*Int.J.Curr.Microbiol.App.Sci* (2020) *9*(9): 1625-1635

*International Journal of Current Microbiology and Applied Sciences*
***ISSN: 2319-7706*** **Volume 9 Number 9 (2020)**
Journal homepage: http://www.ijcmas.com

**Original Research Article** https://doi.org/10.20546/ijcmas.2020.909.202

# Antioxidant Effects of *Aloe vera* as Semen Additive in Cryopreservation of Cattle Bull Semen

**Padamveer Singh[1], Sanjay Agarwal[1*], Harjyote Singh[1], Pawan Kumar Verma[2], A. K. Pandey[1] and Sharad Kumar[3]**

*[1]Divison of Veterinary Gynaecology & Obstetrics, [2]Division of Veterinary Pharmacology and Toxicology, [3]Division of Teaching Veterinary Clinical Complex, F.V.Sc. & A.H., S.K.U.A.S.T.-J., R. S. Pura-181102, Jammu, India*

**Corresponding author*

**Keywords**

Semen, Cattle bull, *Aloe vera*, Oxidative stress

**Article Info**

***Accepted:***
15 August 2020
***Available Online:***
10 September 2020

**A B S T R A C T**

The present study was done at the Frozen Semen Laboratory, U.T. of Jammu & Kashmir, Hukkal, Jammu and Division of V.G.O., F.V. Sc & A.H., SKUAST- J, R.S. Pura, during the period between December 2017 and May 2018. This investigation was carried out as a pioneer study with the objective to study the effect of natural antioxidant *Aloe vera* as a semen additive on cryopreservation of semen. Semen samples (n=10) from mature cattle bull stationed at Frozen semen laboratory, U.T. of Jammu & Kashmir, Hukkal, Jammu, were used to evaluate the effect of *Aloe vera* additive at post-dilution and at post-thaw stage. The semen sample was extended with Tris-Egg-Yolk-Citric-acid-Fructose-Glycerol (TEYCAFG) extender and were split into two groups: Group 1: TEYCAFG without any additive/ control and group 2: TEYCAFG + *Aloe vera* (5µl/ml). Progressive motility, live spermatozoa, acrosomal integrity, sperm abnormality, hypo-osmotic swelling test (HOST) was evaluated at both post-dilution and post-thaw stage, whereas, oxidative stress tests *viz*. malondialdehyde (MDA), catalase (CAT) and superoxide dismutase (SOD) were evaluated at only post-thawed stage. Group 2 i.e. *Aloe vera* group, showed significant ($p<0.05$) increased progressive motility, live spermatozoa, acrosomal integrity and HOST positive spermatozoa, while significant ($p<0.05$) decreased sperm abnormalities in post-thawed semen. In oxidative stress evaluation the MDA level was significantly ($p<0.05$) decreased, whereas, CAT level significantly ($p<0.05$) increased in group 2 in comparison to control group. It was concluded that addition of *Aloe vera* (5µl/ml) as semen additive improves semen quality and minimize oxidative stress to the spermatozoa during cryopreservation of semen.

## Introduction

Artificial insemination is assisted reproductive technology that has made possible the effective use of best breeding bulls, thus greatly improving the genetic potential of the breeding herds (Januskauskas and Zilinskas, 2002). Cryopreservation technique has allowed specific opportunities for the cryopreservation of semen and widespread dissemination of precious genetic resources through sperm banks that

collaborate in breed improvement programme by means of artificial insemination (Shaikh, 2019; Holt, 1999).

During cooling and freezing process, sperm cells are susceptible for harmful effect from unbalanced osmotic pressure, protein denaturation, cellular acidosis, energy loss, membrane breakdown, crystallization of cell body, destabilization of the cytoskeleton and formation of free radicals or reactive oxygen species (Haris *et al.,* 2020; Urgur *et al.,* 2019).

All radicals and non-radicals from oxygen are considered reactive oxygen species (ROS), which has high electron reactivity and instability. The ROS can react with a great number of compounds, acting as donors or receivers of the electron (Agarwal *et al.,* 2005), and they are considered the main promoters of damage to living organisms (Bernard and Krause, 2007). Oxidative stress occurs when there is an imbalance between the production of ROS and the protective action of the antioxidant system, responsible for its neutralization and removal (Pinto *et al.,* 2019; Jedrzejowska *et al.,* 2013).

To avoid oxidative stress, the cells have a defense system compound by antioxidants, which are divided into enzymatic and non-enzymatic substances with low molecular weight (Halliwell and Gutteridge, 1999). However, during semen cryopreservation, antioxidant concentrations are reduced during the dilution stage, which results in the imbalance and consequently in cellular and oxidative stress (Pinto *et al.,* 2019; Bilodeau *et al.,* 2000).

The plasma membrane of sperm cells had supplementary content of unsaturated fatty acids and their cytoplasmic components are deficient in oxidants. Therefore, sperm cells are highly susceptible to lipid peroxidation (LPO) in presence of ROS, leading to impaired function activity (Shaikh, 2019; Shaikh *et al.,* 2016, Hu *et al.,* 2010, Hu *et al.,* 2009).

