Evaluating the efficacy of selective inhibition of Arachidonate 15-lipoxygenase (ALOX15) during human semen cryopreservation in protecting freeze thaw induced sperm damage

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Background
Effect of selective inhibition of ALOX-15 on post thaw sperm motility, morphology, mitochondrial potential, malondialdehyde level DNA fragmentation

Methodology

Results

Effect of selective inhibition of ALOX-15 on post thaw sperm motility, morphology, mitochondrial potential, malondialdehyde level DNA fragmentation

A
B
C
D
E

Results demonstrated non significant improvement in A) sperm motility, B) number of sperm with normal morphology, C) number of sperm with intact mitochondria. In parallel, there was moderate reduction in the D) percentage of sperm with fragmented DNA and E) the level of lipid peroxidation product malondialdehyde in post thaw sperm when ejaculate was incubated with 0.25 µM PD146176 before freezing for 20 min compared to control.

Conclusion
Overall, the selective inhibition of ALOX15 during human sperm cryopreservation did not demonstrate any benefits in improving the sperm functional and genetic integrity

Acknowledgement
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POST-THAW QUALITY OF BEETAL GOAT SPERM CRYOPRESERVED DURING LATE SUMMER AND WINTER SEASONS

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Introduction

Cryopreservation is a complex process, as includes many critical stages and factors that may profoundly affect post-thaw sperm quality. Moreover, photoperiod is another factor that influences the reproductive activity of male. This study was carried out to determine seasonal variations in post-thaw quality of Beetal goat sperm.

Methods

Semen Collection

- Electroejaculation during winter and summer seasons
- Rams (n=03)
- Total ejaculates= 12
- 02 ejaculates per ram per season

Processing

- Pooled ejaculates were diluted with extender (Tris 300 mM, fructose 28 mM, citric acid 95 mM, 15% egg yolk and 5% glycerol).
- Final concentration = 200 × 10^6 sperm/mL
- Cooling to 4˚C over 2hrs
- Freezing and thawing at 35˚C

Attributes

- Motion kinetics
- Plasma membrane integrity (PMI)
- Viability

Results

<table>
<thead>
<tr>
<th>Variables</th>
<th>Winter</th>
<th>Summer</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progressive Motility</td>
<td>8.4±0.5</td>
<td>13.0±0.7</td>
<td>0.005</td>
</tr>
<tr>
<td>Total motility</td>
<td>69.9±0.5</td>
<td>87.7±2.1</td>
<td>0.001</td>
</tr>
<tr>
<td>Viability</td>
<td>28.7±3.4</td>
<td>35.3±2.3</td>
<td>0.18</td>
</tr>
<tr>
<td>Total HOST +ve sperm</td>
<td>30.3±1.8</td>
<td>46.3±5.1</td>
<td>0.044</td>
</tr>
<tr>
<td>VCL</td>
<td>34.4±1.4</td>
<td>38.3±1.4</td>
<td>0.117</td>
</tr>
<tr>
<td>VSL</td>
<td>12.2±0.9</td>
<td>12.8±0.7</td>
<td>0.641</td>
</tr>
<tr>
<td>VAP</td>
<td>18.5±1.3</td>
<td>20.8±0.9</td>
<td>0.228</td>
</tr>
<tr>
<td>LIN</td>
<td>28.5±2.0</td>
<td>28.0±1.6</td>
<td>0.844</td>
</tr>
<tr>
<td>STR</td>
<td>53.8±1.4</td>
<td>51.8±1.6</td>
<td>0.418</td>
</tr>
<tr>
<td>WOB</td>
<td>49.8±2.1</td>
<td>51.3±1.5</td>
<td>0.605</td>
</tr>
<tr>
<td>ALH</td>
<td>1.8±0.0</td>
<td>2.0±0.1</td>
<td>0.018</td>
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<tr>
<td>BCF</td>
<td>4.0±0.3</td>
<td>4.1±0.2</td>
<td>0.766</td>
</tr>
</tbody>
</table>
QUALITY AND FERTILITY OF TREHALOSE SUPPLEMENTED CRYOPRESERVED STALLION SEMEN

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INTRODUCTION
Nonpermeating disaccharide nonreducing sugar a) Non enzymatic scavenger b) Modulates membrane fluidity c) Osmotic effect (1,2)
Enhanced cryoprotection of stallion sperms by Tre supplementation to semen extender.(3,4,5) Tremendous scope to increase the pregnancy rates for AI with equine frozen semen by improving freezability by adding Tre to Freezing Media.

OBJECTIVES
1. To study the effects of Tre supplementation to freezing extender on post-thaw seminal attributes, reactive oxygen species and lipid peroxidation levels of equine semen.
2. Comparison of pregnancy rates in mares inseminated by frozen-thawed semen cryopreserved with Tre.

MATERIAL & METHODS

Post-thaw evaluation
Thawing at 37°C for 30 sec.CASA Progressive Motility(6), Viability(7), Membrane integrity(8), Acrosome integrity(9), Mitochondrial membrane potential(10), DNA integrity(11), Reactive oxygen species(12) and Lipid peroxidation levels(13) were estimated.
Fertility trial
Semen doses were prepared with 50mM Tre concentration based on post-thawed parameters of forty-two ejaculates.

