The "Carryover" Effect of Cryopreservation on Early Development of Human Embryos

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Scientific Questions

Women's fertility preservation has received more attention. Oocyte cryopreservation is an option of fertility preservation. Freeze and thaw affect the oocyte survival.

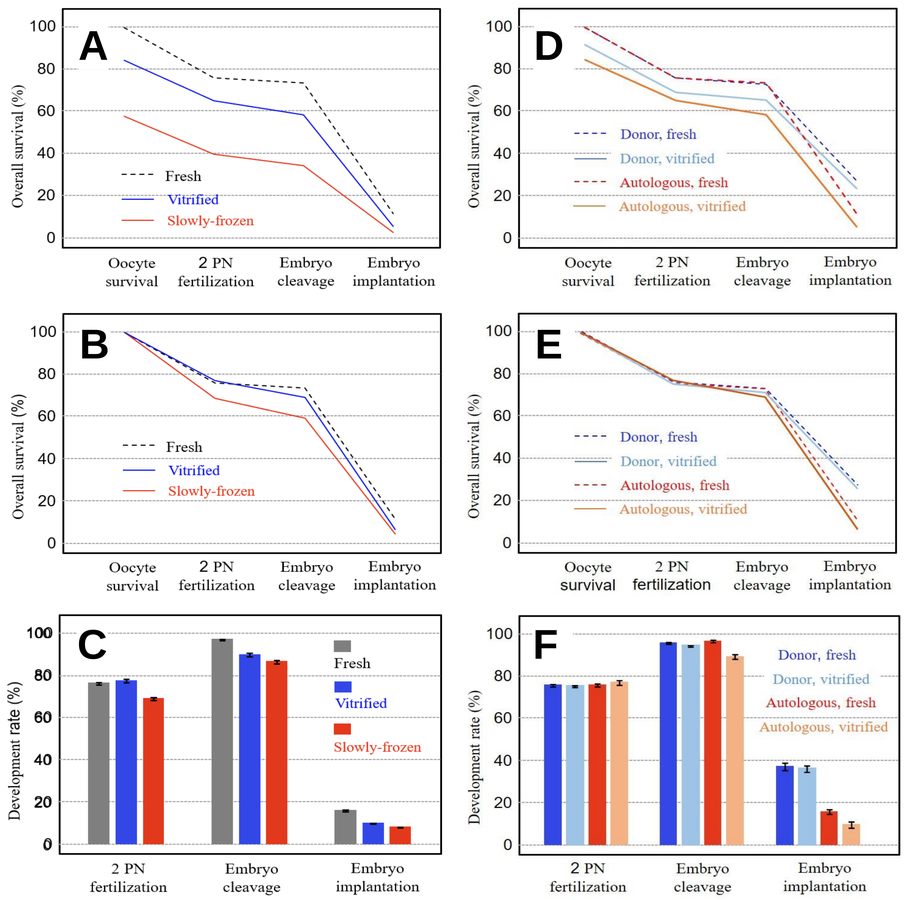
- Does cryopreservation have any longer term "carryover" effect on early development of the embryos derived from the survived oocytes?
- If so, is there any difference between vitrification and slow freezing method?
- Is it related to the technique of cryopreservation or the inherent quality of oocytes?
- What is the possible mechanisms involved?

Methods

Research articles published between 1999 and 2020 was retrieved from public databases (Medline, PubMed and Web of Science).

We have identified a total of 27 studies that are relevant to human oocyte cryopreservation and early development of cryopreserved fertilized oocytes. (embryos). Data in these 27 articles were retrieved for meta-analysis.

Summary of Results

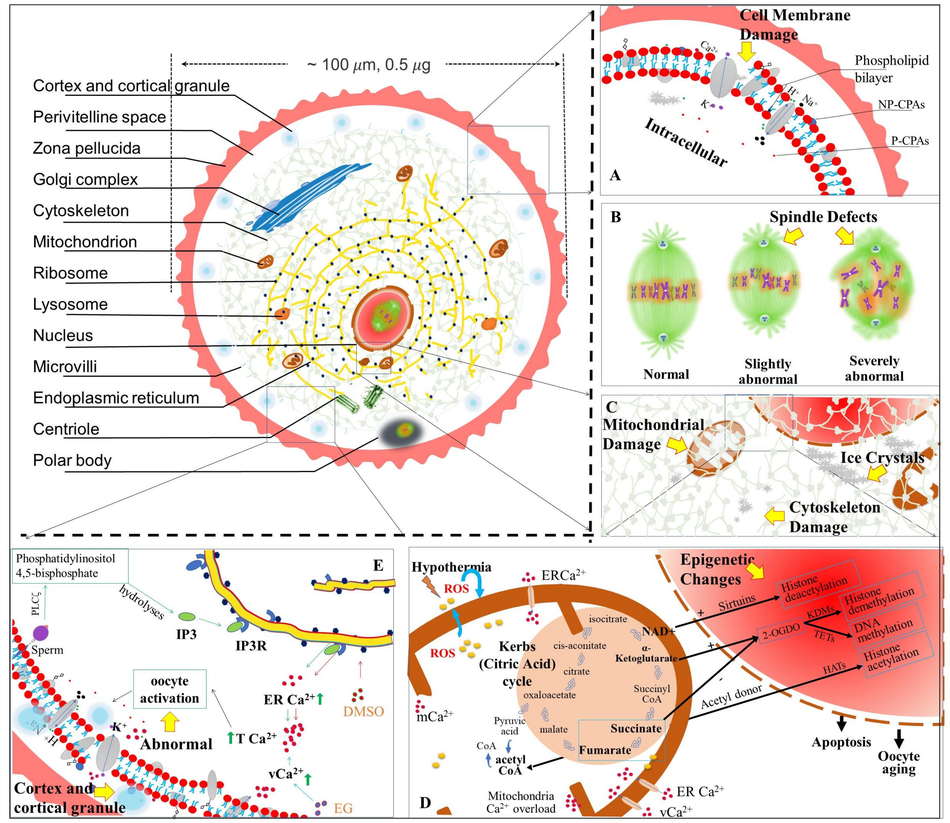


A) Oocyte survival and development upon cryopreservation; **B**) Embryo development as normalized by oocyte survival; **C**) The rate of sequential development into next stage; **D**) Survival and development of autologous and donor oocytes upon vitrification; **E**) Embryo development of vitrified autologous and donor oocytes as normalized by oocyte survival; **F**) The rate of sequential development of vitrified autologous and donor oocytes into next stage.

The carryover effect: Cryopreservation not only damages oocytes, but also decreases the development potential of survived oocytes, especially slowly frozen ones. Autologous vs Donor Oocytes: For vitrified oocytes, the carryover effect is seen only in older autologous oocytes and not in younger donor oocytes.

Mechanisms of the "Carryover" Effect

An overview of the physical and biological criticalities likely associated with the mechanisms of oocyte cryoinjuries that attributes to the "carryover" effect



Conclusion

Cryopreservation affect the early embryo development of survived oocytes. The the "carryover" effect depends on protocols and oocyte sources, and may represent some subtle functional or molecular alterations that are not severe enough to affect normal cell survival, but sufficiently to impair certain developmental and functional expression later. Human oocyte cryopreservation should go beyond the protocol optimization for oocyte survival, and look further into the effects on molecular expression and epigenetic changes.

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OOCYTE AND SPERM CRYOPRESERVATION IN ONCOLOGICAL PATIENTS DURING COVID-19 PANDEMIC

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During the pandemic, most infertility and IVF Units decided to keep fertility preservation active as an urgent procedure. It is well established that chemotherapy is gonadotoxic and impact negatively on quality of life. The American Society of Reproductive Medicine (ASRM) and European Society of Human Reproduction and Embryology (ESHRE) recommend to offer fertility preservation before cancer treatment. Oocyte cryopreservation and sperm cryopreservation are the best treatments of the choices to preserve fertility in cancer patients.

This is a prospective study performed at Infertility and IVF Unit, Sant'Orsola University Hospital, University of Bologna, Italy, from February 2020 to January 2021. **149 cancer patients** underwent gamete cryopreservation to preserve their fertility. Due to the pandemic, realtime (RTPCR) analysis of throat swab specimens for Sars-Cov-2 was introduced for all patients 48 hours before cryopreservation. The viral RNA detection was provided only in case of positive swab and no treatment was interrupted.

59 women underwent ovarian stimulation with gonadotropins followed by oocyte retrieval. Women's basal characteristics were: Age (m±sd) 31.0 ± 7.0 years, FSH (m±sd) 14 ±9IU/l, AMH (m±sd) 2.4 ± 1.3 ng±ml, AFC (m±sd) 9 ±5.