The *Aloe vera* plant has been known and used for centuries for its health, beauty, medicinal and skin care properties. 2000 years ago, the Greek scientists regarded *Aloe vera* as the universal panacea. The Egyptians called Aloe "the plant of immortality". There are over 300 Aloe species, which have been used in cultures like: Greece, Egypt, India, Mexico, Japan and China (Marshall, 1990). Studies on *Aloe vera* have reported for its anti-diabetic, anti-cancer, antibiotic, hypotensive, hepatoprotective and blood purifying properties (Fakhrildin and Sodani, 2014; Kosif *et al.,* 2008; Tiwari and Rao, 2002).

To best of our knowledge, no study has been done for investigation of the role of addition of *Aloe vera* in semen of cross-bred bull. In continuation to our previous reported findings in cryopreservation of cross-bred cattle bull semen (Singh *et al.,* 2020) the present study was designed with the objective to evaluate the oxidative stress to the spermatozoa during cryopreservation of semen and the effects of *Aloe vera* as semen additive.

## Materials and Methods

The present study was undertaken with the objective to determine anti-oxidant effect of *Aloe vera* during cryopreservation of cattle bull semen. Semen samples (n=10) were collected twice a week from mature cattle bull randomly by artificial vagina method. The bulls were kept at Frozen semen laboratory, U.T. of Jammu & Kashmir, Hukkal, Jammu, where collection of the semen, initial evaluation, extension and freezing of the semen took place, while the post-thawed semen samples were evaluated at the Division of Veterinary Gynaecology and Obstetrics

and Division of Pharmacology and Toxicology, F.V.Sc & A.H., SKUAST- J, R.S.Pura. The present study was undertaken during the period between December 2017 and May 2018. The fresh semen samples were evaluated for volume, mass activity (Tomar *et al.*,1966), spermatozoa concentration using Accucell photometer (IMV Technologies, France) and progressive motility (Salisbury *et al.*, 1978). The semen samples qualifying the minimum initial standards were further processed. The qualified samples were extended with tris egg yolk citric acid fructose glycerol (TEYCAFG) kept at 37°C. The semen samples were extended in such a way to yield approximately 40 million spermatozoa/ml (Anel *et al.*, 2003). The extended semen samples were split into two parts. The first part was considered as group 1: TEYCAFG without any additive/ control and group 2: TEYCAFG + *Aloe vera* (5µl/ml). The pH was adjusted within the range 7.2 to 7.4. The semen samples were filled in 0.5 ml capacity, polyvinyl straw (IMV, France). The equilibration was done at 4° C for 4 hours and freezing of the straws was done as per standard protocol of the lab using programmable biofreezer (Digit Cool-530, IMV Technologies, France) with freezing rate 4°C to -10°C @ 5°C, -10°C to -100°C @ 40°C, -100°C to -140°C @ 20°C, after attainment of -140°C temperature straws were plunged in liquid nitrogen and stored in liquid nitrogen container pending future evaluation. Thawing was done at 37° C for 30 seconds for evaluation of post-thawed samples.

The processed semen samples (n=10) were evaluated at two stages post-dilution and post thaw stage. At post-dilution stage the per cent progressive motility, live spermatozoa & acrosomal integrity as per Salisbury *et al.*, (1978), sperm abnormalities (Kumar, 1993) and hypo-osmotic swelling test (Jeyendran *et al.*,1984), whereas, at post-thaw stage in addition to above parameters done at post-dilution stage it was also evaluated for oxidative stress test *viz*. Malondialdehyde (MDA) as described by Shafiq-ur-Rehman (1984); Catalase as per Aebi (1983) and Superoxide dismutase (SOD) as described by Marklund and Marklund (1974). The results were analysed statistically using Analysis of Variance (ANOVA) (Snedecor and Cochran, 1989).

## Results and Discussion

In the present investigation EY-tris (egg yolk-tris-citric acid-fructose-glycerol) was used as control dilutor, EY-tris plus *Aloe vera* (5µl/ml) were used as experimental groups.

## Progressive motility

The per cent progressive motility (Mean ± SE) post-dilution and post-thawing in control group were 67.0 ± 1.52 and 48.0 ± 2.49; whereas in *Aloe vera* added group were 69.0 ± 3.14 and 55.0 ± 2.24, respectively (Table 1). Perusal of the table (1) it was observed that the progressive motility (%) differed non significantly between post-diluted control and *Aloe vera* added group, whereas, in post-thaw a significant ($p<0.05$) difference in progressive motility was observed between control and *Aloe vera* group.

