Kafkas Univ Vet Fak Derg
23 (4): 661-664, 2017
DOI: 10.9775/kvfd.2017.17181

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

Short Communication

Effect of 7-dehydrocholesterol and Cholesterol-loaded Cyclodextrins on Bull Sperm Motility During Short Term Storage [1] [2]

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[1] *This study was supported by grants from TUBITAK, Turkey (TUBITAK-113O775)*
[2] *This study presented in "5th International Scientific Meeting Days of Veterinary Medicine" held between 5-7 September 2014, Ohrid Macedonia as poster presentation*

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Article Code: KVFD-2017-17181 Received: 18.01.2017 Accepted: 08.03.2017 Published Online: 08.03.2017

Citation of This Article

İnanç ME, Tekin K, Olğaç KT, Özen D, Stelletta C, Uysal O, Daşkın A: Effect of 7-dehydrocholesterol and cholesterol-loaded cyclodextrins on bull sperm motility during short term storage. *Kafkas Univ Vet Fak Derg,* 23 (4): 661-664, 2017. DOI: 10.9775/kvfd.2017.17181

Abstract

The study was conducted to determine the effect of 7-dehydrocholesterol loaded cyclodextrin (7-DHCLC) and cholesterol-loaded cyclodextrin (CLC) on changes of bull sperm motility during short term storage (+4°C). Collected ejaculates were pooled and divided into 7 groups, as following one control (C); three 7-DHCLC and the other three CLC concentrations. Diluted semen samples were transferred and stored at +4°C and sperm motility was analysed in determined intervals during three days storage period with computer aided sperm analyse (CASA) system. Motility decreased in all groups; however, 7-DHCLC and CLC groups motility decreased in the next two days gradually. But, CLC 1.5 mg/120x10^6 group were identified as the best groups for protecting motility in short time preservation. In conclusion, adding different rates of 7-DHCLC and CLC to semen extender preserved motility for three days in bull semen stored at +4°C.

Keywords: *Bull sperm, Cholesterol, Motility, Short term storage, 7-dehydrocholesterol*

7-dehidrokolesterol ve Kolesterol İle Doyurulmuş Siklodekstrinlerin Kısa Süreli Saklama Süresince Boğa Spermasının Motilitesine Etkisi

Özet

Bu çalışma, kısa süreli saklama süresince (+4°C) 7-dehidrokolesterol doyurulmuş siklodekstrin (7-DHCLC) ve kolesterol ile doyurulmuş siklodekstrinin (CLC) boğa spermasının motilitesinin korunmasındaki etkisinin değerlendirilmesi için yürütülmüştür. Alınan ejekulatlar birleştirilerek 7 gruba ayrıldı, bu gruplardan biri control (C), üçü 7-DHCLC'nin diğer üçü ise CLC'nin farklı konsantrasyonlarını içeren sulandırıcılarla sulandırıldı. Sulandırılan sperma +4°C'ye transfer edildilerek saklandı ve sperma motilitesi üç gün boyunca bilgisayar destekli sperm analiz cihazında (CASA) değerlendirdi. Motilitenin tüm gruplarda azaldığı görüldü fakat, 7-DHCLC ve CLC gruplarında motilite azalmasının daha yavaş olduğu tespit edildi. Fakat, CLC 1.5 mg/120×10^6 grubunun spermanın kısa süreli saklanmasında motiliteyi koruyan en iyi grup olduğu belirlendi. Sonuç olarak, sperma sulandırıcısına değişik oranlarda 7DHCLC ve CLC'nin eklenmesi +4°C'de boğa sperma motilitesini üç gün boyunca koruduğu tespit edildi.

Anahtar sözcükler: *Boğa sperması, Kolesterol, Motililite, Kısa süreli saklama, 7-dehidrokolesterol*

INTRODUCTION

The plasma membrane of the sperm cells differs from many other cell membranes in its lipid composition. It contains huge amount of polyunsaturated fatty acids (PUFA), especially diPUFA (phospholipids esterified with two PUFA), which is found only in retina, brain and sperm [1]. PUFA in sperm membrane are very vulnerable to oxidative

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stress during sperm preservation and a lot of studies have been carried out on usage of antioxidant during short or long term storage of sperm [2-4]. Stabilization of sperm membrane lipids may be an alternative to antioxidant usage.

