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Preservative effects of cysteine on sperm viability of West African dwarf goat bucks chilled at 4°C

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Abstract

Genetic improvement of goats requires the selection of superior breeding stock and the application of artificial insemination technique. The successes of artificial insemination in goats generally depend on knowledge of semen preservation and insemination techniques. In this study, the preservative effects of cysteine in Tris-pumpkin seed milk based extender on the viability of refrigerated spermatozoa of the West African dwarf (WAD) bucks were investigated. Pooled semen from WAD bucks was diluted in Tris-pumpkin seed milk based extender containing cysteine at 0mM, 2mM, 4mM, 6mM and 8mM/100L respectively. Microscopic assessments of diluted semen samples were carried out on sperm progressive motility, acrosome and membrane integrities, livability, sperm abnormality, arginase and acrosin activities, leukocytes, in vitro sperm capacitation and acrosome reaction after in vitro storage at 4°C. The concentration of malondialdehyde (MDA) in the stored semen was measured in thiobarbituric acid reactive substances. The results showed that Tris based extender supplemented with 6 mM and 8 mM inclusion levels of cysteine had highest ($P<0.05$) percentage sperm progressive motility, membrane integrity and acrosome integrity compared to other inclusion levels and the control group. The results showed highest ($P < 0.05$) percentage live spermatozoa in extender supplemented with 8 mM inclusion levels of cysteine. However, Spermatozoa cryopreserved with Tris based extender supplemented with 6 mM and 8 mM inclusion levels of cysteine had lowest ($P < 0.05$) percentage abnormality. The results showed lowest ($P < 0.05$) concentrations of MDA at, 6 mM and 8 mM inclusion levels of cysteine and lowest ($P < 0.05$) concentrations of leukocyte at 4mM, 6 mM and 8 mM inclusion levels of cysteine. However, Tris-pumpkin seed milk based extender supplemented with 2 mM inclusion levels of cysteine had highest ($P < 0.05$) values for arginase activity compared to other inclusion level and the control. However, 8 mM inclusion of cysteine had highest ($P < 0.05$) percentage of spermatozoa that underwent acrosome reaction and invitro capacitation compared to other inclusion level and the control group. The findings documented the preservative potential of cysteine on sperm viability of chilled semen stored at 4°C.

Keywords: Antioxidants, West African dwarf buck (WAD),cysteine, sperm viability

Effets de conservation de la cystéine sur la viabilité du sperme des boucs de chèvre naine de l'Ouest africains refroidis à 4 ° C

Résumé

L'amélioration génétique des chèvres nécessite la sélection d'actions de reproduction supérieure et de l'application de la technique d'insémination artificielle. Les succès de l'insémination artificielle chez les chèvres dépendent généralement de la connaissance des techniques de préservation du sperme et d'insémination. Dans cette étude, les effets de

conservation de la cystéine dans l'extension à base de lait de graines de tris-citrouille sur la viabilité des spermatozoïdes réfrigérés des boucs de nains d'Afrique de l'Ouest (NAO) ont été étudiés. Le sperme combiné de boucs de NAO a été dilué dans l'extendant à base de lait de trémie Tris-citrouille contenant de la cystéine à 0 mm, de 2 mm, de 4 mm, de 6 mm et de 8 mm / 100l respectivement. Les évaluations microscopiques des échantillons de sperme dilués ont été réalisées sur la motilité progressive du sperme, l'acrosome et les intégrités de la membrane, la vieillabilité, l'anomalie de sperme, l'arginase et les activités d'acrorosine, les leucocytes, la capacité des spermatozoïdes in vitro et la réaction acroqueuse après le stockage in vitro au 4oC. La concentration de malondialdéhyde (MDA) dans le sperme stocké a été mesurée dans des substances réactives de l'acide thiobarbiturique. Les résultats ont montré que l'extension basée sur Tris complétée par des niveaux d'inclusion de 6 mm et de 8 mm de cystéine avait plus haut (p <0,05) pourcentage de motilité progressive, d'intégrité de la membrane et d'intégrité acrosomère par rapport à d'autres niveaux d'inclusion et au groupe témoin. Les résultats ont montré la plus haute (P<0,05) Pourcentage de spermatozoïdes en direct dans l'extension complétée par des niveaux d'inclusion de 8 mm de cystéine. Cependant, Spermatozoacryoprise avec une extension à base de Tris complétée de niveaux d'inclusion de 6 mm et de 8 mm de cystéine avait une anomalie de pourcentage la plus basse (p <0,05). Les résultats ont montré des concentrations les plus basses (p <0,05) de MDA, de 6 mm et de concentrations d'inclusion de la cystéine et des concentrations de leucocyte les plus basses (p <0,05) à 4 mm, 6 mm et 8 mm d'inclusion de cystéine. Cependant, l'extension à base de lait de graine Tris-citrouille complétée par des niveaux d'inclusion de 2 mm de cystéine avait une valeur la plus élevée (P <0,05) pour l'activité d'arginase par rapport à un autre niveau d'inclusion et à la commande. Cependant, l'inclusion de la cystéine de 8 mm avait un pourcentage le plus élevé (p <0,05) de spermatozoïque qui a subi une réaction acrosome et une capacité invitro-invitation par rapport à un autre niveau d'inclusion et le groupe témoin. Les conclusions ont documenté le potentiel de conservation de la cystéine sur la viabilité des spermatozisés stockée à 4oc.

