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The effect of antioxidants on post-thawed Angora goat (*Capra hircus ancryrensis*) sperm parameters, lipid peroxidation and antioxidant activities

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ABSTRACT

The aim of this study was to determine the effects of the antioxidants curcumin, inositol and carnitine on microscopic seminal parameters, lipid peroxidation (LPO) and the antioxidant activities of sperm, following the freeze-thawing of Angora goat semen. Ejaculates were collected via artificial vagina from three Angora goats and microscopically evaluated and pooled at 37 °C. The pooled semen samples were diluted in a Tris-based extender, including curcumin (2.5, 5 or 10 mM), inositol (2.5, 5 or 10 mM), carnitine (2.5, 5 or 10 mM) and no antioxidant (control). The diluted semen was slowly (at a rate of 0.2–0.3 °C/min) cooled to 5 °C and then cryopreserved in 0.25 mL French straws. Frozen straws were thawed individually at 37 °C for 20 s in a water bath, for microscopic sperm evaluation. The freezing extender supplemented with 2.5 mM curcumin led to higher percentage of computer-assisted semen analyzer (CASA) sperm motility (65 ± 3%), when compared to the control, inositol and the 10 mM carnitine ($P < 0.01$) groups, following the freeze-thawing process. The addition of antioxidants did not provide any significant effect on the percentages of post-thaw subjective analyses and CASA progressive motilities, as well as sperm motility characteristics (VAP, VSL, LIN and ALH), compared to the controls. Freezing extenders with antioxidants at three different doses led to lower percentages of acrosome and total sperm abnormalities, when compared to the controls ($P < 0.001$). However, the addition of 5 mM inositol did not induce any difference in total sperm abnormalities, when compared to the controls. The antioxidants also did not show any effectiveness in the elimination of malondialdehyde (MDA) formation and the maintenance of glutathione peroxidase (GSH-PX) activity, when compared to the controls. Superoxide dismutase (SOD) activity was found to be higher in the presence of curcumin at all three dose levels and carnitine at 5 mM, compared to the other groups. Glutathione (GSH) concentration was demonstrated to be maintained at a higher level with the addition of inositol, compared to the other groups. However, these differences in SOD and GSH levels were not significant, compared to the controls. All the antioxidants at all three dose levels resulted in a better protection of the sperm morphology (except for 5 mM inositol with respect to the total sperm abnormalities), compared to the control samples. According to CASA, the best post-thawing sperm motility

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rate was recorded when the freezing extender was supplemented with 2.5 mM curcumin. Further studies are required to obtain more conclusive results regarding the characterization of microscopic and oxidative stress parameters in cryopreserved goat sperm, using the different antioxidants.

1. Introduction

Artificial insemination is an important procedure, which allows the widespread dissemination of semen, even to small flocks, limiting the spread of sexually transmitted diseases, and mainly facilitating genetic improvement programmes. Some of the problems encountered following the use of AI with freeze-thawed semen, include a decrease in certain sperm parameters (e.g. motility, functional integrity and fertilizing capability) and an 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 (LPO). The concentration of polyunsaturated fatty acids in small ruminant sperm membranes is generally higher than in other species, which thus renders the sperm highly vulnerable to oxidative damage, resulting from the production of reactive oxygen species (ROS) – with subsequent loss in membrane and morphological integrity, impaired cell function, along with impaired sperm motility and the induction of sperm apoptosis (Aitken and Fischer, 1994; Gandini et al., 2000). An antioxidant system, comprised of reduced glutathione (GSH), glutathione peroxidase (GSH-Px), catalase (CAT), and superoxide dismutase (SOD), has been described to serve as a defense mechanism against the LPO of semen and has been identified as being important in maintaining sperm motility and viability. This antioxidant capacity of semen may, however, be insufficient in preventing LPO during the freeze-thawing process (Fridovich, 1985). The protective antioxidant systems in sperm are primarily of cytoplasmic origin and sperm discard most of their cytoplasm during the terminal stages of differentiation. Thus, mammalian sperm lack a significant cytoplasmic component, which contains sufficient antioxidants to counteract the damaging effects of ROS and LPO. For this reason, sperm are susceptible to LPO during cryopreservation and thawing process (Storey, 1997). The cryopreservation of mammalian sperm as such produces physical and chemical stress on the sperm membrane – leading to LPO generating excessive ROS – that is highlighted by dead sperm and the breakdown of the antioxidant defense system. ROS mainly demonstrate a detrimental effect on sperm motility, viability and fertilizing ability, due to alterations induced in the sperm plasma membrane (Aitken and Fischer, 1994; Chatterjee et al., 2001). Additives showing anti-oxidative properties may reduce this impact of ROS-induced damage caused by LPO (Donnelly et al., 2000).