90 men underwent spermatozoa rapid cryopreservation. Men's basal characteristics were: Age (m±sd) 34±7 years; Total Sperm count x 106 (m±sd) 52.3±49.6, Sperm x 106/ml 28.1±25.5, Total motility (m±sd) 48.0±26.7 %, Progressive motility (m±sd) 22.2±20.5 %, normal morphology (m±sd) 22.3±11.1 %.

296 oocyte were cryopreserved: 5.5±4.3 (mean±sd per patient). Vitrification with closed devices (High-Security Vitrification™-HSV) was used for oocyte cryopreservation to minimize the risk of viral contamination, including Covid-19. 403 Sperm samples were frozen with slow freezing: 5.7±2.1 (m±sd) per patient. All patients tested negative for RTPCR analysis of throat swab specimens for Sars-Cov-2.

The oncofertility activity must be maintained even in pandemic periods by implementing adequate safety measures to protect the health of patients and healthcare professionals.

Supported by Italian Ministry of Health "Fertility Preservation in gonadotoxic treatments" project code RF-2011-02348826

THE SURVIVAL OF RAT TESTICULAR INTERSTITIAL CELLS IN HYDROXYETHYL STARCH AND DEXTRAN BASED SERUM-FREE MEDIA

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Introduction

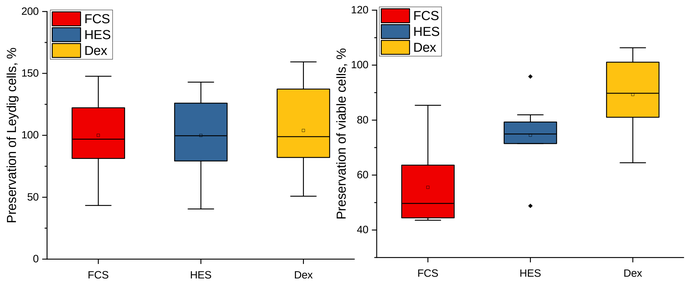
The material of cryopreserved testicular cells can be used for study of testicular defects, preservation of endangered animals, or in reproductive technologies.

Cryopreservation media for the interstitial cells (ICs), in addition to Me_2SO, may contain blood serum or polymers that improve the outcome of cryopreservation. However, the use of serum may impose some problems

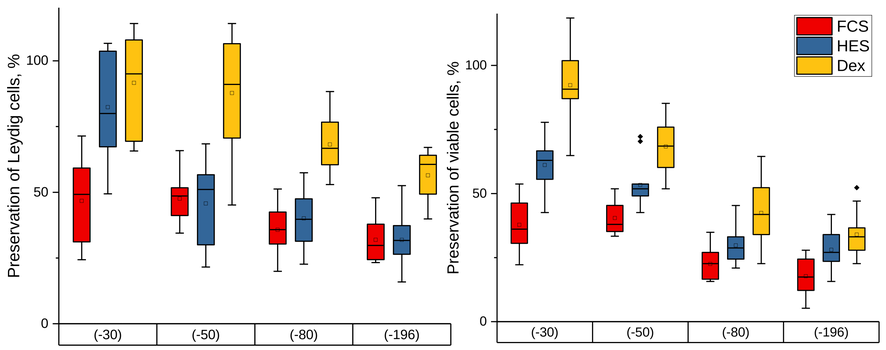
The objective is to investigate cryoprotective properties of serum-free media containing hydroxyethyl (HES) or dextran (Dex) for preservation of ICs.

Results:

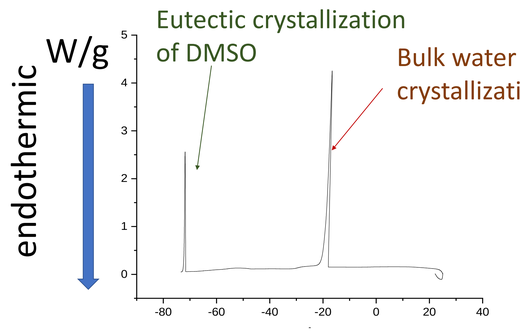
Incubation at +4°C



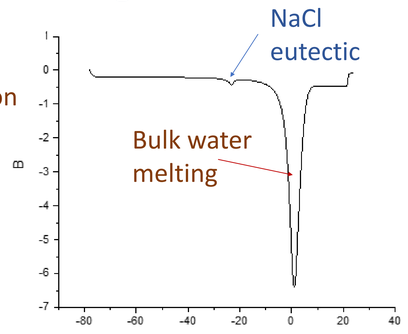
Preservation of cells in different temperature intervals



Cooling of FCS containing medium:



Heating of medium with cells:



- NaCl and DMSO eutectics were not seen when media were supplemented with Dex were used

Materials and Methods

- The viability of ICs was measured using trypan blue dye; Leydig cell count was assessed by histochemical staining on 3β-hydroxysteroid dehydrogenase; The metabolic activity of ICs was measured by MTT-test.
- ICs were incubated at +4°C for toxicity assessment;
- The cells were cooled at a rate of 1°C/ min to the final temperature of -30, -50, -80°C followed by warming or to -80°C followed by their immersion into liquid nitrogen;
- Ham's F12 media containing 100 mg/ml HES starch (Mm. 200 kDa) or Dex (Mm. 40 kDa) in combination with 0.7 M Me_2SO were used for cryopreservation; The medium supplemented with 10% FCS and 1.4 M Me_2SO shown in some researches was used as a control.

Conclusions

- Cryoprotective properties of FCS are not directly linked with the polymeric properties of its proteins. Thus, the mode of cryoprotective action of FCS is distinct from that of developed by HES and Dex based solution.
- HES and Dex based media have lower toxic effect on interstitial cells than the media with FCS.
- The intervals that resulted in the marked cell loss in the FCS based media are (+4 to -30C) and (-50 to -80C) where the water, salt and Me_2SO crystallization may take place.
- The best results were obtained with the media supplemented with 100 mg/ml Dex and 0.7 M Me_2SO

Effect of cryopreservation on morphological parameters, metabolic and antioxidant activities of seminiferous tubules fragments of testes

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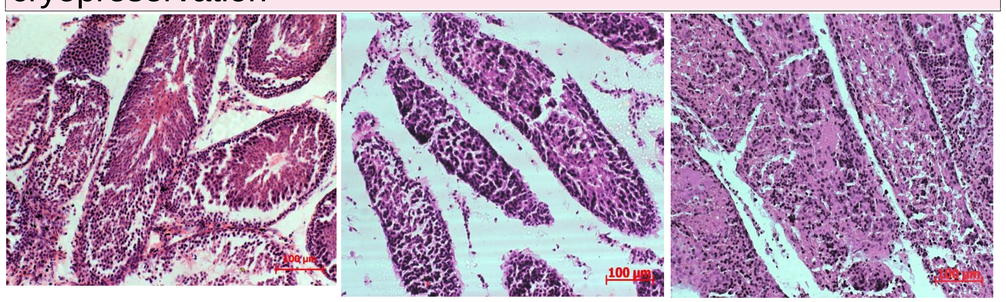
Introduction

One of the current trends in biomedical research is a use of novel biological products and cryotechniques to maintain fertility. The aim of the study was to evaluate the morphological parameters, metabolic and antioxidant activities in the fragments of seminiferous tubules of testes (FSTT) after cryopreservation using fibrin gel and slow cooling rate.

Materials and methods

The samples of immature rat FSTT weighing 75±3 mg were incubated for 20 min at 4°C in medium 1 (0.7 M glycerol+fibrin gel) or medium 2 (0.6 M Me_2SO+fibrin gel). Cryopreservation was made using uncontrolled slow cooling down to –70°C for 40 min followed by immersing into liquid nitrogen. Cryotubes were thawed in a 40°C water bath until a liquid phase appearance. Total metabolic (MTT-test), antioxidant (TAS and ROS) activities and morphological parameters were determined in the samples. The results analyzed using Kruskal-Wallis ANOVA test with multiple comparisons and presented as mean (min-max) values.

Figure. Histological structure of FSTT of immature rats after cryopreservation



Control (intact) Medium 1 Medium 2

Light microscopy; staining with hematoxylin and eosin.

Results

Table 1. Functional activity of FSTT of immature rats after cryopreservation

Sample	MTT, units/mg protein	TAS, mM/mg protein	ROS+, %
Control (intact)	1.25 (1.04-1.32)	19.3 (18.8-20.9)	1.04 (0.68-1.39)
Medium 1	0.94[a] (0.85-1.02)	12.2[a] (10.8-13.5)	3.03[a] (2.69-3.35)
Medium 2	0.72[a,b] (0.54-0.88)	10.2[a,b] (8.3-12.2)	4.24[a,b] (3.5-4.9)

Table 2. Nuclei state in spermatogenic epithelial cells of FSTT of immature rats after cryopreservation

Sample	Nuclei state, %			
	Undamaged	Karyopicnosis	Karyorrhexis	Karyolysis
Control (intact)	84(79-90)	6(4-8)	6(4-10)	4(2-8)
Medium 1	79 (71-88)[a]	10(8-14)[a]	8(5-10)	3(2-4)
Medium 2	58 (54-66)[a,b]	20(16-24)[a,b]	15(10-20) [a,b]	7(5-9) [a,b]

Note: [a] - the difference is significant relative to the control ($p<0.05$; n=5);
[b] - the difference is significant relative to the medium 1 ($p<0.05$; n=5).

