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Ефективність застосування захисних середовищ для довгострокового зберігання штаму вірусу сказу L. Pasteur за різних низьких температур

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Effectiveness of Protective Media Applying for Long-Term Storage of the Rabies Virus L. Pasteur Strain at Various Low Temperatures

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The immunogenicity of rabies biologicals directly depends on the activity of the virus used for their manufacturing, therefore, an urgent problem of the biotechnology industry is the development of individual protective media for storage of rabies vaccine strains at low temperatures.

The aim of the study was to investigate the effectiveness of the protective media applying for long-term storage of the rabies virus L. Pasteur strain at various low temperatures under conditions of rabies vaccine manufacturing.

In the research there was used the rabies virus L. Pasteur vaccine strain, accumulated by permanent Vero cell culture in DMEM-based (Biowest, France) growth medium (GM) supplemented with 0.5% human albumin. The virus was stored at –20, –80 and –196ºC in the following media: 1 – GM without additives: 2 – GM with 5% sucrose, 3 – GM with 5% glycerol, 4 – GM with 5% sucrose and 5% glycerol: 5 – GM with 5% maltose. The infectious activity of the samples was evaluated by the titration method in the BHK-21 cell culture before storage (control), as well as after a week and 3, 6, 12, 18 and 24 months (observation period).

In the studied protective media, the activity of the virus stored at –196ºC was higher than at other temperatures; at –80ºC it was higher than at –20ºC. It was found that after 24 months of storage at –196ºC the high virus preservation (≥ 80%) was provided by GM-based preserving media with the addition of sucrose and glycerol (83% of the initial control), and mixtures of these substances (87%). Temperature mode of –80ºC and medium supplemented with 5% sucrose provided high virus activity levels up to 12 months of storage (82% of the initial rate); –20ºC and protective medium with the addition of 5% sucrose and 5% glycerol mixture provided its high rates up to 6 months (80%). The cryoprotective effect of the media supplemented with sucrose and glycerol is stipulated with their hydration properties and ability to stabilize virions.

Thus, assuming the regulations for the rabies vaccine manufacturing, the temperature of –80ºC and GM-based protective medium with adding the sucrose in 5% final concentration are recommended for the long-term storage of large volumes of rabies virus L. Pasteur strain. The temperature of –196ºC and protective media supplemented with sucrose, glycerol and their mixture in 5% final concentration are recommended for storage of reference samples and small volumes of the virus. For the production aims it is possible to store the virus at –20ºC for up to 6 months in GM supplemented with solutions mixture of sucrose and glycerol in final concentration of 5%.

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Вплив кріопротекторних середовищ на основі сахарози та гліцерину на кінетичні характеристики сперматозоїдів самців кіз зааненської породи

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Effect of Sucrose and Glycerol Based Cryoprotective Media on Kinetic Characteristics of Saanen Goat Spermatozoa

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Goat sperm cryopreservation is a necessary step both for artificial insemination and *in vitro* fertilization. As most of cryoprotectants are cytotoxic, appropriate cryoprotectants concentration and equilibration time before freezing are important to provide high vitality and motility of spermatozoa. The aim of our study was to compare the influence of sucrose and glycerol based cryoprotectant media on the motility of goat spermatozoa during different exposure periods.

The investigation was performed using ejaculates obtained from 5 sexually matured Saanen male goats during non-breeding season by artificial vagina. Every ejaculate was divided into 7 groups which were diluted in 1:1 ratio with cryoprotectant solution based on HEPES buffer with sucrose and glycerol in the following concentrations consequently: group 1 – 0.15M + 5%, group 2 – 0.15M + 7%, group 3 – 0.15 M + 10%, group 4 – 0.29M + 5% , group 5 – 0.29M + 7%, group 6 – 0.29M + 10%, group 7 – control (sperm with HEPES buffer media without cryoprotectants). Sperm motility was evaluated 0, 10, 20 and 30 min after dilution at room temperature (23°C).

Sperm motility decreased over time in the studied groups as well as in the control group. A significant decrease ($p \leq 0.05$) in motility was observed after 10 min of exposure in groups 1–6 compared with group 7, respectively ((76 ± ± 1.4), (76.2 ± 1.7), (73.4 ± 1.5), (60.4 ± 1.8), (55.4 ± 2.7), (51.4 ± ± 1.5) and (79.8 ± 1.6)%). This may be due to dehydration of spermatozoa in a hypertonic solution that can be beneficial while freeze-thawing process. After 20 min a dramatical decrease of motility was observed in groups 4–6 ((49.8 ± 1.3), 4(5.8 ± 2.6) та (42 ± 2.3)%), while this index in groups 1–3 differed from the one of control group ((78.8 ± 3.1)%) within 10% ((72.2 ± 2.6), (71.4 ± 1.1), (69.2 ± 2.4)%). In 30 min the situation was similar as described above. The kinetic characteristics were significantly lower ($p \leq 0.01$) in groups 1–3 compared to control group, respectively: ((70.6 ± ± 2.3), (68.4 ± 1.7), (66.4 ± 1.5), (77.2 ± 2.4)%), however, the kinetic characteristics were almost twice higher than in groups 4–6 ((39.6 ± 1.1), (36.8 ± 1.5), (35 ± 2.5)%).

In conclusion, the sucrose and glycerol based cryoprotectant media had a negative impact on the motility of goat sperm after 10 min of exposure. Sucrose concentration increase in cryoprotective media resulted in significant decrease of the kinetic characteristics of spermatozoa.