Curcumin, a polyphenolic compound abundantly found in the dietary spice turmeric, and which dates back over 5000 years, is a natural antioxidant, and also acts as an anti-apoptotic, anti-inflammatory, antitoxic and anti-cancer agent (Miller et al., 2001; Surh et al., 2001; Goa et al., 2004). Due to its highly efficient cryoprotective and anti-oxidative properties against cold shock and oxidative damage, curcumin has recently been tested by many researchers investigating various cell systems against ROS-induced damage, organ transplantation and cryopreservation (Kunchandy and Rao, 1990; Abuarqoub et al., 2007; Kanitkar and Bhonde, 2008; Mathuria and Verma, 2008). Carnitine on the other hand is biosynthesized by the two essential amino acids, lysine and methionine, in the liver, kidney and brain. L-Carnitine, a vitamin-like compound, is also found in high concentrations in mammalian epididymides and sperm. It plays a role in generating metabolic energy, by facilitating the transport of fatty acids to the mitochondria. The epididymal cells and sperm thus take energy from the carnitine present in epididymal fluid (Ford and Rees, 1990; Lewin et al., 1997). A close correlation between seminal carnitine and semen quality has also been demonstrated in animal models (ram, human, stallion, and rat) (Brooks, 1979; Ruiz-Pesini et al., 2001; Stradaioli et al., 2004). Amongst other compounds in the epididymal fluid, inositol is present in high concentrations, and it plays an important role in maintaining the viability of the epithelial and sperm cells in the epididymis, as an essential growth factor (Cooper, 1998). It has been reported that the motility of frozen–thawed bull sperm can be improved by using inositol in the extender (Reyes-Moreno et al., 2000).

In recent studies, the supplementation of a cryopreservation extender with antioxidants has been shown to provide a cryoprotective effect on bull (Bilodeau et al., 2001; Sarıözkan et al., 2009), ram (Bucak et al., 2008), goat (Bucak et al., 2009), boar (Funahashi and Sano, 2005), canine (Michael et al., 2007) and human (Alvarez and Storey, 1989a) sperm quality. Thus improving semen parameters (e.g. sperm motility, membrane integrity), after thawing. No previous study exists which investigated the roles of the antioxidants curcumin, carnitine and inositol in the cryopreservation extender, against cryodamage of Angora goat semen. This research was therefore conducted to determine the effect of supplementation of the freezing extender with the antioxidants curcumin, inositol or carnitine on sperm motility, morphology, LPO, glutathione (GSH), glutathione peroxidase (GSH-PX) and superoxide dismutase (SOD) activities in post-thawed Angora goat semen.

2. Material and methods

2.1. Chemicals

The antioxidants (curcumin, inositol and carnitine) and other chemicals used in this study were all obtained from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Animals and semen collection

Semen samples from three mature Angora goats (3 and 4 years of age) were used in this study. The goats, belonging to the Lalahan Livestock Central Research Institute, were maintained under uniform optimal nutritional conditions. Ejaculates were collected twice a week from the bucks, with the aid of an artificial vagina, during the natural breeding season (autumn to early winter). Immediately after collection, the ejaculates were incubated in a water bath at 33 °C, until microscopic sperm quality assessments were performed in the laboratory. All semen analyses were performed within approximately 20 min of semen collection.