Mots-clés: antioxydants, bouc de nain de l'Afrique de l'Ouest (NAO), Cystéine, viabilité du sperme

Introduction

Artificial insemination (AI) is one of the major reproductive biotechnologies of the modern era through which rapid genetic improvement in livestock has been achieved in the developed countries. Artificial Insemination with frozen-thawed spermatozoa was introduced in most of the developing countries more than four decades ago, to raise genetic potential of livestock (Andrabi *et al.,* 2008). The success of AI largely depends on the quality of the semen and proper AI practices. The techniques for semen collection and artificial insemination in sheep and goats have been described in detail (Evans and Maxwell, 1987). The techniques and media for storing goat semen were modified (Amoah and Gelaye, 1997) from procedures developed for bull sperm. Some of the problems encountered following the use of stored semen for AI, include a decrease in certain sperm parameters such as motility, functional integrity and fertilizing capability as well as alteration in membrane responses to physiological stimuli of the sperm (Vishwanath and Shannon, 2000). One of the most important factors contributing to poor quality semen has been reported to be oxidative stress, which involves lipid peroxidation (Aitken and Fscher, 1994). The concentration of polyunsaturated fatty acid in small ruminants' sperm membrane or semen is

generally higher than in other species. This renders the sperm highly vulnerable to oxidative damage, resulting from the production of reactive oxygen species (ROS). Consequently there is loss in membrane and morphological integrity, impaired cell function, and impaired sperm motility as well as death of sperm cells (Aitken and Fscher, 1994). An antioxidant system in cells serves as defense mechanism against the lipid peroxidation of semen and also helps to maintain sperm motility and viability. Sperm cells are well equipped with a powerful defense system of antioxidants against reactive oxygen species attack, but an imbalance between the production of ROS and the available antioxidant defenses result in oxidative stress (Sikka, 1996). Moreover, the cells antioxidant capacity system may be insufficient in preventing lipid peroxidation during storage process. Therefore, semen extenders are supplemented with non-enzymatic antioxidants *in vitro*. Antioxidants are the main defense factors against oxidative stress induced by free radicals (Agarwal and Prabakaran, 2005). Additives showing antioxidative properties may reduce this impact of reactive oxygen species-induced damage caused by lipid peroxidation (Donnelly *et al*., 2000).

Materials and methods

Experimental site and animal management

The experiment was carried out at the Goat Unit of Directorate of University Farm, Federal University of Agriculture, Abeokuta, located on latitude 7^{0} 10' N and longitude 3^{0} 2' E, and altitude76m above sea level with a mean annual rainfall of 1,037mm and average temperature of 34.7^{0}C. Six intact WAD goat bucks and one matured teaser doe were used for the experiment. The bucks ranged between 4 and 5 years with average weight of 18kg. The animals were kept under intensive management and maintained under a uniform and constant nutritional regimen with concentrate feed supplemented with guinea grass (*Panicum maximum*).

Semen collection, dilution and storage

Semen samples were collected from six WAD goat bucks with the aid of artificial vagina. Only ejaculates showing >80% motility were pooled. Pooled semen sample (each pool originating from six males) was diluted with Tris-pumpkin seed milk based extender as shown in Table 1. Prior to dilution, the extender was supplemented each with cysteine at 2mM, 4mM, 6mM and 8mM /100ml of the diluents respectively. Following dilution, the semen was dispensed into 5mL tubes sealed and gradually chilled at 4°C for 96 hours. The pH of the pumpkin seed milk extenders (Control: 7.03 and cysteine extender 7.14) was determined using digital pH meter.

