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PROTECTIVE ACTION OF ENVIRONMENTS FOR DILUTION SPERMS OF THE BULLS MADE WITH APPLICATION ALTERNATIVE COMPONENTS

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ABSTRACT: *Reproduction of animals in dairy cattle breeding is based on the wide application of artificial insemination of cattle and son cows the deep-frozen sperm. At deep freezing of sperm, an obligatory component of the cryoprotective environment is the native yolk of egg because of abundance in it of phospholipids and lipopoprotens which, interacting with plasma membranes sperm, strengthen their durability and stability. Being adsorbed by lipophilic and hydrophilic sites of plasma membranes, lipoid complexes almost thicken a cellular membrane that increases stability three times sperm to damaging temperature, osmotic, physical and chemical and to mechanical factors.*

KEY WORDS: Sperm, soy seed, cryoprotective environment, yolk, phospholipids, lipoproteins

INTRODUCTION

Yolk thinners became a basis production technologies of conservation of sperm of animals. However, owing to a number of essential shortcomings, the yolk isn't an ideal component artificial environments. The basic from them is its thermolability that deprives it is reliable to opportunity to sterilize yolk containing environments the public in the ways. Thus it is proved that the yolk in many cases is the carrier of pathogenic and opportunistic microflora [1, 2]. Penetration through a shell of such activators as pullorosis, typhus, plague, aspergillomycosis is established, mycoplasmosis, salmonellosis and number of viral infections [2, 3]. Thus, possibility of a kontamination isn't excluded by microflora of thinners of sperm at production of their ex tempore in the conditions of the breeding enterprise [9]. Application of a yolk as a component of cryotyre-tread environments can cause a microbic blend word of sperm and distribution when carrying out artificial insemination.

Considering the above, there is a need of alternative replacement and an exception of structure of artificial environments of a chicken yolk, search of the protective components from alternative sources of not animal origin.

Earlier it was emitted from soy beans phospholipid – lipositol and it is proved that it protects gametes of a bull from temperature shock at zero temperatures flush with lecithin of a chicken yolk [4-9]. As a result of further researches and stage-by-stage development from seeds of soy the vegetable lipoproteins extract (RAF-1) [10] was received.

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Also in our researches as a protective component the preparation of mitselialny mass of a mushroom of Blakeslea trispora for the first time is tested. Blakeslea trispora biomass is rich in proteinaceous, lipidic substances, carotinoids, water - and fat-soluble vitamins, amino acids, macro - and microcells [5]. Blakeslea biomass trispora is rich in proteinaceous, lipid substances, carotinoids, water - and fat-soluble vitamins, amino acids, macro - and microcells, it is widely used for production of feed additives. It should be noted that one of such fodder additives, derivative of Blakeslea trispora, made by mechano-disaggregation biomass of a mushroom, "Vitagon" was tested by introduction to structure of diets earlier manufacturing bulls, with a positive effect, rather biological characteristics of sperm [5].

Biomass of a mushroom contains in a significant amount phospholipids (2,5%) that was basic for our researches because substantially connect protective effect of the environments applied when cooling and deep freezing of sperm of farm animals with these connections. In lipid composition of Blakeslea trispora biomass phospholipids make 9-12%, and they are the second for quantity after triglycerides. Thus as a part of biomass of a mushroom it is identified 11 phospholipid of connections [5] which allegedly can show protective effect.

Was to establish by the purpose of the real work efficiency of thinners sperms of the bulls made with use of the alternative protective components received from various raw materials of not animal origin.

MATERIALS AND RESEARCH TECHNIQUE

In experiments as raw materials for receiving an alternative anti-shock component in one case used soy seeds, in other biomass of a mitselialny mushroom of Blakeslea trispora. In the first case soy was crushed to a flour consistence, carried out it introduction and glyceric extraction with the subsequent removal of not dissolved substances centrifugation and removal of concentration of glycerin in the environment to 7% by a technique.

In other case for research of protective action of a preparation from a mycelial bimassa of a mushroom of Blakeslea trispora on skilled environment the dry extract from its biomass, received from the fodder preparation "Vitagon" was entered. Extraction carried out with use as an ekstragent of distilled water at to temperature of 70 °C within 2 hours and maceration in the closed laboratory glassware in current of 72 hours in the thermostat at a temperature of 38 °C. In the subsequent made centrifugation and removal of not dissolved part, then drying in the thermostat with use of open cups of Petri. Then dry Blakeslea trispora biomass extracts entered on the environment containing 11% of lactose, 7% of glycerin in concentration of 1%, 2%, 3%.

#Osmotic pressure of the studied thinners (a hydrolyzate of seeds of soy or extract solution) controlled by a cryoscopic method. Osmatic pressure of the environment on the basis of a hydrolyzate of soy made 360 мОсм. Osmatic pressure environments with Blakeslea trispora extracts was at the level of 310, 325, 350 мОсм, according to the studied contsetration. Indicator of concentration of the hydrogen ions (pH) after final dilution of sperm it was close to 7,0. Sterilization the tion of the prepared environments was made by a thermal method (70 °C within 1 hour).