In the present study in control group the per cent progressive motility of diluted semen (Table 1) was 67.0 ±1.52 which was in nearly similar to the findings of Pathak *et al.*, 1990; 65.30 ± 1.20 per cent in diluted semen of cattle bull. Though the higher per cent progressive motility in the diluted semen was reported by Rao *et al.*, 1996; 76.90 ± 0.87 and Gupta *et al.*, 1990; 73.23 ± 0.38. In the present study per cent post thaw motility in control group (Table 1) was 48.0 ± 2.49 which was less than reported by Kishore (2009), 80.74; Pawshe *et al.*, (2016), 61.7 ±

2.6; , Uysal *et al.,* (2007), 55.5 ± 2.5 and Ulfina and Raina (2003), 55.34 ± 1.02, while it was higher than reported by Hu *et al.,* (2010), 36.88 ± 1.53 and Li *et al.,* (2016), 14.7 ± 1.4. The main reason for the decrease in the per cent post thaw progressive motility might be due to cryopreservation damage, ROS production and damage caused due to formation of ice crystal formation in mitochondria and Axomemes during cryopreservation that impairs sperm motility.

## Live spermatozoa

The per cent live spermatozoa (Mean ± SE) post-dilution and post-thawing in control group were 74.4 ± 1.82 and 50.8 ± 1.17; whereas in *Aloe vera* group were 76.2 ± 2 and 59.0 ± 1.65, respectively (Table 1). Perusal of the table (1) it was observed that the live spermatozoa (%) differed non significantly between post-diluted control and *Aloe vera* group, whereas, in post-thaw a significant ($p<0.05$) difference in live spermatozoa was observed between control and *Aloe vera* group.

In the present study in the control group values of per cent live spermatozoa in diluted semen (Table 1) was 74.4 ± 1.82, which was in agreement with the findings of Keshava (1996), 74.28; Abdel-khalek *et al.,* (2008), 73.0 ± 1.3 and Ulfina and Raina (2003), 72.44 ± 0.51. In the present study the values of per cent live spermatozoa of post thaw semen of control group (Table 1) was 50.8 ± 1.17 which was less than reported by Bhalde *et al.,* (1991), Rao *et al.,* (2017) and Abdel-khalek *et al.,* (2008) who reported 57.64 ± 0.78, 56.24 ± 0.01 and 66-73 per cent, respectively. Decreasing propotion of live spermatozoa in post thaw semen may be due to cryo damage caused to formation of ice crystals in extra and intracellular environment, increasing solute concentration (Mazur, 1984) and sperm susceptibility for freezing and thawing temperature, ROS production and lipid peroxidation (Bucak *et al.,* 2008)

## Acrosomal integrity

The per cent acrosomal integrity (Mean ± SE) post-dilution and post-thawing in control group were 79.7 ± 1.52 and 53.9 ± 0.94; whereas in *Aloe vera* group were 81.2 ± 1.34 and 67.6 ± 1.43, respectively (Table 1). Perusal of the table (1) it was observed that the acrosomal integrity (%) differed non significantly between post-diluted control and *Aloe vera* group, whereas, in post-thaw a significant ($p<0.05$) difference in acrosomal integrity was observed between control and *Aloe vera* group.

The evaluation of acrosomal integrity is important for determination of semen quality as enzyme necessary for fertilization present in the acrosomal cap. In the present study the values of per cent acrosomal integrity of diluted semen of control group (Table 1) was 79.7 ± 1.52, which was in agreement with the finding of Lone *et al.,* (2017), 80.17 ± 3.26, whereas it was less than the finding of Rao *et al.,* (2017), 85.77 ± 0.01. In the present study the values of per cent acrosomal integrity of post thaw semen of control group (Table 1) was 53.9 ± 0.94 which was in agreement with the finding of Rao *et al.,* (2017), 56.24 ± 0.01 in crossbred bull, however it was higher than the finding of Sandeep *et al.,* (2015) and Paudel *et al.,* (2010) which were reported as 36.25 ± 0.91 and 31.5 ± 1.3, respectively.

## Sperm abnormalities

The per cent sperm abnormalities (Mean ± SE) post-dilution and post-thawing in control group were 7.2 ± 0.63 and 16.8 ± 0.61; whereas in *Aloe vera* group were 7.9 ± 0.97 and 14.2 ± 0.96, respectively (Table 1). Perusal of the table (1) it was observed that the sperm abnormalities (%) differed non

significantly between post-diluted control and *Aloe vera* group, whereas, in post-thaw a significant ($p<0.05$) difference in sperm abnormalities was observed between control and *Aloe vera* group.

In the present study the values of per cent sperm abnormalities in diluted semen of control group (Table 1) was 7.2 ± 0.63, which was in agreement with the finding of Rao *et al.,* (2017), 7.28 ± 0.02 in crossbred bull, whereas, it was higher than as reported by Mittal *et al.,* (2014), 4.91 ± 0.14 in bhadawari bull, while it was lower than as reported by Gupta *et al.,* (1990), 9.14 ± 0.09 in cattle bull. In the present study per cent post thaw sperm abnormalities in control group (Table 1) was 16.8 ± 0.61 which was in agreement with the finding of Rao *et al.,* (1999), range of 15.66 ± 0.39 to 16.75 ± 0.37 in Jersey bulls, while it was higher than as reported by Mittal *et al.,* (2014), 7.75 ± 0.17 in Bhadawari bull; Sariozkan *et al.,* (2009a), 14.93 ± 1.07 in cattle bull and Sariozkan *et al.,* (2009b), 15.0 ± 1.1 in cattle bull, whereas lower than the finding of Gupta *et al.,* (1990) and Rao *et al.,* (2017) who reported as 17.63 ± 1.99 and 18.36 ± 0.04 in crossbred bull, respectively.

