Research Report

Effects of a semen extender treated with coenzyme Q10-ubiquinol on the quality of thawed ram sperm

Eduardo Arroyo,[a‡] Warwick Bayly,[a] Swarnita Chakraborty,[b] Desmond Leadon,[c] Ahmed Tibary[a]
[a]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, WA, USA
[b]Department of Mathematics and Statistics, College of Arts and Sciences, Washington State University, Pullman, WA, USA
[c]Swordlestown Little Stud, Naas County, Kildare, Ireland

Abstract

Objective was to determine whether addition of coenzyme Q_{10} - ubiquinol to glycerol extender improved cryopreserved ram sperm quality. Semen was collected (electroejaculation) from mature Suffolk rams (n = 6) 6 times during the breeding season. Pooled semen from each session was processed and cryopreserved in extenders with 150 µmol/liter of ubiquinol (treated) and without (control). Thawed cryopreserved semen was evaluated using computer-assisted sperm analysis for motility and kinetic parameters. Sperm viability was determined via plasma membrane integrity. After thawing, measurements were obtained at 10, 20, 60, and 120 minutes. In treated samples, there were increases in progressive motility ($p < 0.0001$), curvilinear velocity ($p = 0.002$), straight line velocity ($p = 0.003$) and time average velocity ($p = 0.001$), and sperm tended ($p = 0.07$) to have more viability. Furthermore, there were time-related decreases in all variables in treated control and samples. Ubiquinol improved cryopreserved ram sperm quality.

Keywords: Antioxidants, reactive oxygen species, sperm, small ruminants

Introduction

Artificial insemination has been beneficial to the livestock industry, as it allows dissemination of valuable genetics and hastens improvement in genetic makeup of production animals.[1] Semen processing, storage, and transport should meet high quality standards to maximize conception rates.[2] Manipulation and storage of semen impairs sperm function. Mammalian sperm are highly susceptible to oxidative stress due to high concentrations of polyunsaturated fatty acids in cell membranes and lack of cytoplasmic antioxidants. Oxidative stress and accumulation of radical oxygen species (ROS) are major factors contributing to poor sperm function and low conception rates.[3] During mitochondrial redox reactions via oxidative phosphorylation, ~ 5% oxygen is transformed into ROS.[4] Freezing, thawing, and procedures associated with semen storage induce oxidative stress damage to sperm. These may be factors contributing to cryodamage and alteration of sperm function and structure. Oxidative stress is partially responsible for reductions in postthaw sperm motility, viability, membrane and acrosome integrity, mitochondrial function, DNA integrity, and finally, fertility in stallions.[5] Several approaches have been used to limit ROS formation in semen, including systemic supplementation with antioxidants or their addition to semen extenders.[6]

In men, reduced sperm motility was attributed to a relative deficiency of Coenzyme Q10 (CoQ_{10}).[4] Additionally, energy use by sperm depends on CoQ_{10} availability.[7] CoQ_{10} exists in 2 forms: ubiquinone and ubiquinol. Ubiquinol is the reduced, electron-rich bioactive form of CoQ_{10} and a potent nonenzymatic antioxidant. Most CoQ_{10} in mammalian body (> 90%) exists as ubiquinol. It protects lipids, proteins, and DNA by minimizing first stages of lipid peroxidation and participates in recycling of secondary antioxidants like α-tocopherol.[8] These reactions result in temporary oxidation of ubiquinol to ubiquinone that serves as an electron carrier in mitochondrial respiratory chain. This function is associated with ROS production and reduction of ubiquinone back to ubiquinol.[9] CoQ_{10} is naturally present in every body cell, including sperm midpiece.[10] CoQ_{10} has enhanced postthaw

‡Current affiliation: Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA

CONTACT Eduardo Arroyo **eduardoarroyo@ufl.edu**

Citation: Clinical Theriogenology 2024, 16, 10820, http://dx.doi.org/10.58292/CT.v16.10820

sperm quality in men,[11] stallions,[12] bulls,[11] goat bucks,[13] roosters,[14] and rams.[15]