RESULTS

MATERIAL & METHODS

Post-thaw seminal attributes

<table>
<thead>
<tr>
<th>Gr</th>
<th>PM (%)</th>
<th>SV (%)</th>
<th>MI (%)</th>
<th>AI (%)</th>
<th>MMP (%)</th>
<th>DI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>42.51a ±0.98</td>
<td>48.36a ±0.89</td>
<td>32.65b ±0.43</td>
<td>72.87b ±0.5</td>
<td>45.75a ±0.93</td>
<td>87.21a ±0.28</td>
</tr>
<tr>
<td>T1</td>
<td>54.76b ±1.11</td>
<td>60.03c ±1.28</td>
<td>39.62d ±0.61</td>
<td>76.89d ±0.49</td>
<td>59.16d ±1.11</td>
<td>90.25c ±0.44</td>
</tr>
<tr>
<td>T2</td>
<td>40.61a ±0.98</td>
<td>48.01a ±1</td>
<td>30.65a ±0.45</td>
<td>70.16a ±0.46</td>
<td>45.44a ±0.82</td>
<td>87.09a ±0.29</td>
</tr>
</tbody>
</table>

Conception rate
Pregnancy rates in control and Tre (50mM) groups were 46.66%(7/15) and 66.66%(10/15), respectively with overall mean pregnancy rates were 56.66%(17/30). NS difference between two groups.

CONCLUSION
Tre supplementation to freezing extender improves post-thaw quality of equine semen.
50 mM Tre is suitable concentration for cryopreservation of equine semen while using Lactose-Glucose-EDTA-egg yolk as SE.

ACKNOWLEDGEMENT

Authors are thankful to Dr. S.C.Mehta Incharge EPC NRCE Bikaner, Pro. R.K.Joshi Dean CVAS Navania for providing facilities and Dr. L.K. Gautam for statistical analysis.

LITERATURE CITED

CONTRIBUTION OF METABOLIC PATHWAYS IN RESISTANCE OF ALGINATE ENCAPSULATED MESENCHYMAL Stromal CELLS TO STORAGE AT AMBIENT TEMPERATURE

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Introduction

Mesenchymal stromal cells (MSCs) implementation requires convenient and efficient storage methods:

Clinical center(s)
Tissue sample collection
Cell-based therapy
Research center(s)
Cell Isolation & Expansion
Researches

Storage at ambient temperature may solve problems associated with cryopreservation. But some questions are still open:

• the period of effective storage;
• the most beneficial for storage state of MSCs (monolayer, suspension, alginate encapsulated);
• cell properties during storage

Storage at 22 °C

Viability of MSCs by FDA/EB staining

<table>
<thead>
<tr>
<th>Monolayer</th>
<th>Suspension</th>
<th>AMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial viability (0 day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 days</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results:

Cell cycle analysis (Premo™ FUCCI Cell Cycle Sensor)

Culture

Cell Isolation & Expansion
Research center(s)

Monolayer
Suspension
AMS

Mitochondrial membrane potential by JC1-test

Low mitochondrial membrane potential (ΔΨm)
High mitochondrial membrane potential (ΔΨm)

ROS level by Abcam Cellular ROS Kit

Basal H₂O₂-induced

Monolayer
AMS

Alginate encapsulation significantly supported MSCs viability during storage, while it was decreased dramatically in both monolayer and suspension.

Alginated encapsulation decreased basal metabolic activity in MSCs in preculture period. Metabolic activity decrease in AMS during subsequent storage was smooth compared to the sharp decrease in monolayer and suspension.

Conclusion:

Alginated encapsulation results in cell cycle arrest, metabolic activity & ROS level decrease, intracellular pathways modulation, antioxidant defense activation, which increase the resistance of encapsulated MSCs to storage at ambient temperature.
INTRODUCTION

The Cellular Therapy Laboratory (CTL) of the South African National Blood Service (SANBS) is responsible for the processing and storing of Haematopoietic Stem Cell Transplant (HSCT) products. HSCT products are stored in the vapour phase in liquid nitrogen (LN) freezers. Prior to the release of HSCT products for reinfusion, viability of the storage vials, aliquoted at the time of processing and stored under the same conditions, is performed using flow cytometric methods. HSCT viability release criteria is ≥50% for CD45 and ≥70% for CD34. Discrepancies between vial and product viability are well known, with vial viability often underestimating product viability. HSCT products require rapid infusion post thawing, however, our clinical units are geographically distant from our laboratory, therefore routine product viability testing is not possible in our setting.

METHODS

HSCT products that are no longer required for patient use, are useful for assessing true product viability. This study assessed product viability (CD45 and CD34) of ten redundant HSCT products stored in CTL. Written consent for the use of these products was received from the treating physician.