## Hypo-osmotic swelling test (HOST)

The hypo-osmotic swelling test positive spermatozoa per cent (Mean ± SE) post-dilution and post-thawing in control group were 71.0 ± 1.10 and 45.4 ± 0.93; whereas in *Aloe vera* group were 72.5 ± 1.89 and 55.1 ± 0.99, respectively (Table 1). Perusal of the table (1) it was observed that the hypo-osmotic swelling test positive spermatozoa (%) differed non significantly between post-diluted control and *Aloe vera* group, whereas, in post-thaw a significant ($p<0.05$) difference in hypo-osmotic swelling test positive spermatozoa was observed between control and *Aloe vera* group.

In the present study the per cent hypo-osmotic swelling test (HOST) positive spermatozoa of diluted semen in control group (Table 1) was 71.0 ± 1.10 which was in agreement with the findings of Rao *et al.,* (2017) and Lone *et al.,* (2017) who reported as 72.54 ± 0.01 in crossbred bull and 70.91 ± 5.92 in buffalo bull, however lower values of per cent hypo-osmotic swelling test (HOST) of diluted semen was observed by Kumar *et al.,* (2018) who reported as 62.05 ± 0.80 in pure Jersey bulls and 62.11 ± 0.89 in crossbred Jersey bulls. In the present study the values of per cent hypo-osmotic swelling test (HOST) of post thaw semen of control group (Table 1) was 45.4 ± 0.93, which was agreement with the finding of Kumar *et al.,* (2018), 47.27 ± 1.05 in pure Jersey bulls; 45.94 ± 1.33 crossbred Jersey bulls and Sariozkan *et al.,* (2009a) who reported as 43.70 ± 1.96 in cattle bull, while lower values were observed by Paudel *et al.,* (2010), Rao *et al.,* (2017) and Taraphdar (1999) who reported as 39.6 ± 1.3 in crossbred bulls, 38.73 ± 0.01 in crossbred bull and 36.52 in cattle bull, respectively. Whereas, higher finding was observed by Lone *et al.,* (2017), 51.30 ± 4.43 in buffalo bull and Sohail *et al.,* (2015), 49.97 ± 3.62 in sahiwal cattle bull. The structural changes produced in the post thaw sperm cells membrane are primarily linked to altered abilities for energy sourcing which later on influence both cellular metabolism and other sperm functions (Dziekonska *et al.,* 2009; Gillan *et al.,* 2004).

## Malondialdehyde (MDA)

The MDA levels (Mean ± SE) post-thaw semen samples in control group and *Aloe vera* group were 3.49 ± 0.19 and 1.71 ± 0.04, respectively (Table 2). Perusal of the table (2) it was observed that MDA levels in post-thaw semen samples differed significantly ($p<0.05$) between control and *Aloe vera* group.

In the present study MDA level (nmol $H_2O_2$ produced/ $10^8$ spermatozoa) in post thaw semen of control group (Table 2) was 3.49 ± 0.19 which was lower than the value observed by Paudel *et al.*(2010), 712.1 ± 49.1 nmol MDA level/$10^9$ spermatozoa in crossbred bull and Lone *et al.*, (2017), 496.02 ± 39.28 nmol MDA level/$10^9$ spermatozoa in buffalo bull. The finding of other scientists could not be compared with our finding due to disparity in method of evaluation and units of measurement.

## Catalase

The catalase levels (Mean ± SE) post-thaw semen samples in control group and *Aloe vera* added group were 1.18 ± 0.36 and 1.41 ± 0.48, respectively (Table 2). Perusal of the table (2) it was observed that catalase levels in post-thaw semen samples differed significantly ($p<0.05$) between control and *Aloe vera* group.

In the present study catalase activity (µmol $H_2O_2$ decomosed/ min/ $10^8$ spermatozoa) in post thaw semen of control group (Table 2) was 1.18 ± 0.31 which was higher than finding of Zhao *et al.*, (2015), 1.16 ± 0.82 (U/ml) in Qinchuan bulls, whereas lower than the finding of Sharma *et al.*, (2016) who reported as 23.36 ± 0.25 in rainy season, 24.25 ± 0.30 winter season and 24.22 ± 0.56 in summer season in buffalo bull. There was reduction in the catalase activity with the level of freezing.

CAT activity reduced at post thaw stage when compared to pre freeze stage (Lone *et al.*, 2017). CAT is an enzymatic antioxidant found in all living organisms exposed to oxygen, which decomposes harmful peroxides and converts them into water and oxygen (Chelikani *et al.*, 2004).

## Superoxide dismutase (SOD)

The SOD levels (Mean ± SE) post-thaw semen samples in control group and *Aloe vera* added group were 0.95 ± 0.06 and 1.26 ± 0.06, respectively (Table 2). Perusal of the table (2) it was observed that SOD levels in post-thaw semen samples differed significantly ($p<0.05$) between control and *Aloe vera* group.