Several ubiquinone preparations are commercially available but have poor water solubility and limited bioavailability. CoQ_{10} is absorbed from the intestine as ubiquinol. Intestinal absorption is variable and affected mainly by CoQ_{10} formulation type.[16] Recently, a water-dispersible preparation of ubiquinol was developed and provided better bioavailability compared to conventional CoQ_{10} preparations.[17] Our objective was to determine whether adding water-dispersible ubiquinol preparation to a ruminant semen extender improved motion parameters and viability of frozen-thawed ram sperm. We hypothesized that addition of water-dispersible ubiquinol to freezing extender improves sperm motility and viability compared to similar extender without ubiquinol.

Materials and methods

In accordance with WSU IACUC policy # 21, this research is exempt because it utilized tissues collected from animas for a routine breeding soundness examination that occurred for clinical purposes and no animals were manipulated for the purpose of this research.

Animals and study location

Six mature Suffolk rams aged 1-4 years, in good physical condition and with proven fertility were used. Rams were raised in Pacific Northwest of US (46° 44′ 32.4708″, 117° 1′ 3.234), housed separately from ewes, and received a daily diet consisting of alfalfa hay, grass hay, barley, commercial sheep concentrate, canola meal, and ground corn. Diet contained, on a dry matter basis, 2.97 megacalorie metabolizable energy/kg, 12.6% crude protein, 0.8% calcium, 0.31% phosphorus, 0.19% magnesium, 1.71% potassium, and 0.92% sodium.[18] Rams had access to mineral blocks and fresh water.

Experimental design

Semen was collected via electroejaculation on 6 days during the September-October breeding season. The low-voltage setting of a ram ejaculator (Lane Manufacturing, Denver, Co, USA) with a probe length of 6 inches was utilized. Each ejaculate was evaluated for volume and concentration. Only ejaculates of > 1 ml with a concentration of > 2 x 10^9 sperm/ml, total motility > 50%, and abnormal sperm < 10%, determined with a computer-assisted sperm analyzer, were used. Ejaculates from each collection day were pooled and divided into 2 equal aliquots and then diluted to achieve the final cryopreservation concentration of 200 x 10^6/ml. Dilutions were made with a commercial semen extender containing 20% egg yolk. Either 0 µmol/liter (control) or 150 µmol/liter of a 30% solution (treated) of water-dispersible ubiquinol (Petroeuroasia, Shizuoka, Japan) was added to extender and homogenized for 30 minutes before use.

For cryopreservation, semen samples plus Triladyl® were transferred into a warm water bath (30°C) and then cooled to 5°C at an approximated rate of 0.27°C/minute. Semen was equilibrated for 4 hours at 5°C then loaded into 0.5 ml straws. After equilibration, the straws were placed 4 cm above the liquid nitrogen surface for 20 minutes and then plunged into liquid nitrogen. Frozen semen straws were stored for 30 days in liquid nitrogen until postthaw evaluation.

Semen evaluation

All ejaculates fulfilled inclusion criteria. Semen evaluations were replicated 6 times on each stored sample. Cryopreserved straws were thawed at 37°C for 30 seconds. Samples were kept at 37°C and evaluated for sperm motility, kinetics, and viability at 10, 20, 60, and 120 minutes after thawing.

Sperm motion parameters were determined using computerized assisted semen analysis (CASA SpermVision®, Mofa®, Verona, WI, USA) equipped with a monochrome video camera (Imi Tech, IMB-12 FT). For analysis, 3 µl of sample was placed on a prewarmed (37°C) calibrated slide (Leja®, IMV Technologies, Maple Grove, MN, USA). Seven fields were randomly evaluated under a phase contrast microscope 20 x positive low objective (AXIO 10, Carl Zeiss) with a warmed stage. The acquisition frame rate was set at 75 frames/second. Output criteria of CASA included the proportion of motile sperm and the proportion of sperm with progressive motility (PM). Sperm motion characteristics were analyzed as follows: if the average velocity was below 10 µm/second, sperm were regarded as immotile, whereas velocity minimums for slow, medium and rapid sperm were 10, 25 and 50 µm/second, respectively. Sperm motion velocity was measured in 3 ways: a) straight line velocity (VSL) is the time-average velocity of a sperm head along the straight line between its first and last detected positions; b) curvilinear velocity (VCL) is the average velocity measured over the actual point-to-point track followed by the sperm; and c) time-average velocity (VAP) measures sperm head along its spatial average trajectory (i.e. smoothed version of VCL). Linearity (LIN) is the linearity of the curvilinear trajectory calculated as VSL/VCL × 100. Straightness (STR) is the LIN of the sperm average path calculated as VSL/VAP × 100. Minimal LIN was 50%, and minimal STR for progressive fast sperm was 70%. Amplitude of lateral head displacement (ALH) is the maximum lateral displacement of a sperm head about its spatial average trajectory (i.e. track width).