RESULTS

The HSCT products were older than 1 year with a range of 1-5 years and a mean of 1.7 years. Upon flow cytometric viability analysis, it was found that all products passed the release criteria, with a range of 50-90% for CD45 (mean: 70%) and 96-100% (mean: 96%) for CD34. There was no correlation between age of product and viability of product (correlation coefficient: 0.019309).

<table>
<thead>
<tr>
<th>Sample No</th>
<th>Days in Freezer</th>
<th>CD34%</th>
<th>CD45%</th>
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<tr>
<td>1</td>
<td>712</td>
<td>96</td>
<td>69</td>
</tr>
<tr>
<td>2</td>
<td>933</td>
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<td>4</td>
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<tr>
<td>5</td>
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<td>95</td>
<td>72</td>
</tr>
<tr>
<td>7</td>
<td>375</td>
<td>87</td>
<td>84</td>
</tr>
<tr>
<td>8</td>
<td>1857</td>
<td>94</td>
<td>50</td>
</tr>
<tr>
<td>9</td>
<td>343</td>
<td>97</td>
<td>66</td>
</tr>
<tr>
<td>10</td>
<td>855</td>
<td>97</td>
<td>56</td>
</tr>
</tbody>
</table>

CONCLUSION

This study showed that HSCT products stored for more than one year still fulfill the release criteria, indicating the robust processing and LN storage procedures in SANBS CTL. Annual testing of samples with increasing ages will be beneficial in determining maximum validated LN storage time periods in this facility.
ABSTRACT

Cryospray is a method of destructing cancerous lesions occurring on skin. Cryogen is sprayed on the affected area and ablation is achieved through rapid freezing of the cell.

An Eulerian-Lagrangian mathematical model is used to simulate the behavior of cryogen spray.

The validity of the present model has been confirmed with experimental results of evaporating liquid nitrogen spray gushing out form the commercial cryogen.

Simulation result predicts 30 mm as most optimized spraying distance on the basis of the area enclosed by lethal front and freezing front.

INTRODUCTION

Cryotherapy is an advanced application that utilizes therapeutic effect of low temperature in the area of cancer treatment.

Rapid increment in cancer cases globally and inability of conventional techniques to treat diseases compelled researcher to seek options of cancer treatment.

Cryospray is the type of cryotherapy which deals with superficial tumor.

In cryospray cryogen exchanges heat and mass with surrounding compared to cryosurgery in which cryogen circulates in closed cannulas and only exchanges heat with surrounding. It makes the imaging of cryospray a challenging task as compared to cryosurgery.

Thus, surgeon estimates the dimension of necrotic zone through his experience which makes cryospray more surgeon oriented process rather than comprehensive process.

In this perspective present study aim at the development of numerical model that will acknowledge all the parameters involved in the cryospray process.

METHODOLOGY/FORMULATION

The Eulerian-lagrangian approach is used to describe the thermo-fluid behavior of liquid nitrogen droplets present in atmospheric air. Continuous phase (atmospheric air) is described by Eulerian framework while dispersed phase (liquid nitrogen droplets) is described using Lagrangian framework.

The whole simulation is divided into two parts first atmospheric air flow field is calculated before adding the injection source, after obtaining the stable flow field of atmospheric air liquid nitrogen droplets are introduced into the atmospheric air for further calculation.

In simulation, interphase coupling method is used to calculate the influence of energy transfer on the atmospheric air due to the evaporation of nitrogen droplets and vice versa.

BOUNDARY CONDITIONS

The temperature of the surrounding air is taken as 300 K.

The velocity of surrounding air is taken as 0.1 m/s at inlet condition.

The outlet boundary condition are specified as fully developed outflow condition. The exit flow pressure is set at atmospheric pressure.

The gravitational acceleration is set as 9.81 m/s².

Mass flow rate of liquid nitrogen is determined experimentally.

Input velocity of droplet is calculated as 5 m/s.

Rosin-Rammler diameter distribution varies in a range of 20 μm to 100 μm.

The exit boundary condition for droplet is set as escape.

Both phases air and droplet are two way coupled which means droplets can have effect on surrounding and vice versa.

CONCLUSION

Proposed approach explores the phenomena of heat and mass transfer through cryogen spray.

It has been observed that as the distance from the nozzle exit increases the minimum temperature inside the spray core decreases.

Overall decrement of 30 °C is recorded in the spray core for the spraying distance of 80 mm.

Curves of velocity with respect to radial distance flattens as the spraying distance increases.

On the basis of area enclosed by lethal front and freezing point it can be concluded that spraying distance of 30 mm is providing the most optimized results.
METHODICAL APPROACHES TO CRYOPRESERVATION OF CELLULAR SPHEROIDS

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The search for optimal methods of cryopreservation of spheroids is a topical issue in cryobiology. Numerous cryopreservation protocols for cell suspensions, i.e. single cells, do not solve the problem of cryosensitivity of cells in multicellular spheroids, which have a three-dimensional structural organization and complicated intercellular interactions.

The aim of the study was to determine the filtration coefficients for water molecules and permeability of cell membranes for dimethyl sulfoxide (Me₂SO) into spheroids at different terms of cultivation.

Using the theoretical calculation proposed in the work, it is possible to determine such parameters.

