Scientific Works. Series C. Veterinary Medicine. Vol. LXI (2)
ISSN 2065-1295; ISSN 2343-9394 (CD-ROM); ISSN 2067-3663 (Online); ISSN-L 2065-1295

EFFECTS OF COENZYME Q10 ON SPERM VIABILITY DURING STORAGE OF BOAR SEMEN AT 17°C

Laura PARLAPAN PÎNDARU, Mihai CENARIU, Emoke PALL, Ioan Ștefan GROZA

University of Agricultural Science and Veterinary Medicine,
Faculty of Veterinary Medicine, Cluj-Napoca, Calea Mănăștur 3-5, Romania,
Email: parlapan_laura@yahoo.com

Corresponding author email: *parlapan_laura@yahoo.com*

Abstract

Currently, there is a growing interest on cryopreservation of boar semen even if the sperm membrane of swine shows high sensibility to this process. Previous studies have shown that the cryoconservation has an oxidative degradation effect, associated with the production of reactive oxygen species (ROS). ROS cause lipid peroxidation in sperm membranes and a variety of physical and chemical changes of sperm cells that predispose to DNA damage and apoptosis. The only alternative to counteract the effects of ROS is the addition of various antioxidants in lipid storage extenders. Starting from this premise, this work investigates Coenzyme Q10 effects on the viability of preserved boar semen during liquid storage. Semen was randomly divided into 5 groups and treated with different concentration of Coenzyme Q10 and storage at 17ºC for 5 days. The viability of semen was evaluated every day, using flow cytometer FACSCanto II (BD Biociencias) systems. The samples for FACS were labeled with Hoechst 33342, florescein-isothiocyanate conjugated with peanut agglutinin and propidium iodide. These experiments indicate that supplementation of Coenzyme Q10 to the semen extender can increase the sperm characteristics and prolong the survival of liquid storage semen, which may have potential benefits in reproductive biotechnology field.

Key words*: Boar Semen, Coenzima Q10, viability, flow cytometry.*

INTRODUCTION

Artificial insemination (AI) is an important biotechnology widely used in swine breeding programs, playing a key role in improving productivity (Bailey et al., 2008;). Ideally, in terms of biosecurity, international trade, for a better distribution of genetic material of high semen quality and for minimizes boar transportation, AI should be performed with semen extended in the liquid state and storage for 1 to 5 days at 15-20 ºC (Johnson et al., 2000, David Martin-Hidalgo et al., 2013). In order to preserve sperm cells for a few days, their metabolic activity should be reduced by lowering the temperature of semen dilution (Waberski et al., 2011).

Previous studies have revealed that swine sperm membrane shown a high sensibility to low temperatures. This is due to the high content of polyunsaturated fatty acids (PUFAs) (Awda et al., 2009; Waterhouse et al., 2004), as well as a lack of significant protective enzymes (antioxidants) in the seminal plasma. Also, lowering the temperature has a powerful oxidative effect, associated with production of reactive oxygen species (ROS) and lipid peroxidation in sperm membranes (Umut et al., 2014). When ROS are produced excessively, they may display a variety of physical and chemical changes of the sperm cells, which predispose at damaging effects on sperm motility (Kao et al., 2008), plasma membrane integrity (Alvarez et al., 1992), DNA integrity (Kadirvel et al., 2009; Waberski et al., 2011) and fertilizing capability (Kasimanickam et al., 2007). The only alternative to counteract the harmful effects of SRO is the addition of antioxidants in liquid storage extenders (Grossfeld, 2008; Bathgate, 2011).

Antioxidants are the main defense factors against oxidative stress induced by SRO by accepting or donating an electron to eliminate the unpaired condition (Agarwal et al., 2005).

Coenzyme Q10 (CoQ_{10}) is lipid-soluble molecule found in mitochondrial membrane of every mammal cells, having an important role in the synthesis of adenosine triphosphate (ATP) (Gürkan et al., 2005; Begum et al., 2009). Also, the recent studies have revealed that the deficiency in CoQ_{10} may be the cause of asthenozoospermia in some male (Lewin et al., 2007), and incubation of sperm cells with CoQ_{10} will improve the pattern of bull sperm cells motility (Ibrahim et al., 2011; Thakur et al., 2013).

