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Research properties of the environment on basis of vegetable components from extract soy at the cryopreservation of sperm of bulls

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ABSTRACT

By cryobiological, immunological and biophysical methods it is established that in the mechanism of temperature shock of cell the leading role is assigned to osmosis, and instant movement of water through it at the expense of an equilibration of osmotic pressure that is the reason of destruction of a cell membrane deprives a cytoplasmic membrane of properties of semipermeability. It was studied the fortification mechanism sperm when cooling and it is established that the fortificant accumulates on surfaces of a cell and creates a hydrophobic phase that slows down the osmotic action connected with an balancing of concentration gradients in ''cell-wednesday'' system. It is established, also, that the protective layer is created on a cytoplasmatic membrane on an extent of 2-5 minutes from the moment of its contact with an anti-shock component – fortificanty and then strongly is kept on it even at reusable washing of cells by isotonic environments. These theoretical research were taken as a basis of a technique of determination of anti-shock properties of cryoresistance various the bioactive substances and biochemical connections by search, development and improvement of environments for a cryopreservation of sperm of different types of animals. When freezing sperm in nitrogen, an obligatory component of the cryoprotective environment is the native yolk of

eggs containing many phospholipids and lipoproteins which at interaction with plasma membranes sperm modify them by increase of resistant and stability. Being adsorbed by lipophilic and hydrophilic sites of plasma membranes lipid complexes almost by 2-3 times thickencell membrane that gives stability to sperm to extreme temperature, osmotic, immunological, physical and chemical and mechanical to damages.

1. Introduction

In this regard yolk environments became a basis of production technologies of conservation of sperm of animals. However, a yolk on the negative to indicators concerning sperm (a non-standard, thermolability, a carriage of bacteria, immunospecificity and toxicity) isn't an ideal component of cryoprotective environments that results in need of search of its effective substitutes, first of all from phytogenesis sources. Earlier (Milovanov and Monograph, 1962) from beans of soy phospholipid – lipozitol was emitted and is proved that it protects sperm from temperature shock at zero temperatures, flush with lecithin of a chicken yolk. However, according to researches (Semyonova) use of vegetable phospholipid in the form of a spirit extract at a cryopreservation of sperm doesn't create cryoprotective effect, comparable with application of a native yolk in this connection these researches didn't gain further development. Further by (Milovanov and Monograph, 1962) same authors it was experimentally proved that more effective for protection sperm from temperature shock are not free phospholipids of a yolk, but their lipoproteins complexes with the contents to 50% of proteins. Exploring this question (Ostashko) for the first time was it is frozen sperm of a bull in the environment of the soy made on the basis of a lipoproteins extract (RAF-1) of seeds that opened prospect for creation cheap, thermostable, environmently friendly and effective environments for sperm of animals. Our further researches were directed on studying of anti-shock properties of a vegetable fortificant (RAF-2) in relation to sperm of manufacturing bulls. The purpose of researches was increase of sanitary and hygienic level of artificial insemination of females and protection of a uterine livestock against spread of the diseases which are transferred a yolk of eggs through frozen or cooled to 0 0C sperm of producers by replacement in structure cryoprotective environments of a yolk a component of the phytogenesis allocated from soy seeds.

2. Materials and methods

Object of researches - native, frozen and deconservation sperm of bulls, skilled and control cryoprotective environments, yolk of eggs, soy hydrolyzate and other components of environments, equipment for freezing of sperm and artificial insemination. For researches used sperm, the standard laktose-yolk-glycerol environment No 1 and don't yolk laktose-citrate-glycerol environment No 2 which are intended for a sperm cryopreservation on the Republican Center of livestock breeding JSC "ASIL TYLIK" technology. As raw materials for receiving an anti-shock component used soy seeds. At production of experimental environments seeds of soy dried up to 1% humidity then milled them to a condition of flour. Flour was dissolved distiled water in the ratio 1:3 extractive weight was mixed on an extent of 1 hour at the room temperature on a magnetic mixer, and then warmed up on a water bath at a temperature of 65 of 0C. Within 30 minutes. After that extractive mix was centrifuged at 7 thousand turns on an extent of 20 minutes. The deposit was deleted, and supernatant liquid was used in experiences.