These overpowering lipids in many membranes are built with phospholipids and cholesterol. Membranes also contain carbohydrate-lipid complexes (glycolipids), with the most common cholesterol and phospholipids that build the capacity of the lipid substance of the membrane. When the temperature of the membranes is lowered, the phospholipids are converted from liquid phase to the gel state. Each type of lipid species performs this phase change at different temperatures; therefore, the membrane being composed of complex different lipids, undergoes the phase change over a relatively extensive temperature range. Cholesterol provides the fluidity of membranes, along with the fatty acid chains of the phospholipids, when the temperature decreases [5].

Plasma membrane can be affected at any stage of semen preservation; causes such as cell death, which are seen as the result of the formation of ice crystals, may induce many stresses on sperm [5]. The spermatozoon membrane acts as a physical barrier and its main function is to protect the cell from damage during low temperatures. Damage contains loss of lipids from the membrane, peroxidation of membrane lipids as a result of formation of reactive oxygen species (ROS) and phase transition, from the fluid phase to the gel phase, as temperature is decreased and membrane destabilization occurs due to lateral lipid rearrangement. These damages can be decreased by adding lipids, in the form of egg yolk, to the sperm prior to cooling and freezing. Egg yolk is the main membrane protective agent in semen extenders [6]. The main disadvantage is of having a protective agent of an animal source in extenders used for short term preservation or cryopreservation is the risk of disease transportation [7] and microbial contamination which allows production of endotoxins [8]. This has raised a problem to international semen transport because of biosecurity issues and for this reason we are working on alternatives to egg yolk [9].

Cholesterol can easily interaction from the plasma membranes of cells using cyclodextrins. Cyclodextrins are cyclic oligosaccharides which are main degradation yields of starch. Methyl-beta-cyclodextrin which is one of the most used cyclodextrins, can solubilise hydrophobic molecules, such as cholesterol [5] and can transport cholesterol into or out of membranes down a concentration gradient. 7-dehydrocholesterol is a cholesterol conjugate and consists of cholesterol upper stage (intermediate product) in biochemical diagram which means that it is one of the cholesterol conjugates and 7-DCLC formed before cholesterol is produced. Amorim et al.[10] cryopreserved bull semen with cholesterol conjugates (heptanoate, palmitate, pelorganate and stearate) and obtained increased percentages of sperm motility after thawing. However, to our knowledge, there are no studies demonstrating semen preservation with 7-DHCLC in any animal species.

In the light of the above, these experiments were conducted to determine the effect of 7-DHCLC and CLC treatment on bull sperm motility at short term preservation.

MATERIAL and METHODS

Chemicals

All chemicals used in this study were obtained from Sigma Chemical Company (St. Louis, MO, USA).

Semen Collection

Ejaculates were collected from three Simmental bulls (2-4 years of age), regularly used for breeding purpose which were held in the International Center for Livestock Research and Training Ankara-Turkey under uniform breeding conditions. Totally, 30 ejaculates were taken from the three bulls using artificial vagina. The ejaculates from bulls were kept at 34°C with aid of water bath until their evaluation in the laboratory. The evaluation was performed within 15 min after collection; each ejaculate was evaluated to determine percentages of total and progressive motility as well as concentration. Only ejaculates containing sperm with >75% motility and >1.0×10^9 sperm/mL concentration were used in current study. Ejaculates provided these criterias were pooled in order to decrease individual differences.

Cyclodextrin Preparation

Methyl-beta- cyclodextrin was loaded with 7-dehydrocholesterol and cholesterol as explained by Purdy and Graham [5].

Semen Processing

The total semen volume was obtained from the graded conical tube soon after collection, and its concentration was determined using photometer (IMV, France). Fresh total and progressive motility was evaluated subjectively by a phase contrast microscopy (Olympus BX43) at 37°C. For short-time semen preservation, we used Tasdemir et al.[4]'s Tris-based extender (T) without cryoprotectant and egg yolk. Each pooled ejaculate was equally split into seven groups. These groups were: one as control (C); three different (1.5, 2.5, 3.5 mg/120×10^6) 7-DHCLC and three different (1.5, 2.5, 3.5 mg/120×10^6) CLC treatments. Control were diluted T extender without any additives. 7-DHCLC and CLC groups were initially incubated with CLC or 7-DHCLC for 15 min at 22°C, prior to diluted with T, then diluted with T to final concentration of 100×10^6 sperm/ml. Diluted semen cooled slowly in the water bath (22°C), inside the cold box (4°C) for four h. Then semen samples

were transferred to the refrigerator at +4°C and kept in the throughout the replications.