Sperm viability was assessed using the SYBR14/PI stain (Minitube, Germany) to test sperm membrane integrity, following manufacturer's instructions. Briefly, 100 µl of semen was mixed with 10 µl of warm SYBR14/PI fluorescent stain and incubated for 10 minutes at 37°C. After incubation, 5 µl droplet of the stained sample was placed over a slide, covered with a coverslip, and analyzed under epifluorescence microscopy (400 x). Red-stained sperm head was considered to have a nonintact membrane (nonviable, PI positive), and green-stained sperm were regarded as having intact membranes (viable, SYBR14 positive). Two hundred sperm were evaluated per sample for frozen-thawed samples.

Data analyses

As data were normally distributed (Shapiro-Wilk test), parametric tests were used. Effects of time of incubation, diluent, and their interactions on CASA measurements of sperm motility kinetics (PM, VSL, VCL, VAP, LIN, STR, ALH) and sperm viability were analyzed by a general linear model randomized complete block design with repeated measures analysis of variance. If significant treatment x time interactions were identified, pair-wise tests for the interaction effects

Citation: Clinical Theriogenology 2024, 16, 10820, http://dx.doi.org/10.58292/CT.v16.10820

were conducted with the Tukey-Kramer adjustment to identify significant pairs of interactions. Analyses were performed using the SAS 9.4 studio online platform (SAS 2022 Institute, Cary, NC, USA) to test treatment effects of extender with ubiquinol on sperm parameters; $p < 0.05$ was considered significant. Values are expressed as mean ± standard deviation.

Results

Mean values of sperm motility and kinetics are reported (Table 1). Postthaw progressive motility was higher ($p < 0.0001$) in extender containing ubiquinol compared to control. VSL ($p = 0.003$), VCL ($p = 0.002$), and VAP ($p = 0.001$) were higher in treated samples. There was an effect of treatment with ubiquinol on ALH ($p = 0.029$) but not on LIN ($p = 0.67$) or STR ($p = 0.68$). The values declined over time for control and treated samples (Table 1).

Values for viability (plasma membrane integrity) at each time point are reported (Table 2). There was no significant extender treatment effect on the percentage of sperm with intact membranes, although the difference approached significance ($p = 0.073$).

Discussion

Addition of 150 µmol/liter of ubiquinol to extender improved PM and motion kinetics parameters (VAP, VCL, VSL, ALH) after thawing. Viability of samples exposed to extender plus ubiquinol tended to be higher; however, the improvement ($p = 0.073$) did not meet the $p < 0.05$ criterion for significance. Concentration of ubiquinol (150 µmol/liter) used was based on a stallion semen report wherein 75 µmol/liter of CoQ_{10}-ubiquinone had positive effects on certain parameters of sperm motion.[19] We tested a higher concentration of ubiquinol because of the possibility that egg yolk in the extender could bind with ubiquinol and make it less available to sperm.[20]

Although causes of subfertility and infertility in male reproduction are multifactorial, oxidative stress has an important role.[21] Use of exogenous antioxidants improved the quality and lifespan of poor-quality sperm such as in conditions associated with asthenozoospermia and teratozoospermia.[22] Addition of CoQ_{10} and other antioxidants to diet and semen extenders had positive effects on sperm quality.[22–24] Ubiquinol is the antioxidant form of CoQ_{10} and has been widely used to enhance reproductive health and prevent neurodegenerative diseases and musculoskeletal disorders in people.[24] Several CoQ_{10} preparations are available, differing mainly in their redox state and degree of bioavailability, due primarily to their relative insolubility in water. We used a synthesized water-dispersible formulation of ubiquinol preparation that has high availability and stability once in solution.[17]

