2021) reported that motility percentage of Barki ram semen sample were gradually increased while adding *Moringa oleifera* leaves extract to semen extender as compared to control group. Fafo *et al.* (2016) reported that citrate-egg yolk extender with 5% moringa leaf extract (MLE) diluent maintained significantly higher boar sperm motility and viability over 24 hours preservation at 18-20°C compared to control group. The reason for this improvement might be due to reducing the amount of ROS (Reactive Oxygen Species) by *Moringa oleifera* leaves extract which having natural antioxidants (Luqman *et al.*, 2012).

The mean percentage of live sperm among the groups *i.e.*, 100 µg/ml (T1), 200 µg/ml (T2) and 300 µg/ml (T3) *Moringa oleifera* leaves extract were increased significantly ($P < 0.01$) as compared to control (C) at 24 and 48 hours of preservation. The mean percentage of live sperm found to be significantly ($P < 0.01$) higher in T3 group as compared to control (C), T1 and T2 at different hours of preservation. The mean percentage of live sperm in different hours of preservation were decreased significantly ($P < 0.01$) at control (C), 100 µg/ml (T1), 200 µg/ml (T2) and 300 µg/ml (T3) *Moringa oleifera* leaves extract. The present finding was lower than that reported by the Dina *et al.* (2021). Dina *et al.* (2021) reported that viability index of Barki ram semen sample were gradually increased while adding *Moringa oleifera* leaves extract to semen extender as compared to control group. According to El-Sheshtawy and El-Nattat (2020) addition of different levels of *Moringa oleifera* extract after chilling and cryopreservation was able to reduce free radicals and enhanced live sperm percentage in cooled and cryopreserved cattle bull semen.

The mean percentage of intact plasma membrane among the groups *i.e.*, 100 µg/ml (T1), 200 µg/ml (T2) and 300 µg/ml (T3) *Moringa oleifera* leaves extract were increased significantly ($P < 0.01$) as compared to control (C) at 24 and 48 hours of preservation. The mean percentage of intact plasma membrane found to be significantly ($P < 0.01$) higher in T3 group as compared to control (C), T1 and T2 at different hours of preservation. The mean percentage of intact plasma membrane in different hours of preservation were decreased significantly ($P < 0.01$) at control, T1, T2 and T3 group. The present finding was lower than that reported by the Perumal *et al.* (2024). Perumal *et al.* (2024) reported that 500 µg/ml of *Moringa oleifera* leaf extract showed significantly better sperm motility, viability, membrane integrity and antioxidant capacity of buck semen as compared to control. The present finding was higher than that reported by the Dina *et al.* (2021). Dina *et al.* (2021) reported that intact plasma membrane index was gradually increased compared to

control group. According to El-Sheshtawy and El-Nattat (2020) addition of different levels of *Moringa oleifera* extract after chilling and cryopreservation was able to reduce free radicals and enhanced the percentage of intact plasma membrane in cooled and cryopreserved semen.

The mean percentage of intact acrosome among the groups *i.e.*, 100 µg/ml (T1), 200 µg/ml (T2) and 300 µg/ml (T3) *Moringa oleifera* leaves extract were not significant at 0, 24 and 48 hours of preservation. The mean percentage of intact acrosome in different hours of preservation were decreased significantly ($P < 0.01$) at control (C), T1, T2 and T3 group. The mean percentage of intact acrosome found to be significantly ($P < 0.01$) higher in T3 group as compared to control (C), T1 and T2 at different hours of preservation. The present finding was in close agreement with the observation reported by Perumal *et al.* (2024). Iqbal *et al.* (2021) investigated the effects of *Moringa oleifera* leaf extract (MLE) in tris extender on post-thaw quality, antioxidant capacity and fertility of water buffalo semen and they reported that 15% MLE showed significantly improved intact acrosome compared to control. According to El-Sheshtawy and El-Nattat (2020) addition of different levels of *Moringa oleifera* extract after chilling and cryopreservation was able to enhanced acrosomal integrity of cooled and cryopreserved semen.

The mean percentage of normal sperm among the groups *i.e.*, T1, T2 and T3 groups were decreased significantly ($P < 0.01$) as compared to control (C) at 0, 24 and 48 hours of preservation.

