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Short Paper

The effect of adding pyridoxine to soybean lecithin-based extender on goat semen quality parameters after the freeze-thawing process

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Abstract

Background: Supplementing the semen extender with various antioxidants can increase the quality of semen. **Aims:** The aim of the present study was to investigate the addition of pyridoxine (vitamin B_6), a phenolic compound with antioxidant properties, to soybean lecithin extender on motility and quality indices of goat sperm after freezing-thawing process. **Methods:** Semen was collected at weekly intervals from four Mahabadi goats, mixed, and was divided into 7 subsamples. They were then diluted with the basal extender supplemented with 0, 2, 4, 6, 8, 10, and 12 mM pyridoxine. Following freeze-thawing process, quality parameters such as sperm motility characteristics, viability, plasma membrane integrity, and malondialdehyde content were determined. **Results:** The results showed that pyridoxine at the level of 6 mM caused the highest total motility rate ($P<0.05$). Progressive sperm motility was highest at the 4, 6, and 8 mM pyridoxine ($P<0.05$). Although the control group showed the least progressive motility, it was not statistically significantly different from the 12 mM level. Among the pyridoxine levels, the 6 mM level recorded the best performance in term of plasma membrane integrity, sperm viability, and decreasing malondialdehyde concentration compared to the control group ($P<0.05$). **Conclusion:** The findings indicated that soybean lecithin extender supplemented with 6 mM pyridoxine can improve motility and quality parameters such as viability, plasma membrane integrity, and reduce oxidative stress of goat sperm after thawing.

Key words: Antioxidant, Malondialdehyde, Soybean lecithin extender, Sperm, Vitamin B_6

Introduction

Artificial insemination (AI) is the most effective reproductive technology for the genetic improvement of livestock (Holt, 2000; Leboeuf *et al*., 2000). Cryopreservation is the preferred method for long-term storage of semen, through which sperm can be used for multiple inseminations in females (Anger *et al*., 2003). Despite much research, the quality of frozen sperm has not improved to the desired extent. While the sperm of some species are more tolerant of cooling processes, goat and sheep sperm are prone to the destructive effects of cooling and freezing (Evans and Maxwell, 1987). Loss of asymmetry of bilayer phospholipids in mammalian sperm as one of structural changes along with the plasma membrane damages lead to increased sensitivity to lipid peroxidation (Anghel *et al*., 2010).

Reactive oxygen species (ROS) are constantly produced in the body of mammals and must be removed by antioxidants. Imbalance in oxidant-antioxidant activity is involved in free radical damages (Mahfouz *et al*., 2009). Sperm mitochondrion is also a major source of ROS production and is strongly involved in inducing aging and apoptosis (Koppers *et al*., 2011).

In vitro studies show that the addition of antioxidants to semen extenders improves sperm quality (Atessahin *et al*., 2008; Bucak *et al*., 2009; Eidan, 2016). Pyridoxine (vitamin B_6) is a water-soluble vitamin and belongs to the B family of vitamins. Pyridoxine plays role in more than 100 enzymatic reactions (Bowling, 2011), and has strong antioxidant activity (Tunali, 2014). Its protective action is partly due to inhibiting free radicals produced inside and outside of the mitochondria (Kannan and Jain, 2004). Compared to vitamin C, pyridoxine neutralizes singlet oxygen radicals (Ehrenshaft *et al*., 1999). Therefore, the normal level of vitamin B_6 in males seems to be important for maintaining semen quality and normal sperm parameters. It has been shown that diluting goat semen with extenders containing egg yolk or milk can be harmful to sperm cells (Purdy, 2006), while soybean lecithin extender has been suggested as an appropriate alternative (Vidal *et al*., 2013). However,

optimization of the recently suggested extender by the addition of different levels of antioxidants, such as pyridoxine, is required to dilute goat semen. The aim of the present study was to investigate the effect of soybean lecithin-based extender supplementation with different pyridoxine levels on post-thaw parameters of goat sperm.

Materials and Methods

Chemicals

All of the chemicals in this research were acquired from Sigma Aldrich (USA).