Ubiquinol-treated samples had a higher PM at each time point. These results agree in part with another study on ram semen that had a higher progressive motility after storage for 5 days at room temperature in samples to which 50 µmol/liter ubiquinol was added.[15] Better PM associated with using ubiquinol-treated extender was likely due to its protective antioxidant effect on sperm plasma membrane. However, an effect on the mitochondrial respiratory chain and the synthesis of ATP might have also contributed. Higher postthaw PM observed in samples containing ubiquinol was also similar to a study on goat semen; addition of 1 µM of CoQ_{10} significantly improved motility of

Table 1. Postthaw ram sperm motion characteristics of semen frozen in Triladyl extender, without and with ubiquinol

	Triladyl				Triladyl + Ubiquinol			
	10 minutes	20 minutes	60 minutes	120 minutes	10 minutes	20 minutes	60 minutes	120 minutes
PM	30.83 ± 7.62^{aA}	32.54 ± 8.62^{bB}	25.63 ± 5.24^{cC}	24.14 ± 7.38^{dD}	46.05 ± 1.79^{bA}	44.98 ± 1.77^{cB}	40.01 ± 2.36^{dC}	31.27 ± 4.08^{eD}
VCL µm/second	96.53 ± 4.3^{aA}	87.06 ± 6.32^{bB}	87.06 ± 4.02^{cC}	87.47 ± 6.52^{eE}	100.39 ± 3.45^{bA}	94.10 ± 5.1^{cB}	92.64 ± 4.24^{dC}	94.97 ± 4.69^{eD}
VAP µm/second	30.84 ± 7.63^{aA}	32.55 ± 8.69^{bB}	25.63 ± 5.24^{cC}	24.15 ± 7.38^{eE}	46.05 ± 1.80^{bA}	44.98 ± 1.77^{cB}	40.02 ± 2.37^{dC}	31.28 ± 4.08^{eD}
VSL µm/second	44.82 ± 2.34^{aA}	41.41 ± 2.39^{bB}	39.71 ± 1.79^{cC}	39.74 ± 2.19^{eE}	46.40 ± 1.85^{bA}	44 ± 1.66^{cB}	42.25 ± 1.17^{dC}	43.27 ± 1.98^{eD}
LIN (%)	0.46 ± 0.01^{aA}	0.46 ± 0.01^{aB}	0.45 ± 0.01^{aC}	0.45 ± 0.01^{aD}	0.46 ± 0.01^{aA}	0.46 ± 0.01^{aB}	0.45 ± 0.02^{aC}	0.45 ± 0.01^{aD}
STR (%)	0.78 ± 0.02^{aA}	0.78 ± 0.02^{aB}	0.77 ± 0.01^{aC}	0.77 ± 0.02^{aD}	0.78 ± 0.01^{aA}	0.78 ± 0.01^{aB}	0.77 ± 0.02^{aC}	0.78 ± 0.01^{aD}
ALH (µm)	4.73 ± 0.41^{aA}	4.63 ± 0.47^{aB}	4.53 ± 0.47^{aC}	4.57 ± 0.31^{aD}	5.08 ± 0.32^{aA}	4.86 ± 0.39^{aB}	4.94 ± 0.46^{aC}	4.7 ± 0.37^{aD}

$^{a-e}$means without a common lowercase superscript in a row differed ($p < 0.05$) between treatments.

$^{A-E}$means without a common uppercase superscript in a row differed ($p < 0.05$) between times of treatment.

