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CONSTRUINDO SABERES, FORMANDO PESSOAS E TRANSFORMANDO A PRODUÇÃO ANIMAL

EFFECT OF CHOLESTEROL-LOADED CYCLODEXTRIN PRETREATMENT ON FREEZABILITY OF RABBIT SPERMATOZOA

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Resumo: Este estudo objetivou avaliar o impacto do tratamento prévio com CLC sobre parâmetros pós-congelamento em esperma de coelhos. As amostras de sêmen (2 ejaculados/macho) foram coletados de dez doadores regulares da raça Nova Zelândia Branca, através de vagina artificial. A mistura de ejaculados foi primeiro diluída utilizando-se diluidor básico (1:1 v/v) e então dividida em duas alíquotas. Uma alíquota foi tratada com CLC (3mg por 120×10^6 espermatozoides) enquanto que, na segunda alíquota, o mesmo volume de diluente básico foi adicionado. Ambas as alíquotas foram incubadas a 35 °C por 15 minutos. Após, as alíquotas foram novamente diluídas (1:1 v/v) com diluente para congelamento (diluente básico adicionado de gema de ovo e acetamina). As amostras foram então resfriadas a 4 °C e congeladas seguindo um protocolo padrão. Os recipientes foram então descongelados sendo após avaliados os parâmetros de qualidade. Os resultados mostraram que a porcentagem de motilidade pós congelamento do esperma vivo após HOST foi maior no grupo tratado com CLC ($P<0.05$). Contudo os parâmetros de viabilidade espermática, intactos, intactos vivos, integridade do acrossoma e anormalidades morfológicas permaneceram

similares (P>0.05) entre os grupos. Em conclusão, o pré-tratamento com CLC poderia beneficiar a qualidade do sêmen pós congelado, beneficiando sua motilidade e capacidade de permanência após exposição às condições severas de HOST em coelhos.

Keywords: CLC, Cryopreservation, Rabbit, Sperm

Introduction

Recently rabbits gained popularity as meat animal in many countries; therefore, rabbit production has achieved commercial status focusing on meat production. Additionally transgenic rabbits are being considered as suitable model to study various genetic and acquired human diseases. This trend has encouraged the use of biotechnologies including artificial insemination (AI) to improve rabbit genetics for higher meat and fur production. However, AI is usually performed with fresh or chilled semen with higher fertility and prolificacy. On the other hand, AI with frozen thaw sperm is rarely practiced in rabbit. Perhaps the low adaptability of AI is due to lower fertility and/or prolificacy in rabbit. In contrast to the other mammalian species, the rabbit sperm plasma membrane is rich in cholesterol content and hence more resistant to the cold shock. In spite of higher resistance to cold shock, the low sperm survival rate after freezing and thawing is still a major constrain in the success of AI in rabbit. It is known that cholesterol depletion due to lipid phase transition in the plasma membrane during freezing causes membrane destabilization (Crockett 1998). In this regard it is anticipated that pretreatment of sperm with cholesterol would reduce the cryo-induced damage. During the last decade a considerable reach has been carried out on the beneficial effect of cholesterol-loaded cyclodextrin (CLC) on in on survival rate and quality of frozen thawed sperm in variety of species (Ahmet et al., 2013) The encouraging results of previous studies envisage that by uploading cholesterol in plasma membrane

before freezing, the detrimental effect of cryopreservation on sperm can be minimized. In this background present study was designed aiming to determine impact of CLC pretreatment on post-thaw quality parameters of rabbit sperm.

Material and methods

The study was carried out at Department of Reproduction and AI, Faculty of Veterinary Medicine, Adnan Menderes University, Aydin, Turkey. Ten regular semen donor New Zealand white rabbit bucks were used in this reach. The bucks were housed in individual cages, with free access to water and food. Before starting the experiment an approval for the use of these animals was acquired from Ethical Committee. The CLC stock solution by dissolving 50 mg CLC powder in 1ml of basic extender (125 mM glucose, 105 mM lactose, 91 mM trehalose and 10 mM HEPES). CLC working solution (3 mg BSA/ml of CLC stock solution) was prepared as reported previously (Aksoy et al., 2010). The semen samples (2 ejaculates/buck) were collected through artificial vagina following a standard protocol. The gel in the ejaculates was removed immediately after collection.

The samples were pooled by mixing two or three individual ejaculates in each replicates. The ejaculates which fulfilled the minimum criteria (motility > 50% and abnormalities < 10 %) used. The samples were first diluted with basic extender (1:1 v/v) and then split into two aliquots. One aliquot was treated with CLC (3mg per 120×106 spermatozoa) whereas, in second aliquot an equal volume of basic extender was added. Both the aliquots incubated at 35 ^{o}C for 15 min. Afterwards they were further diluted (1:1 v/v) with freezing extender (basic extender plus 40% egg yolk and 12% w/v acetamide). The final concentrations of egg yolk and acetamide were 20% and 6% respectively. The samples were then cooled to 4 ^{o}C and then frozen in liquid introgen. After freezing straws from each replicate was thawed and evaluated for quality parameters. Sperm progressive motility, viability and abnormalities were evaluated according to the previously described techniques (Ugur et al. 2016). Sperm plasma membrane integrity (Intact, live-intact and live) was

assessed by a modified hypoosmotic swelling test (HOST) in combination with eosin (HE-test) staining as reported earlier (Aksoy et al. 2010). The data were analyzed by using SPSS version 17; SPSS Inc., Chicago, IL. Independent-samples t-test was used to compare the sperm quality parameters between CLC and control groups. The P value (< 0.05) was set to find significant difference between CLC treated and control groups.