Animal

Semen was collected from four Mahabadi goats, an Iranian dual-purpose breed for the consumption of milk and meat, aged 3 to 4 years. Semen was collected using an artificial vagina twice a week for four weeks during the breeding season. The animals were housed on a farm at the University of Teheran under the same condition and constant dietary regime. During each semen sampling, the samples complied with initial quality (motility > 80% and abnormality < 10% and concentration $\geq 4 \times 10^9$ sperm/ml) were mixed to remove individual results and aliquoted into 7 subsamples to impediment treatments.

Semen processing

Semen samples were diluted with soybean lecithin-based extender supplemented by various concentrations of pyridoxine (0, 2, 4, 6, 8, 10, and 12 mM) to reach a final concentration of 400×10^6 sperm per ml. The base diluent consisted of Tris buffer 30.7 g/L, fructose 12.6 g/L, citric acid 16.4 g/L, and soybean lecithin 1.5% (w/v) with 5% (w/v) glycerol. The base solution's final pH and osmolarity were 6.9 and 425 mOsm/kg, respectively. The procedure for cooling and freezing was based on the method outlined by Baghshahi *et al*. (2014). Briefly, diluted semen samples were transferred into 15 ml plastic Falcon tubes, stored for 2 h at 4°C, and then packed into 0.25 ml French straws (IMV, Laigle, F-61300, France). Afterwards, the straws were placed at a distance of 3 cm from the liquid nitrogen surface for 15 min, immersed in the liquid nitrogen, and stored until sequence evaluations. For post-thawing evaluations, frozen straws were thawed in a water bath at 37°C for 30 s.

Computer-assisted semen analyzer (CASA)

A contrast phase microscope (Labomed Lx400, LA) connected to CASA software (VideoTesT, Sperm 3.1 St. Petersburg, Russia) was used for sperm motility assessments. Each sample of semen was diluted with a Tris-based extender at the ratio of 1:4 and transferred to 1 ml of plastic tubes and incubated for 10 min at 37°C. For each sample, a 3-µL drop was placed on the slide and five fields were selected for analysis, randomly. Sperm motility characteristics included the total motility (%), progressive motility (%), straight-line velocity (VSL, µm/s), curvilinear velocity (VCL, µm/s), amplitude of the lateral head displacement (ALH, µm), linearity index [LIN= (VSL/VCL)×100], average path velocity (VAP, µm/s), straightness coefficient [STR= (VSL/VAP)×100], mean angular displacement (MAD, degrees), beat cross frequency (BCF, Hz), and WOB= wobble (VAP/VCL).

Plasma membrane integrity

The sperm plasma membrane integrity was examined by the hypo-osmotic swelling test (HOST). To prepare the hypo-osmotic swelling solution (100 mmol/kg), 0.9 g of fructose and 0.49 g of sodium citrate were dissolved in 100 ml of distilled water. Then, 30 µL of semen was mixed with 300 µL of the hypo-osmotic solution and incubated for 60 min at 37°C. After incubation, 15 µL of the mixture was placed on a warm slide and covered with a coverslip. A total of 200 sperm per slide were examined in five fields of view using a phase-contrast microscope with a magnification of 400×. Sperm with swollen and curved tails were recorded as intact plasma membranes, while those with straight tails were identified as damaged plasma membranes (Revell and Mrode, 1994).

Sperm viability

Sperm viability was measured using eosin-nigrosin dye (Björndahl *et al*., 2003). To prepare this dye, 0.67 g of eosin Y yellow and 0.9 g of sodium chloride were dissolved in 100 ml of distilled water under heating. A 10 µL drop of thawed semen was mixed on a warm slide with two drops (20 µL) of stain and allowed to air-dry. At least 200 sperm were counted in at least four microscopic fields of view on each slide at 400× magnification. Because live sperm with a healthy membrane do not absorb the dye, the whole or partially stained sperm head was considered as the dead sperm.