Table 2. Postthaw viability of ram sperm frozen in Triladyl extender, without and with ubiquinol

	Triladyl				Triladyl + Ubiquinol			
	10 minutes	**20 minutes**	**60 minutes**	**120 minutes**	**10 minutes**	**20 minutes**	**60 minutes**	**120 minutes**
Viability (%)	60.31 ± 2.65^{A}	50.05 ± 3.16^{B}	28.02 ± 3.23^{C}	31.89 ± 2.06^{D}	80.19 ± 3.70^{E}	68.97 ± 2.22^{B}	49.66 ± 2.53^{F}	35.76 ± 2.51^{D}

$^{A-F}$means without a common uppercase superscript differed ($p < 0.05$) between times of treatment.

frozen-thawed samples. However, no details were given regarding the method of addition of CoQ_{10} to extender. In addition, studies on cattle and buffalo bulls,[25] stallions,[26] boars,[27] and rams[28] also reported that CoQ_{10}-supplemented extenders improved sperm motility supporting current results.

Some decrease in PM of frozen-thawed samples overtime was expected, regardless of whether control or treated semen was used. For the treated extender, reduction in PM may be due to the progressive utilization of bioactive ubiquinol, as the greatest decrease in PM occurred between 60 and 120 minutes. This decrease in PM after 60 minutes, despite ubiquinol presence, may not be practically relevant, because frozen-thawed semen have to be used within 10 minutes to achieve good fertilization rates.[29]

Addition of ubiquinol to semen extender increased VSL, VCL, VAP, and ALH; VCL and VAP are positively correlated with sperm quality. There are no standardized values for farm animals' sperm kinetics. Nonetheless, ram VCL is higher than bulls or men.[30] Frozen-thawed ram sperm undergo high oxidative stress during incubation as evident by reduction in kinetics parameters over time.[31] In our study, ubiquinol-treated frozen-thawed samples had higher values for PM and most motion kinetics parameters at specific time points compared to controls. This may suggest that the addition of water-dispersible ubiquinol had a mitigating effect on the oxidative stress imposed on ram semen by freeze-thaw process.

Addition of ubiquinol to semen extender did not increase ($p = 0.073$) plasma membrane integrity. There was a high variance in results for this parameter might explain lack of significant treatment effect. Nonetheless, mean percentage of sperm with intact membranes was numerically higher at each analysis time following addition of ubiquinol to extender. Maintenance of plasma membrane integrity was expected with ubiquinol addition. CoQ_{10} regenerated α-tocopherol and increased its action against ROS damage to plasma membrane.[9] Freezing and thawing of semen impose substantial stress on sperm plasma membrane integrity and functionality. Increases in ROS exacerbate oxidative stress that leads to irreversible cell damage.[32] DNA damage induced by high concentrations of ROS may hasten sperm apoptosis leading to reduced numbers of live sperm.[33] Addition of CoQ_{10} to semen extenders improved sperm plasma membrane integrity and viability in bulls,[25] bucks,[13] men,[11] and roosters.[34] Although there was no significant difference between treatment and control groups, percentage of viable cells tended to remain higher in ubiquinol-treated samples at each time point. Due to lack of direct measurement of free radicals, ROS, and total antioxidant capacity,[15] we can only speculate that the maintenance of plasma membrane integrity indirectly reflected a reduction in lipid peroxidation and DNA fragmentation due to ubiquinol addition, as those parameters were also not directly measured.

Conclusion

There were positive effects of water-dispersible ubiquinol's addition to semen extender on cryopreserved ram sperm. Sperm PM and most kinetics parameters were higher in ubiquinol-treated extender. There was also a trend towards better viability. Addition of water-dispersible form of ubiquinol to extenders may improve frozen/thawed stored sperm quality. Lack of measurements of lipid peroxidation, DNA fragmentation, total antioxidant capacity, and content of ROS are major limitations of the study; further research is warranted.

Conflict of interest

None to report.