Motility Analysis

Computer aided sperm analysis (CASA) system (SCA®, Barcelona, Spain) was used to examine of motility and kinetic parameters. The sperm motility properties were set as static, slow >50 µm/s, medium >75 µm/s, fast >100 µm/s. Five µl of rewarmed semen was put onto slide and covered with a coverslide then motility were analysed with a 10 x objective at 37°C. Progressive motility (%) and total sperm motility (%) were recorded. For each evaluation, at least 200 to most 300 sperm were analysed in six microscopic fields. Sperm motility was evaluated for three days (0, 1, 2, 6, 24, 48 and 72. h) in short tem storage.

Statistical Analysis

Descriptive statistics for each variable obtained from ten experimental replications were calculated and presented as mean ± standard error of mean. The main effect of "treatment", "time" and the interaction term of "treatment x time" upon the fertility parameters were modelled by using the GLM procedure for repeated measures of SPSS 14.01 (SPSS Inc., Chicago, IL, USA). Post hoc testing was carried out for significant interactions and was performed using simple effect analysis. A probability value of less than 0.05 was considered significant, unless otherwise noted.

RESULTS

The CASA result of progressive and total motility of the pooled semen, over the three days are given in *Table 1* and *Table 2*. There was no significant difference between 7-DHCLC and CLC groups in terms of progressive motility ($P>0.05$); however statistically significant difference was detected when this were compared with control groups ($P<0.001$) at 1 h. Sperm motility rates reduced in each group; however, CLC and 7-DHCLC 1.5 mg/120×10^6 were maintained motility higher than control group.

DISCUSSION

Storage process causes damage to both intracellular and extracellular sperm membranes starting with dilution and continuing until warming procedures. Plasma membranes are considered to be in the lamellar liquid crystalline phase at normal body temperatures with the

Table 1. *Mean (±SE) CASA progressive motility of semen treated with CLC or 7-DHCLC (1.5, 2.5 or 3.5 mg/120x10^6)*

Type	Group	Time						
		0 hour	1 hour	2 hour	6 hour	24 hour	48 hour	72 hour
Progressive	CLC 1.5	31.2±4.12^{a,A}	30.4±7.13^{ab,A}	30.02±7.01^{a,AB}	29.5±8.10^{a,AB}	25.4±5.81^{a,B}	17.67±2.92^{a,C}	11.28±1.56^{a,D}
	CLC 2.5	34.1±3.31^{a,A}	26.9±4.32^{ab,A}	26.27±4.66^{ab,B}	25.1±6.38^{ab,AB}	16.1±4.03^{b,C}	8.15±5.20^{bc,D}	5.05±3.88^{bc,E}
	CLC 3.5	36.1±6,23^{a,A}	28.4±5.53^{ab,A}	27.72±5.14^{ab,B}	25.5±2.74^{ab,B}	17.7±3.27^{b,C}	9.88±4.62^{b,D}	5.22±2.63^{bc,E}
	7DHCLC 1.5	33.2±4.21^{a,A}	31.1±3.11^{ab,A}	30.68±3.06^{a,B}	23.8±2.78^{ab,C}	14.9±6.56^{bc,D}	9.23±3.96^{bc,E}	6.05±2.21^{b,F}
	7DHCLC 2.5	38.4±5.00^{a,A}	27.0±3.00^{ab,A}	26.63±2.91^{ab,A}	22.0±6.20^{b,B}	16.9±3.88^{b,C}	8.38±4.59^{bc,D}	6.43±3.39^{b,D}
	7DHCLC 3.5	36.0±2.45^{a,A}	24.0±3.75^{ab,B}	23.58±3.75^{b,A}	22.6±4.88^{b,BC}	16.0±5.59^{bc,D}	11.00±3.93^{bc,E}	7.53±2.76^{b,F}
	Control	34.3±3.26^{a,A}	15.4±1.19^{c,B}	15.23±1.14^{c,B}	15.3±1.19^{c,B}	10.7±0.91^{c,B}	5.25±0.31^{c,C}	2.73±1.63^{c,D}

^{a,b,c} Different superscripts within the same column demonstrate significant differences among groups ($P<0.001$)
^{A,B,C} Different superscripts within the same line demonstrate significant differences during storage period in same group ($P<0.001$)

Table 2. *Mean (±SE) CASA total motility of semen were treated with CLC or 7-DHCLC (1.5, 2.5 or 3.5 mg/120x10^6)*