Sperm lipid peroxidation

According to the method of Placer *et al*. (1966), malondialdehyde concentration in semen samples was determined as an indicator of lipid peroxidation. For this purpose, 1 ml of semen and 2 ml of 20% thiobarbituric acid were mixed and then centrifuged at 900 g for 15 min. Then, 1 µL of the supernatant was incubated with 2 ml of 0.67% thiobarbituric acid for 10 min at 100°C. After cooling, the absorbance at 532 nm was read using a spectrophotometer.

Statistical analysis

Data were expressed as mean±SEM. The Shapiro-Wilk test was used to check the data distribution. Statistical analyses were done using the general linear modeling (GLM) method of the SAS Statistical software version 9.4 (SAS Institute, Cary, NC, USA). The means were compared using Tukey's test and the values of $P<0.05$ were considered statistically significant.

Results

Results of the total and progressive motility of sperm

are shown in Fig. 1. Addition of different levels of pyridoxine improved the sperm total and progressive motility (P<0.05). Pyridoxine at 6 mM had the highest total motility (58.37%) compared to other levels (P<0.05). However, the level of 12 mM was not statistically different from the control group. The progressive motility percentage was the highest at 4, 6, and 8 mM (P<0.05). Although the control group had the least progressive motility, there was no significant difference between control and 12 mM groups.

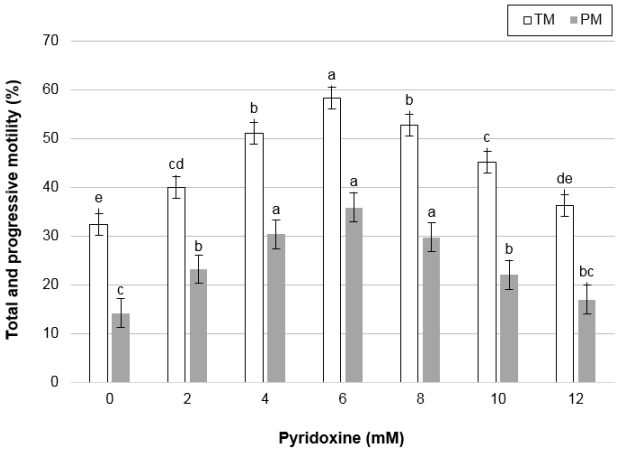

Fig. 1: Effect of different levels of pyridoxine on total and progressive motility of goat sperm after thawing. Bars with different superscript letters are significantly different at level of P<0.05

The 6 mM group had the highest VSL (92.32 µm/s) and 12 mM pyridoxine with 27.07 had the lowest VSL compared to other groups (P<0.05; Table 1). In addition, the 6 mM pyridoxine showed the highest VCL (127.71 µm/s) compared to other groups (P<0.05; Table 1). The VAP was the highest at 6 mM (P<0.05; Table 1). The 6 mM and the control had the highest and lowest MAD (22.68 and 7.78, respectively; P<0.05). The highest and lowest ALH were recorded for the 6 mM and 12 mM pyridoxine (5.67 µm and 1.45 µm, respectively) (P<0.05; Table 1). The 6 mM pyridoxine had the highest BCF compared to other groups (P<0.05). The observations also showed that the control group had the lowest BCF, although it was not significantly different from the levels of 12 mM (Table 1). The results showed that the level of 12 mM had the lowest linear coefficient (P<0.05). However, no significant difference was observed between other treatments (Table 1). The highest WOB was related to the 6 mM level (P<0.05; Table 1). Straightness coefficient at 6 mM was calculated to be 0.71, showing a significant difference with the control group (Table 1).

Viability

The effect of adding pyridoxine in soybean lecithin-based extender on viability after freezing-thawing was significant (Fig. 2, P<0.05). Among pyridoxine levels, 6 mM with 76.6% and control with 34.57% had the highest and lowest viability percentage after cryopreservation, respectively (P<0.05).