2.3. Semen extending, freezing and thawing

The volume of the ejaculates was measured in a conical tube, graduated at 0.1 mL intervals, and the ejaculate concentration was determined using a haemocytometer (Smith and Mayer, 1955). Sperm motility was estimated using phase-contrast microscopy (400× magnification) at 37 °C. Only ejaculates between 1 and 2 mL in volume, containing sperm with >80% progressive motility and an ejaculate concentration of higher than 2.5×10^9 sperm/mL were pooled. Pooling of semen was performed to balance the sperm contribution of each male and eliminate individual buck differences. In total 6 pooled ejaculates were included in the study.

A Tris-based extender (Tris 254 mM, citric acid 78 mM, fructose 70 mM, egg yolk 15% (v/v), glycerol 6% (v/v), pH 6.8) was used as the base extender (cryopreservation diluent). Each pooled ejaculate was split into 10 equal aliquots and diluted at 37 °C with the base extender containing curcumin (2.5, 5 or 10 mM), inositol (2.5, 5 or 10 mM), or carnitine (2.5, 5 or 10 mM), and no antioxidant (control), respectively. Given a total of 10 experimental groups, with a final semen concentration of approximately 4×10^8 sperm/mL (single step dilution), in 15-mL polypropylene centrifuge tubes. The diluted semen samples were then aspirated into 0.25 mL French straws, sealed with polyvinyl alcohol powder and equilibrated at 5 °C for 3 h. After equilibration, the straws were frozen in liquid nitrogen vapor, (4 cm above the liquid nitrogen), for 15 min and then plunged into liquid nitrogen for storage. After being stored for 1 month, the frozen straws were thawed individually (37 °C for 20 s) in a water bath, for microscopic semen evaluation.

2.4. Semen evaluation

2.4.1. Microscopic sperm parameter analyses

Subjective motility was assessed with the aid of a phase-contrast microscope (100× magnification), using a warm stage maintained at 37 °C. A wet mount was made using 5-μL semen, placed directly on a microscope slide and covered by a cover slip. Sperm motility estimations were performed at three different microscopic fields for each semen sample. The mean of the three successive estimations was recorded as the final sperm motility score. Besides recording the subjective sperm motility, a computer-assisted sperm motility analysis (CASA, Version 12 IVOS, Hamilton-Thorne Biosciences, Beverly, MA, USA) was also used to analyze sperm motion characteristics. CASA was set up as follows: phase contrast; frame rate – 60 Hz; minimum contrast – 70; low and high static size gates – 0.6–4.32; low and high intensity gates – 0.20–1.92; low and high elongation gates 7–91; default cell size – 10 pixels; default cell intensity −80. Thawed semen was diluted (5 μL semen + 95 μL extender) in a Tris-based extender (without egg yolk and glycerol) and evaluated immediately after dilution. A 4-μL sample of the diluted semen sample was then put on a pre-warmed 20 μm slide (Leja Products B.V., Nieuw-Vennep, Holland) and the sperm motility characteristics determined using a 10× objective, at 37 °C. The following sperm motility values were recorded: motility (%), progressive motility (%), VAP (average path velocity, μm/s), VSL (straight linear velocity, μm/s), ALH (amplitude of lateral head displacement, μm) and LIN (linearity index (LIN = (VSL/VCL) × 100). For each evaluation, 10 microscopic fields were analysed to include at least a total of 300 sperm cells.

For the assessment of sperm abnormalities, at least three drops of each sample were added to Eppendorf tubes containing 1 mL Hancock solution (62.5 mL formalin (37%), 150 mL sodium saline solution, 150 mL buffer solution and 500 mL double-distilled water) (Schafer and Holzmann, 2000). One drop of this mixture was then placed onto a slide and covered with a cover slip. The percentage sperm abnormalities (acrosome and total abnormalities) was determined by counting a total of 200 sperm under phase-contrast microscopy (1000× magnification, oil immersion).