The calculation of these parameters is based on the change of spheroids volume during the time after their contact with 1 M DMSO solution and NaCl solution of different toxicity.

CONCLUSIONS

It is proved that the exposure time of spheroids in 1 M DMSO and the value of the energy of transfer of water molecules and DMSO probably increases 2 times on the 14th and 21st day of cultivation compared to the 7th day of cultivation. Based on the obtained parameters and physical and mathematical modeling of spheroid dehydration processes during cooling, the optimal cooling rate was determined for 7 days spheroids: 2 deg. /min with cooling to -80°C and subsequent immersion in nitrogen; and for spheroids on 14th and 21st day: 1 deg. /min to -40°C and subsequent immersion in nitrogen.

The optimal exposure time in 1 M DMSO

Predicted kinetics of change in spheroid volume during freezing depending on various cooling rates: 0.5, 1, 2, 5 °C/min.
Introduction
In this study, we assumed that the thermal field parameters in the ice spot on the skin surface, caused by the cryoapplicator, are equivalent to those of underlying tissue frozen volume both during freezing and warming.

Purpose
To assess the practical application of infrared thermal imaging for real-time monitoring of freezing and warming of biological tissues in vivo.

Materials & Methods
Cryoaблation of the skin in 30 rats was performed with exposure durations of 0.5, 1 and 2 minutes. The liquid nitrogen-cooled contact cryoprobe was used.

Results, Discussion

Ice spot diameters at various cryoexposure durations and subsequent warming.

Ice spot minimal temperatures during natural warming after various cryoexposure durations.

Ice spot diameter and its minimal temperature after cryoexposure durations of 1 min.

The dynamics of ice spot diameter and its center temperature are similar. The feature of the freezing/thawing cycle includes the specific stages, cryoexposure duration depended parameters. During natural thawing, the quasi-stable stages (QSS) are present in both dynamics, apparently associated with structural ice rearrangements in the frozen tissue.

Acknowledgements
This work was supported by the National Academy of Sciences of Ukraine funding by the grant 0121U108515

To conclude we have shown that infrared imaging can be used for real-time monitoring of skin temperature field dynamics during cryotherapy, including the examination of the necrotic and cryoscopic isotherm positions.
As artificial sleeping is increasingly considered for use as a stand-alone therapeutic agent, our study was to examine the effect of its administration on E. coli infected animals and to elucidate the biochemical parameters of the body at different stages of infection.

Methods and materials

The experiments were performed with 2-months rats, they were led in for 3 hours in the hypobiosis (Bakhmetev-Jaya-Anjus method with lowering the animals' body temperature);

We have used a sealed chamber with a volume of 3 dm³ at temperature 3–5 °C.

Infection's stages were initial symptoms (I), progressive symptoms (II) and total depletion of the body (III stage);

We evaluated the antioxidant status and the oxidative status in the liver tissues and the pattern of redistribution of protein fractions in the blood.

Conclusions

- SOD and catalase activities of biological samples have shown that hypobiosis stimulates the body to fight E. coli (I and II stages) by activating SOD and catalase in liver tissues on the principle of additive synergism.
- Analyzing the glutathione antioxidant system of the liver of a sick body in conditions of artificial sleep, it was found that the dynamics of its indicators in general duplicates the picture of SOD and catalase in the same conditions for I and II stages.
- While the introduction of hypobiosis in rats with E. coli infection on stage III is accompanied by depletion of glutathione resource in liver tissues and low glutathione reductase activity.
- The effect of hypobiosis is manifested by a decrease in the level of MDA in the liver of patients at all stages of E. coli infection.
- The proteinogram showed an increase in the level of γ-globulins and a decrease in the level of albumin.
- The obtained results indicate a real prospect of using a 3-hours of hypobiosis for the treatment of E. coli infection at all stages of the disease as a modern cryogenic agent.
Introduction
Sucrose is often used both as a primary cryoprotectant and in complex cryoprotective media. In particular, sucrose is a component of many media for cryopreservation of mammalian embryos by vitrification.

Purpose
To study low-temperature phase transitions in sucrose-containing solutions of glycerol (Gl), 1,2-PD, 1,3-PD, ethylene glycol (EG), and Me\textsubscript{2}SO.

Materials and methods

Low temperature differential scanning calorimetry (DSC)
DSC thermograms were recorded at the heating stage (0.5 grad/min) after rapid cooling of the solutions by immersion into liquid nitrogen (~200\(^\circ\)C/min).

Preparation of cryoprotectant solutions
Cryoprotectant solutions (30\% concentration) were prepared with Dulbecco’s nutrient medium supplemented with sucrose (1 M).

Cryopreservation of mouse embryos
2-cell and 8-cell mouse embryos in cryoprotective medium were vitrified by immersion into liquid nitrogen using plastic straws and preserved 3-7 days. Thawing was performed in water bath (38\(^\circ\)C). To remove the cryoprotectant there was used a 10-min equilibration in 0.5M sucrose solution. Then the embryos were three times washed-out with physiological medium, transferred into CO\textsubscript{2} -incubator for culturing. Rate of embryo viability was estimated by their developmental capacity to the stage of extended blastocyst.