The study of functional characteristics of FSTT showed that the indicators of metabolic and antioxidant activities (MTT-test, TAS) in the samples cryopreserved in medium 1 were respectively 1.3- and 1.2-fold higher versus medium 2 ($p<0.05$). A decreased content of ROS+ cells was found in cryopreserved FSTT (medium 1) by 1.4 times versus medium 2 ($p<0.05$). Histological study showed a more pronounced protective effect after using medium 1 (reduced number of pyknotic nuclei, nucleus fragmentation and karyolysis). Results showed that the application of 0.7 M glycerol prevented the development of necrosis in spermatogenic epithelium compared to 0.6 M Me_2SO. However, the studied parameters did not reach the initial values after freezing-thawing in all experimental groups.

Conclusion. The obtained results can be used to develop the effective cryopreservation methods for seminiferous tubules using fibrin gel.

ENDOCRINE FUNCTION OF CRYOPRESERVED OVARIAN TISSUE GRAFTS UNDER PROTECTION OF 3M DIMETHYLSULFOXIDE (Me_2SO)

Victoria Kiroshka, Iryna Rula, Tetayna Gurina

Introduction

Cryopreservation of ovarian tissue is becoming an increasingly common method to maintain hormonal and reproductive functions in women after chemotherapy and radiotherapy.

Materials and methods

In this work fragments of rat's ovarian tissue were cryopreserved under protection of 3M dimethylsulfoxid (Me_2SO)) with 200 mm sucrose by slow freezing with initiation of crystallization (IC) – and without initiation. Me_2SO was gradually added –removed at 22 ˚C.

Samples were frozen slowly in a freezer («Cryoson», Germany) at 2°C/min to -7°C, ice crystal formation (seeding) was induced manually with prechilled forceps. The specimens were held at this temperature for 10 min, and then cooled at 0.3°C/min to -40°C, followed by 10°C/min to -140°C, plunged immediately into liquid nitrogen. The scheme of the experiment is shown in **Fig.1.**

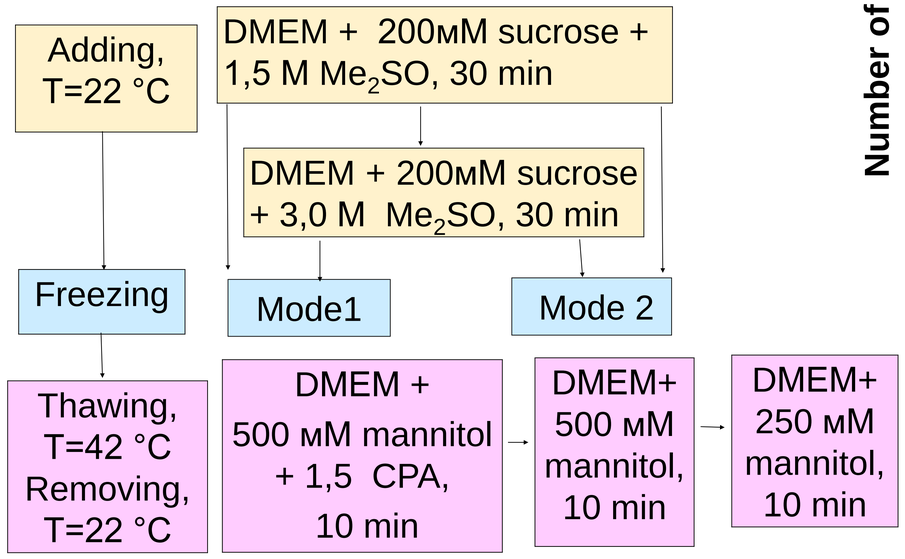


Fig.1. The scheme of the experiment

Experimental animals for estimation of cryopreserved ovarian tissue function were divided into the following groups: intact animals – C1; ovariectomized – C2; recipient animals with fresh ovarian tissue grafts – C3, recipient animals with cryopreserved ovarian tissue grafts– 4 (with IC), recipient animals with cryopreserved ovarian tissue grafts – 5 (without IC). The data were represented as M±m ($p<0,05$).

Results

It was shown, that follicular density in grafts of cryopreseved ovarian tissue (group 4) was similar to that at the control level (4.0±0.5 per mm^2, versus 4.2±0.4 (C1)). Whereas in the group 5, this index decreased to 1.1±0.2 per mm^2 **(Fig.2)**.

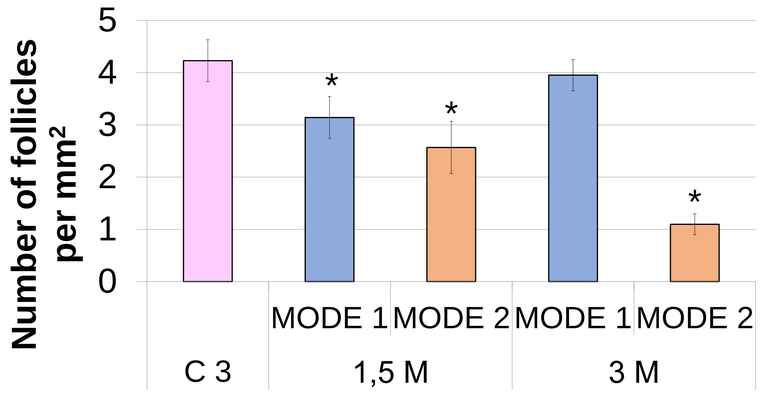


Fig.2. Follicular density
Note: * – $p<0,05$ in comparison with **C3**

Conclusions

Our results suggest that the deliberate seeding of extracellular ice improves the outcome of ovarian tissue freezing, due to reducing a cytotoxic effect of the cryoprotectant (3M Me_2SO).

Sex hormone levels in group 4 corresponded to the physiological norm and were as follows: estradiol (E2) – 25.5±3.2 pg/ml; progesterone (PR) – 16.2±2.9 ng/ml, which was not significantly different from the level of hormones in C3 group (E2 – 28.1±5.6 pg/ml; PR – 19±3.8 ng/ml). Freezing of ovarian tissue using 3M Me_2SO without IC led to damage of its structure and a decrease in E2 (15.5±4.2 pg/ml) and PR (7.6±3.6 ng/ml) levels **(Fig.3)**.

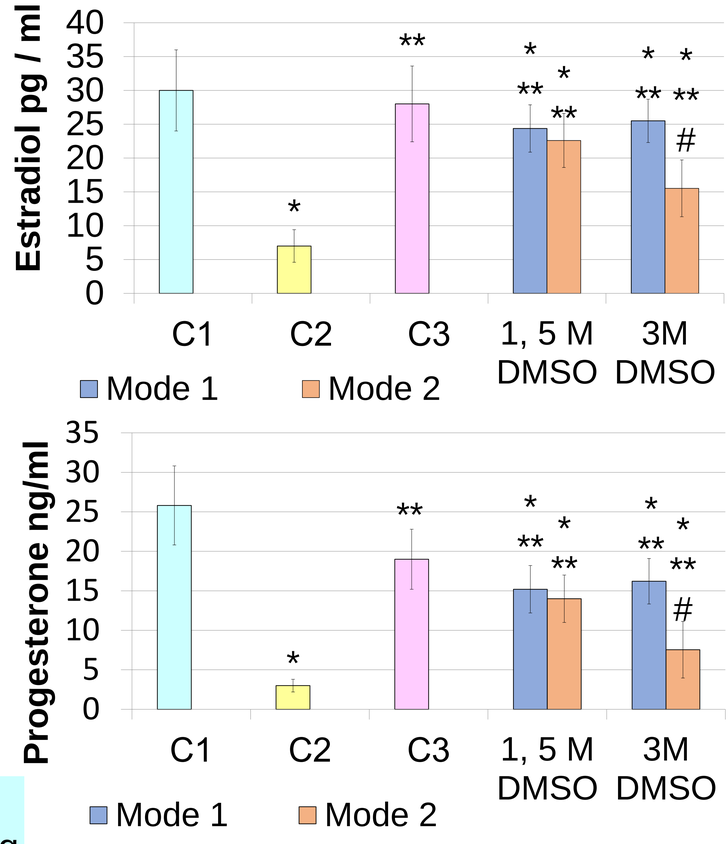


Fig. 3. Sex hormone level in recipient animal to 30th day after transplantation
Note: *– $p < 0,05$ in comparison with **C1**
** – $p<0,05$ in comparison with **C2**
# – $p<0,05$ in comparison with **C3**

DOES CRYOSTIMULATION PREVENT DESYNCHRONOSIS-INDUCED CHANGES IN ERYTHROCYTES' SPHERISITY INDEX?