The mean percentage of normal sperm in different hours of preservation were increased significantly ($P < 0.01$) in T3 group as compared to control (C), T1 and T2 groups. The present finding was higher than that reported by the Perumal *et al.* (2024). Perumal *et al.* (2024) reported that 500 µg/ml *Moringa oleifera* extract significantly improve normal sperm compared to other doses and the control. The present finding was in close agreement with the observation reported by the Dina *et al.* (2021). Dina *et al.* (2021) reported that *Moringa oleifera* leaves extract significantly ($P < 0.01$) decreased sperm abnormalities as compared to control group.

The mean value percentage of lipid peroxidation among the groups *i.e.*, T1, T2 and T3 were decreased significantly ($P < 0.01$) as compared to control (C) at 24 and 48 hours of preservation except at 0 hour in all the groups. The mean value of lipid peroxidation in different hours of preservation were increased significantly ($P < 0.01$) at control (C), T1, T2 and T3 group. Perumal *et al.* (2024) looked into how *Moringa oleifera* leaf extract helps boost antioxidant levels. It might even lower free radicals. This could make sperm better at fertilizing eggs and it helps protect important parts of sperm, like flagella, plasma and mitochondrial membranes. The extract has lots of good stuff like vitamins, minerals and antioxidants. Some of these are beta-sitosterol, quercetin and vitamin C. They all work together to fight off those pesky ROS and stop lipid peroxidation. By

doing this, they control oxidative stress. Dina *et al.* (2021) found that adding *Moringa oleifera* leaf extract (MOLE) at 300 and 600 mg/mL, or a mix of vitamin E and selenium at 2.5 and 5 mg/mL, to semen extenders made a big difference. It really boosted the antioxidant enzyme activities like catalase, SOD, GPx, GR and TAC in buffalo semen after it was frozen (Dina *et al.*, 2021). These additions also lowered the enzyme activities of ACP and ALP and reduced MDA levels. That showed there was less oxidative stress going on. The highest dose of 600 mg/mL MOLE led to the best antioxidant levels and the lowest signs of oxidative stress and the levels of ascorbic acid didn't change at all across any of the treatments when compared to the controls.

The conception rate among the groups *i.e.*, 100 µg/ml (T1), 200 µg/ml (T2) and 300 µg/ml (T3) *Moringa oleifera* leaves extract were increased significantly ($P < 0.01$) as compared to control (C) group. The present findings for conception rate treated with *Moringa oleifera* leaves extract were significantly ($P < 0.01$) higher as compared to that obtained in the control group. This could indicate superior keeping quality of the preserved boar semen after addition of *Moringa oleifera* leaves extract to the extender that was used for insemination. The present finding was higher with the observation reported by Chutia *et al.* (2014). The present finding was lower with the observation reported by Bresciani *et al.* (2013). Variations obtained in the present study from the findings of the earlier studies might be due to quality of extenders used, semen additives used, processing techniques applied, physiological status of the animals, methods and timing of AI and environmental and managemental factors.

The mean percentage of litter size at birth among the groups *i.e.*, 100 µg/ml (T1), 200 µg/ml (T2) and 300 µg/ml (T3) *Moringa oleifera* leaves extract were increased significantly ($P < 0.01$) as compared to control (without *Moringa oleifera* leaves extract) group. The present findings for litter size at birth treated with *Moringa oleifera* leaves extract were higher as compared to that obtained in the control group. This could indicate superior keeping quality of the preserved boar semen after addition of *Moringa oleifera* leaves extract to the extender that was used for insemination. The present finding was higher with the observation reported by Chutia *et al.* (2014). The present finding was lower with the observation reported by Feng *et al.* (2019). Variations obtained in the present study from the findings of the earlier studies might be due to quality of extenders used, processing techniques applied, physiological status of the animals, methods and timing of AI and environmental and managemental factors.

CONCLUSION

The supplementation of *Moringa oleifera* leaves extract in BTS extender at concentration of 100 µg/ml, 200 µg/ml and 300 µg/ml significantly improved the Large White Yorkshire boar's semen quality during short term preservation at 18°C as compared to control. The conception rate and average

litter size at birth among the groups were increased significantly ($P < 0.01$) as compared to control (C). The significantly ($P < 0.01$) higher conception rate and litter size at birth was recorded in T3 group *i.e.* 300 µg/ml *Moringa oleifera* leaves extract supplemented semen. In conclusion, the concentration of 300 µg/ml *Moringa oleifera* leaves extract was found to be better among other concentrations for preservation of boar semen at 18°C in a BOD incubator.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Institution Animal Ethics Committee of College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram, India.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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