Type	Group	Time						
		0 hour	1 hour	2 hour	6 hour	24 hour	48 hour	72 hour
Total	CLC 1.5	78.1±5.38^{a,A}	77.2 ± 8.86^{a,A}	77.05±8.93^{a,A}	72.48±12.49^{a,AB}	60.7±7.23^{a,B}	46.87±6.04^{a,C}	41.10±2.15^{a,D}
	CLC 2.5	75.3±6.23^{a,A}	67.6 ± 7.73^{b,B}	67.48±7.78^{b,B}	63.5±11.60^{ab,BC}	52.6±15.71^{ab,C}	29.13±8.57^{b,D}	28.27±9.65^{b,D}
	CLC 3.5	73.2±4.35^{a,A}	65.8±6.13^{bc,B}	65.63±6.15^{bc,B}	53.5±18.43^{bc,C}	37.0±6.12^{c,D}	25.37±3.25^{b,E}	22.53±2.68^{b,E}
	7DHCLC 1.5	77.2±3.45^{a,A}	74.9±4.74^{a,A}	74.75±4.49^{a,A}	63.4±15.04^{ab,B}	46.4±13.49^{bc,C}	30.82±15.07^{b,D}	25.32±8.06^{b,E}
	7DHCLC 2.5	72.1±4.34^{a,A}	60.4±4.67^{bc,B}	60.07±4.66^{c,B}	60.3±5.10^{ab,B}	55.5±6.24^{ab,B}	28.93±9.75^{b,C}	24.40±6.89^{b,D}
	7DHCLC 3.5	70.5±6.2^{1a,}A	63.8±6.65^{c,B}	62.25±4.66^{bc,B}	61.6±4.80^{ab,BC}	51.5±11.02^{ab,C}	29.67±7.93^{b,D}	24.75±6.21^{b,E}
	Control	70.3±4.38^{a,A}	42.4±1.37^{d,B}	42.08±1.40^{d,B}	42.1±1.36^{c,B}	39.9±1.21^{c,B}	8.58±1.99^{c,C}	6.98±2.21^{c,C}

^{a,b,c} Different superscripts within the same column demonstrate significant differences among groups ($P<0.001$)
^{A,B,C} Different superscripts within the same line demonstrate significant differences during storage period in same group ($P<0.001$)

acyl chains relatively irregular. At low body temperatures, the lamellar gel phase is formed, in which case the lipid acyl chains are highly regular. In a cell membrane, the mixture of sterols, proteins and lipids causes complex thermal phase behaviour [11]. Membrane complexity increase stress susceptibility that result in membrane disorderliness, membrane lipid/protein structure, and osmotic changes across the membrane. Therefore, sperm membranes may be altered its intrinsic structure resulting in cell death.

In this research, mixed semen groups was observed during 3 days at 4°C and the highest total motility was 77.2±8.86% in CLC 1.5; the lowest total motility was 42.4±1.37% in control (P<0.001) at 1 h. In progressive motility, there were no significant differences between 7-DHCLC and CLC groups (P>0.05); but these groups were statistically different from control (P<0.001) at 1 h. The highest progressive motility was 30.4±7.13% in CLC 1.5; the lowest motility was 15.4±1.19% in control. These motility results were similar to Vyas et al.[12] who was collected 166 ejaculates in 5 hybrid bull semen and at 0 h motility was 71.03%. After semen collection, they kept samples in refrigerator and evaluated the motility at 24, 48, 72 and 96 h and observed 60.69, 50.47, 41.75, 30.13% respectively. Motility rates reduced in each group also during our experiment with similar rates; however the groups CLC and 7-DHCLC 1.5 mg/120×10^6 decreased their rates in 2 days gradually. Similar results were obtained by Franceshini et al.[13] investigated the effect on the motility of the pig sperm storage at 4°C for 4 days and reported motility decrease.

In our research, we added 7-DHCLC and CLC to T solution for external preservation. It's well known that cholesterol modulates the fluidity of membranes by interacting with the fatty acyl chains of the phospholipids similarly with egg yolk in extender [5] and maintains the phospholipids in a random, lamellar arrangement as temperature decreases. However, cholesterol to phospholipid ratio is an important determinant of membrane fluidity and stability at low temperature. Besides, different animal species exhibit different sperm membrane composition, such as different cholesterol/phospholipid ratio and degree of hydrocarbon chain saturation, which can affect how the sperm responds to cooling and, subsequently, can confer different sperm sensitivities to low temperature across various species [14].

In conclusion, adding different rates of CLC and 7-DHCLC in T can be used for maintain bull sperm alive for 3 days at +4°C. Although CLC 1.5 mg/120×10^6 treatment was identified as the best for short time preservation, 7-DHCLC reduced cell damage at low temperatures compared to control. 7-DHCLC can be used in tris extender instead of cholesterol loaded cyclodextrin (CLC). Also, 7-DHCLC and CLC may be considered a good alternative to egg yolk, which is animal sourses, in bull semen short term preservation.

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