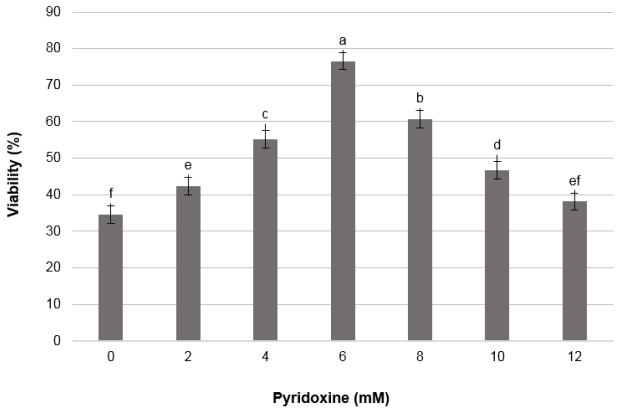

Fig. 2: Effect of different levels of pyridoxine on goat sperm viability after thawing. Bars with different superscript letters are significantly different at level of P<0.05

Plasma membrane integrity

Addition of pyridoxine to soybean lecithin-based extender improved the sperm plasma membrane integrity after thawing (P<0.05; Fig. 3). The highest percentage of plasma membrane integrity was observed in 6 mM group (87.50%; P<0.05).

Malondialdehyde concentration

Figure 4 shows the malondialdehyde concentration in different treatments. Pyridoxine at 6 mM with the highest decrease in malondialdehyde concentration had the best

Table 1: Effect of different levels of pyridoxine on motility characteristics of goat sperm after thawing

Pyridoxine (mM)	Parameters								
	VSL	VCL	VAP	MAD	ALH	BCF	LIN	WOB	STR
0	44.03±4.82^{d}	72.48±5.47^{cd}	43.3±1.77^{c}	7.78±1.51^{e}	3.24±0.43^{d}	0.71±0.47^{d}	0.61±0.11^{a}	0.60±0.05^{c}	1.02±0.13^{a}
2	51.04±4.93^{c}	75.9±4.96^{cd}	47.68±3.89^{c}	12.76±1.7^{d}	3.33±0.48^{d}	1.83±0.57^{c}	0.67±0.04^{a}	0.63±0.03^{c}	1.07±0.03^{a}
4	69.02±2.01^{b}	94.77±3.52^{b}	83.36±13.82^{b}	18.61±2.84^{bc}	5.03±0.36^{ab}	2.9±0.16^{ab}	0.73±0.04^{a}	0.88±0.12^{b}	0.85±0.18^{ab}
6	92.32±4.41^{a}	127.71±9.51^{a}	129.85±8.94^{a}	22.68±0.55^{a}	5.67±0.32^{a}	3.33±0.28^{a}	0.72±0.04^{a}	1.02±0.02^{a}	0.71±0.04^{b}
8	67.85±2.49^{b}	91.89±5.43^{b}	77.6±3.12^{b}	19.68±1.61^{ab}	4.33±0.41^{bc}	2±0.68^{bc}	0.74±0.05^{a}	0.85±0.03^{b}	0.88±0.05^{ab}
10	51.7±1.44^{c}	79.62±5.88^{c}	51.23±2.49^{c}	15.1±1.94^{cd}	3.62±0.33^{cd}	1.25±0.43^{cd}	0.65±0.07^{a}	0.65±0.05^{c}	0.88±0.05^{ab}
12	27.07±0.94^{e}	65.6±3.96^{d}	36.38±5.12^{c}	12.2±1.28^{d}	1.45±0.4^{e}	1.28±0.36^{cd}	0.41±0.03^{b}	0.56±0.10^{c}	1.01±0.05^{a}
SEM	1.69	2.91	3.43	0.87	0.19	0.22	0.03	0.03	0.05

VSL: Straight-line velocity (µm/s), VCL: Curvilinear velocity (µm/s), VAP: Average path velocity (µm/s), MAD: Mean angular displacement (degrees), ALH: Amplitude of the lateral head displacement (µm), BCF: Beat cross frequency (Hz), LIN: Linearity index [(VSL/VCL)×100], WOB: Wobble [(VAP/VCL))×100], STR: Straightness coefficient [(VSL/VAP)×100], and SEM: Standard error of the mean. $^{a, b, c, d}$ Different letters in each row indicate a significant difference at P<0.05

performance compared to the control group (P<0.05), although there was no significant difference among 4, 6, and 8 mM. Also, the control group and 12 mM had the highest malondialdehyde concentration (P<0.05).