Semen evaluation

Sperm motility

Sperm motility was determined as described by Bearden and Fuquay (1997). Briefly, chilled semen was placed in Clifton Water bath (Model: 74178 by Nickel Electro Ltd, Weston-S-Mare Somerset, England) at 37^{0}C and accessed for sperm motility using CelestronPenta View microscope (LCD-44348 by RoHS, China) at 400X magnifications. A semen mount of 5µL was placed directly on a microscope slide and covered with cover slip. For each sample, five microscopic fields were examined to observe progressive sperm motility and the mean of the five successive evaluations was recorded as the final motility score.

Table 1: Chemical composition of Tris pumpkin seed milk based extender

Composition	Quantity (g/100mL)
Trishydroxyaminomethane	2.42
Citric acid	1.36
Glucose	1.0
Penicillin	0.028
pupkin seed milk	20
Distil water	76

Source: Daramola and Adekunle, 2016

Acrosome integrity

The percentage of spermatozoa with intact acrosomes was determined according to Ahmad *et al.,* (2003).Briefly, 50µL of each semen sample was added to a 500µL formalin citrate solution (96 mL 2.9% sodium citrate, with 4 mL 37% formaldehyde) and mixed carefully. A small drop of the mixture was placed on a microscope slide and a total of 200 spermatozoa were counted in at least three different microscopic fields for each sample, using CelestronPenta View LCD microscope (400X magnifications).Intactness of acrosome characterized by normal apical ridge of spermatozoa.

Sperm membrane integrity

Hypo-osmotic swelling test (HOST) assay as described earlier (Jeyendran*et al.,* 1984) was used to determine sperm membrane integrity and this was done by incubating 10µL semen in 100µL Hypo-osmotic solution (fructose and sodium citrate) at 37^0C for 30minutes, 0.1mL of the mixture was spread over a warm slide, covered with a cover slip and observed under CelestronPentaView LCD digital microscope (400 X magnifications). Two hundred spermatozoa (200) were counted for their swollen characterized by coiled tail, indicating intact plasma membrane.

Sperm abnormality

Sperm abnormality was evaluated as described by Bearden and Fuquay (1997) with the use of eosin-nigrosin smears. A thin smear of mixture of semen and eosin-nigrosin solution was drawn across the slide and dried. Abnormality of sperm cells located in the head, midpiece and tail were observed under CelestronPenta View LCD microscope (400X magnifications).

Live sperm

Live sperm was evaluated as described by Bearden and Fuquay (1997) with the use of eosin-nigrosin stain. A thin smear of mixture of semen and eosin-nigrosin solution were drawn across the slide and dried. Semen samples were examined under a digital microscope (CelestronPenta®) LCD view at 400X magnification for live spermatozoa. Spermatozoa that appear white were recorded as live spermatozoa and those that pick up the stain were recorded as dead spermatozoa.

Malondialdehyde (MDA) concentrations

The MDA concentration as an index of lipid peroxidation in the stored semen was measured in thiobarbituric acid reactive substances (TBARS) according to Yagi(1998). For this assay, 0.1mL of sperm suspension was incubated with 0.1 mL of 150 mMTris-HCl (pH7.1) for 20 minutes at 37°C. Subsequently, 1mL of 10% trichloroacetic acid (TCA) and 2 mL of 0.375% thiobarbituricacid was added followed by incubation in boiling water for 30min. Thereafter, it was centrifuged for 15min at 3000 rpm inside the blank tube and the absorbance was read with UV spectrophotometer (SW7504 model by Surgifriend Medicals, England) at 532nm. The concentration of MDA was calculated as follows: The concentration of malondialdehyde MDA (nmol/mL) = $AT - AB/1.56 \times 10^5$; Where: AT = the absorbance

of the semen sample, AB = the absorbance of the blank, 1.56×10^5 molar absorptivity of MDA.

Arginase activity

Arginase activity was carried out according to the procedure of Lowry *et al.* (1951). Briefly, 0.1g bovine serum albumin (BSA) as standard in 10mL of water was used. The tubes containing 1mL alkaline copper reagent (a mixture of *copper sulfate reagent,* sodium dodecyl sulfate *solution*, and *sodium hydroxide solution* (1:2:1) and 0.1mL supernatant sample were mixed and incubated for 10min at room temperature. After this, 4mLfolinCiocalteu's phenol reagent was added to the tubes, mixed and incubated for 5min at 55°C. The absorbance of the samples was recorded at 650nm in spectrophotometer (UV spectrophotometer, SW7504 model by Surgifriend Medicals, England).