The level of Superoxide dismutase (Unit/ $10^8$ spermatozoa) in present study in post thaw semen of control group (Table 2) was 0.95 ± 0.06 which was lower than the finding of Nair *et al.*, (2006) who reported as 3.50 ± 0.19 in cattle and 1.98 ± 0.09 in buffalo bulls, however it was higher than the finding of Lone *et al.*, (2017) who reported as 0.16 ± 0.03 (units/mg protein) in buffalo bulls. Superoxide is a free radical which is converted to oxygen and hydrogen peroxide by the dismutation action of antioxidant enzyme superoxide dismutase (Tariq *et al.*, 2015).

## Effects of *Aloe vera*

In the present study in *Aloe vera* added group the per cent progressive motility, live spermatozoa, acrosomal integrity, sperm abnormalities and hypo-osmotic swelling test (HOST) positive spermatozoa of diluted semen and post-thawed semen were recorded as 69.0 ± 3.14 and 55.0 ± 2.24; 76.2 ± 2 and 59.0 ± 1.65; 81.2 ± 1.34 and 67.6 ± 1.43; 7.9 ± 0.97 and 14.2 ± 0.96; 72.5 ± 1.89 and 55.1 ± 0.99, respectively (Table 1).

In attempt to study the oxidative stress MDA, Catalase and SOD levels were studied. In *Aloe vera* added group the (Mean ± SE) of MDA, Catalse and SOD levels of post-thawed samples were recorded as 1.71 ± 0.04, 1.41 ± 0.48 and 1.26 ± 0.06, respectively (Table 2).

**Table.1** The effect of *Aloe vera* on different physio-morphological characteristics at post-dilution and post-thaw semen of cross-bred bulls

| Parameter (%) | Stage | Control | *Aloe vera* (5µl/ml) |
|---|---|---|---|
| **Progressive motility** | Post-dilution | 67.0 ± 1.52[a] | 69.0 ± 3.14[a] |
| | Post-thaw | 48.0 ± 2.49[a] | 55.0 ± 2.24[b] |
| **Live spermatozoa** | Post-dilution | 74.4 ± 1.82[a] | 76.2 ± 2[a] |
| | Post-thaw | 50.8 ± 1.17[a] | 59.0 ± 1.65[b] |
| **Acrosomal integrity** | Post-dilution | 79.7 ± 1.52[a] | 81.2 ± 1.34[a] |
| | Post-thaw | 53.9 ± 0.94[a] | 67.6 ± 1.43[b] |
| **Sperm abnormalities** | Post-dilution | 7.2 ± 0.63[a] | 7.9 ± 0.97[a] |
| | Post-thaw | 16.8 ± 0.61[a] | 14.2 ± 0.96[b] |
| **HOST** | Post-dilution | 71.0 ± 1.10[a] | 72.5 ± 1.89[a] |
| | Post-thaw | 45.4 ± 0.93[a] | 55.1 ± 0.99[b] |

Values are given as mean ± SE of 10 animals unless and otherwise stated
Values bearing different superscripts in a row differ significantly ($p<0.05$)

**Table.2** The effect of *Aloe vera* on malondialdehyde (MDA), catalase (CAT) and superoxide dismutase (SOD) activity in post-thawed semen of cross-bred bulls

| Oxidative stress test | Control | *Aloe vera* (5µl/ml) |
|---|---|---|
| **MDA (nmol/$10^8$ Spermatozoa)** | 3.49 ± 0.19[a] | 1.71 ± 0.04[b] |
| **CAT (µmol/$10^8$ Spermatozoa)** | 1.18 ± 0.36[a] | 1.41 ± 0.48[b] |
| **SOD (U/ $10^8$ Spermatozoa)** | 0.95 ± 0.06[a] | 1.26 ± 0.06[a] |

Values are given as mean ± SE of 10 animals unless and otherwise stated
Values bearing different superscripts in a row differ significantly ($p<0.05$)

It was observed that in *Aloe vera* added group the per cent progressive motility, live spermatozoa, acrosomal integrity, sperm abnormalities and hypo-osmotic swelling test (HOST) positive spermatozoa of diluted semen differed non significantly between post-diluted control and *Aloe vera* added group, whereas, in post-thaw a significant ($p<0.05$) difference in per cent progressive motility, live spermatozoa, acrosomal integrity, sperm abnormalities and hypo-osmotic swelling test (HOST) positive spermatozoa was observed between control and *Aloe vera* group (Table 1). In the present study the in post-thawed sample the MDA, Catalse and SOD levels differed significantly ($p<0.05$) between control and *Aloe vera* group (Table 2).

*Aloe vera* is a pharmaceutical plant which can be useful for curing various diseases and improving body's physiology. It can be used as a natural antioxidant with high potential of reducing fats oxidation and oxidative stresses (Vinson *et al.*, 2005). Though multiple uses of *Aloe vera* as antioxidant, therapeutic agents etc. has been reported in humans and animals, but its use as semen additive and that to as an antioxidant is completely missing.