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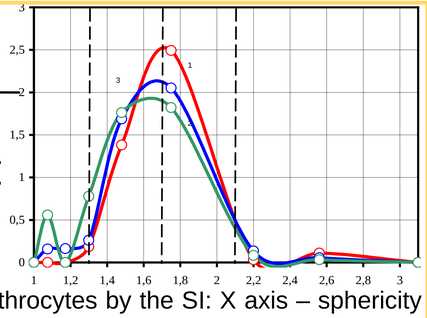
Introduction and aim.

Recently, whole-body cryostimulation (WBC) has been successfully used in clinical and preventive medicine. Circadian desynchronosis (CD) provokes the development of numerous pathologies, so it requires timely detection, treatment and prevention. Since the transformation of blood erythrocytes is altered by certain pathologies development, we **aimed** to study the effect of WBC on the erythrocyte shapes ratio in blood of rats with modelled CD.

Methods.

Experiments were performed in 6 (adult) and 18-months-old (old) outbred male white rats (n=5). One WBC (-120°C, 90 s) session (WBC+CD) was applied the day before CD initiation, which was caused by a single 12 hrs' light-time period prolongation. Erythrocyte transformation was evaluated by determining their distribution in the population by the sphericity index (SI). The data were statistically analysed by the Kruskal-Wallis test.

Predominant shapes of erythrocytes	SI interval
Flattened discocytes	2.1 – 3
Normal discocytes	1.7 – 2.1
Stomatocytes	1.3 – 1.7
Spherocytes	1 – 1.3



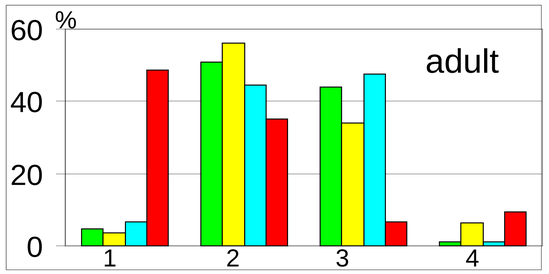
Examples of the distribution density of erythrocytes by the SI: X axis – sphericity index; Y axis – distribution density of erythrocytes (ρ)

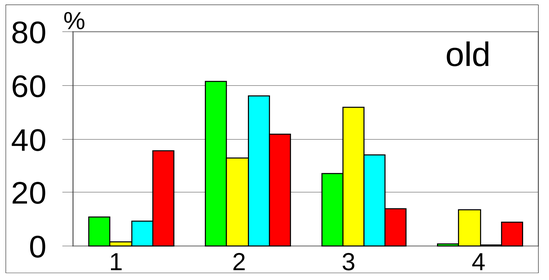
Results.

At CD, the percentage of both normal and flattened discocytes decreased (2 and 5 times, respectively) in old animals. In adult animals, the number of normal discocytes increased. In old animals the proportion of stomatocytes increased, as well as the one of spherocytes enhanced in both age groups.

WBC in adult rats decreased the discocytes percentage (due to normocytes) and altered erythrocytes proportion towards their rise (due to stomatocytes). In old animals, the discocytes percentage decreased (due to normocytes), and that of altered forms increased (due to stomatocytes).

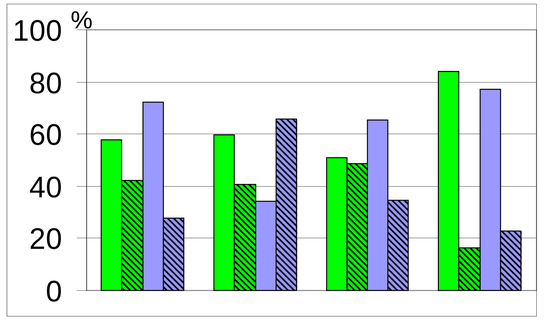
After preventive WBC when the CD was initiated, the discocytes percentage increased (due to flattened discocytes 10 and 3 times, respectively) and the percentage of altered shapes decreased (due to stomatocytes) in adult and old rats.





CONTROL CD WBC WBC+CD

1 - Flattened discocytes, 2 - Normal discocytes, 3 - Stomatocytes 4 - Spherocytes



Adult, all discocytes and altered shapes
Old, all discocytes and altered shapes

Conclusions.

Therefore, the preventive WBC, regardless of animals' age, positively affect erythron when the CD develops: the discocytes proportion in blood grows due to an increase in highly resistant flattened discocytes with the reduction of the altered shapes percentage.

CHANGES IN AUTONOMIC REGULATION OF HEART IN ANTARCTIC WINTERERS

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Introduction and Aim

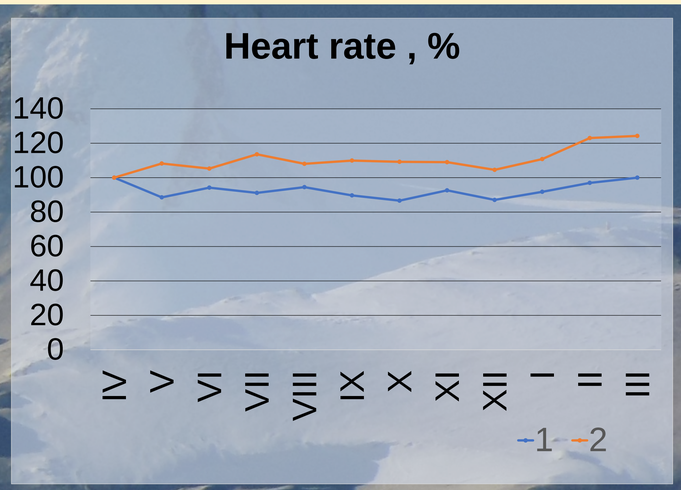
Recently, there has been an increased interest in medical and physiological research related to human performance in polar environments. The adaptation to cold is often evaluated by autonomic regulation of the organism. With this aim the heart rate variability (HRV) analysis is usually used.
In our study we aimed to investigate the individual characteristics of human HRV during the year-round wintering at the Ukrainian Antarctic station "Akademik Vernadsky".

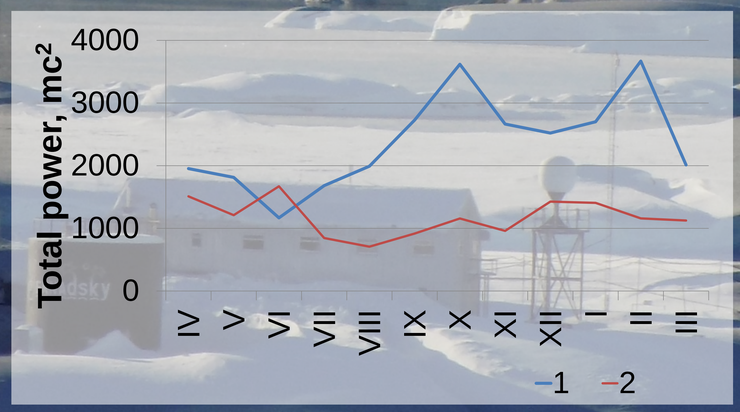
Methods

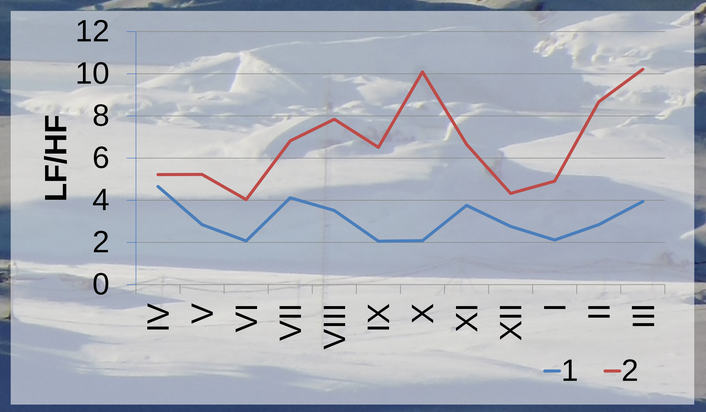
The study comprised 35 winterers of the 21th, 23th and 24th Ukrainian Antarctic expeditions. These were 33 men and 2 women, aged 22-63 (mean age of 38.4 years). All participants were informed about the goals and objectives of the study and gave their consent. In the morning the ECG and blood pressure (BP) were monthly recorded in a sitting position. The data were statistically analyzed by the Mann-Whitney U test.