Leukocytes

Peroxidase test as recommended by WHO (1992) was used as follows: A stock solution was prepared by mixing 50mL distilled water with 50mL 96% ethanol plus 125mg benzidine. The working solution was obtained by adding 5µL 30% H_2O_2 to 4 mL of stock solution. Twenty (20)µL of working solution was mixed with 20µL of cryopreserved semen in a small test tube. After incubation for 5 min at room temperature, 20µL of working solution was mixed with 20µL of phosphate-buffered saline. Then, 10µL was placed in a haemocytometer, and peroxidase-positive cells (dark brown round cells) were counted.

In vitro acrosome reaction

Following cryopreservation, spermatozoa were thawed by plunging straws into a water bath (37°C) for 1min and the proportion of acrosome reaction was determined as described by Tardif *et al.* (*1999*) with modification as follows: Samples of cryopreserved spermatozoa were washed with non-culture medium (Phosphate-Buffered Saline (PBS), and the pellets were re-suspended in culture medium (Calcium chloride dihydrate 265mg/L, Magnesium chloride anhydrous 46 mg/L, Potassium chloride 200 mg/L, Sodium chloride 8000 mg/L, Sodium dihydrogen phosphate anhydrous 50mg/L, D-Glucose 1000 mg/L). Immediately after the inclusion of 0.9% wt/vol PBS (15 µg/ml), the acrosome reaction was induced by incubating spermatozoa for 20 min with progesterone (2.5mg/mL) at 38.5°C (5% CO_2 in air; 100% humidity). To determine the proportion of spontaneous acrosome reaction, progesterone was omitted but an equal volume of PBS was added. Spermatozoa were observed in an upright Carl Zeiss Fluorescent Microscope (Primo Star, Germany) equipped with phase contrast and epifluorescence optics, and 100 cells were counted per slide. Spermatozoa with intense fluorescence over the acrosome were classified as acrosome intact and those with no fluorescence or a dull fluorescence along the equatorial segment as acrosome reacted.

In vitro capacitation

In vitro capacitation of the spermatozoa was evaluated using the CTC fluorescence assay as described by Collin *et al.* (*2000*). In brief, CTC (750 µM*)* was prepared in 20 mM Tris buffer containing 130 mM NaCl and 5 mM DL-cysteine (final pH 7.8). Sperm suspension (5µl) was mixed with 5µL of CTC solution on a warmed slide (37° C). After 30 sec, 5µL of 0.2% glutaraldehyde in 0.5 M Tris pH 7.4 was added. Finally, 5µlof 90% glycerol and 10% PBS (pH was adjusted to 8.6) were added to retard fluorescence fading. After adding a cover slip, slide was examined with an upright Carl Zeiss Fluorescent Microscope (Primo Star, Germany) equipped with phase contrast and epifluorescence optics, and 100 cells were counted per slide. The proportion of cryopreserved spermatozoa that exhibited pattern B according to the

CTC assay was determined. Spermatozoa characterized by bright anterior head and faint fluorescence in the post-acrosomal region were classified as capacitated spermatozoa while non capacitated sperm had bright uniform fluorescence over the head.

Statistical analysis and model

Data obtained were subjected to analysis of variance (ANOVA) using SAS 1999. While Duncan Multiple Range Test (Duncan, 1955) was used to separate significantly different means. The model that was used to analyze the data is stated below:

