The influence of polysaccharides of plants and fungi on the safety of reproductive cells of animals during hypothermic storage

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Abstract. The relevance of preserving the genetic resources of the animal and plant world necessitates the search for biologically active substances for the preservation of reproductive cells. Under conditions of hypothermic storage, the osmosis of the extracellular environment, stabilization of membranes and structures of the cytoskeleton of cells can be provided by polysaccharides. Xylotrophic Basidiomycete *Hericium erinaceus* (Bull.: Fr.) Pers. is a source of polysaccharides with high biological activity. Apple pectin is widely used in various spheres of human life. The effect of polysaccharides of different concentrations on the viability of spermatozoa of Holstein bulls under hypothermic (+4°C) storage and on the intensity of lipid peroxidation (LPO) processes and antioxidant activity was studied. The data obtained in the work indicate the prospect of using *H. erinaceus* polysaccharides as a component to maintain the functional usefulness of bovine sperm gametes under hypothermic (+6°C) storage.

Keywords: apple pectin, polysaccharides, *Hericium erinaceus*, sperm, refrigeration, hypothermic storage.

Hericium comb *(Hericium erinaceus* (Bull.: Fr.) Pers.) - a representative of agaricoid xylotrophic fungi is known as a valuable edible and medicinal species in Europe and South America, artificially cultivated and used in traditional medicine in East Asia. More than 35 polysaccharides with anticancer, immunomodulatory, hypolipidemic, antioxidant and neuroprotective activity have been isolated from the fruit bodies and mycelium of this fungus [1, 2]. A large number of medical products and drugs are known, patented in China [3], USA, Japan and

Korea [4], which contain only *H. erinaceus* as an active ingredient.

Apple pectin has found its application in many industries (food, aerospace, pharmaceutical). Its biological effect is also being studied. The introduction of apple pectin into the diet of rats reduces the degree of myocardial damage by inhibiting apoptosis [5]. Pectin is effective for normalizing weight in obesity [6].

Currently, research is actively continuing aimed at expanding the boundaries of the practical use of polysaccharides. The relevance of preserving the genetic resources of the animal and plant world necessitates the search for biologically active substances for the preservation of reproductive cells. Analysis of literature [7, 8] data suggests that polysaccharides can have a positive effect during hypothermic storage of reproductive cells. Under anaerobic and aerobic conditions, sugars are not only an energy substrate for the cell, but also stabilize the protein-lipid complexes of cell membranes and cytoskeleton structures upon cooling [9, 10].

The aim of this study was to assess the ability of the polysaccharides of the fungus *H. erinaceus* BP16 and apple pectin AU-701 to influence the preservation of the functions of bovine spermatozoa under hypothermic storage.

Objects and methods

In this work, we used a polysaccharide fraction from the fruit bodies of an artificially cultivated fungus *H. erinaceus* BP16 (natural isolate BP16, the nucleotide sequence of the ITS1_5.8S_ITS2 fragment was deposited at NCBI under number MK809367), which was obtained as a result of extraction with hot water followed by ethanol precipitation. The dry matter mass yield is 2.8%. According to gas-liquid chromatography, carbohydrate chains consist of galactose (9.62%), glucose (9.14%), arabinose (6.79%), mannose (5.01%), rhamnose (2.35%), fucose (2.68%), xylose (0.3%) residues.

Used commercial apple pectin "Classic AU-701" (Herbstreith & Fox KG, Germany). The molecule has a linear structure and contains 91% galacturonic acid. The rest of the molecule (9%) consists of neutral monosaccharides. The percentage of each type of monosaccharide in apple pectin is: galactose - 2.4%, arabinose - 0.3%, rhamnose - 1.4%, glucose - 1.6%, xylose - 2.9%. The degree of esterification of pectin is 38-40%.

Spermatozoa of bulls-producers of the Holstein breed were obtained in production conditions (JSC "KirovPlem"). Freshly obtained sperm with a mobility of 7-9 points was diluted 1:1 with lactose-citrate-yolk medium for bovine semen. After 5 minutes, lactose-citrate-yolk medium with glycerin (control group) or lactose-citrate-yolk medium with glycerol and polysaccharide (experimental groups) was slowly added dropwise to the resulting mixture in a ratio of 2:3. The final concentration of glycerol in the sperm medium was 4.4%, the polysaccharides of *H. erinaceus* 0.4%, or apple pectin 0.12%. The mixture was poured into polymer conical microtubes (0.5 ml

each), which were kept at + 6°C from 1 to 11 days.