Results

<table>
<thead>
<tr>
<th>Samples</th>
<th>T\textsubscript{g}, (^\circ)C</th>
<th>T\textsubscript{c}, (^\circ)C</th>
<th>T\textsubscript{m}, (^\circ)C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Me\textsubscript{2}SO</td>
<td>-108.2±0.5</td>
<td>-85.3±0.5</td>
<td>-34.6±0.5</td>
</tr>
<tr>
<td>EG</td>
<td>-109.1±0.5</td>
<td>-86.9±0.5</td>
<td>-25.8±0.5</td>
</tr>
<tr>
<td>Gl</td>
<td>-76.0±0.5</td>
<td>-54.0±0.5</td>
<td>-11.7±0.5</td>
</tr>
<tr>
<td>1.2-PD</td>
<td>-72.2±0.5</td>
<td>-51.6±0.5</td>
<td>-13.9±0.5</td>
</tr>
<tr>
<td>1.3-PD</td>
<td>-83.0±0.5</td>
<td>-62.1±0.5</td>
<td>-15.1±0.5</td>
</tr>
</tbody>
</table>

*0.5 min of 2-cell embryo exposure in Dulbecco’s physiological medium at room temperature: \(^\ast\) – statistically significant difference compared to the control, p<0.05

Conclusions
The addition of sucrose to cryoprotective solutions leads to an increase in the glass transition temperature and the disappearance of crystallization and melting of eutectic compositions.
**Introduction**

During cryopreservation, goat spermatozoa are exposed to different types of physical, chemical, osmotic, and oxidative stresses that have adverse effects on the quality and fertility of sperm. Since, providing a cryo-medium supplement for freezing sperm can enhance the artificial insemination efficacy and afterward, the reproductive performance of goats. Despite the antioxidant properties of Ferulago angulata extract (FAE), the effect of this natural antioxidant as an additive for the semen extender has not been studied.

**Aim**

In this study, we evaluated the effect of FAE supplementation into freezing extender on the cryopreserved goat semen quality and fertility ability after thawing.

**Methods**

In experiment 1, the treatments consisted of basic extender containing different concentrations (0.00, 0.002, 0.005, and 0.01%, w/v) of FAE. After determination of the potential effective concentration of FAE using assessment of the sperm motility, in experiment 2, semen samples (15 ejaculates/3 goats) were pooled, diluted with Bioxcell® extender, and supplemented with 0.002% FAE. Control diluent contained no additives. Following equilibration, the straws were exposed to liquid nitrogen (LN2) vapor and then plunged into LN2. After thawing, sperm quality and fertility were evaluated.

**Results**

Our results showed that the sperm motility and plasma membrane integrity after thawing significantly improved by FAE compared to the control group. The sperm viability was not different between the two groups. ROS production level significantly decreased by FAE compared to the control. FAE supplement improved the hatched blastocyst rate in embryos derived from frozen/thawed goat sperm compared with the control group. No significant differences were observed in cleavage and blastocyst rates of embryos among FAE and control groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cont</th>
<th>FAE 0</th>
<th>FAE 0.002%</th>
<th>FAE 0.005%</th>
<th>FAE 0.01%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motility (%)</td>
<td>a</td>
<td>ab</td>
<td>abc</td>
<td>abcb</td>
<td>b</td>
</tr>
<tr>
<td>Viability (%)</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Membrane Integrity</td>
<td>a</td>
<td>a</td>
<td>ab</td>
<td>abc</td>
<td>bc</td>
</tr>
<tr>
<td>ROS Production</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>ab</td>
</tr>
</tbody>
</table>

**Conclusion**

As an overall conclusion of this study, the addition of FAE (0.002%, w/v) to the freezing extender improved goat sperm quality and fertility after the freeze-thaw procedure. Hence, FAE as a natural antioxidant can increase sperm cryotolerance and post-thaw persistence.

**Acknowledgments**

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THE EFFECT OF DIFFERENT CRYOPROTECTANT CONCENTRATIONS DURING CRYOPRESERVATION OF SEMEN FROM WINDSNYER BOARS

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Introduction

Windsnyer pig breed is known with its unique characteristics of being able to perform better in harsh environmental conditions and are often possess valuable traits such as disease tolerance, longevity and able to survive under cheap feed sources (Umesiobi, 2000; Broekhuijse et al., 2012). Therefore, there is a need to conserve the Windsnyer boar semen for future breeding purposes. Cryopreservation is the preferred method to store boar semen for ages. Cryoprotectants are used during cryopreservation with the aim of reducing sperm injury and osmotic stress caused by cold shock.

Objective

To determine the suitable cryoprotectant concentration for cryopreservation of Windsnyer boar semen.

Materials and methods

(A) Windsnyer boars, (B) Incubator, (C) Cryoprotectants, (D) Cooling of the straws with LN2 vapour, (E) Liquid nitrogen tank (F) Fluorescent microscope & (G) Sperm Class Analyzer.