$Y_{ij} = \mu + A_i + L_j + \Sigma_{ij}$

Where,

Y_{ij} = Dependent variables

μ = Population mean

A_i = Effect due to i^{th} cysteine,

L_j = Effect due to j^{th} level of inclusion, j = 0, 2, 4, 6, 8

Σ_{ij} = Experimental Error

Results

Results in Table 2 showed higher (P<0.05) spermmotility, acrosome integrity, membrane integrity and live sperm in extenders supplemented with cysteine compared to the control. Spermatozoa chilled with 6mM and 8mM of cysteine had the highest (P<0.05)percentage motility, acrosome integrity and membrane integrity compared to 2mM, 4mM inclusion levels of cysteine and the control. However, the results showed highest(P<0.05) percentage of live spermat 8mM inclusion level of cysteine compared to the control. The results showed lower (P<0.05) percentage abnormalities at all levels of cysteine supplementation compared to the control. However, lowest (P<0.05) percentage abnormalities were observed at 6mM and 8mM inclusion levels of cysteine compared to the control. Seminal oxidative stress parameters of semen cryopreserved with varying concentrations of cysteine as antioxidant is presented in Table 3. The results showed that semen cryopreserved with tris based extender supplemented with different cysteine level had lower (P>0.05) MDA concentrations compared with the control group. However, semen cryopreserved with cysteine at6 and 8mM levels had lower (P>0.05) MDA concentrations compared to 2 and 4mM and the control while lower (P>0.05) leukocytes was obtained in 4, 6 and 8mM compared to other levels and the control . However, higher arginase activity (P>0.05) was obtained in cysteine supplemented groups as compared the control group. The results in Table 4 showed higher (*P* < 0.05) percentage of spermatozoa cryopreserved with Tris based extenders supplemented with 6mM and 8mM cysteine that underwent acrosome reaction and capacitation. More spermatozoa (*P* < 0.05) cryopreserved with Tris based extenders supplemented with cysteine induced acrosome reaction and capacitation compared to control group.

TABLE 2: Effect of Tris-pumpkin seed milk based extenders supplemented with cysteine on viability of Wad buck chilled at 4°C

	Extenders				
Parameters	**0Mm**	**2mM**	**4mM**	**6mM**	**8mM**
Motility (%)	31.10 ± 6.26^d	61.00 ± 2.77^c	73.00 ± 2.64^b	82.00 ± 2.49^a	87.00 ± 1.53^a
Acrosome Integrity (%)	59.50 ± 3.30^c	69.00 ± 5.26^b	67.00 ± 1.00^b	72.50 ± 1.26^a	74.50 ± 1.50^a
Membrane Integrity (%)	59.50 ± 2.36^d	74.00 ± 1.41^c	78.50 ± 0.50^b	83.50 ± 2.06^a	87.50 ± 0.50^a
Live Sperm (%)	70.00 ± 4.08^d	75.00 ± 8.66^c	82.50 ± 2.50^b	85.00 ± 5.00^b	92.50 ± 4.79^a
Abnormality (%)	4.00 ± 0.01^a	1.50 ± 0.28^c	2.75 ± 0.48^b	0.25 ± 0.25^d	0.50 ± 0.28^d

$^{a, b, c, d}$Values within rows with different superscripts differ significantly (P < 0.05), SE: standard error.

TABLE 3;Effect of Tris-pumpkin seed milk based extenders supplemented with cysteine on seminal oxidative stress parameters of Wad buck chilled at 4°C

	Extenders				
Parameters	**0mM**	**2mM**	**4mM**	**6mM**	**8mM**
MDA Concentration (nmol/mL)	0.58 ± 0.01[a]	0.22 ± 0.03[b]	0.20 ± 0.01[b]	0.11 ±0.03[c]	0.15 ± 0.03[c]
Arginase Activity (units/mg protein)	0.83 ± 0.01[e]	1.94 ± 0.01[c]	2.00 ± 0.02[b]	2.87 ± 0.01[a]	1.85 ± 0.01[d]
Leukocytes (x10mL)	0.63 ± 0.04[a]	0.66 ± 0.04[a]	0.26 ± 0.02[b]	0.26 ± 0.03[b]	0.26 ± 0.03[b]

[a, b, c,d]Values within rows with different superscripts differ significantly ($P < 0.05$), SE: standard error.

TABLE 4; Effect of Tris-pumpkin seed milk based extenders supplemented with cysteine on invitro acrosome reaction (%) and capacitation (%) of Wad buck spermatozoa chilled at 4°C

	Extenders				
Parameters	**0mM**	**2mM**	**4mM**	**6mM**	**8mM**
Acrosome reaction (%)	51.00 ± 3.00[c]	73.00 ± 1.91[b]	76.00 ± 2.83[b]	77.00 ± 1.91[b]	86.00 ± 3.46[a]
Capacitation (%)	55.00 ± 6.61[d]	78.00 ± 1.15[c]	81.00 ± 5.26[b]	84.00 ± 2.83[b]	94.00 ± 1.15[a]

[a, b, c,d]values within rows with different superscripts differ significantly ($P < 0.05$)