MATERIALS AND METHODS

Sperm-rich ejaculate fractions (SERFs) were collected from 5 sexually mature and healthy boars (1-3 years of age) by using the gloved-hand method (2 ejaculates for each boar). After sperm collection, the SERFs were extended in Beltsville Thawing Solution (BTS) (1:1, v/v) and evaluated for microscopically semen characteristics. After the evaluation, were selected only the SERFs containing more that $250x10^6$ sperm/mL, 80% sperm with normal morphology and 70% motile, and dispensed into 50 ml plastic tubes, cooled to 17-18°C, packed in insulated containers and transported to the laboratory.

At the laboratory the SERFs were diluted BTS in 2 ml Eppendorf tube at a final concentration of $25x10^6$ sperm/mL and subsequently analyzed (day 0). After that, semen samples were treated without or with different concentration of CoQ10 (3 µM, 6 µM, 8 µM, 1 m) and preserved at 17ºC for 5 days. Sperm viability as well as acrosomial membrane integrity was analyzed with flow cytometry at days 0, 1, 3, and 5 of preservation.

The sperm viability and acrosome membrane integrity was assessed after staining the sperm with Hoechst 33342 (H-42) as DNA marker, florescein-isothiocyanate conjugated with peanut agglutinin (FITC- PNA) as marker for acrosome status, and Propidium Iodide (PI) as viability marker.

Aliquots of 75 µL of each semen sample (1,5x106 spermatozoa/mL) were incubated for 10 minutes at 37°C in the dark (according to the method described by Martinez-Alborcia et al., 2012) with 4 µl H-42 (0.05 mg/ml in PBS), 2 µl PI (PI, 0.5 mg/ml in PBS, Molecular Probes Europe BV, Leiden, The Netherlands) and 4 µl PNA-FITC (200 µg/ml in PBS). Just before flow cytometry analysis, 400 µL of PBS was added to each simple. Spermatozoa cells were analyzed and the percentage of live with damaged or reacted acrosome and death sperm was recorded.

To evaluate sperm viability and acrosomial integrity was used a FACSCanto II (BD Biociencias) flow cytometer and the digital data were processed by BD FACSDiva software. Samples analyzed by FACS were excited by an Argon-ion laser of488 nm, and the fluorescence spectra of PNA-FTC and PI were detected by a 670 nm LA filter respectively by a 530/30 nm BP filter. A total 10.000 of gated events were collected per sample with a sample running rate of 100-800 events/sec. Florescence data were collected in the logarithmic way.

Statistical analyses were performed using SPSS software, version 12.0.1, with Post-hoc test to corroborate statistical significance, p-values of <0.05 was considered as statistically significant. The differences between the viability of groups of sperm storage with different concentration of CoQ_{10} were compared and results were expressed as mean ± SEM

RESULTS AND DISCUSSIONS

In the present study, the sperma cells analyzed with FACSCanto II flow cytometer were characterized into four categriers (Figure no.1): (1): live sperm cells with intact acrosome (PI-/FITC- PNA-); (2): sperm cells with intact plasma membrane and acrosome damage (PI-/ FITC-PNA +); (3): sperm cells with damaged intact plasma membrane and acrosome intact (PI + / FITC- PNA-); (4): dead sperm cells with acrosome damage.

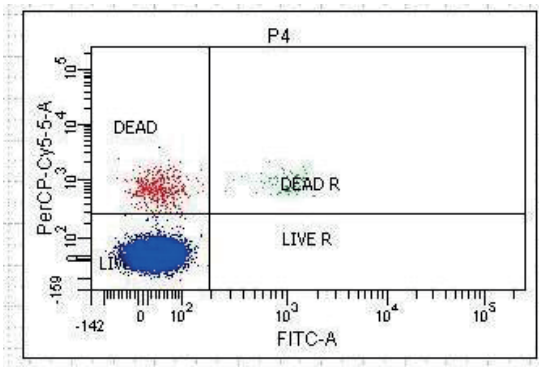

Figure no.1. Flow cytometry histogram

The mean values for sperm viability before and after liquid storage at the 17°C from the all boars used in this study are summarized in Table no. 1. All of the SERFs had a good sperm quantity and quality before storage, with more than $250x10^6$ sperm/mL, 80% sperm with normal morphology, 75% viable sperm cells and 70% motile.

For semen stored at 17 ° C only with BTS, research has shown that sperm maintain a viabilititate up to 70.87% ± 1.78 after 5 days of storage, the percentage of membrane degradation acrozomial, in this case, being 7.3 ± 1.2%.