Fakhrildin and Sodani (2014) worked on human sperm and observed a beneficial role of antioxidative phenolic compound in *Aloe vera* on human semen and its effects on lipid peroxidation in their study 5µl of *Aloe vera* extract was mixed with 1 ml of each semen sample and then the sperm parameter

including concentration, motility, morphology, agglutination, viability and HOS test were recorded.

This was the only report of *Aloe vera* where it was used as semen additive. It formed the basis of our curiosity to use *Aloe vera* in our experiment and to evaluate its antioxidant property and it was observed that though the *Aloe vera* added diluted semen didn't showed any improvement in the semen parameters, but the significant ($p<0.05$) improvement was recorded in semen parameter i.e. per cent progressive motility, live spermatozoa, acrosomal integrity, sperm abnormalities, hypo-osmotic swelling test (HOST) positive spermatozoa as well as MDA, Catalase, SOD levels.

In the absence of the reports of *Aloe vera* use as semen additive our readings could not be compared and discussed, but its beneficial effect as semen additive in post-thawed semen is noticeable and further studies should be done with other possible concentrations and complete semen analysis.

In conclusion, it was the pioneer study in which due to antioxidant property of *Aloe vera* it was tried as a semen additive. Due to its beneficial effects in the cryopreserved semen it may be used for the cryopreservation of the semen. The parameters of semen quality improved in the *Aloe vera* added cryopreserved semen in comparison of control group. The oxidative stress to the semen is also minimized after addition of the *Aloe vera*. The *in vitro* fertility assessed by hypo-osmotic swelling test also indicates for the better quality and fertility in *Aloe vera* added cryopreserved semen, however, the larger number of the semen samples along with other concentrations of *Aloe vera* coupled with field fertility trials should be done in the cattle population for its final validation.

## References

Abdel-Khalek, A.E., Aboul-Ela, S.M.B., Fawzy, A.Q. and Dandooush, E. 2008. Semen quality of holstein and buffalo bulls after filtration using sepahdex column. J. Bio. Sci., 15: 91-97.

Aebi, H. 1983. Catalase. In: Methods in Enzymatic Analysis. Bergmeyer HU (ed). Academic Press, New York. pp 276-286.

Agarwal, A., Gupta, S. and Sharma, R.K. 2005. Role of oxidative stress in female reproduction. Reprod. Biol. Endocrinol., 3: 28-49.

Anel, L., DePaz, P., Alvarez, M., Chamorro, C.A., Boixo, J.C., Manso, A., Gonzalez, M., Kaabi, M. and Anel, E. 2003. Field and *in vitro* assay of three methods for freezing ram semen. Theriogenology, 60(7): 1293 -1308.

Bernard, K. and Krause, K.H. 2007. The NOX family of ROS-Generating NADPH oxidases: Physiology and Pathophysiology. Physiol. Rev., 87: 245-313.

Bhalde, R.M., Hukeni, V.B. and Deopaskas, V.L. 1991. Post thaw keeping quality of frozen bull semen maintained at room/ chilled temperature. Indian J. Anim. Reprod., 12(1): 87-90.

Bilodeau, J.F., Chaterjee, S. and Sirrard, M. 2000. Levels of antioxidant defenses are decreased in bovine spermatozoa after a cycle of freezing and thawing. Mol. Reprod. Dev., 55: 282-288.

Bucak, M.N., Atessahin, A. and Yuce, A. 2008. Effect of anti-oxidants and oxidative stress parameters on ram semen after the freeze-thawing process. S. Rum. Res., 75(2): 128-134.

Chelikani, P., Fita, I., and Loewen, P.C. 2004. Diversity of structures and properties among catalases. Cel. Mol. L. Sci., 61: 192–208.

Dziekonska, A., Fraser, L. and Strzeżek, J. 2009. Effect of different storage temperatures on the metabolic activity of

spermatozoa following liquid storage of boar semen. J. Anim. and Feed Sci., 18: 638-649.

Fakhrildin, M.B. and Sodani, F.I.J. 2014. Effect of *Aloe vera* on its *in vitro* human sperm parameters for asthenozoospermic patients. J. Thi-Qar. Sci., 5(1): 8-13.

Gillan, L., Maxwell, W.M.C. and Evans, G. 2004. Preservation and evaluation of semen for artificial insemination. Reprod. Fertil. Dev., 16: 447-454.

Gupta, H.P. Saxena, V.B. and Tripathi, S.S. 1990. A rapid method for evaluation of semen quality in bulls. Indian J. Anim. Sci., 60(3): 329-330.

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

Haris, F.Z., Ondho, Y.S. and Samsudewa, D. 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.

Holt, W.V. 1997. Alternative strategies for long-term preservation. Reproduction, Fertility and Development, 9: 309-319.

Hu, J.H., Li, Q.W., Jiang, Z.L., Yang, H., Zhang, S.S. and Zhao, H.W. 2009. The cryoprotective effect of trehalose supplementation on boar spermatozoa quality. Reproduction in Domestic Animals, 44(4): 571-575.