Discussion

In the study, we investigated the cysteine as an antioxidant on sperm viability, oxidative stress, and fertilizing ability parameters in WAD buck goat sperm chilled at 4°C. Mammalian spermatozoa are extremely vulnerable to lipid peroxidation (LPO), which happens as a result of partially reduced oxygen molecules such as superoxide, hydrogen peroxide, and hydroxyl radicals oxidizing membrane lipids. The LPO caused by ROS attacks, created by the univalent reduction of oxygen, impaired sperm function, such as motility, functional membrane integrity, and fertility, via oxidative stress and the generation of cytotoxic aldehydes (Aitken *et al.,* 1993). Antioxidant supplementation can help to prevent this process (Sikka, 2004) as amino acids are charged molecules (Anchordoguy *et al.,*1988) that electrostatically interact with the phosphate groups of sperm plasma membrane phospholipids, generating a coating on the sperm surface that protects it from temperature shocks. They may also contribute to sperm osmolarity and play a favourable influence in sperm viability (Billard and Menezo, 1984). The result revealed that cysteine inclusion in a Tris-pumpkin seed milk based extender considerably increases sperm motility, membrane and acrosome integrity. The axoneme and related dense fibers of the sperm mid-pieces, which are important for sperm motility, are coated by mitochondria, which create energy through oxidative phosphorylation. Large quantities of ROS can decrease sperm motility and increase aberrant sperm production, causing axonemal damage due to ATP depletion (De Lamirande and Gagnon, 1992; Garner and Hafez 1993). In contrast to this ROS effect, it is hypothesized that 6mM and 8mM inclusion levels of cysteine have a protective effect on the functional integrity of the axosome and mitochondria. The findings were consistent with (Funahashi and Sano, 2005; Mustafa *et al.,* 2007). The percentage of viable sperms detected in the current investigation is consistent with previous findings (Perumal *et al.,* 2011). Sperm plasma membrane plays an important function in fertilization and the percentage of plasma membrane integrity of WAD buck was higher in the extender containing 6mM and 8mM inclusion levels of cysteine. It was found that buffalo semen have considerably higher proportion of sperm cells with intact plasma membrane

after freeze–thawing with tris-citric acid diluent supplemented with 5mM cysteine (El-Sheshtawy *et al.,*2008), while a larger percentage of sperm cells with functional plasma membrane was reported in stored bovine (Sariözkan *et al.,* 2009) and ovine (UysalandBucak, 2007) sperm in Bioxcell® and tris-based diluent supplemented with 2.0 mM and 5.0 mM cysteine, respectively. It has been proposed that cysteine supplementation in extender protects membrane integrity by scavenging ROS molecules (Alvarez and Storey, 1983) directly and/or indirectly in the semen-extender complex, which can disrupt the sperm membrane during the cryopreservation process (Bucak *et al.,* 2008). Intracellular MDA concentration is a stress indicator and the optimal MDA concentration obtained at 6mM and 8mM inclusion levels of cysteine was due to a sudden change in temperature during the storage process. During sperm storage, leukocytes and sperm are important producers of reactive oxygen species (ROS) (Agarwal *et al.,* 2003; Garrido *et al.,* 2004). Excessive ROS produced by leukocytes or sperm reduces plasma and organelle membrane fluidity and damages membrane function, ion gradients, and receptor-mediated signal transduction (Sikka *et al.,* 1996). High levels of leukocytes, particularly active leukocytes, are detrimental to sperm function (Henkel, 2011). In this investigation, the reduced concentration of leukocytes following cysteine addition compared to the control group indicated that cysteine's antioxidative capacity was successful in lowering faulty sperm or excessive ROS produced by leukocytes or sperm. The fundamental function of arginase in the testes is to regulate NO concentration (Nathan, 1997), and excessive NO generation impairs gonad spermatogenesis (Taneli *et al.,* 2005). Increased seminal plasma arginase activity with the addition of cysteine may have a favourable effect on sperm cell division, growth, and differentiation. Sperm function tests, such as acrosome response and capacitation, are more accurate indicators of fertility than standard sperm characteristics (Katsuki *et al.,* 2005). In this investigation, Tris-pumpkin seed milk based extender supplemented with cysteine improved *in vitro* acrosome response and sperm capacitation compared to the control group, demonstrating that this antioxidant may improve the fertilizing capacity of cryopreserved sperm. At varying inclusion levels, there were equivalent improvements in functional, fertilizing, and seminal oxidative stress end points of goat semen cryopreserved using Tris-pumpkin seed milk based extender supplemented with cysteine.

Conclusion

In conclusion, this study clearly suggested that cryopreservation of WAD goat buck sperm supplemented with 6 mM and 8 mM inclusion levels of cysteine in Tris pumpkin milk based extender showed an improved viability, oxidative stress and fertilizing ability parameters.Preservation of semen samples obtained from WAD goat bucks with tris-pumpkin seed milk based extender supplemented with cysteine as antioxidant is satisfactory for AI programme.

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