Results

We found that winterers developed 2 types of autonomic response during wintering. In group 1, there was a decrease in heart rate, systolic BP, and with an increase in both total power and its basic constituents (HF and LF) we found a decrease in the LF/HF ratio. Group 2 vice versa demonstrated an increase in heart rate, no significant changes in BP, reduced total power, HF, LF, accompanied with a rise in LF/HF ratio. The distribution by groups did not depend on age, sex and previous wintering experience.







Since we previously have found similar changes after the Cold Pressor Test, we can hypothesize that the cold crucially affects the changes in autonomic regulation, but this statement requires further studies, because there are certain limitations of the method and impossibility of comprehensible distinguishing of the contributions of various extreme physical and psychophysiological factors.

1 INTRODUCTION

- *Solanum lycopersicum* L. is one of vegetables of the world economic importance. However, infections of tomato plants with large number of viruses can lead to enormous crop losses. Our objective was to determine the post-thaw survival rates of viral tomato shoot tips.

Yellow leaf curl virus

Institute of Vegetable and Melon Growing of the National Academy of Agrarian Sciences of Ukraine 1

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Natalia Bashtan

Cryopreservation of viral tomato (Solanum lycopersicum L.) shoot tips

Researchers:

Natalia Bashtan, Anna Mozgovska,
Tetiana Miroshnichenko, Tetiana Ivchenko 1

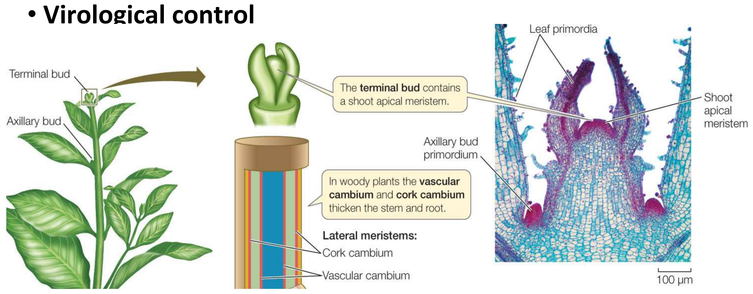
Nadiia Shevchenko, Galyna Kovalenko 2

5 Results of research

- **It was shown that the rate of regenerated plants made 78 % for the control group. The shoot tips regrowth was 35% after 88% PVS3 treated, in other groups this value was within 66 and 83%.**
- **No regrowth of cryopreserved shoot tips was observed in case of aluminum foil strips and cryovials.**
- **After cryopreservation in aluminium pans for DSC the survival rate was 74% for modified PVS1, 70% for PVS2 and 62% for PVSN.**
- **To conclude, the use of aluminium pans for DSC did not change the shoot tips survival rate compared with non-cooled explants.**
  In the perspective, we plan to assess the viral load in the cryopreserved speciments.

2 Basic methods for restricting vegetable plants infections by viruses :

- **The culture of apical meristems**
- **Chemotherapy**
- **Thermotherapy**
- **Virological control**

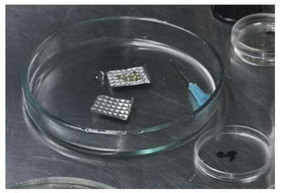


3 MATERIALS AND METHODS

The in vitro culture of tomato was obtained from infected by ToMV, TYLCV plants of local Ukrainian Irishka cultivar. The shoot tips were isolated from plantlets and transferred into a liquid MS, supplemented with 12 % sucrose and exposed at dark for 24 hour. The shoot tips were treated with loading solution for 20 min and dehydrated for 40 min at 22°C in different plant vitrification solutions (PVS): PVS1 mod, PVS2, 88% PVS3, PVSN. Dehydrated samples dived into three groups and transferred to sterile aluminum foil strips (15×30 mm), in 1.2 ml cryovials or in 20 µl hermetic aluminium pans for differential scanning calorimeter (DSC) and were directly immersed into liquid nitrogen. The specimens were warmed in MS medium, supplemented with 12% sucrose at 25°C (in case of aluminium strips and pans) or in water bath at 40°C (for criovials).

4 CRYOPROTECTING OF TOMATO MERISTEMS:

The shoot tips were isolated from plantlets and transferred into a liquid MS



Dehydrated samples transferred to sterile aluminum foil strips

Hermetic aluminium pans, 20 µl

Storage of frozen food articles: Problems and solutions

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Submission ID 24

INTRODUCTION

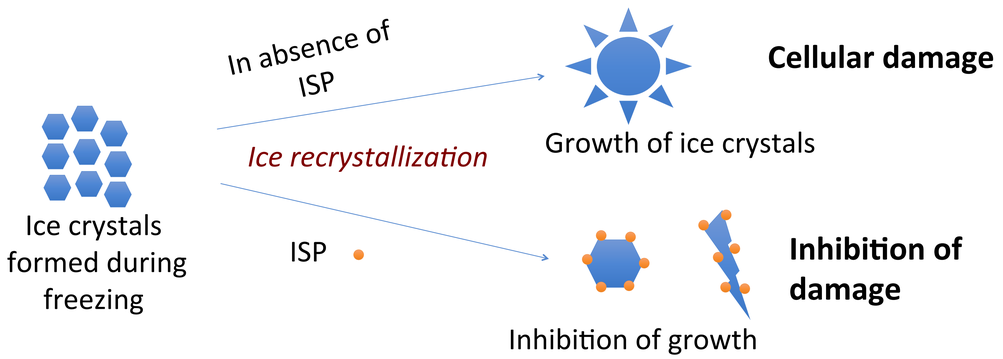
In densely populated lower and lower middle income countries, the frozen food items can prove very useful for the fulfillment of food requirements.

Frozen food

Freeze burn

→ **Damage to food textue**

Ice structuring proteins (ISPs) might provide solution due to their ice recrystallization inhibition activity

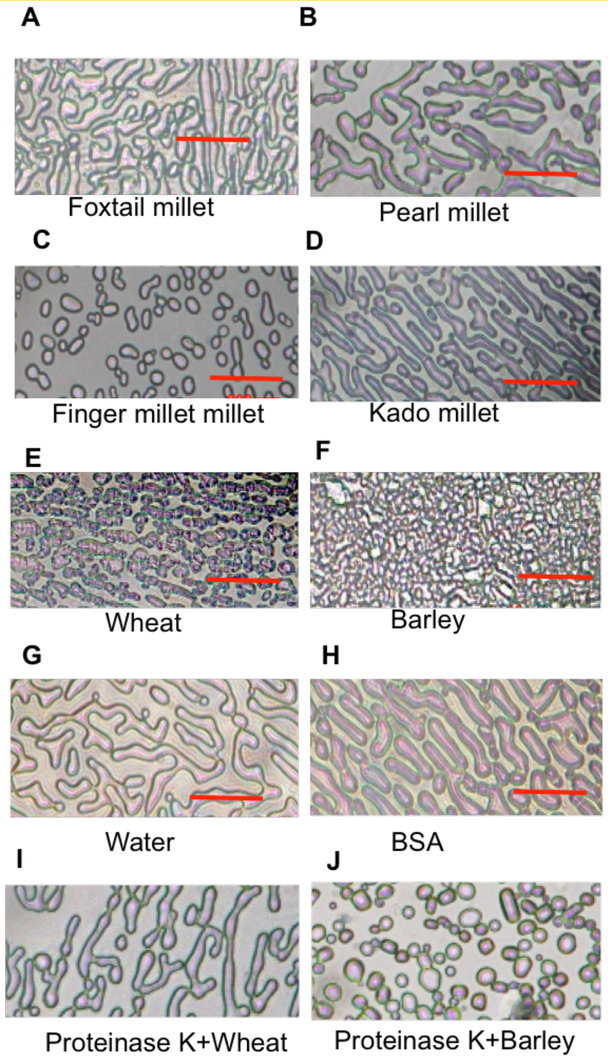


ICE RECRYSTALLISATION INHIBITION ACTIVITY (IRI)

The plants growing in cold and harsh climatic conditions of Hmalayas exhibit ice structuring properties.