Hu, J.H., Zan, L.S., Zhao, X.L., Li, Q.W., Jiang, Z.L., Li, Y.K. and Li, X. 2010. Effects of trehalose supplementation on semen quality and oxidative stress variables in frozen-thawed bovine semen. J. Aim. Sci., 88(5): 1657-1662.

Januskauskas, A. and Zilinskas, H. 2002. Bull semen evaluation post-thaw and relation of semen characteristics to bull's fertility. Veterinarija IR Zootechnika T., 17(39): 1-12.

Jedrzejowska, R.W., Wolski, J.K. and Hilczer, J.S. 2013. The role of oxidative stress and antioxidants in male fertility. Cent. Eur. J. Urol., 66: 60-67.

Jeyendran, R.S., Vander ven, H.H., Perez-Pelaez, M., Carbo, B.G. and Zanfeld, L.J.D. 1984. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. J. Repro. Fertil., 70: 219-228.

Keshava. P. 1996. Studies on seminal attributes and their association with expected breeding value of dairy bulls. M.Sc. Thesis, NDRI (Deemed University), Karnal, Haryana.

Kishore, A. 2009. Effect of taurine and other additives on preservability of bull semen, PhD thesis, NDRI, Karnal.

Kosif, R., Akta, G., Oztekin, A. 2008. Microscopic examination of placenta of rats prenatally exposed to Aloe barbadensis: A Preliminary Study. Int. J. Morphol., 26(2): 275-281.

Kumar, H., Srivastava, S., Kumar, R., Kumar, R. and Singh, K.D. 2018. Effect of ascorbic acid on storage capacity of Murrah bull epididymal spermatozoa at refrigerator temperature. Int. J. Curr. Microbiol. App. Sci., 7: 4380-4386.

Kumar, M. 1993. Sexual behaviour pattern in sahiwal and murrah bulls. M.Sc. thesis, NDRI, Karnal.

Li, Y., Kalo, D., Zeron, Y. and Roth, Z. 2016. Progressive motility-a potential predictive parameter for semen fertilization capacity in bovines. Zygote, 24(1): 70-82.

Lone, S.A., Prasad, J.K., Ghosh, S.K., Das, G.K., Balamurugan, B. and Verma, M.R. 2017. Study on correlation of sperm quality parameters with antioxidant and oxidant status of buffalo bull semen during various stages of cryopreservation. Andrologia, 50(4): 1297.

Marklund, S. and Marklund, G. 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J.Biochem., 47(3): 469–474.

Marshall, J.M. 1990. *Aloe vera* gel. What is the

evidence? Pharma J., 24: 360.

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

Mittal, P.K., Anand, M., Madan, A.K., Yadav, S. and Kumar, J. 2014. Antioxidative capacity of vitamin E, vitamin C and their combination in cryopreserved Bhadavari bull semen. Vet. World, 7: 1127-1131.

Nair, S.J., Brar, A.S., Ahuja, C.S., Sangha, S.P.S. and Chaudhary, K.C. 2006. A comparative study on lipid peroxidation, activities of antioxidant enzymes and viability of cattle and buffalo bull spermatozoa during storage at refrigeration temperature. Reprod. Sci., 96(1): 21-29.

Pathak, N., Bunzamin, B.R., Mohan, G. and Sahni, K.L. 1990. Libido in relation to other reproductive trait among the crossbred bulls. Indian J. Anim. Sci., 60(11): 52-54.

Paudel, K.P., Kumar, S., Meur, S.K. and Kumaresan, A. 2010. Ascorbic acid, catalase and chlorpromazine reduce cryopreservation-induced damages to crossbred bull spermatozoa. Reprod. domest. Anim., 45(2): 256-262.

Pawshe, M., Bibin, B., Becha ,Unnikrishnan, M.P., Harshan, H.M. and Ghosh, K.N.A. 2016. Bovine semen thawed at 28ºc or 37ºc can be used for ai till 90 min post-thaw. Thaw. Bo. sem., 32: 9-11.

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

Rao, A.V.N., Sreemanarayana, O. and Rao, C.V. 1996. Studies on sex behaviour and seminal traits in Ongole, Jersey, Jersey x Ongole and Murrah bulls. Indian Vet. J., 73: 284-287.

Rao, K.B., Naidu, K.V., Singh, V., Rao, A.S. and Suresh, J. 1999. Studies on semen characteristic of Punganur bulls. Indian Vet. J., 51: 889-900.

Rao, T.K.S., Mohanty, T.K. and Bhakat, M. 2017. Assessment of antioxidants for preservation of crossbred bull semen in Tris based extender. Indian J. Anim. Res., 51(6): 993-997.

Salisbury, G.W., Van Denmark, N.L. and Lodge, J.R. 1978. Principles and techniques of freezing spermatozoa. In: Physiology of Reproduction and Artificial Insemination of Cattle. Saliburg, G.A. (edt.), San Francisco: W.H Freeman Co. pp: 454-459.