The results reported for the storage of semen with the addition of 3μM and 6 μM CoQ10, both for assessing the viability and integrity acrosomial are not significant between the 2 treatments applied there not being noticed as a clear improvement of these parameters.

Significant differences appear when stored semen with the addition of 8 μM and 1 mM CoQ10. In this case, after 5 days of storage, the values obtained. In the evaluation of the viability of 79.89 ± 3.76% and 82.24 ± 3.56% of that had been read, with only 4.1 ± 0.4% and 4.0 ± 0.5% acrosomial degraded sperm membrane.

The only scientific data linking coenzyme Q reproductive apply to the human, canine and bull species. The action of various concentration of CoQ10 on bull semen motility, show that the CoQ10 has a beneficial effect and improve the sperm movement, making it an ideal antioxidant for incubation of the sperm prior to may be as an ideal antioxidant for storage of the semen prior to assisted reproductive technology (Ibrahim et al., 2011).

Several researchers demonstrated that, in patients with a proven interfil, prolonged treatment with 2, 3 or even 6 months with coenzyme Q administered orally at a dose of 300mg, improved semen parameters. Also, Lewin and Lavon showed that in vitro incubation of high concentrations of CoQ semen samples asthenospermic can significantly increase their motility and in vivo administration of CoQ can have positive effects in terms of fertilization networks in patients with low rates of fertilization after intracytoplasmic sperm ICSI infection. As regards the frozen semen CoQ use by dogs, Neagu (2011)., have shown that this substance has the ability to improve the post-thaw motility porgresivă. But in this case, the results are influenced by individual factors and must be linked to both the values of membrane integrity, mitochondrial potential and degree of lipid perozidare.

Table no. 1. Spermatozoa viability and the integrity of acrosome after addition of different CoQ_{10} concentrations to boar semen doses preserved at 17ºC for 7 days

	Cell viability (PI-/FITC-PNA-)*				Acrosome integrity (PI-/FITC-PNA+)*			
	Day 0	Day 1	Day 3	Day 5	Day 0	Day 1	Day 3	Day 5
BTS	88.44±1.03	87.38±3.40	74.00±1.63	65.87±1.78	3.4±0.6	3.9±0.8	5.1±0.5	7.3±1.2
CoQ_{10} 3µM		88.49±2.56	78.65±2.15	74.21±1.26		3.6±0.5	3.9±0.3	4.2±0.2
CoQ_{10} 6µM		88.67±1.68	85.47±3.56	76.65±1.65		3.5±0.8	3.4±0.4	4.0±0.6
CoQ_{10} 8µM		87.94±2.56	84.12±2.94	77.89±3.76		3.5±0.5	3.4±0.2	4.1±0.4
CoQ_{10} 1mM		88.23±1.69	85.57±1.26	80.24±3.56		3.4±0.2	3.5±0.6	4.0±0.5

*Boar seminal doses were preserved at 17ºC during 5 days in BTS in absence or presence of different concentrations of CoQ_{10} (3 µM, 6 µM, 8 µM and 1 mM). Percentages of spermatozoa cell viability ans spermatozoa acrosomal integrity were measured by flow cytometry as described in Materials and Methods. Results are expressed as mean ± standard error of the mean (SEM).

CONCLUSIONS

Although, little is known about the mechanism by which CoQ_{10} influences the viability of sperm cells, we can say that it can be a great antioxidant for sperm preservation prior to AI. The storage of boar semen in the presence of different concentrations of CoQ10 (3 µM, 6 µM, 8 µM and 1 mM) had a positive effect on the percentage of viable sperm and of acrosomal integrity, compared with BTS alone at any storage time or doses. But only the highest doses of CoQ10 used (8 µM and 1 mM) caused a statistically significant increase in the percentages of cell viability and acrosomal integrity on day 3 and 7.

However, there is a big interest in what extent would be affected the sperm if the storage time are raised above 7 days, and the concentrations of CoQ_{10} over the 1 mM. This could be determined trough further research.

ACKNOWLEDGEMENTS

This paper was published under the frame of European Social Found, Human Resources Development Operationl Programme 2007 2013, project no. POSDRU/159/1.5/136893

REFERENCES

Agarwal A., Prabakaran S.A., Said T.M., 2005, Prevention of oxidative stress injury to sperm. Journal of Andrology 26, 654-660.

Alvarez JG, Storey BT., 1992, Evidence for increased lipid peroxidative damage and loss of superoxide dismutase activity as a model of sublethal cryodamage to human sperm during cryopreservation. J Androl, 13, 232-241.

