Effects of coenzyme Q10 (CoQ_{10}) on equine semen quality after cryopreservation

A. Ruiz, S. Waqas, W. Bayly, A. Tibary
Comparative Theriogenology, Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Center for Reproductive Biology, Washington State University, Pullman, WA

Mammalian spermatozoa are highly susceptible to free radicals because of a high concentration of polyunsaturated fatty acids in their membranes and a lack of cytoplasmic antioxidants. Coenzyme Q_{10} (CoQ_{10}) is a powerful antioxidant when reduced (C_0Q_{10}-ubiquinol), preventing lipid peroxidation in sperm membranes. CoQ_{10} concentrates in sperm mitochondria and has been shown to improve sperm motility and fertility after oral supplementation in infertile men. Addition of CoQ_{10} to semen extender prior to cooling and cryopreservation has also been shown to improve equine sperm motility parameters. The aim of the present work was to determine if post-thaw incubation with CoQ_{10} has any effect on equine sperm quality.

Frozen semen from 6 stallions of various breeds and fertility was used. Semen was cryopreserved using a standard protocol: semen was collected, extended to 50 million/ml spermatozoa in INRA 96®, centrifuged (600 g for 10 minutes) and the supernatant discarded. Sperm aliquots were suspended to 200 $x10^6$ sperm/ml in E-Z Freezing "LE"® extender, loaded into 0.5 mL straws and cryopreserved 4 cm above liquid nitrogen for 20 minutes before being plunged into liquid nitrogen. The experiment was conducted in 5 replicates with two straws per stallion per replicate. Semen was thawed at 37°C for 30 seconds, split into 3 aliquots and incubated at 37°C with no CoQ_{10} addition (control group), with extender containing CoQ_{10} (Sigma-Aldrich Company, St. Louis, MO) at 40 µg/ml (Treatment 1) or CoQ_{10} at 80 µg/ml (Treatment 2). Post-thaw sperm total motility (TM) and progressive motility (PM) were assessed using CASA (SpermVision®, Mofa, Verona, WI), viability (V) was assessed using SYBR-PI stain and plasma membrane functionality (PMF) using HOST, at 0, 1 h, and 2 h following incubation.

Data were analyzed by RM ANOVA with CoQ_{10} treatment as main factor using SAS. Significance was set at $p<0.05$. Results are expressed as average for all stallions. Sperm quality decreased significantly during incubation in all samples. There was no significant effect of treatment on any of the sperm parameters studied (Table).

In conclusion, post-thaw addition of CoQ10 to the extender did not have any effect on motility, viability and plasma membrane integrity of equine sperm. It is possible that CoQ_{10} was not incorporated in sperm in this form. Future studies will focus on the nutritional CoQ_{10} supplementation and its effect on semen quality parameters.

Table: Effects of treatment and time on semen quality parameters

Time	Treatment	TM	PM	V	PMF
0h	Control	32.26 +/- 1.25^{a}	20.45 +/- 0.85^{a}	29.64 +/- 1.55^{a}	16.93 +/- 1.09^{abc}
	Treatment 1	31.49 +/- 1.70^{ab}	19.50 +/- 1.10^{a}	27.21 +/- 1.22^{ab}	15.86 +/- 1.04^{abc}
	Treatment 2	30.89 +/- 1.95^{ab}	19.14 +/- 1.09^{ab}	27.44 +/- 1.23^{ab}	17.96 +/- 1.18^{ab}
1h	Control	28.37 +/- 1.45^{abc}	15.97 +/- 1.19^{bc}	27.05 +/- 1.66^{ab}	17.40 +/- 0.63^{a}
	Treatment 1	27.18 +/- 1.59^{bcd}	15.32 +/- 1.17^{cd}	26.11 +/- 1.21^{ab}	16.16 +/- 0.91^{abc}
	Treatment 2	28.83 +/- 1.86^{abcd}	15.48 +/- 1.07^{c}	26.18 +/- 1.32^{ab}	16.26 +/- 0.94^{abc}
2h	Control	25.06 +/- 1.41^{cde}	13.23 +/- 1.04^{cde}	25.74 +/- 1.22^{ab}	15 +/- 0.93^{bc}
	Treatment 1	24 +/- 1.45^{de}	12.30 +/- 0.96^{de}	25.46 +/- 1.53^{ab}	14.53 +/- 0.96^{c}
	Treatment 2	22.52 +/- 1.30^{e}	11.47 +/- 0.78^{e}	25.21 +/- 1.39^{b}	14.66 +/- 1.14^{bc}

a,b,c Different superscript letters within the same column indicate significant differences, $P< 0.05$.

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