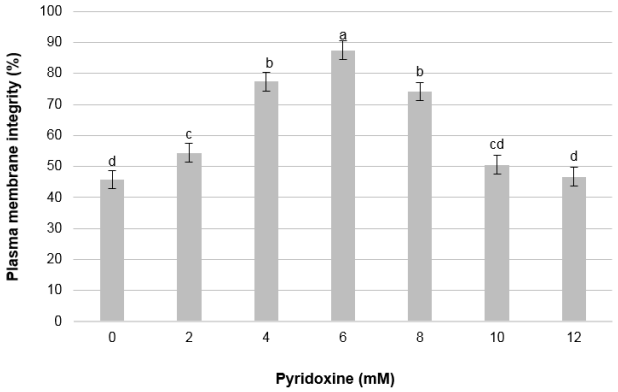

Fig. 3: Effect of different pyridoxine levels on plasma membrane integrity of goat sperm after thawing. Bars with different superscript letters are significantly different at level of P<0.05

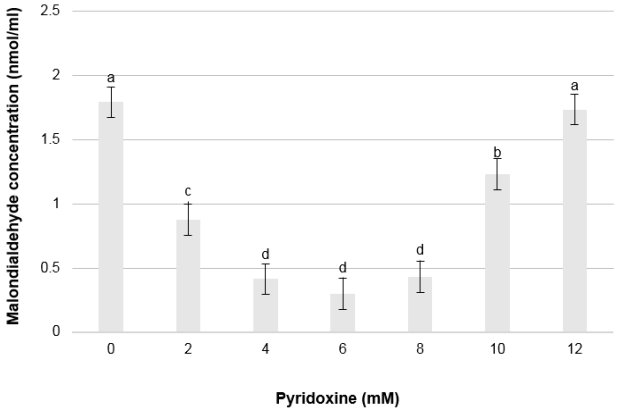

Fig. 4: Effect of different levels of pyridoxine on goat semen malondialdehyde concentration after thawing. Bars with different superscript letters are significantly different at level of P<0.05

Discussion

The present study showed that the addition of pyridoxine could improve the motility and quality parameters of goat sperm after the freezing-thawing process. Probably, part of the beneficial effects of pyridoxine on sperm motility and quality parameters is through reducing the oxidants concentrations produced in the freezing process. In line with these results, it has been shown that vitamin B_6 deficiency in rats and humans can alter the glutathione system and thus affect the antioxidant defense mechanism of sperm against oxidative damage, which ultimately leads to changes in sperm parameters (Banihani, 2017). Similarly, Daramola *et al*. (2017) found that supplementation of egg yolk-based extender with 4 and 6 mM pyridoxine increased motility, acrosomal, and membrane integrity of goat sperm (Daramola *et al*., 2017). The improvement of these parameters was accompanied by a decrease in malondialdehyde, indicating the beneficial effect of pyridoxine on sperm viability, and this can be attributed to its strong antioxidant properties. Besides, extender supplementation with pyridoxine was able to improve motility, capacitation, and acrosome reaction of goat sperm during cryopreservation, which could speculatively be ascribed to its ability to enter mitochondria as a water-soluble antioxidant (Daramola *et al*., 2015; Daramola *et al*., 2016). They showed that the addition of 8 mM pyridoxine to a coconut milk-based extender was able to maintain the optimal viability of goat sperm after thawing (Daramola *et al*., 2016). These conflicting results regarding the optimal dose of pyridoxine can be due to the various amounts of vitamins in eggs or milk, depending on their diet.

It has been reported that most common antioxidants cannot enter the mitochondria, as the main site of ROS production, due to the physicochemical properties of mitochondria. Enzymatic antioxidants such as superoxide dismutase and catalase are not able to cross cell membranes and therefore are not able to reduce intracellular ROS (Ramis *et al*., 2015). Vitamin E and coenzyme Q, which are highly lipophilic, also tend to remain in cell membranes and cannot protect against intracellular ROS (Ramis *et al*., 2015). Therefore, it is necessary to use the antioxidants that can enter the mitochondria to remove intracellular ROS. The protective action of pyridoxine is partly by inhibiting free radicals produced inside and outside the mitochondria (Kannan and Jain, 2004). Compared to vitamin C, pyridoxine neutralizes singlet oxygen radicals (Ehrenshaft *et al*., 1999).