2.4.2. Biochemical assays

Biochemical assays were performed on the sperm samples immediately after thawing, following centrifugation and washing.

2.4.2.1. Malondialdehyde (MDA) concentrations. Concentrations of MDA, as indices of LPO in the semen samples, were measured using the thiobarbituric acid reaction (Placer et al., 1966). The quantification of the thiobarbituric acid reactive substances was determined by comparing the absorption with the standard curve of malondialdehyde equivalents, generated by the acid-catalyzed hydrolysis of 1,1,3,3-tetramethoxypropane. MDA concentrations were expressed as nmol/mL.

2.4.2.2. GSH, GSH-PX and SOD activities. The GSH content of the sperm was measured using the method of Sedlak and Lindsay (1968). The samples were precipitated using 10% trichloracetic acid and then centrifuged at 1000 × *g* for 5 min. The reaction mixture contained 0.5 mL supernatant, 2.0 mL Tris–EDTA buffer (0.2 mol/L; pH 8.9) and 0.1 mL, 0.01 mol/L 5,5′-dithio-bis-2-nitrobenzoic acid. This solution was kept at room temperature for 5 min, and then read at 412 nm using a spectrophotometer. GSH values were expressed as nmol/mg protein.

The GSH-Px activity was determined according to the method of Lawrence and Burk (1976) The reaction mixture consisted of 50 mM potassium phosphate buffer (pH

Table 1
Mean (±SE) sperm parameters in pooled semen supplemented with different antioxidants of three Angora goats following freeze-thawing.

Groups	Control	Curcumin (mM)			Inositol (mM)			Carnitine (mM)			*P*
		2.5	5	10	2.5	5	10	2.5	5	10	
Subjective motility (%)	49.0 ± 3.1	60.0 ± 4.6	53.8 ± 3.2	53.8 ± 1.3	50.0 ± 2.7	46.0 ± 1.9	56.0 ± 2.9	60.8 ± 2.0	58.3 ± 2.8	54.2 ± 4.6	–
CASA motility (%)	43.9 ± 2.9^{bc}	65.3 ± 3.2^{a}	53.3 ± 4.5^{ab}	58.0 ± 5.4^{ab}	49.4 ± 7.1^{bc}	38.8 ± 1.6^{c}	50.8 ± 3.4^{bc}	57.2 ± 1.3^{ab}	55.3 ± 2.4^{ab}	45.5 ± 6.0^{bc}	**
Progressive motility (%)	15.7 ± 2.1	16.5 ± 2.0	15.8 ± 2.2	14.5 ± 1.2	17.0 ± 3.8	12.8 ± 1.1	16.4 ± 2.4	14.5 ± 1.8	15.8 ± 1.9	12.2 ± 1.4	–
VAP (μm/s)	97.5 ± 4.5	87.6 ± 4.2	86.4 ± 5.1	74.9 ± 6.7	99.7 ± 6.6	96.2 ± 5.5	96.9 ± 4.3	94.8 ± 8.0	95.8 ± 7.6	75.0 ± 12.9	–
VSL (μm/s)	80.4 ± 4.9	69.9 ± 6.2	69.1 ± 7.0	59.5 ± 4.7	82.2 ± 7.3	79.8 ± 5.9	81.4 ± 4.9	77.2 ± 8.4	79.5 ± 8.8	72.5 ± 5.6	–
LIN (%)	39.5 ± 1.1	37.8 ± 2.4	35.8 ± 2.8	40.5 ± 4.6	39.6 ± 1.5	40.2 ± 1.7	40.4 ± 1.0	38.7 ± 1.3	39.8 ± 2.0	37.3 ± 1.1	–
ALH (μ)	8.5 ± 0.2	8.3 ± 0.4	8.3 ± 0.4	8.3 ± 0.4	8.8 ± 0.3	8.5 ± 0.3	8.5 ± 0.3	8.9 ± 0.3	8.7 ± 0.2	9.1 ± 0.4	–
Acrosome abnormality (%)	13.9 ± 0.9^{a}	7.8 ± 1.5^{b}	10.0 ± 1.5^{b}	7.5 ± 1.7^{b}	8.6 ± 0.8^{b}	9.0 ± 0.6^{b}	8.4 ± 0.8^{b}	7.3 ± 0.8^{b}	8.7 ± 0.9^{b}	7.3 ± 1.0^{b}	***
Total abnormalities (%)	25.7 ± 1.0^{a}	17.3 ± 2.2^{b}	18.5 ± 3.0^{b}	18.8 ± 1.9^{b}	18.4 ± 1.2^{b}	21.2 ± 1.4^{ab}	17.8 ± 1.4^{b}	17.0 ± 1.9^{b}	20.7 ± 1.1^{b}	19.3 ± 0.6^{b}	***