Sandeep, P.S., Virmani, M. and Malik, R.K. 2015. Effect of vitamin c on the seminal and biochemical parameters of Murrah buffalo bull semen during different stages of freezing. Haryana Veterinarian, 54(1): 15-18.

Sariozkan, S., Bucak, M.N., Tuncer, P.B., Ulutas, P.A. and Bilgen, A. 2009a. The influence of cysteine and taurine on microscopic-oxidative stress parameters and fertilizing ability of bull semen following cryopreservation. Cryobiology, 58(2): 134-138.

Sariozkan, S., Bucak, M.N., Tuncer, P.B., Ulutas, P.A. and Bilgen, A. 2009b. influence of various antioxidants on microscopic-oxidative stress indicators and fertilizing ability of frozen thawed bull semen. Acta Vet. Brno., 78: 463-469.

Shafiq-ur-Rehman. 1984. Lead induced regional lipid peroxidation in brain. Toxicology Letters, 21(3): 333-337.

Shaikh, K.Q. 2019. Estimation of oxidative stress in indigenous bull's semen during cryopreservation following incorporation of trehalose. International Journal of Life Sciences, Special Issue A13: 117-121.

Shaikh, K.Q., Nakhashi, H.C., Suthar, B.N., Sutaria, P.T. and Sharma, V.K. 2016. Comparison of fresh semen parameters with frozen thawed semen following incorporation of trehalsoe. Life Sciences Leaflets, 76: 107-115.

Sharma, L., Pandey, V., Nigam, R., Saxena, A.,

Swain, D.K. and Yadav. B. 2016. Association of oxidative status and semen characteristics with seminal plasma proteins off buffalo semen. Iranian J. Vet. Res., 17(4): 226-230.

Singh, P., Agarwal, S., Singh, H., Singh, S., Verma, P.K., Butt, M. S. and Sharma, U. 2020. Effects of Ascorbic acid as antioxidant semen additive in cryopreservation of cross-bred cattle bull semen. Int. J. Microbiol. App. Sci., 9(07): 3089-3099.

Snedecor, G. W and Cochran, W. G. 1989. Statistical Methods, 8th edn., Iowa State University Press, USA.

Sohail, M.U., Shahzad, A.H., Iqbal, S., Shabbir, M.S., Iqbal, Z., Abbas, S. and Arshaad, T. 2015. Ascorbic acid inclusion in semen extender improves the post-thawed semen quality of Sahiwal cattle (*Bos indicus*). Pak. J. Zool., 47(6): 1571-1577.

Taraphdar, S. 1999. Studies on initial semen quality, freezability and fertility performance of Murrah buffalo bulls. M.Sc. thesis NDRI (Deemed Univrsity), Karnal, Haryana.

Tariq, M., Khan, M.S., Shah, M.G., Nisha, A.R., Umer, M., Hasan, S.M., Rahman, A. and Rabbani, I. 2015. Exogenous antioxidants inclusion during semen cryopreservation of farm animals. J. Chem. and Pharma. Res., 7(3): 2273-2280.

Tiwari, A.K. and Rao, M. 2002. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. Curr. Sci., 83: 30-38.

Tomar, N.S., Mishra, B.S. and Johari, C.B. 1966. Seasonal variations in reaction time and semen production, and prediction of some semen attributes on initial motility of spermatozoa in Hariana and Murrah bulls. Indian J. Dairy Sc., 19(1):87-93.

Ulfina, G. and Raina, V.S. 2003. Seminal attributes related to age and scrotal circumference in dairy bulls. Indian J. Dairy Sci., 56(6): 376-379.

Urgur, M.R., Abdelrahman, A.S., Evans, H.C., Gilmore, A.A, Hitit, M., Arifiantini, R.I., Purwantara, B., Kaya, A. and Memili, E. 2019. Advances in cryopreservation of bull sperm. Front. Vet. Sci., 6(268): 1-15.

Uysal, O., Buck, M.N., Yavas, I. and Varisli. O. 2007. Effect of various antioxidants on the Quality of frozen thawed bull semen. J. Anim. Vet. Ad., 6(12): 1362-1366.

Vinson, J.A., Al Kharrat, H. and Andreoli, L. 2005. Effect of *Aloe vera* preparations on the human bioavailability of vitamin C and E. Phytomedicine, 12: 760-765.

Zhao, X.L., Li, Y.K., Cao, S.J., Hu, J.H., Wang, W.H., Hao, R.J. and Zan, L.S.2015. Protective effects of Ascorbic acid and Vitamin E on antioxidant enzyme activity of freeze-thawed semen of Qinchuan bulls. Genet. Mol. Res., 14(1): 2572-2581.

**How to cite this article:**

Padamveer Singh, Sanjay Agarwal, Harjyote Singh, Pawan Kumar Verma, A. K. Pandey and Sharad Kumar. 2020. Antioxidant Effects of *Aloe vera* as Semen Additive in Cryopreservation of Cattle Bull Semen. *Int.J.Curr.Microbiol.App.Sci.* 9(09): 1625-1635.
doi: https://doi.org/10.20546/ijcmas.2020.909.202