Osmotic pressure of extract was measured by a cryoscopic method, concentration of hydrogen ions – a cryoscopic method in accordance. Compensation of osmotic pressure was carried out additional entering of sucrose into the received extracts then used them in quality bases for cryoprotective environments. The general control was the laktose-yolk-glycerol environment. Diluted with the received samples of environments the studied sperm tests in the ratio 1:1, stood them at the room temperature on an extent of 5 minutes, after that diluted sperm with the don't yolk laktose-citrate-glycerol environment No 2 in the ratio 1:10 (Pavlenko, 1981). The sperm processed thus was investigated on resistance to cold shock (Pavlenko and Pavlenko) and ability to freezing in the

liquid nitrogen technology. Thus studied influence of freezing on quality indicators sperm after thawing, survival of sperm at to temperature 38 ^{0}C.

3. Results

It is established that at extraction of an anti-shock component bean in all used samples concentration of hydrogen ions made 6.2-6.4 units of soy and corresponded to the level of this indicator of the yolk environment which was control. At a cryoscopy of the extracts received by the described technique it is established that osmotic pressure in soy extract made 8.2 units and was at the level of the control environment that is explained by the high contents in these beans of osmotic active agents. At the first stage of researches the resistance coefficient is defined to temperature shock by the standard technique.

Definition of an indicator of cryoresistance to temperature shock of sperm the diluted environment on the basis of a vegetable fortificant (RAF-2).

Environments for sperm dilution	**Resistance indicator to temperature shock (R)**
Vegetable fartificant	0.83
Control yolk	0.74
Control negative	0.24

Researches of anti-shock properties of the specified fortificant established his ability to protect sperm from temperature shock. In conditions instant difference of temperatures in the range from 28 ^{0}C to 0 ^{0}C. Thus the cryoresistance coefficient in the skilled environment made R=0.83; in control – 0.74 and 0.24 – in negative control. These data testify to high efficiency of a vegetable fortificant as a protector to the temperature to shock. Similar dependence of efficiency of use vegetable fortificant on the basis of extract of soy it is marked out also and at deep freezing of sperm of rams in liquid nitrogen. After freezing thawing in control there were 42% full sperm, in soy extract – 41%. The survival at a temperature of 38 of ^{0}C made: 6.0 hours, and the indicator of absolute survival made 17.0 units.

Biological indicators of quality of sperm after freezing thawing with application of a vegetable fortificant.

Indicators of quality of sperm (a)	**Lactose-yolk-glacirol environment**	**Vegetable fartificant**
Mobility of sperm after dilution (a)	7.3 ±0.02	7.3 ±0.01
Mobility of sperm after freezing-thawing	4.5±0.002	4.7±0.001
Survival in hours (t)	7.7±0.4	9.3±0.05
Indicator absolute survival (Sa)	21.2 ± 1.2	23.8±1.1

The analysis of experimental data testifies that the studied the vegetable fortificant on the basis of extract of soy is capable to protect sperm bulls from temperature shock at a hypothermia, both in zones of above zero temperatures, and in zones of subzero temperatures. According to the received results high protective action from cryodamages sperm a vegetable fortificant from soy extract which can be equivalent substitute of a native yolk in cryoprotective environments is established. The received results testify to high efficiency of use of anti-shock components of a phytogenesis at a cryopreservation of sperm of manufacturing bulls.

4. Conclusions

1. It is established that the lipoprotein extract from soy shows properties of protection sperm from temperature shock in the conditions of instant temperature drop with 28 ^{0}C to 0 ^{0}C flush with a native yolk.
2. Direct dependence of osmotic pressure in extracts from temperature of an exposition and conditions of extraction is established. Optimum parameters of extraction are provided at continuous mixing of extractions mix on an extent of 1 hour, its exposition at a temperature of 65 of ^{0}C with the subsequent division of mix into fractions. The soy extract compensated on osmotic pressure by sucrose in presence of a cryoprotector of glycerin (5%) provide high survival sperm when freezing in liquid nitrogen. Thus survival sperms I made 20.0

units and authentically I didn't differ from use for freezings of the standard cryoprotective environment with the contents in it 30% of a yolk.

3. Use of a vegetable fortifikant of plasma membrane instead of a native yolk gives the chance to avoid pollution of sperm and sexual ways of a female causative agents of the infectious diseases which are transferred with yolk to apply easy and reliable ways of sterilization, to avoid use of expensive dietary yolk, and also to solve a problem of the centralized production of cryoprotective environments and delivery to their breeding enterprises.

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