Results and discussion

The effect of CLC pretreatment on rabbit semen quality after freezing and thawing is presented in Table 1. The results showed that CLC pretreatment enhanced pot-thaw sperm motility and capability to remain live after exposure to harsh condition in HOST. The higher post thaw motility of CLC treated sperm is present study is in consistent with previous study which elucidated that CLC supplementation not only enhances motility but also increases quality of motility in rabbit spermatozoa (Nishijima et al., 2014).

Table: Effect of CLC pretreatment on rabbit semen quality after freezing and thawing.

	CLC	Control
Motility (%)	31.11 ± 4.84[a]	19.44 ± 2.11[b]
Viability (%)	68.33 ± 4.79	59.66 ± 6.04
HOST parameters		
Intact (%)	21.50 ± 3.20	16.33 ± 3.24
Live-intact (%)	7.05 ± 0.91	5.83 ± 1.23
HOST-live (%)	28.33 ± 2.23[a]	19.50 ± 2.89[b]
Reacted Acrosome (%)	24.22 ± 2.99	31.22 ± 3.31
Morphologic abnormalities (%)	29.33 ± 3.36	32.00 ± 2.50

Different superscripts within the same line are different (P < 0.05).
HOST: Hypo-osmotic swelling test

The rabbit sperm are considered rich in cholesterol/phospholipid ratios which is similar to the human sperm. Therefore, it is generally anticipated that protective

impact of CLC would not be as much obvious as it is observed in other species having lower cholesterol to phospholipid ratio. In contrast to this perception, the results of present study proved that beneficial impact of CLC is not limited to the species having lower cholesterol to phospholipid ratio.

It is well know that during cryopreservation procedure ROS production induces capacitation like changes and premature acrosome reaction in frozen thawed sperm. Moreover, sperm experience wide range of osmotic changes throughout freezing and thawing process which further intensify the cryo-induced damages. It has been elaborated that CLC pretreatment enhances membrane fluidity, osmotic tolerance in a wide range and suppresses premature acrosome reaction (Aksoy et al., 2010). In addition CLC pretreatment is known to protect the sperm against in vitro induced oxidative stress which laid down the assumption that cholesterol incorporation in sperm membrane attenuated the oxidative damage either through stabilizing the membrane or maintaining the antioxidant enzyme defense system (Naseer et al. 2015). These studies suggests different mechanisms including increased membrane fluidity, enhanced osmotic tolerance, reduced premature capacitation/acrosomal reaction and lowered oxidative stress might be involved in protective influence of CLC on sperm during freezing and thawing.

Conclusion

In conclusion, CLC pretreatment could improve post-thaw sperm quality parameters by enhancing their motility and capability to remain live after exposure to harsh condition of HOST in rabbits.

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References

Ahmad E.; Aksoy M.; Serin I.; Kucuk N.; Ceylan A.; Ucan, U. 2013.Cholesterol-loaded cyclodextrin pretreatment of ram spermatozoa protectsstructural integrity of plasma membrane during osmotic challenge andreduces their ability to undergo acrosome reaction in vitro. Small Ruminant Research 115:77-81.

Aksoy M.; Akman O.; Lehimcioglu N.C.; Erdem H. 2010. Cholesterol-loadedcyclodextrin enhances osmotic tolerance and inhibits the acrosome reaction inrabbit spermatozoa. Animal Reproduction Sciences 120:166-172.

Crockett E.L. 1998. Cholesterol function in plasma membranes from ectotherms: membrane-specific roles in adaptation to temperature. American Zoologist 38: 291-304.

Naseer Z.; Ahmad E.; Aksoy M.; Kücük N.; Serin I.; Ceylan A.; Boyacıoglu M.; Kum C. 2015. Protective effect of cholesterol-loaded cyclodextrin pretreatmentagainst hydrogen peroxide induced oxidative damage in ram sperm. Cryobiology 71:18-23.

Nishijima K.; Yamaguchi S.; Tanaka Mai.; Sakai Y.; Koshimoto C.; Morimoto M.; Watanabe T.; Fan J.; Kitajima S. 2014. Effects of cholesterol-loaded cyclodextrins on the rate and the quality of motility in frozen and thawed rabbit sperm. Experimental Animals 63: 149-154.

Ucan U.; Kucuk N.; Ahmad E.; Nasee Z.; Aksoy M.; Serin I.; Ceylan A. 2016. Effect of different sugars supplemented to the extender in combination with cholesterol-loaded cyclodextrin (CLC) on post-thaw quality of ram spermatozoa. Small Ruminant Research 136:243-246.