References

1. Gibbons AE, Fernandez J, Bruno-Galarraga MM, et al: Technical recommendations for artificial insemination in sheep. Anim Reprod 2019;16:803-809. doi: 10.21451/1984-3143-AR2018-0129
2. Salamon S, Maxwell WM: Storage of ram semen. Anim Reprod Sci 2000;62:77-111. doi: 10.1016/S0378-4320(00)00155-X
3. Aitken RJ, Baker MA, De Iuliis GN, et al: New insights into sperm physiology and pathology. In: Habenicht UF., Aitken RJ. (editors). Fertility Control. Handbook of Experimental Pharmacology, vol 198. Berlin, Springer: 2010; 198:99–115. doi: 10.1007/978-3-642-02062-9_7
4. Amidi F, Pazhohan A, Shabani Nashtaei M, et al: The role of antioxidants in sperm freezing: a review. Cell Tissue Bank 2016;17:745-756. doi: 10.1007/s10561-016-9566-5
5. Aitken RJ, Lambourne S, Gibb Z: The John Hughes Memorial Lecture: aspects of sperm physiology – oxidative stress and the functionality of stallion spermatozoa. J Equine Vet Sci 2014;34:17-27. doi: 10.1016/j.jevs.2013.10.120
6. Gvozdjáková A, Kucharská J, Dubravicky J, et al: Coenzyme Q_{10}, α-tocopherol, and oxidative stress could be important metabolic biomarkers of male infertility. Dis Markers 2015;2015:827941. doi: 10.1155/2015/827941
7. Bailey JL, Bilodeau JF, Cormier N: Semen cryopreservation in domestic animals: a damaging and capacitating phenomenon. J Androl 2000;21:1-7. doi: 10.1002/j.1939-4640.2000.tb03268.x
8. Ernster L, Dallner G: Biochemical, physiological and medical aspects of ubiquinone function. Bba-Mol Basis Dis 1995;1271:195-204. doi: 10.1016/0925-4439(95)00028-3
9. Turunen M, Olsson J, Dallner G: Metabolism and function of coenzyme Q. Biochim Biophys Acta 2004;1660:171-199. doi: 10.1016/j.bbamem.2003.11.012