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Sperm of bulls was diluted environment by a two-moment technique: at the beginning in ratio 1:1 first environment with their endurance at the room temperature within 5 minutes, then final dilution second environment – lactose-citrate-glycerol thinner (environment № 2) in the ratio 1:8 [8,10]. In skilled groups as the first environment used lactose-glycerol environments containing a hydrolyzate of soy or mushroom biomass extracts Blakeslea trispora. In control used the similar lactose-glycerol environment, but with addition of 30% of a yolk. Hus, studied stability of sperm to temperature or cold to shock on the basis of resistance coefficient (at sharp temperature drop in the positive range), according to a technique, and also resistance of sperm to action of low temperatures at deep freezing on the standard methods. Mobility, survival, an indicator was studied absolute survival and cryoresistance of sperm after freezing thawing when using in thinners of experimental protective components, comparing them to a yolk.

The main results of the research

In experiments at artificial induction temperature shock the percent спермиев a bull which preserved mobility and made 75% for a soy hydrolyzate, and 42,4-52,6% for environment with a mushroom extracts Blakeslea trispora. When using a yolk this indicator was in control at the level of 74,8%, and in negative control where protective components – 24,6% weren't entered. Thus, environment on the basis of extract of soy protected sperm from temperature shock at the level from the yolk. Environment with extracts from a mushroom Blakeslea trispora didn't show the necessary level of protective action, though had the expressed protective effect (table 1).

table 1

Resistance of sperm of bulls to an artificial induction of the temperature (cold) shock in the environments containing extracts of soy and biomass mycelial mushroom of Blakeslea trispora.

Environment	Percent sperm, kept mobility (coefficient resistance to cold shock)
Environmet with water and glycerolextract of soy	75,0±1,50 (0,75)
Environment with dry extracts from Blakeslea trispora, in concentration, % 1.0	42,4±2,45 (0,42)
2.0	50,6±2,65 (0,51)
3.0	50,6±2,65 (0,51)
Control, environment with a yolk	74,8±1,49 (0,75)
Control negative (without the additional protective components)	24,6 ±4,18 (0,25)

Biological indicators of sperm after freezing thawing at application in protective environments of extracts from a mycelial mushroom of Blakeslea trispora were insufficiently high also conceded to the corresponding indicators received at use of the standard thinner containing an egg yolk. Most the good result on mobility of sperm was received at 3% of concentration of dry extract – 3,5±0,5 balls. An indicator of survival and an absolute measure of survival of sperm at a temperature of 38 °C in experimental groups considerably (more than twice) conceded to a control indicator. Thus, the protective effect of extracts from Blakeslea trispora, was insufficient for practical application in

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thinners of sperm of bulls, at least at the technological capabilities of processing of the above raw materials which are available for us.

table 2

Studying of biological indicators of sperm of bulls after freezing in experimental environments on the basis of a hydrolyzate of soy showed that mobility and survival sperm didn't concede to that at use yolk of thinners (n=10)

Environments for cryoconservation sperms with use	Mobility sperm (a), points	Survival, at 38 0C (t), hours	Indicator of absolute survival, (Sa) c.u.	Cryoresistance (CR)
Hydrolyze soy	5,0±0,011	8,9±0,8	23,9±1,8	0,625
Yolk(control)	4,8±0,015	7,7±0,4	21,2±1,2	0,60
Reliability (p) with control	<0,5	<0,5	<0,5	<0,5

table 3

Biological indicators of sperm of bulls after freezing thawing in the environment containing dry extracts from biomass of a mycelial mushroom Blakeslea trispora (n=20)

Environments for cryoconservation sperms with use dry extract from Blakeslea trispora in concentration	Mobility sperm (a), points	Survival, at 38 0C (t), hours	Indicator of absolute survival, (Sa) c.u.	Cryoresistance (CR) %
1%	2,3±0,2	2,5±0,5	5,1±0,9	28,7
2%	2,8±0,2	2,7±1,3	6,4±0,6	35,0
3%	3,5±0,5	2,6±0,6	6,4±0,6	43,7
Conrol,yolk	4,3±0,3	7,3±1,4	22,2±1,9	53,7

The discussion of the received data and the conclusion

As a result, perhaps, to note that the Wednesdays received about application of the alternative protective components which are giving in to sterilization can be very effective. Thus the thinners received on the basis of extracts of soy are capable to protect gametes of bulls not worse than the environment made with use of a yolk of egg, excluding thus possibility of a microbic contamination of sperm. The protective environments received with use of dry extracts from biomass of a mycelial mushroom of Blakeslea trispora didn't provide protective effect on level, necessary for practical application, but perhaps are perspective at further improvement of technology of allocation of anti-shock components and them and application.

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Summary
These studying of protective action of the components received from seeds of soy and biomass of a mitsel mushroom of Blakeslea trispora are given. Possibility of successful use of some of them as a part of environment for dilution is proved sperms, instead of a chicken yolk.

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