Results

Figure 1: Comparison of the cryoprotectant concentrations on the sperm motility.

Figure 2: Comparison of the cryoprotectant concentrations on the sperm progressive motility.

Figure 3: Comparison of the cryoprotectant concentrations on the sperm rapid motility.

Conclusion

The 16% Glycerol was found to be suitable cryoprotectant concentration to cryopreserved semen of Windsnyer boars.

Acknowledgement

Table 2: Comparison of the cryoprotectant concentrations on post-thaw sperm morphology of Windsnyer boar semen (Mean±SD).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Concentrations (%)</th>
<th>Sperm morphology parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Live (%)</td>
<td>Dead (%)</td>
</tr>
<tr>
<td>Raw semen</td>
<td>71.3±5.1*</td>
<td>23.8±3.2*</td>
</tr>
<tr>
<td>Cryoprotectant free</td>
<td>22.3±5.8*</td>
<td>77.8±6.4*</td>
</tr>
<tr>
<td>Glycerol</td>
<td>4</td>
<td>31.8±6.7*</td>
</tr>
<tr>
<td>Ethylene glycol</td>
<td>8</td>
<td>28.7±3.2*</td>
</tr>
<tr>
<td>Propanediol</td>
<td>12</td>
<td>29.0±3.9*</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>49.5±8.3*</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>29.0±7.6*</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>22.9±9.3*</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>20.6±4.7*</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>17.3±6.6*</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>25.7±9.9*</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>21.7±6.9*</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>35.3±9.5*</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>34.7±9.1*</td>
</tr>
</tbody>
</table>
**EFFECT OF DIFFERENT CONCENTRATIONS OF GLUTATHIONE ON FROZEN-THAWED SEMEN FROM KOLBROEK BOARS**

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**INTRODUCTION**

Kolbroek is a South African indigenous pig breed. It is well known that cryopreserved semen is not routinely used in the pig industry due to cell cryodamage that occurs during freezing and thawing procedures. Compared with liquid preservation, the primary problem related with semen cryopreservation is the reduction in semen quality. However, cryopreservation is considered as an effective technique to preserve gametes. Focusing on trying to understand the underlying issues of cryodamage by introducing the use of antioxidants is one way to improve cryopreservation technology. Glutathione is an antioxidant that protects sperm cells from oxidative stress, maintains redox balance and acts as a defence mechanism against lipid peroxidation.

**OBJECTIVE**

The aim of the study was to evaluate the effect of supplementation of semen extender with different glutathione concentrations (0, 1, 5 and mM/L) on the quality of frozen-thawed Kolbroek sperm.

**CONCLUSION**

In conclusion, BTS supplemented with different glutathione concentrations in the freezing extender had reduced sperm motility traits as compared to raw semen from boars. However, 5 mM was the optimum concentration of glutathione to be added to the BTS extender for cryopreserving semen from Kolbroek boars.

**MATERIALS AND METHODS**

**Table 1: Sperm motility of cryopreserved Kolbroek boars (mean±SD)**

<table>
<thead>
<tr>
<th>TREATMENTS</th>
<th>TM%</th>
<th>PM%</th>
<th>NPM%</th>
<th>SLW%</th>
<th>MED%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw semen</td>
<td>90.7±2.5a</td>
<td>27.07±4.5a</td>
<td>56.10±12.7a</td>
<td>14.1±9.8ab</td>
<td>57.9±9.1a</td>
</tr>
<tr>
<td>Control</td>
<td>25.7±9.6c</td>
<td>9.8±3.2b</td>
<td>15.9±9.9b</td>
<td>9.1±7.1b</td>
<td>8.8±4.7b</td>
</tr>
<tr>
<td>1 Mm</td>
<td>23.9±9.9f</td>
<td>7.1±2.6a</td>
<td>16.8±9.9ab</td>
<td>13.0±8.6ab</td>
<td>6.0±3.1a</td>
</tr>
<tr>
<td>5 Mm</td>
<td>33.6±10.9g</td>
<td>8.1±3.3c</td>
<td>25.6±10.2b</td>
<td>19.5±9.4a</td>
<td>7.8±3.1b</td>
</tr>
<tr>
<td>10 Mm</td>
<td>23.1±5.8c</td>
<td>7.5±3.5bc</td>
<td>15.5±6.4c</td>
<td>11.6±6.2b</td>
<td>5.7±2.1b</td>
</tr>
</tbody>
</table>