Awda, B. J., Mackenzie-Bell, M., & Buhr, M. M., 2009, Reactive oxygen species and boar sperm function. Biol. Reprod, 81(3), 553-561.

Bailey JL, Lessard C, Jacques J, Breque C, Dobrinski I, Zeng W, Galantino-Homer HL., 2008, Cryopreservation of boar semen and its future importance to the industry. Theriogenology. 70 (8), 1251-1259.

Bathgate R., 2011, Antioxidant Mechanisms and their Benefit on Post-thaw Boar Sperm Quality. Reproduction in Domestic Animal, 46 (Suppl. 2), 23–25.

Begum H, Moniruddin A, Nahar K., 2009, Environmental and nutritional aspects in male fertility. J Med 10:16−19.

David Martin-Hidalgo, Ana Hurtado de Llera, Heiko Henning, Ulrike Wallner, Dagmar Waberski,Maria J. Bragado, Maria C. Gil, Luis J. Garcia-Marin, 2013, The Effect of Resveratrol on the Quality of Extended Boar Semen During Storage at 17ºC, Journal of Agricultural Science; Vol. 5, No. 8, 231-242.

Grossfeld R., Sieg B., Struckmann C., Frenzel A., Maxwell W.M., Rath, D., 2008, New Aspects Of Boar Semen Freezing Strategies. Theriogenology. 70: 1225-1233.

Gürkan AS, Bozda–Dündar O., 2005, COENZYME Q10. J Fac Pharm, Ankara 34:129− 154.

Johnson, L. A., Weitze, K. F., Fiser, P., & Maxwell, W. M., 2000, Storage of boar semen. Anim Reprod. Sci., 62(1-3), 143-172.

Kadirvel G, Kumara S, Kumaresan A., 2009, Lipid peroxidation, mitochondrial membrane potential and DNA integrity of spermatozoa in relation to intracellular reactive oxygen species in liquid and frozen-thawed buffalo semen. Anim Reprod Sci, 114, 125-134.

Kao SH, Chao HT, Chen HW, Hwang TI, Liao TL, Wei YH., 2008, Increase in oxidative stress in human sperm with lower motility. Fertil Steril, 89, 1183-1190.

Kasimanickam R, Kasimanickam V, Thatcher CD, Nebel RL, Cassell BG., 2007, Relationships among lipid peroxidation, glutathione peroxidase, superoxide in dismutase, sperm parameters, and competitive index dairy bulls. Theriogenology, 67, 1004-1012.

Lewin A, Lavon H., 1997, The effect of coenzyme Q10 on sperm motility and function. Mol Biotech 18:213−219.

Lewin A., Lavon H., 1997, Effect of Coenzyme Q10 on sperm motility and function, Molec. Aspects Med., Vol. 18, 213-219

Martinez-Alborcia MJ, Valverde A, Parrilla I, Vazquez JM, Martinez EA, 2012, Detrimental Effects of Non-Functional Spermatozoa on the Freezability of Functional Spermatozoa from Boar Ejaculate. PLoS ONE 7(5).

Neagu V.R., 2011, Researches regarding collection, processing and cryopreservation of canine semen, PhD thesis.

Siti Fatimah Ibrahim, Farah Hanan Fathihah Jaffar, Khairul Osman, Syarifah Faezah Syed Mohamed, Chew Fang Nang, Nur Hilwani Ismail, Mohd Iswadi Ismail, 2011, Bull spermatozoa motility: optimization of Coenzyme Q10 and Alpha-Lipoic acid concentration, IIOABJ; Vol. 2; Issue 5; 8–13.

Thakur Krishna Shankar Rao, Niranjan Kumar, Navinkumar B. Patel, Indrasen Chauhan and Shailendra Chaurasia, 2013, Sperm selection techniques and antioxidant fortification in low grade semen of bulls: Review, Vet World 6(8): 579-585.

Waberski, D., Schapmann, E., Henning, H., Riesenbeck, A., & Brandt, H., 2011, Sperm chromatin structural integrity in normospermic boars is not related to semen storage and fertility after routine AI. Theriogenology, 75(2), 337-345.

Waterhouse, K. E., De Angelis, P. M., Haugan, T., Paulenz, H., Hofmo, P. O., & Farstad, W., 2004, Effects of in vitro storage time and semen-extender on membrane quality of boar sperm assessed by flow cytometry. Theriogenology, 62(9), 1638-1651.