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

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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 analysis (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/120×10^6 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

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...
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 [4].

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 [1], 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 >1.0×10⁹ sperm/mL concentration were used in current study. Ejaculates provided these criteria 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/120×10⁶) 7-DHCLC and three different (1.5, 2.5, 3.5 mg/120×10⁶) CLC treatments. Control were diluted T extender without any additives. 7-DHCLC and CLC groups were initially incubated with CLC 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/120×10⁶) 7-DHCLC and three different (1.5, 2.5, 3.5 mg/120×10⁶) 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 100×10⁶ 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 term 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/120×10⁶  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/120x10⁶)

<table>
<thead>
<tr>
<th>Type</th>
<th>Group</th>
<th>Time</th>
<th>0 hour</th>
<th>1 hour</th>
<th>2 hour</th>
<th>6 hour</th>
<th>24 hour</th>
<th>48 hour</th>
<th>72 hour</th>
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<tbody>
<tr>
<td>Progressive</td>
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</tr>
<tr>
<td>CLC 1.5</td>
<td></td>
<td>31.2±4.12aA</td>
<td>30.4±7.13abA</td>
<td>30.02±7.01aB</td>
<td>29.5±8.10aB</td>
<td>25.4±5.81abC</td>
<td>17.67±2.92cdC</td>
<td>11.28±1.56cDE</td>
<td></td>
</tr>
<tr>
<td>CLC 2.5</td>
<td></td>
<td>34.1±3.31aA</td>
<td>26.9±4.32abA</td>
<td>26.27±4.66abA</td>
<td>25.1±6.38abA</td>
<td>16.1±4.03bcC</td>
<td>8.15±5.20bcD</td>
<td>5.05±3.88bcE</td>
<td></td>
</tr>
<tr>
<td>CLC 3.5</td>
<td></td>
<td>36.1±6.23aA</td>
<td>28.4±5.53abA</td>
<td>27.72±5.14abA</td>
<td>25.5±2.74abA</td>
<td>17.7±3.27bcC</td>
<td>9.88±4.62bcD</td>
<td>5.22±2.63bcE</td>
<td></td>
</tr>
<tr>
<td>7DHCLC 1.5</td>
<td></td>
<td>33.2±4.21aA</td>
<td>31.1±3.11abA</td>
<td>30.68±3.06abA</td>
<td>23.8±2.78abC</td>
<td>14.9±5.65bcD</td>
<td>9.23±3.96bcD</td>
<td>6.05±2.21bcF</td>
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<tr>
<td>7DHCLC 2.5</td>
<td></td>
<td>38.4±5.00aA</td>
<td>27.0±3.00abA</td>
<td>26.63±2.91abA</td>
<td>22.0±6.20abA</td>
<td>16.9±3.88bcC</td>
<td>8.38±4.59bcD</td>
<td>6.43±3.39bcD</td>
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<tr>
<td>7DHCLC 3.5</td>
<td></td>
<td>36.0±2.45aA</td>
<td>24.0±3.75abB</td>
<td>23.58±3.75abB</td>
<td>22.6±4.88bcC</td>
<td>16.0±5.59bcD</td>
<td>11.0±3.93bcD</td>
<td>7.53±2.76bcF</td>
<td></td>
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<tr>
<td>Control</td>
<td></td>
<td>34.3±3.26aA</td>
<td>15.4±1.19abB</td>
<td>15.23±1.14abB</td>
<td>15.3±1.19abB</td>
<td>10.7±0.91abC</td>
<td>5.25±0.31bcC</td>
<td>2.73±1.63bcD</td>
<td></td>
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</tbody>
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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 treated with CLC or 7-DHCLC (1.5, 2.5 or 3.5 mg/120x10⁶)

<table>
<thead>
<tr>
<th>Type</th>
<th>Group</th>
<th>Time</th>
<th>0 hour</th>
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<th>2 hour</th>
<th>6 hour</th>
<th>24 hour</th>
<th>48 hour</th>
<th>72 hour</th>
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<tr>
<td>Total</td>
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<td></td>
</tr>
<tr>
<td>CLC 1.5</td>
<td></td>
<td>78.1±5.38aA</td>
<td>77.2±8.86abA</td>
<td>77.05±8.93abA</td>
<td>72.48±12.49abA</td>
<td>60.7±7.23abA</td>
<td>46.87±6.04bcA</td>
<td>41.10±2.15bcD</td>
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<tr>
<td>CLC 2.5</td>
<td></td>
<td>75.3±6.23aA</td>
<td>67.6±7.73abA</td>
<td>67.48±7.76abA</td>
<td>63.5±11.60bcC</td>
<td>52.6±15.71bcC</td>
<td>29.13±8.57bcD</td>
<td>28.27±9.65bcD</td>
<td></td>
</tr>
<tr>
<td>CLC 3.5</td>
<td></td>
<td>73.2±4.35aA</td>
<td>65.8±6.13abA</td>
<td>65.63±6.15abA</td>
<td>53.5±18.43bcC</td>
<td>37.0±12.12D</td>
<td>25.3±3.25bcD</td>
<td>22.5±2.68bcE</td>
<td></td>
</tr>
<tr>
<td>7DHCLC 1.5</td>
<td></td>
<td>77.2±3.45aA</td>
<td>74.9±4.74abA</td>
<td>74.75±4.94abA</td>
<td>63.4±15.04abA</td>
<td>46.4±13.49bcC</td>
<td>30.82±15.07bcD</td>
<td>25.32±8.06bcE</td>
<td></td>
</tr>
<tr>
<td>7DHCLC 2.5</td>
<td></td>
<td>72.1±4.34aA</td>
<td>60.4±4.67abA</td>
<td>60.07±4.66abA</td>
<td>60.3±5.10abA</td>
<td>55.5±6.24bcA</td>
<td>28.93±9.75bcC</td>
<td>24.40±6.89bcD</td>
<td></td>
</tr>
<tr>
<td>7DHCLC 3.5</td>
<td></td>
<td>70.5±5.2aA</td>
<td>63.8±6.65abB</td>
<td>62.25±4.66abB</td>
<td>61.6±4.80bcC</td>
<td>51.5±11.02bcC</td>
<td>29.67±7.93bcD</td>
<td>24.7±6.21bcE</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>70.3±4.38aA</td>
<td>42.4±1.37bcA</td>
<td>42.08±1.40bcA</td>
<td>42.1±1.36abA</td>
<td>39.9±1.12abC</td>
<td>8.58±1.99bcC</td>
<td>6.98±2.21bcC</td>
<td></td>
</tr>
</tbody>
</table>

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)
acetyl 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 71.0%3. 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/120×10⁶ decreased their rates in 2 days gradually. Similar results were obtained by Franceschini 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/120×10⁶ 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 sources, in bull semen short term preservation.

REFERENCES