**Table 2: Sperm velocity rates of cryopreserved Kolbroek boars (mean±SD)**

<table>
<thead>
<tr>
<th>TREATMENTS</th>
<th>RAP(μm/sec)</th>
<th>VCL(μm/sec)</th>
<th>VSL(μm/sec)</th>
<th>VAP(μm/sec)</th>
<th>LIN%</th>
<th>STR%</th>
<th>WOB%</th>
<th>HYPERACTIVE%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw semen</td>
<td>19.9±12.2a</td>
<td>154.8±43.0a</td>
<td>63.6±12.1a</td>
<td>78.6±13.8a</td>
<td>43.2±11.3a</td>
<td>80.3±4.6a</td>
<td>53.4±12.4a</td>
<td>5.1±3.8a</td>
</tr>
<tr>
<td>Control</td>
<td>7.8±2.5b</td>
<td>157.4±32.9c</td>
<td>64.5±23.9a</td>
<td>78.0±19.4a</td>
<td>41.0±10.5b</td>
<td>79.9±12.9a</td>
<td>50.2±7.6b</td>
<td>2.1±2.2a</td>
</tr>
<tr>
<td>1 mM</td>
<td>4.8±2.7b</td>
<td>145.9±32.4a</td>
<td>60.8±21.8a</td>
<td>76.9±18.2a</td>
<td>43.1±9.1b</td>
<td>78.6±13.8a</td>
<td>53.9±7.9a</td>
<td>1.7±1.6a</td>
</tr>
<tr>
<td>5 mM</td>
<td>6.2±3.6b</td>
<td>177.6±56.4a</td>
<td>52.3±97.1a</td>
<td>70.9±22.5a</td>
<td>31.9±13.5a</td>
<td>69.2±19.4a</td>
<td>43.9±10.6a</td>
<td>1.8±1.5a</td>
</tr>
<tr>
<td>10 mM</td>
<td>5.7±4.1b</td>
<td>150.1±49.8a</td>
<td>37.9±27.2a</td>
<td>63.3±20.1a</td>
<td>31.9±18.4a</td>
<td>63.6±21.7a</td>
<td>47.8±13.8a</td>
<td>2.1±2.5a</td>
</tr>
</tbody>
</table>
EFFECTS OF RUTIN ON THE QUALITY OF ROOSTER SPERM DURING CRYOPRESERVATION

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Department of Animal Science, College of Agriculture, University of Tabriz, Tabriz, Iran;

BACKGROUND

Rutin is known as a plant pigment (flavonoid) found in green tea, passion flower, buckwheat, and apple. In this experiment, rutin has been selected because of the interesting findings of flavonoids in genotoxicity tests. It is demonstrated that flavonoids show a double-edge action, because they can perform as prooxidants or antioxidants, depending on concentration. The strong antioxidative capacity of rutin has been confirmed by several experiments, mainly for outstanding scavenging activity. The purpose of the current study was to estimate the effect of different levels of rutin on post-thawed rooster semen quality.

MATERIALS AND METHODS

Ejaculates were collected using the dorsolateral massage method and collection was always accomplished by the same person and with the same conditions. After collection, the ejaculates were then transferred to the laboratory by a thermal flask containing water at a temperature 37°C for primary evaluation. To reduce individual differences and achieve satisfactory sperm for analysis, in each replicate, the ejaculates of the ten roosters were concisely inspected and ejaculates with ≥ 30 × 106 spermatozoa/ml, ≥ 96% motility and, ≥ 90% normal morphology were then pooled. The diluted semen was gradually cooled to 4°C and then cryopreserved in 0.25 mL French straws. Frozen straws were thawed at 37°C for 30 s in a water bath, for post-thawed sperm evaluation.

RESULTS

Rutin at level 0.6 mM resulted in the highest total and progressive motilities percentages, in comparison to other treatments (P < 0.05). Our results revealed that rutin at level 0.6 mM led to higher GPx, mitochondria activity, and membrane integrity in comparison to the control group (P < 0.05).

CONCLUSION

It can be deduced that addition of rutin at level 0.6 mM improved the post thawing quality and oxidative variables of rooster semen.

KEYWORDS

cryopreservation; sperm; rooster; extender
Changes in the redox state of cytochromes in mouse embryos during cooling with different cryopreservation protocols

by K.A. Okotrub¹, V.I. Mokrousova, S.Ya. Amstislavsky², and N.V. Surovtsev¹

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Electron Transport Chain

Interrambrane space
Complex I
Complex III
Complex IV
2H₂O

Complex II
Phenylalanine
NADH+4
Fumarate
Succinate

Matrix

Cytocromes are haemoproteins that participate in the work of respiratory electron transport chain (ETC), the fundamental process of cellular respiration. The intensity of resonance Raman lines of cytochrome haem depends on the cytochrome redox state. We applied this effect to characterize the activity of respiratory electron transport chain (ETC) in mouse early embryos and yeast cells during freezing.

Green light radiation induces the formation of reactive oxygen species in biological cells resulting in oxidative stress and oxidation of cytochromes. Oxidation process leads to photobleaching of cytochrome Raman lines. We applied this effect to characterize the activity of ETC in biological cells during freezing and compared the cytochrome redox state of cytochromes in embryos cooled to -170°C using different cryopreservation protocols: slow program freezing, vitrification with penetrating cryoprotectants, vitrification without penetrating cryoprotectants [Jin & Mazur, Sci. Rep. 2015].

Results

Examples of mouse embryo Raman spectrum evolution during exposition to laser radiation at different temperatures.

Raman spectra of frozen and vitrified mouse embryos measured at -170°C.