The crops cultivated in Himalayas may prove to be a continuous edible source of ISPs

RESULTS

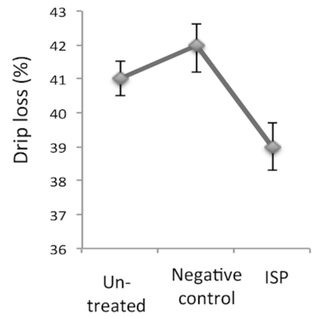


IRI activity in terms of retardation of growth of ice crystals in Himalayan

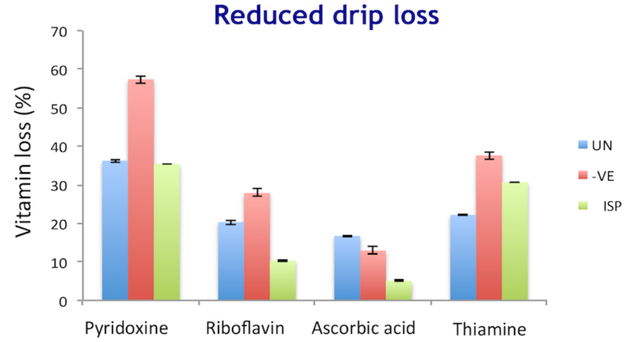
* Himalayan wheat and barley exhibited remarkable IRI activity

Pretreatment with ISP

Freeze storage (15 days)



Reduced drip loss



Reduced vitamin loss

- Pretreatment of peas with ISP before freeze-storage reduced the freeze-induced drip loss.
- The reduced drip loss resulted in loss of vitamin content ensuring the nutrition content of frozen peas.

Future direction: Use of edible sources for food industry and identification of ISPs in Himalayan grains

Acknowledgements

This study was supported by Department of Health and Research (DHR), Young Scientist Scheme under the Human Resource Development (Grant no. YSS/2019/00002/OPRCYSS), India and CSIR-IHBT), India (in-house grant number MLP0201).

Germination of *Stipa capillata L.* Before and After Low Temperature Storage

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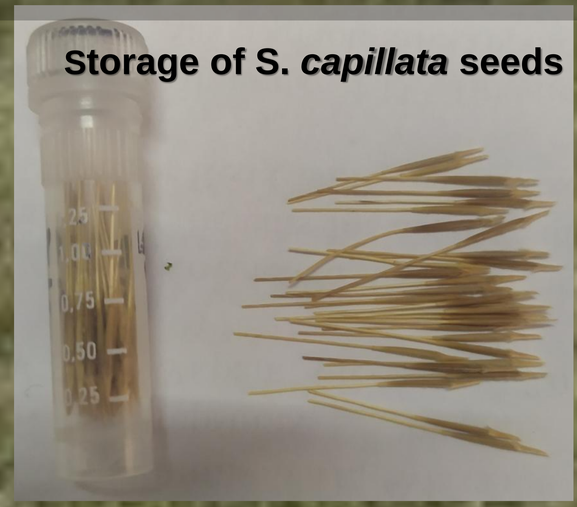
Unique herbaceous communities formed the richest chernozem soils concentrated in Ukraine for thousands of years. Now pastures and plowings occupy the most part of these lands. The natural vegetation of virgin steppe, preserved in a few protected areas, is very diverse and can be represented by various plant communities, where such herbs, as feather grasses (*Stipa* L.) predominate. Some of *Stipa* genus are listed in different Red Data Books and needed to be especially protected by cryopreservation techniques. Seeds of feather grasses are resistant to dehydration and therefore must remain viable after cryogenic storage.

The seeds of *Stipa capillata* L. were collected on August 29, 2019 in Buzk's Gard National Nature Park, coordinates – 47.840198, 31.120537.
The collected seeds were stored at a room temperature and a low relative humidity (30%) or at –20 and –196°C for 18 month prior to their use in germination tests. Seed coats nicked with a scalpel and the seeds were sown in Petri dishes. Experiments were carried out at 20°C in a darkness.
Our experiments showed that *Stipa capillata* seeds have a low germination both in control (10.46±7.45%) and after low temperature storage at -20°C (12±8.18%). The germination of *S.capillata* seeds after liquid nitrogen exposure significantly increased up to 30.28±10.73%.



Dithioerythritol improves frozen-thawed sperm quality in rooster

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Background

It was previously reported that dithioerythritol protected lymphocytes from toxin-induced apoptosis and oxidative harm. Using dithioerythritol in bull and human sperm during liquid storage or freezing appeared to increase motility. There has been no prior research into the effects of dithioerythritol on the quality of rooster sperm after they have been thawed. Our theory is that there is a dithioerythritol concentration that is ideal for improving the production of rooster sperm after thawing.

Materials and Methods

Sperm collection was performed once a week during two months from roosters. Semen was collected and put in a water bath (37 °C) for initial laboratory evaluation. The sperm samples were diluted with Lake Extender, then aspirated into 0.25 ml French straws to obtain a final concentration of 400 × 106 sperm/ml, sealed with polyvinyl alcohol powder, and equilibrated at 4 °C for 3 hours. The straws were suspended in liquid nitrogen gas, 4 cm above the liquid nitrogen, for 7 minutes in a cryobox containing liquid nitrogen after equilibration. The straws were then stored by submerging them in liquid nitrogen.

Results

In contrast to the control sample, the extender supplemented with 0.4 mM dithioerythritol resulted in higher progressive motility ($P < 0.05$). In comparison to the control, the 0.4 mM dithioerythritol resulted in a higher percentage of mitochondrial activity and membrane integrity. The extender supplemented with 0.4 mM dithioerythritol resulted in a lower percentage of apoptotic sperm, comparing to the control group.

Conclusion

The addition of 0.4 mM dithioerythritol to Lake Extender greatly increased the condition of rooster sperm after freeze thawing, according to our findings.

Keywords

dithioerythritol: sperm: post-thawed; rooster

Enhancement of post-thawed sperm quality in rooster by butylated hydroxyltoluene

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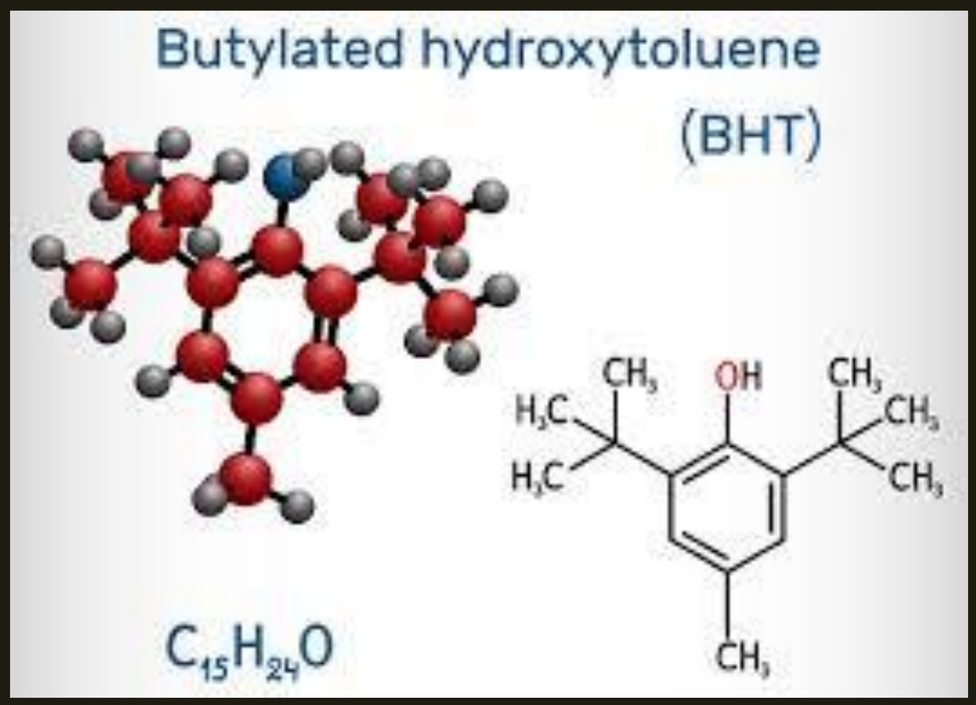
Background

The addition of antioxidants to the cooling and freezing media will effectively reduce sperm damage caused by reactive oxygen species (ROS) during the cryopreservation phase. Butylated hydroxyltoluene (BHT) is a synthetic vitamin E analog that inhibits autooxidation by converting peroxy radicals to hydroxyperoxides. Despite the interest in the use of BHT in spermatozoa cryopreservation, no previous studies have focused on the effects of BHT supplemented extenders on rooster semen cryopreservation. As a result, the aim of this study was to see how adding BHT affects the post-thaw properties of rooster spermatozoa.

Methods & Materials

After a 2-week habituation period, semen samples were obtained weekly from the roosters using the dorsoabdominal massage process. To obtain a conclusive concentration of 400 ×106 spermatozoa/mL, sperm samples from treated roosters were diluted with Lake extender and then aspirated into 0.25 mL French straws. For around 7 minutes, the straws were mounted 4 cm above the liquid nitrogen. They were then stored at –196 °C using liquid nitrogen.



