QUALITATIVE INDICATORS OF RAM SPERM IN CASE OF USING REDUCED GLUTATHIONE AND BSA AS A COMPONENT OF THE EXTENDER FOR CRYOPRESERVATION

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The addition of 5 μM of reduced glutathione and 15 mg/ml of BSA to LYTCGE leads to an increased activity of antioxidant enzymes (SOD and GPO) and LDH in the sperm after equilibration by 12,0, 24,4 and 17,5 % respectively, after freezing-thawing – by 19,7, 28,6 and 24,1 %, as well as a decreased content of TBA-active products and a decreased activity of AST in the sperm plasma after equilibration by 22,2 and 12,1% respectively, after thawing – by 27,4 and 16,3%, which indicates a decreased damage to sperm membranes. The obtained results can serve as a basis for in-depth research on improving the extender for cryopreservation of ram sperm and its testing in the practice of sheep breeding.

The efficiency of sperm cryopreservation of largely depends on the composition of synthetic extenders. To prevent excessive accumulation of toxic products of lipid peroxidation in the ram sperm, a number of researchers propose to use different antioxidants during the process of equilibration and chilled and deep freezing storage [7, 2, 1, 6]. In the literature, bovine serum albumin and thiol compounds are reported to have a positive impact on the biological full value of the animals sperm [4, 5]. However, there are no data on the effect of the combined use of BSA and glutathione in the environments on ram sperm deep freezing

The purpose of work was to determine the antioxidant and cryoprotection effect of reduced glutathione and BSA as part of the lactose-yolk-tris-citrate-glycerine extender (LYTCGE) for ram sperm freezing.

Research resources and methods. Studies were performed in the peasant farm organization "Saldobosh" in Khust district, Zakarpattia region. The experiment involved 18 ejaculates of six rams of the Ukrainian Carpathian Mountain breed. The freshly obtained ejaculates with the activity of 80-90% were diluted 1:3 and divided into two groups: control and experimental. In the experimental group, 5 μM of reduced glutathione and 15 mg/ml of BSA were introduced into the LYTCGE. After

dilution, the sperm was equilibrated (4°C, 2,5 hours) and frozen in liquid nitrogen. After dilution, equilibration and thawing, biochemical studies of sperm and diluted plasma according to methods described in the "Reference book" were conducted [3]. To separate the sperm cells from the diluted seminal plasma, the sperm was centrifuged at 3000 rpm for 20 minutes. The activity of lactate dehydrogenase (LDH) and antioxidant enzymes (superoxide dismutase (SOD), glutathione peroxidase (GPO) and catalase (CAT) was determined in the sperm cells. The activity of aspartate aminotransferase (AST), and the quantity of TBA-active products were determined in the diluted sperm plasma.

Statistical analysis of the obtained digital data was performed with the help of the computer program "Microsoft Excel 2003" using Student's criterion.

Research results. Researching the antioxidant defence system of the ram sperm during its chilled storage, an increased activity of antioxidant enzymes in experimental samples of sperm was reported. The activity of GPO and SOD in the experimental group sperm after equilibration was higher by 24,4% ($p<0,01$) and 12,0 % compared to that in the control group (table 1).

Similarly, after thawing, the activity of GPO and SOD in the experimental samples was higher by 28,6% ($p<0,01$) and 19,7% ($p<0,01$) as opposed to the control group. At the same time, the difference between the experimental and control samples in terms of the activity of CAT was insignificant and amounted to 2,9 % after equilibration and 12,5 % after sperm thawing.

Since it is known that LDH catalyzes the reversible oxidation of lactate into pyruvate in the presence of NAD and is therefore an enzyme that connects respiration and glycolysis, we evaluated the changes of carbohydrate metabolism in the ram sperm in the process of sperm cryopreservation in terms of the LDH activity.