a, b, c: Different superscripts within the same row demonstrate significant differences (**$P < 0.01$, ***$P < 0.001$).
Sperm motion characteristics in computer-assisted semen analysis (CASA). VAP: average path velocity, μm/s. VSL: Straight linear velocity, μm/s. LIN: Linearity index, % (LIN = (VSL/VCL) × 100). ALH: Amplitude of lateral head displacement, μm.

Table 2
Mean (±SE) malondialdehyde (MDA), superoxide dismutase (SOD), GSH-PX, and GSH activity in pooled semen supplemented with different antioxidants from three Angora goats following freeze-thawing.

Groups	Control	Curcumin (mM)			Inositol (mM)			Carnitine (mM)			*P*
		2.5	5	10	2.5	5	10	2.5	5	10	
MDA(nmol/mL)	2.0 ± 0.1	2.0 ± 0.1	1.87 ± 0.09	2.0 ± 0.2	2.2 ± 0.2	2.2 ± 0.0	2.0 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	–
SOD (U/mg protein)	143.3 ± 10.8^{abc}	182.2 ± 28.8^{a}	212.3 ± 26.8^{a}	183.0 ± 27.5^{a}	78.8 ± 21.6^{c}	98.0 ± 21.2^{bc}	86.5 ± 25.3^{c}	132.6 ± 33.2^{abc}	168.2 ± 29.8^{ab}	137.6 ± 25.3^{abc}	**
GSH-Px (U/g protein)	35.0 ± 2.9	43.7 ± 6.8	38.4 ± 4.0	38.0 ± 2.4	29.3 ± 4.5	30.9 ± 2.8	30.5 ± 2.6	31.7 ± 3.6	28.2 ± 3.6	32.9 ± 2.0	–
GSH (nmol/mg protein)	3.4 ± 0.5^{ab}	3.2 ± 1.2^{ab}	2.6 ± 0.3^{b}	2.3 ± 0.4^{b}	4.1 ± 0.6^{ab}	5.2 ± 0.7^{a}	5.1 ± 0.6^{a}	2.2 ± 0.2^{b}	2.3 ± 0.5^{b}	2.2 ± 0.3^{b}	**

a, b, c: Different superscripts within the same row demonstrate significant differences (**$P < 0.01$, ***$P < 0.001$).

7.0), 1 mM EDTA, 1 mM sodium azide (NaN_3), 0.2 mM reduced nicotinamide adenine dinucleotide phosphate (NADPH), 1 EU/mL oxidized glutathione (GSSG)-reductase, 1 mM GSH, and 0.25 mM H_2O_2. An enzyme source (0.1 mL) was added to 0.8 mL of the above mixture, and this mixture was incubated at 25 °C for 5 min before the initiation of the reaction induced by the addition of 0.1 mL peroxide solution. The absorbance at 412 nm was recorded for 5 min using a spectrophotometer. The activity was then calculated from the slope of the lines as micromoles of NADPH oxidized per minute. The blank (zero) value was subtracted from each value. The GSH-PX activity was expressed as international units (U/g) protein for the sperm samples.