10. Lançoni R, Celeghini ECC, Giuli V, et al: Coenzyme Q-10 improves preservation of mitochondrial functionality and actin structure of cryopreserved stallion sperm. Anim Reprod 2021;18:e20200218. doi: 10.1590/1984-3143-ar2020-0218
11. Gualtieri R, Barbato V, Fiorentino I, et al: Treatment with zinc, d-aspartate, and coenzyme Q10 protects bull sperm against damage and improves their ability to support embryo development. Theriogenology 2014;82:592-598. doi: 10.1016/j.theriogenology.2014.05.028
12. Nogueira BG, Sampaio BF, Souza MI, et al: Coenzyme Q10 and alpha-tocopherol prevent the lipid peroxidation of cooled equine semen. Reprod Domest Anim 2015;50:1003-1010. doi: 10.1111/rda.12627
13. Yousefian I, Emamverdi M, Karamzadeh-Dehaghani A, et al: Attenuation of cryopreservation-induced oxidative stress by antioxidant: Impact of coenzyme Q(10) on the quality of post-thawed buck spermatozoa. Cryobiology 2018;81:88-93. doi: 10.1016/j.cryobiol.2018.02.005
14. Raouf SM, Taha AT: Effects of COQ10 with vitamin E supplementation on semen quality and seminal plasma parameters of broiler breeder males. Iraqi J Vet Sci 2021;35:65-70. doi: 10.33899/ijvs.2020.126287.1289
15. Wang Y, Kang Y, Zhang L, et al: Coenzyme Q(10) improves the quality of sheep sperm stored at room temperature by mitigating oxidative stress. Anim Sci J 2022;93:e13708. doi: 10.1111/asj.13708
16. Pastor-Maldonado CJ, Suarez-Rivero JM, Povea-Cabello S, et al: Coenzyme Q(10): novel formulations and medical trends. Int J Mol Sci 2020;21;8432. doi: 10.3390/ijms21228432
17. Uchida Y, Wakimoto K, Takahashi H, et al: The bioavailability of reduced coenzyme Q10 water-dispersive powder after single oral administration. J Altern Complement Med 2014;11:103-105. doi: 10.1625/jcam.11.103
18. Salah N, Sauvant D, Archimède H: Nutritional requirements of sheep, goats and cattle in warm climates: a meta-analysis. Animal 2014;8:1439-1447. doi: 10.1017/S1751731114001153
19. Carneiro JAM, Canisso IF, Bandeira RS, et al: Effects of coenzyme Q10 on semen cryopreservation of stallions classified as having good or bad semen freezing ability. Anim Reprod Sci 2018;192:107-118. doi: 10.1016/j.anireprosci.2018.02.020
20. Khalifa TA, Lymberopoulos AG, El-Saidy BE: Testing usability of butylated hydroxytoluene in conservation of goat semen. Reprod Domest Anim 2008;43:525-530. doi: 10.1111/j.1439-0531.2007.00947.x
21. Kumaresan A, Das Gupta M, Datta TK, et al: Sperm DNA integrity and male fertility in farm animals: a review. Front Vet Sci 2020;7:321. doi: 10.3389/fvets.2020.00321
22. Balercia G, Mosca F, Mantero F, et al: Coenzyme Q(10) supplementation in infertile men with idiopathic asthenozoospermia: an open, uncontrolled pilot study. Fertil Steril 2004;81:93-98. doi: 10.1016/j.fertnstert.2003.05.009
23. Masoudi R, Sharafi M, Shahneh AZ: Effects of CoQ10 on the quality of ram sperm during cryopreservation in plant and animal based extenders. Anim Reprod Sci 2019;208:106103. doi: 10.1016/j.anireprosci.2019.06.015
24. Hernandez-Camacho JD, Bernier M, Lopez-Lluch G, et al: Coenzyme Q(10) supplementation in aging and disease. Front Physiol 2018;9:44. doi: 10.3389/fphys.2018.00044
25. Saeed AM, El-Nagar HA, Wafa WM, et al: Effect of coenzyme Q10 as an antioxidant added to semen extender during cryopreservation of buffalo and cattle semen. J Anim Poult Prod 2016;7:403-408. doi: 10.21608/jappmu.2016.48748
26. Yousefian I, Zare-Shahneh A, Zhandi M: The effect of coenzyme Q10 and α-tocopherol in skim milk–based extender for preservation of Caspian stallion semen in cool condition. J Equine Vet Sci 2014;34:949-954. doi: 10.1016/j.jevs.2014.04.002
27. Pindaru L: Effects of coenzyme Q10 on sperm viability durign storage of boar semen at 17°C. Sci Works 2016;61:32-36.
28. Daghigh Kia H, Bolooki Z, Vaseghi Dodran H, et al: Effect of adding coenzyme Q10 and Ellagic acid during cryopreservation on post-thaw quality of ram semen. Iran J Appl Anim Sci 2017;7:445-451.
29. Gillan L, Evans G, Maxwell WM: Capacitation status and fertility of fresh and frozen-thawed ram spermatozoa. Reprod Fertil Dev 1997;9:481-487. doi: 10.1071/R96046
30. Castellini C, Dal Bosco A, Ruggeri S, et al: What is the best frame rate for evaluation of sperm motility in different species by computer-assisted sperm analysis? Fertil Steril 2011;96:24-27. doi: 10.1016/j.fertnstert.2011.04.096
31. Balasuriya A, Serhal P, Doshi A, et al: Processes involved in assisted reproduction technologies significantly increase sperm DNA fragmentation and phosphatidylserine translocation. Andrologia 2014;46:86-97. doi: 10.1111/and.12052
32. Srivastava N, Pande M: Mitochondrion: features, functions and comparative analysis of specific probes in detecting sperm cell damages. Asian Pac J Reprod 2016;5:445-452. doi: 10.1016/j.apjr.2016.10.008
33. Aitken RJ: Reactive oxygen species as mediators of sperm capacitation and pathological damage. Mol Reprod Dev 2017;84:1039-1052. doi: 10.1002/mrd.22871
34. Masoudi R, Sharafi M, Pourazadi L: Improvement of rooster semen quality using coenzyme Q10 during cooling storage in the Lake extender. Cryobiology 2019;88:87-91. doi: 10.1016/j.cryobiol.2019.03.003