Conclusions

- Changes in the photobleaching rate and increase of cytochrome Raman lines intensity observed in yeast cells and mouse embryos indicate on the changes in ETC state at about -50°C.
- Difference spectra analysis revealed photoinduced reduction of b type cytochromes and oxidation of c type cytochromes in mouse embryos at temperatures below -50°C. This effect can be interpreted as an oxidant-induced reduction effect indicating on downregulation of bc1 complex of the ETC in frozen cells.
- We observed that embryos vitrified with penetrating cryoprotectants demonstrate the lowest concentration of cytochrome c in reduced redox state.

Intensity ratios reflecting redox state of cytochromes in embryos cooled using different cryopreservation protocols.

For c & b type cytochromes, the intensity of the Raman line at 600 and 750 cm⁻¹ in Fe²⁺ state is ~10 times greater than in Fe³⁺.

Cryopreservation of domestic cat preimplantation embryos: effects of in vitro exposure to linoleic acid

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Introduction Felidae species are the focus of wildlife conservation activity. The domestic cat (Felis silvestris catus) is considered as a model species for the applying technologies of Genome Resources Bank to wildlife felids

Embryos of Carnivora species are characterized by high lipid content; for such embryos rich in lipid droplets (LDs), tolerance to freezing and cryopreservation (CRYO) is low. The problem can be solved by a change in the composition of intracellular lipids.

The aim This work is aimed to study the effect of linoleic acid (LA) in vitro exposure on the intracellular lipids and the onset of their phase transition (T*) during freezing, as well as the efficiency of embryo cryopreservation.

Results The addition of LA to the medium during in vitro culturing (IVC) of cat embryos did not influence the overall number of lipids assessed by fluorescent intensity of the Nile red (Fig.1). However there was found an increasing of the intracellular lipid unsaturation degree and a decrease in the onset of lipid T* as compared to control (Fig.2).

Fig.1. Optical slices of cat’s embryos labeled with Nile Red. CLSM. Photon count mode

Fig.2. Dependence of the lipid’s T* on the level of their unsaturation.

Table 1. No. of interphase nuclei after cryopreservation

<table>
<thead>
<tr>
<th>Group</th>
<th>Morula</th>
<th>Blastocyst</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>36.9±3</td>
<td>62.6±5.4</td>
</tr>
<tr>
<td>LA</td>
<td>27.7±2.7*</td>
<td>91.8±10.7**</td>
</tr>
</tbody>
</table>

Conclusion The addition of LA to the culture medium during IVC led to an increased unsaturation of intracellular lipids of in cat' embryos. The observed change in the degree of lipid unsaturation led to a decrease in their T*, and caused an improvement of the embryo development after CRYO.
FORMATION OF A CRYOBANK OF HIGH PRODUCING COWS’ EMBRYOS IN THE CONDITIONS OF THE INDUSTRIAL DAIRY COMPLEX

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¹ Agricultural limited liability company agricultural firms «Petrodolynske», ² Institute of Animal Breeding and Genetics nd. a. M.V.Zubets of National Academy of Agrarian Science of Ukraine, ³ Luhansk National Agrarian University

INTRODUCTION

In the conditions of intensive milk production there is a conflict between the physiological needs of lactating cows and the genetically determined ability to produce a large amount of milk. The consequence of this is the problem of reproduction of high producing cows, which arise due to reduced immunity and the development of polymorbidity.

It is known that during intensive operation, most cows have less than three calves, which significantly reduces the profitability of the industry due to the purchase of additional heifers to repair the dairy herd. Premature culling of highly productive cows eliminates valuable genotypes from the population, which significantly reduces the effect of selection.

MATERIALS AND METHODS

The aim of the study was to test the creation and rational use of a cryobank of embryos obtained from discarded record-breaking cows.

The study was conducted on the basis of a breeding dairy complex for breeding Holstein cattle of European selection with an average productivity per lactation of 10,000 kg of milk (Private Joint Stock Animal "Agro-Soyuz", Dnipro region). In vivo removal of preimplantation embryos from cows removed from the dairy herd due to various reasons was performed. After hormonal stimulation of superovulation from 22 donors, only 114 embryos suitable for transplantation were obtained (5.2 units per cycle). Evaluation of embryos was performed in vitro in the field of view of the light microscope MBS-10 (29x) on a set of morphological features. Cryopreservation of embryos was performed in sequins by passive cooling in the neck of the Dewar vessel using 1.0 M glycerol.

RESULTS

For the planned rational use of embryos from the formed cryobank, 41 embryos after 30 days in liquid nitrogen were deconserved (in a water bath at 370°C for 30 seconds at a rate of 3.00°C per minute) and transplanted to synchronized recipient heifers. Control of engraftment was performed by diagnosing the uterine cavity of recipients on day 35 with an ultrasound scanner (“Tringa”). 39.0% of pregnancies (16 goals) were obtained, which testified to the possibility of obtaining additional pregnancies from culled cows for one cycle of hormonal induction of superovulation according to the scheme "embryos-cryopreservation-deconservation-transplantation".

CONCLUSION

Thus, the production study confirmed the possibility of increasing the selection, economic, scientific potential of culled high-producing cows by forming a cryobank of embryos.