SOD activity was analysed using the technique of Sun and Zigman (1978). A volume of 0.4 mL semen, 0.1 mL of 0.9% NaCl and 0.4 mL of chloroform was centrifuged for 10 min at 2000 × *g*. The reaction mixture contained 20 mL 3 mmol/L xanthine, 10 mL 0.6 mmol/L EDTA, 10 mL 0.15 mmol/L nitroblue tetrazolium (NBT), 6 mL 400 mmol/L sodium carbonate, and 3 mL 1 g/L BSA for 20 tests. An aliquot (50 μL) of xanthine oxidase (20 μmol/2 mL) was added to 2.45 mL of this reaction mixture and incubated at 25 °C for 20 min. Subsequently, the reaction was stopped by adding 1 mL 0.8 mmol/L $CuCl_2$ to the solution. The spectrophotometric absorbance was recorded at 560 nm (Techcomp Spectrophotometer UV 7500, Hong Kong). SOD activity was expressed as U/mg protein.

2.5. Statistical analysis

The study was repeated 6 times and the results expressed as the mean ± SEM. Means were analyzed using a one-way analysis of variance, followed by a Duncan's post hoc test to determine significant differences in all the parameters recorded between groups using the SPSS/PC computer programme (Version 12.0, SPSS, Chicago, IL, USA). Differences with values of $P < 0.05$ were considered to be statistically significant.

3. Results

3.1. Microscopic sperm parameters (sperm motility and motion characteristics, acrosome and total abnormalities)

The effect of the different antioxidants on the standard semen parameters following the freeze-thawing process was evaluated in 6 independent experiments. As set out in Table 1, supplementation with 2.5 mM curcumin led to higher CASA sperm motility values (65 ± 3%), compared to the control, inositol and the 10 mM carnitine groups ($P < 0.01$) following the freeze-thawing process. The addition of antioxidants did not provide any significant effect on the percentage of post-thaw sperm subjective evaluations and the CASA progressive motilities, as well as the sperm motion characteristics (VAP, VSL, LIN and ALH), compared to the controls. All cryopreservation diluents with antioxidants at three different doses, led to lower percentages of acrosome and total sperm abnormalities, when compared to the controls ($P < 0.001$). However, the addition of 5 mM inositol did not cause any difference in the total abnormalities, when compared to the control samples.

3.2. MDA, GSH, GSH-PX and SOD activities in frozen–thawed goat semen

As set out in Table 2, the antioxidants did not show any effectiveness in the elimination of MDA formation and the maintenance of GSH-PX activity. SOD activity was found to be higher in the presence of curcumin at all three different dose levels and carnitine (5 mM), compared to the other groups. The maintenance of GSH activity also demonstrated to be higher following the addition of inositol, compared to the other groups. However, these differences in SOD and GSH activities were not significant.

4. Discussion

A variety of biochemical and functional changes occur in the sperm cells during the cryopreservation and thawing process. Ice crystallization and lipid peroxidation induce oxidative stress, which leads to the formation of ROS during cryopreservation. In vivo physiological concentrations of ROS are involved in providing membrane fluidity, and maintaining the fertilizing ability and acrosome reaction of sperm (Bell et al., 1993; Aitken and Fischer, 1994). However, a high production of ROS increases the sensitivity of the sperm, resulting in damage to the sperm structure, morphology and function – accompanied by decreased sperm motility, viability and fertilizing ability (Alvarez and Storey, 1989b; Bilodeau et al., 2001). Antioxidant mechanisms comprising of GSH, GSH-PX and SOD exist as a defense against lipid peroxidation in the semen, and are important in maintaining sperm motility and viability (O et al., 2006). This antioxidant capacity in the sperm cell may, however, be insufficient in preventing LPO during the freeze-thawing process (Storey, 1997). Generally, dietary supplementation with L-carnitine and curcumin has been used to increase sperm motility and concentration in some species. The reduction of carnitine in epididymal plasma has been shown to lead to a decrease in the fertilizing ability of sperm (Stradaioli et al., 2004; Sahoo et al., 2008). In this study, while carnitine was not effective regarding the sperm motility characteristics, the elimination of MDA formation and maintenance of antioxidant activities, it provided a cryoprotective effect on the sperm acrosome and total sperm morphology.