Daramola *et al*. (2015) showed that the presence of 6 mM pyridoxine could improve the efficiency of vitamin C or E on the quality parameters of sperm after thawing (Daramola *et al*., 2015), which may be due to the entry of pyridoxine into the mitochondria and scavenging of ROS.

The declining trend of beneficial effects of dose-dependent pyridoxine can be due to the adverse effects of high levels of pyridoxine on reproductive cells. It is reported that high oral intake of pyridoxine caused atrophy of the testes, epididymis, prostate gland, and seminal vesicles and decreased the number of mature sperm in the testes and epididymis of rats (Kaido *et al*., 1991; Mori *et al*., 1992). Another study also showed that the main disorder caused by an overdose of pyridoxine was on adult sperm in rats. Overdose of pyridoxine reduces sperm motility and causes severe changes in the sperm nuclei, making the sperm nucleus smaller, shorter, and smoother. It is reported that rat sperm are more sensitive to pyridoxine than testes (Ide *et al*., 1992).

Our findings showed that supplementation of pyridoxine (6 mM) in soybean lecithin extender could improve motility and quality parameters such as viability, plasma membrane activity, and could reduce the oxidative stress in goat sperm after thawing. The results also showed that high doses of pyridoxine could impair sperm motility and quality parameters.

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Conflict of interest

The authors have no conflicts of interest to declare.

References

Anger, JT; Gilbert, BR and Goldstein, M (2003). Cryopreservation of sperm: indications, methods and results. J. Urol., 170: 1079-1084.

Anghel, AH; Zamfirescu, S; Coprean, D and Nadolu, D (2010). Antioxidant additives effect on cytological parameters of refrigerated ram semen. Archive. 53: 294-298.

Atessahin, A; Bucak, MN; Tuncer, PB and Kızıl, M (2008). Effects of anti-oxidant additives on microscopic and oxidative parameters of Angora goat semen following the freeze-thawing process. Small Rumin. Res., 77: 38-44.

Baghshahi, H; Riasi, A; Mahdavi, AH and Shirazi, A (2014). Antioxidant effects of clove bud (*Syzygium aromaticum*) extract used with different extenders on ram spermatozoa during cryopreservation. Cryobiology. 69: 482-487.

Banihani, SA (2017). A systematic review evaluating the effect of vitamin B_6 on semen quality. J. Urol., 15: 1-5.

Björndahl, L; Söderlund, I and Kvist, U (2003). Evaluation of the one-step eosin-nigrosin staining technique for human sperm vitality assessment. Hum. Reprod., 18: 813-816.

Bowling, FG (2011). Pyridoxine supply in human development. Semin. Cell Dev. Biol., 22: 611-618.

Bucak, MN; Sarıözkan, S; Tuncer, PB; Ulutaş, PA and Akçadağ, Hİ (2009). Effect of antioxidants on microscopic semen parameters, lipid peroxidation and antioxidant activities in Angora goat semen following cryo-preservation. Small Rumin. Res., 81: 90-95.

Daramola, JO; Adekunle, EO; Iyasere, OS; Oke, OE; Sorongbe, TA; Iyanda, OA; Kehinde, AR; Aluko, SP; Olaoye, IO; Gbadebo, OE and Falolu, LI (2016). Effects of coconut milk alone or supplementation with pyridoxine in tris-extenders on viability of buck spermatozoa during vitrification. Small Rumin. Res., 136: 208-213.

Daramola, JO; Adekunle, EO; Oke, OE; Onagbesan, OM; Iyasere, OS; Williams, TJ; James, IJ; Oyewusi, IK and Oyewusi, JA (2015). Effects of pyridoxine supple-mentation or in combination with other antioxidants on motility, *in vitro* capacitation and acrosome reaction of goat buck spermatozoa during cryopreservation. Small Rumin. Res., 131: 113-117.