Results

In comparison to the other groups, BHT at a concentration of 1 mM resulted in the maximum of total and progressive motility ($P < 0.05$). Furthermore, as compared to the other groups, 1 mM of BHT provided the highest percentage of membrane integrity. Comparing to the other groups, BHT at a concentration of 1 mM decreased malondialdehyde formation ($P < 0.05$).

Conclusions

The addition of 1 mM BHT to Lake extender greatly increased the condition of rooster sperm after freeze thawing, according to our findings.

Key words

BHT; sperm; post-thawed; rooster

IMPACT OF RAFFINOSE, GLUCOSE OR TREHALOSE ALONG WITH DIFFERENT CRYOPROTECTIVE AGENTS IN TRIS BASED EXTENDER ON POST THAW QUALITY OF RAM SPERMATOZOA

Muhammad Saleem Akhtar, Muhammad Sajid, Ejaz Ahmad, Muhammad Salman Waqas, Huma Jamil, Muhammad Saqib

The objective of this study was to know the effect of raffinose, glucose or trehalose combined with glycerol or dimethyl sulfoxide (DMSO) on the post thaw quality of ram spermatozoa extended with Tris based semen extender

Methodology

he semen of four healthy, mature, 2 to 3 years old Lohi rams was collected through electro-ejaculation twice a week for four successive weeks. After initial evaluation, the samples were pooled and were divided in six aliquots, i.e extended (@1:10) with one of the extenders Glycerol Raffinose (GR), Glycerol Glucose (GG), Glycerol Trehalose (GT), DMSO Raffinose (DR), DMSO Glucose (DG), DMSO Trehalose (DT).

Results

The results showed the progressive motility, motion kinetics and plasma membrane integrity of sperm cryopreserved in extenders having glycerol were higher ($P<0.05$) than the sperm cryopreserved in extender having DMSO. The acrosome integrity of sperm cryopreserved in extender having either glycerol or DMSO was similar ($P>0.05$). When the combination of CPA (glycerol or DMSO) with one of the sugar raffinose, glucose or trehalose was compared, it was observed that the post thaw progressive motility, motion kinetics and viability of sperm cryopreserved in extender having raffinose, glucose or trehalose in combination with glycerol (GR, GG or GT) was higher ($P<0.05$) compared with the sperm cryopreserved in extender having raffinose, glucose or trehalose in combination with DMSO (DR, DG or DT). No difference ($P>0.05$) was observed between sugar type either in combination with glycerol or DMSO. The acrosome integrity of sperm cryopreserved in DR was lower ($P<0.05$) than GR, GG, GT or DT. It was also observed that interaction between CPA type and sugars was non-significant ($P>0.05$), which indicated that both CPAs and sugars has individual effect on the post thaw semen quality in Lohi ram. In conclusion, the raffinose, glucose and trehalose have best combination with glycerol than DMSO to preserve the quality of Lohi ram sperm during freezing and thawing.

Increase of DNA fragmentation evaluated through the alkaline Comet is concomitant with a decrease in the quality of frozen-thawed bovine sperm

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Introduction

Even if sperm **cryopreservation** is the most efficient procedure for long-term storage, it is widely known to detrimentally affect sperm quality and functionality parameters. Moreover, the impact of **sperm DNA fragmentation (SDF)** on reproductive outcomes has been largely studied.

The aim of this study was to evaluate the potential correlation of Single- and Double-Strand DNA fragmentation and the intensity of DNA damage assessed through the alkaline Comet assay with sperm quality parameters in cryopreserved bull sperm.

Materials and methods

Frozen-thawed bull ejaculates (n=29)

Alkaline Comet assay
- Sperm DNA fragmentation

CASA system
- Total and progressive motility

Flow cytometry
- Sperm viability (SYBR/PI)
- Plasma membrane lipid disorder (M540/YO-PRO-1)
- Intracellular levels of $O_2^{\cdot-}$ (HE/YO-PRO-1)
- Intracellular levels of H_2O_2 (H_2DCFDA/PI)
- DNA condensation (CMA3/YO-PRO-1)

Shapiro-Wilk (normality), Levene (homoscedasticity) and Spearman (correlation) tests were run ($P \leq 0.05$).

Figure 1. Experimental design.

This work was supported by the Ministry of Science and Innovation, Spain (RYC-2014-15581, AGL2017–88329-R, PRE2018-083488), and the Regional Government of Catalonia, Spain (2017-SGR-1229).

Results

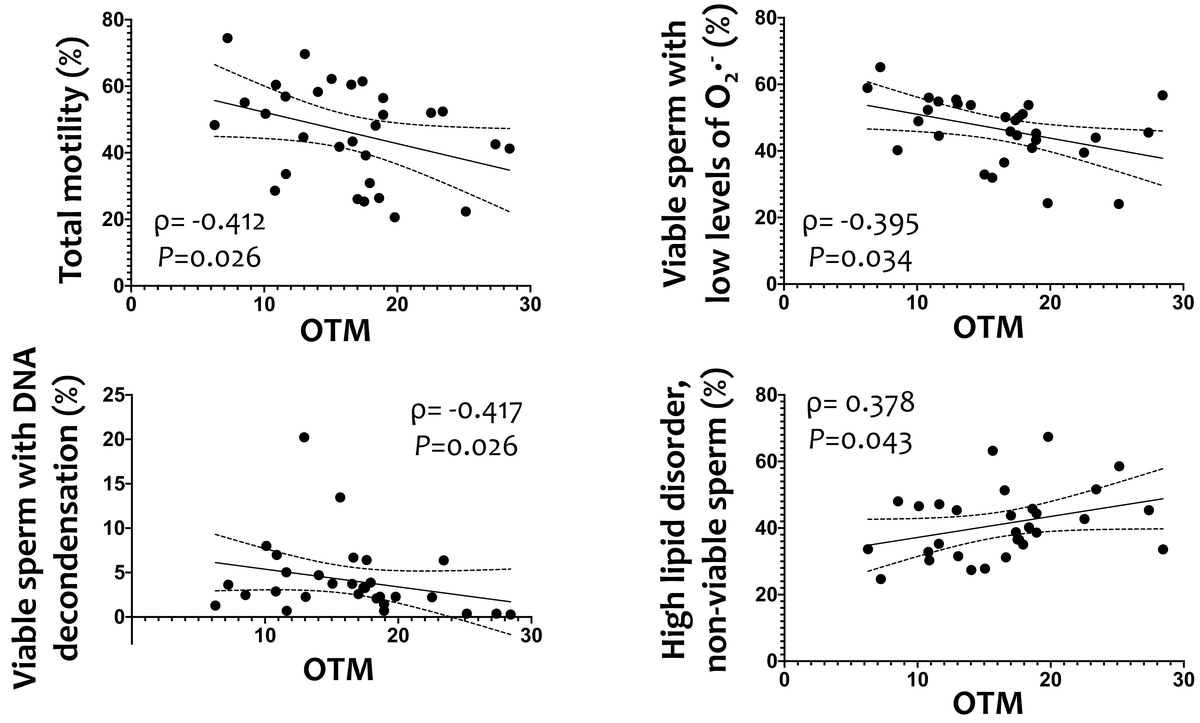

Figure 2. Correlation of sperm quality parameters with sperm DNA fragmentation in terms of olive tail moment (OTM).

Discussion

Our results evidence that the decrease in sperm quality following freeze-thawing is concomitant with an increase in SDF evaluated through the alkaline Comet assay. Additional studies evaluating the correlation of SDF with sperm quality parameters after different post-thaw incubation times and with fertility outcomes could contribute to elucidate the relevance of DNA integrity in cryopreserved samples.

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Effects of melatonin on the rooster sperm quality during cryopreservation

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[2]Department of Animal Science, College of Agriculture, University of Tabriz, Tabriz, Iran;

Background

Several exogenous antioxidants have been added to the freezing medium in a number of trials, and the obtained results were positive. Beneficial impacts on the production and viability of sperm after cooling at 5 °C for 6–12 hours was found in a research on the roosters supplementing with oral melatonin. The current study was carried out to assess the quality of sperm after cryopreservation of rooster sperm in Lake extender supplemented with melatonin.

Materials and methods

Abdominal massage was used for semen collection eight times a month. The collected semen samples were transferred to the laboratory in a water bath (37 °C) during 5 minutes for quality assessment. Diluted semen was aspirated into 0.25 ml French straws to achieve a final concentration of 100 × 106 sperm/ straw. After that, the straws were sealed with polyvinyl alcohol powder and equilibrated for 3 hours at 4 °C. Then, they were plunged into liquid nitrogen for storage.

Results

In comparison to the control sample, supplemented extenders with 1 µM melatonin showed higher total motility, progressive motility, viability, membrane functionality, mitochondrial activity, and lower lipid peroxidation ($P < 0.05$).

Conclusion

Supplementing Lake extender with 1 µM melatonin increases the cryo-survival of rooster sperm and may be used to further achievements in reproductive targets.