Sperm viability indices were determined before and after hypothermic exposure. The number of gametes was determined by microscopy in a Goryaev chamber (with the addition of 3% sodium chloride) according to the generally accepted method and was expressed in million/ml. The level of biological usefulness of gametes was determined by the method of light microscopy by the indicator of their mobility in 3% sodium citrate (10 points - all spermatozoa in the field of view move progressively forward). Sperm viability was assessed using the hypoosmotic swelling test (HOS test) for twisting and swelling of their tails (per 100 tested spermatozoa, expressed as a percentage). The intensity of lipid peroxidation in the sperm medium was assessed by the chemiluminescent method on a BKhL-07 biochemiluminometer (LLC "Medozons", Russia) for 30 sec. Recorded I max (mV) - the maximum intensity of a rapid flash, reflecting the potential ability of a biological object to free radical oxidation; S (mV×sec) - light sum for 30 sec, reflecting the content of radicals RO_2; tg(-2α) - the tangent of the slope of the time axis curve (characterizes the maximum slope of the curve, with the sign "-"), the higher the value of the tan (-2α) index, the higher the activity of the enzymatic systems of cells that regulate the content of hydroperoxides.

The results of the study were subjected to statistical analysis using the "BioStat 2009 Professional 5.8.4" software (AnalystSoft, USA). To assess the differences, the nonparametric Wilcoxon test was used at $p<0.05$. The research results are presented as median, 25th and 75th centiles (*Me*, *Q*1-*Q3*).

Results and discussion

Using the chemiluminescent method, it was found (figure) that the presence of the polysaccharide fraction of *H. erinaceus* in the sperm medium contributes to an increase in the intensity of LPO processes (by the value of I max, S) and antioxidant activity (by the tg (2α) indicator) equally and through all storage periods. Apple pectin has no significant effect on the above processes. Probably, this difference in the effects of polysaccharides is due to the peculiarities of the structure of their molecules.