Daramola, JO; Adekunle, EO; Oke, OE; Onagbesan, OM; Williams, TJ; Iyasere, OS; James, IJ; Oyewusi, IK and Oyewusi, JA (2017). Effects of pyridoxine in combination with different antioxidants on viability and oxidative stress parameters of cryopreserved goat buck semen. Arch. Zootec., 66: 15-21.

Ehrenshaft, M; Bilski, P; Li, MY; Chignell, CF and Daub, ME (1999). A highly conserved sequence is a novel gene involved in de novo vitamin B_6 biosynthesis. Proc. Natl. Acad. Sci., 96: 9374-9378.

Eidan, SM (2016). Effect on post-cryopreserved semen characteristics of Holstein bulls of adding combinations of vitamin C and either catalase or reduced glutathione to Tris extender. Anim. Reprod. Sci., 167: 1-7.

Evans, G and Maxwell, WC (1987). *Salamons' artificial insemination of sheep and goats*. 2th Edn., Butterworths, Sydney, Australia. P: 194.

Holt, W (2000). Basic aspects of frozen storage of semen. Anim. Reprod. Sci., 62: 3-22.

Ide, Y; Kaido, M and Koide, O (1992). Changes in spermatozoa due to large doses of pyridoxine (vitamin B_6). Pathol. Int., 42: 861-869.

Kaido, M; Mori, K; Ide, Y; Inoue, N and Koide, O (1991). Testicular damage by high doses of vitamin B_6 (pyridoxine) in rats: a light and electron microscopical study. Exp. Mol. Pathol., 55: 63-82.

Kannan, K and Jain, SK (2004). Effect of vitamin B_6 on oxygen radicals, mitochondrial membrane potential, and lipid peroxidation in H_2O_2-treated U937 monocytes. Free Radic. Biol. Med., 36: 423-428.

Koppers, AJ; Mitchell, LA; Wang, P; Lin, M and Aitken, RJ (2011). Phosphoinositide 3-kinase signalling pathway involvement in a truncated apoptotic cascade associated with motility loss and oxidative DNA damage in human spermatozoa. Biochem. J., 436: 687-698.

Leboeuf, B; Restall, B and Salamon, S (2000). Production and storage of goat semen for artificial insemination. Anim. Reprod. Sci., 62: 113-141.

Mahfouz, MM; Zhou, SQ and Kummerow, FA (2009). Vitamin B_6 compounds are capable of reducing the superoxide radical and lipid peroxide levels induced by H_2O_2 in vascular endothelial cells in culture. Int. J. Vitam. Nutr. Res., 79: 218-229.

Mori, K; Kaido, M; Fujishiro, K; Inoue, N and Koide, O (1992). Effects of megadoses of pyridoxine on spermatogenesis and male reproductive organs in rats. Arch. Toxicol., 66: 198-203.

Placer, ZA; Cushman, LL and Johnson, BC (1966). Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Anal. Biochem., 16: 359-364.

Purdy, P (2006). A review on goat sperm cryopreservation. Small Rumin. Res., 63: 215-225.

Ramis, MR; Esteban, S; Miralles, A; Tan, DX and Reiter, RJ (2015). Protective effects of melatonin and mitochondria-targeted antioxidants against oxidative stress: a review. Curr. Med. Chem., 22: 2690-2711.

Revell, SG and Mrode, RA (1994). An osmotic resistance test for bovine semen. Anim. Reprod. Sci., 36: 77-86.

Tunali, S (2014). The effects of vitamin B6 on lens antioxidant system in valproic acid-administered rats. Hum. Exp. Toxicol., 33: 623-628.

Vidal, AH; Batista, AM; Cordeiro, E; Silva, B; Gomes, WA; Pelinica, MA; Silva, SV; Medalena, M and Guerra, P (2013). Soybean lecithin-based extender as an alternative for goat sperm cryopreservation. Small Rumin. Res., 109: 47-51.