EFFECT OF SUPPLEMENTATION OF TREHALOSE IN EY-FREE POLYVINYL ALCOHOL EXTENDER ON DOG SPERM CRYOPRESERVATION

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Introduction

Loss of water during cryopreservation lead to a detrimental effects on sperm membranes.
Trehalose is a disaccharide commonly found in high concentrations in some organisms and help them to survive the conditions of complete dehydration. Therefore, in this study, we investigated the cryoprotective effect of trehalose supplementation in egg yolk-free extender on dog sperm cryopreservation.

Materials and methods

Spermatozoa was collected and diluted with extender 1 supplemented with 0, 10, 15, 20 or 30 mM trehalose (2×10^8 sperm/mL). Sperm was incubated at 4°C for 1 h. After that, extender 2 containing extender 1 and 1 M glycerol was added to the diluted sperm (1×10^8 sperm/mL), loaded in 0.5 mL straws and incubated for another 30 min. Straws were then cooled further over LN_2 vapor (7 cm over the surface of LN_2) for 20 min and plunged directly into LN2. After thawing (37°C for 25 s), sperm progressive motility (CASA), viability (SYBR-14/PI), apoptosis (Annexin v/PI) and reactive oxygen species (H2DCFDA/PI) were evaluated. Thereafter, the optimum concentrations of trehalose were selected and the gene expression of SMCP, BAX, BCL2, NOX5, SMOX, OGG1 and ROMO1 were evaluated after freezing-thawing.

Results

Table 1: Effect of trehalose supplementation in EY-free extender on apoptotic index and ROS

Apoptotic variables (%)	Trehalose (mM)				
	0	10	15	20	30
Annexin V-/PI+(dead and late necrotic)	1.0±0.1	1.1±2.1	0.9±2.8	0.9±0.7	1.1±1.5
Annexin V+/PI+(dead and PS translocated)	30.4±0.1	34.2±2.4	34.1±3.3	34.1±0.9	35.9±1.9
Annexin V-/PI-(live and non-PS translocated)	59.7±0.1	56.2±1.8	56.7±2.6	56.1±0.8	54.0±1.5
Annexin V+/PI- (live and PS translocated)	9.0±0.1	8.5±2.0	8.3±2.7	8.7±0.6	9.0±1.4
PS translocation index	13.06±0.1	13.14±1.8	12.74±2.7	13.5±0.8	14.3±1.7
ROS (%)	43.1±4.7	38.4±1.6	39.9±4.3	41.8±5.9	42.6±3.9

Data was expressed as mean value±SE

Table 2: Effect of trehalose supplementation in EY-free extender on genes expression

Trehalose (mM)	Genes						
	SMCP	BCL2	BAX	NOX5	SMOX	OGG1	ROMO1
0	0.21±0.2	0.74±0.4	0.37±0.2	0.23±0.1	0.44±0.3	0.36±0.3	0.57±0.3
20	0.39±0.1	0.90±0.4	0.85±0.4	0.19±0.1	0.20±0.1	0.07±0.0	0.63±0.2
30	0.23±0.1	0.76±0.2	0.65±0.2	0.11±0.1	0.19±0.1	0.05±0.0	0.36±0.1

Data was expressed as mean values±SE.

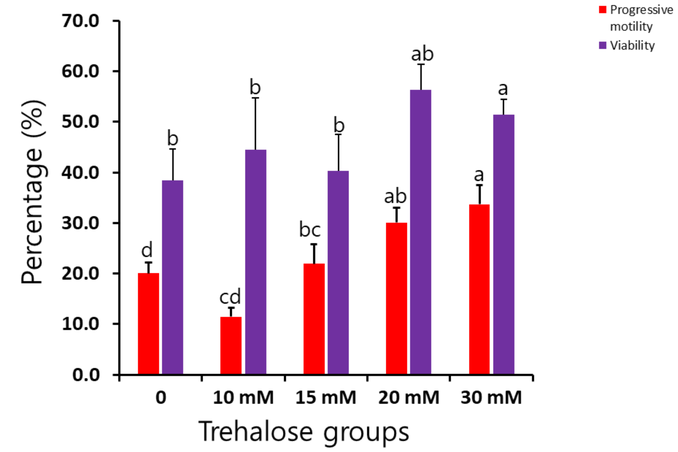

Figure 1: Effect of trehalose supplementation in EY-free extender on progressive sperm motility and viability. Data was expressed as mean value±SE. [a, b, c, d] different superscripts indicate the significant difference between groups (P<0.05).

Conclusion

We conclude that the addition of trehalose in EY-free PVA extender could successfully improve sperm motility, viability without significant effect on expression of genes related to motility, ROS and apoptosis.

CRYO 2021

FREEZING OF BOVINE SEMEN IN A EXTENDER WITH SODIUM CASEINATE

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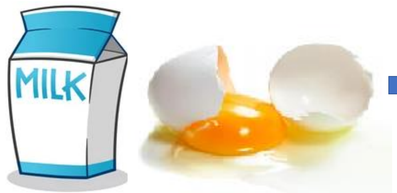
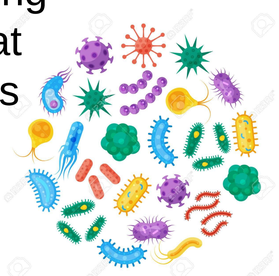
[2] Faculty of Agricultural Sciences. Universidad Nacional de Colombia sede Medellín. Antioquia. Colombia. GIBA research group

Introduction



Conventionally used for cryopreservation of bovine semen.

However. alternatives are being sought to reduce the risks that these could generate. such as microbiological alteration of semen and transmission of diseases



Sodium caseinate (SC)

Obtained by precipitation of caseins from milk. The cryoprotective component of milk is most likely casein micelles. which are their main proteins

Objective

The aim of this study was to evaluate SC in the extending and freezing of bovine semen.

Material and methods

Five bulls

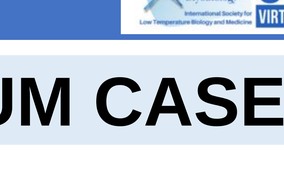
15 ejaculates

Extender: tris-glycerol-citric acid + 8% v/v of low-density lipoprotein (LDL)

Treatments

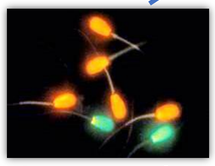
SC 3% w/v (SC3)
SC 4% w/v (SC4)
SC 5% w/v (SC5)
Control

Freezing (30 x 10^6 cell/mL)

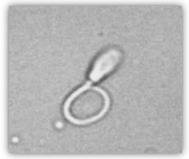


Thawed semen

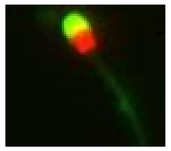
Motility and kinetics



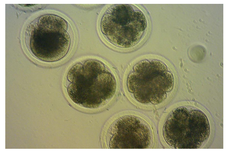
Structural integrity of membrane



Functional integrity of membrane



Acrosome integrity



In vitro fertilizing capacity (IVF)

Statistical analysis: linear models and comparison of means by the Tukey test

Results

	TM	PM	VCL	VAP	VSL	ALH	BCF	FIM	SIM	AI
Control	62.7^{b}	28.9^{ab}	45.3^{a}	27.4^{ab}	19.7^{ab}	2.0^{a}	4.6^{b}	47.3^{a}	39.6^{a}	98.2^{a}
CS3	68.9^{a}	33.0^{a}	44.7^{a}	29.2^{a}	22.3^{a}	1.9^{ab}	5.4^{a}	49.6^{a}	38.9^{a}	98.3^{a}
CS4	48.2^{c}	20.0^{c}	38.5^{b}	23.3^{b}	17.4^{b}	1.8^{b}	4.5^{b}	37.1^{b}	30.9^{b}	97.3^{a}
CS5	58.5^{b}	25.5^{b}	38.7^{b}	23.8^{b}	17.7^{b}	1.8^{b}	4.8^{ab}	45.0^{a}	38.9^{a}	98.0^{a}

TM: Total motility(%) PM: Progressive motility(%) VCL: Curvilinear speed(µm/s) VAP: Average pathway velocity(µm/s)VSL: Straightline velocity(µm/s) ALH: Amplitude of lateral head displacement(µm/s) BCF: Beat cross frequency(Hz) FIM: Functional integrity of membrane(%) SIM: Structural integrity of membrane(%) AI: Acrosomal integrity (%) Results are presented as mean. Different letters within columns indicate statistically significant difference ($p < 0.05$).

No differences were found for IVF ($p > 0.05$)

Conclusion

Supplementation of freezing extender with 3% sodium caseinate can improve the post-thaw quality of cryopreserved bovine semen.

Acknowledgment