Table1

The activity of LDH and enzymes of antioxidant protection in the ram sperm

Indicators	After dilution	After equilibration		After freezing-thawing	
		C	E	C	E
SOD, IU/mg proteine	48,2±2,82	38,2±2,01	42,8±2,02	29,4±1,96	35,2±1,90*
GPO, umol / min.? mg proteine	0,53±0,06	0,45±0,05	0,56±0,04*	0,35±0,03	0,45±0,05*

CAT, umol / min.? mg proteine	0,30±0,04	0,35±0,03	0,34±0,04*	0,40±0,04	0,35±0,03
LDH, nmol / min.? mg proteine	25,4±2,81	16,0±1,44	18,8±2,54	7,9±0,63	9,8±1,24*

In particular, the activity of the enzyme in the control sample sperm after equilibration decreased by 37,0 % ($p<0,01$) and after freezing-thawing accounted for 68,9 % ($p<0,001$) compared to the initial level of diluted sperm. Concurrent with the release of LDH from the sperm, chilling and freezing-thawing of the sperm lead to inactivation of the enzyme, related to oxidation of sulfhydryl groups of lactate dehydrogenase. Simultaneously, in the experimental samples the activity of LDH in the sperm after equilibration was higher than that in the control samples by 17,5 %, while after conservation it exceeded that of the control group by 24,1 % ($p<0,01$), which indicates a decrease in the damage of cells.

Estimating the intensity of peroxidation of lipids in the process of ram sperm cryopreservation, an increased content of final metabolites LPO (TBA-active products) in the experimental sperm samples was determined. Thus, the content of TBA-active products in the control sperm samples after equilibration increased by 10,8 % ($p<0,05$), and after freezing-thawing — by 29,2 % ($p<0,05$) compared to the initial level of diluted sperm (table 2).

Table2

The content of TBA-active products and activity of AST in the ram sperm plasma

Indicators	After dilution	After equilibration		After freezing-thawing	
		C	E		
TBA-active products, nmol / ml	6,5±0,32	7,2±0,33	5,6±0,29**	8,4±0,44	6,1±0,21***
AST, nmol / min. ? mg protein	4,7±0,68	6,6±0,53	5,8±0,42	8,6±0,47	7,2±0,46*

The addition of glutathione with BSA to the extender resulted in a decreased amount of TBA-active products in the diluted seminal plasma after equilibration by 22,2 % ($p<0,01$), after thawing – 27,4 % ($p<0,001$) compared to the control extender.

The activity of AST in sperm plasma also increased in the process of ram sperm cryopreservation, though the rate growth in the samples was different. In

particular, in the control samples, after equilibration the activity of AST was higher by 40,4 % ($p<0,01$), while after thawing by – 83,0 % ($p<0,001$) compared to the initial level of diluted sperm. The introduction of glutathione with BSA into the extender resulted in a decreased amount of AST in the seminal plasma after equilibration by 12,1 %, after thawing - by 16,3 % ($p<0,05$) compared with the control extender, indicating a decreased damage of sperm.

Thus, the study on biochemical characteristics of ram sperm, as markers of cell damage, reported the positive effect of the addition of reduced glutathione with BSA to the extender for cryopreservation, characterized by an increased activity of enzymes of antioxidant protection and LDH in the sperm, as well as a decreased content of TBA-active products and the activity of AST in the sperm plasma, which indicates a decreased damage to sperm membranes.

Conclusions and prospects for further research. The addition of 5 μM of reduced glutathione and 15 mg/ml of BSA to LYTCGE leads to an increased activity of antioxidant enzymes (SOD and GPO) and LDH in the sperm after equilibration by 12,0, 24,4 and 17,5 % respectively, after freezing-thawing – by 19,7, 28,6 and 24,1 %, as well as a decreased content of TBA-active products and a decreased activity of AST in the sperm plasma after equilibration by 22,2 and 12,1% respectively, after thawing – by 27,4 and 16,3%, which indicates a decreased damage to sperm membranes. The obtained results can serve as a basis for in-depth research on improving the extender for cryopreservation of ram sperm and its testing in the practice of sheep breeding.