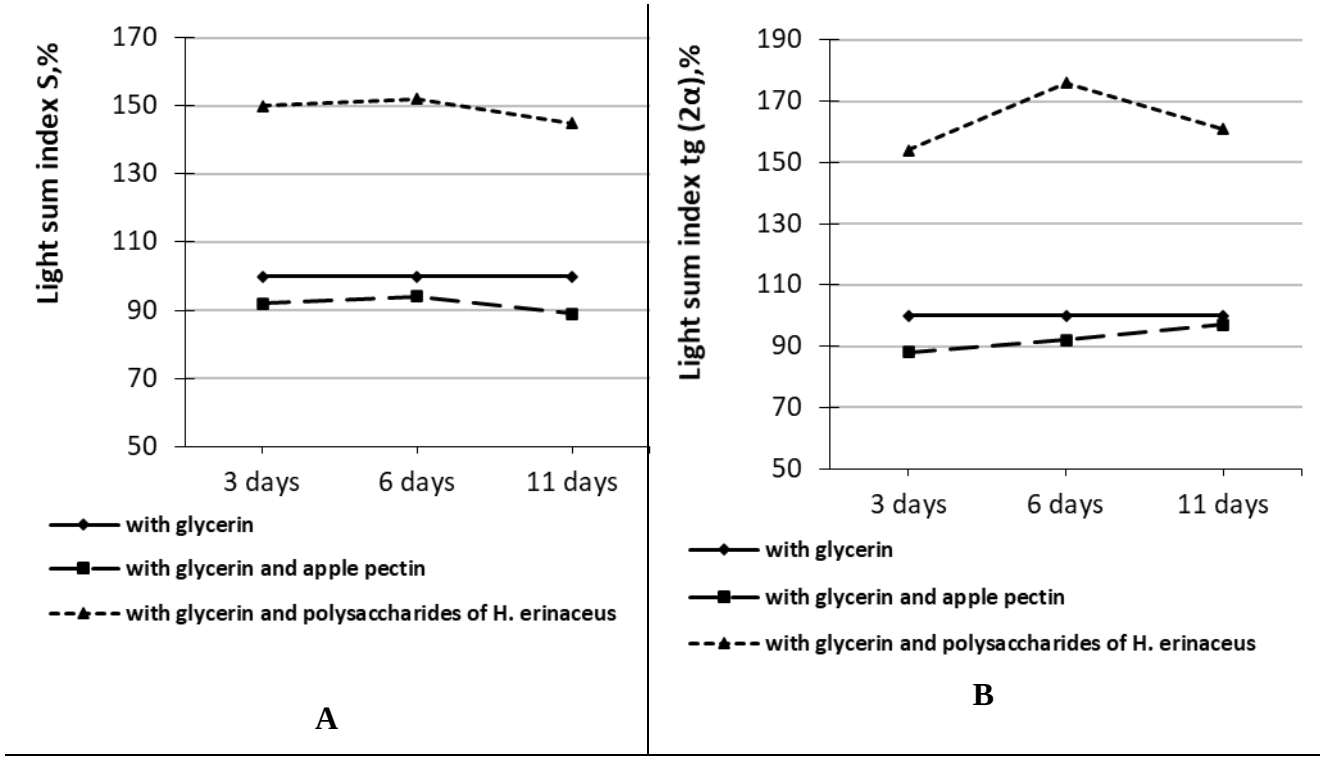

Figure. Indicator (in% to the level of glycerol) S of the intensity of lipid peroxidation (diagram A) and antioxidant activity tg (2α) of bovine spermatozoa (diagram B) subjected to hypothermic (+6°C) storage for 3, 9 and 10 days in the medium glycerin (4.4%) and polysaccharide fraction of *H. erinaceus* 0.4% or apple pectin 0.12%

In gametes stored in a glycerol medium (4.4%) at +6°C, the ability of sperm to progressively move at a level of 40% (allowable for fertilization) remained for 6 days, when the polysaccharide fraction of *H. erinaceus* was introduced into the glycerol medium 0.4% - 7 days, with the addition of apple pectin 0.12% for 5 days (table 1). Probably, this effect in the polysaccharide fraction of *H. erinaceus* is due to its revealed and described above antioxidant effect at the indicated concentration.

Table 1

Motility index of spermatozoa (*Me, Q1-Q3*), subjected to hypothermic (+6°C) storage for 3-9 days in glycerol (4.4%) and *H. erinaceus* polysaccharides (0.4%) or apple pectin AU-701 (0.12 %)

Series	Storage periods (days)				
	3	6	7	8	9
Glycerol	78 (62.5 – 83.5)	44 (25.0 – 67.0)	22 (15.25 – 53.0)	11 (0.0 – 29.75)	12 (0.0 – 44.0)
+ 0.4% polysaccharide fraction of *H. erinaceus*	78 (64.0 – 89.0)	50 (36.75 – 67.0)	44 (24.75 – 74.25) *	22 (8.25 – 51.5) *	18 (8.25 – 54.25)
+ 0.12% apple	78	29.5	38	13	13

pectin AU-701	(67-89)	(22-56)	(19.75-61.5)	(11-61.5)	(13-52.5)

Notes: data are presented as a percentage in relation to the level before storage, taken as 100%; * – the difference with the value of the indicator "sperm with glycerol" with the corresponding storage periods is statistically significant at $p< 0.05$

Sperm viability, according to the HOS test, at all storage periods corresponded to the level of sperm in glycerol, i.e. the presence of polysaccharides in the medium did not affect the effect of glycerol for this indicator (table 2). We believe that under conditions of hypothermic storage of sperm at + 6°C for 9 days, the membrane did not receive significant damage, therefore, under hypoosmotic stress, the inflow of fluid into the cell did not cause its uncontrolled swelling to the degree of rupture of the membrane.

Table 2

Indicator of resistance of spermatozoa to hypoosmotic swelling (HOS-Test) (*Me, Q*1-*Q3*), subjected to hypothermic (+6°C) storage for 3-9 days in glycerol (4.4%) and *H. erinaceus* polysaccharides (0.4%) or apple pectin AU-701 (0.12%)

Storage solution	Storage periods (days)				
	3	6	7	8	9
Glycerol	83 (76.0-91.0)	69 (60.5 – 80.0)	40 (28.0-56.0)	35 (31.0-51.5)	45 (42.0-64.0)
+ 0.4% polysaccharide fraction of *H. erinaceus*	83 (78.75-98.5)	78 (45.5-84.0)	29 (27.0-69.5)	41 (39.75-48.75)	38 (35.0-55.0)
+ 0.12% apple pectin AU-701	71 (57.25-94.5)	70 (38-81)	43.5 (35-64)	51.5 (49-66)	50.5 (34-50.5)

Notes: data are presented as a percentage in relation to the level before storage, taken as 100%;

The composition and physicochemical properties of preserving media are decisive in the preparation of semen for hypothermic storage or freezing. In the light of modern concepts, the search for new effective components for preservative solutions should be focused primarily on natural compounds. The data obtained in this work indicate the possibility of using *H. erinaceus* BP16 polysaccharides as a component to maintain the functional usefulness of bovine sperm gametes under hypothermic (+6°C) storage.

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