These results recorded are in contrast to those reported by Neuman et al. (2002), stating that MDA levels in sperm are decreased by feeding carnitine to roosters. This difference may have resulted from carnitine in the present study being added to the cryopreservation extender, instead of being used as a dietary supplement. Dietary carnitine supplementation in the stallion improved sperm morphology (Stradaioli et al., 2004), while it was not effective on boar sperm motility (Kozink et al., 2004). Similarly, current findings demonstrated a marked protection in post-thawed sperm acrosome and total morphology, and no improvement in sperm motility when the cryopreservation extender was supplemented with carnitine.

The cryoprotective, LPO-reducing and radical scavenging properties of curcumin have been documented in various cell types (Kanitkar and Bhonde, 2008; Vinson, 1998). A study showed that curcumin prevented or scavenged cryopreservation-induced ROS formation in the Islets of Langerhans – thus leading to better morphology and integrity of islets post-thawing (Kanitkar and Bhonde, 2008). In the present study, curcumin (2.5 mM) led to a better CASA motility score, compared to the control, inositol (2.5, 5, 10 mM) and carnitine (10 mM) groups.

SOD, a well-known enzymatic biological antioxidant, generally scavenges the ROS, such as superoxide union and hydroxyl radicals, and thus controls oxidative stress in mammalian sperm (Fridovich, 1985). In this study, in addition to the determination of the improving the effect of curcumin on sperm motility and morphology, SOD activity was found to be higher in the presence of curcumin at the three different doses – demonstrating its anti-oxidative action. However, LPO levels did not change significantly in the presence of curcumin, compared to the controls and the other treatments. These results thus indicate that LPO does not seem to be an effective factor in influencing the survival and antioxidant potential of sperm following the freeze-thawing process. It is well known that GSH is able to react with many ROS, directly and as a co-factor for GSH-PX, which catalyses the reduction of toxic H_2O_2 and hydroperoxides (Bilodeau et al., 2001). Results in this study show inositol to have cryoprotective and anti-oxidative properties, resulting in higher antioxidant GSH activities and the acrosome integrity and intact morphology rates, compared to the other groups. Inositol did not improve sperm motility characteristics following freeze-thawing. This data is in agreement with the findings of Sánchez-Partida et al. (1997).

5. Conclusions

A freezing extender with antioxidants was superior in the protection of the acrosome and total morphology integrity of sperm ($P < 0.001$) and curcumin (2.5 mM) as such led to higher percentages of CASA motility, when compared to the controls. However the antioxidants examined in this study did not improve the post-thaw sperm motility – either for the subjective, CASA progressive motility, or any of the sperm characteristics evaluated using CASA. It could be suggested that all the antioxidants at the three different doses resulted in the best protection of sperm morphology, except for 5 mM inositol with respect to total abnormalities. For CASA the best post-thawed sperm motility rate was achieved when the cryopreservation extender was supplemented with 2.5 mM curcumin. These antioxidants helped elevate the antioxidant capacity, but did not eliminate MDA production. Confirmation and clarification of the contradictory results reported require further investigation. Supplementation with these antioxidants prior to the cryopreservation process may be recommended to facilitate the enhancement of sperm cryopreservation techniques for the goat breeding industry. Furthermore, future research should focus on the molecular mechanisms of the cryoprotective effects of the antioxidants curcumin, inositol and carnitine, during cryopreservation.

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