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Підготовка сперми барана для екстракорпорального запліднення із застосуванням мінімального градієнта

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Preparation of Ram Sperm Using Minimal Application Gradient for *In Vitro* Fertilization

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Selection of spermatozoa for fertilization using additional reproductive technologies is one of the key aspects of the success of biotechnological approaches. Selecting the most active fraction of morphologically correct sperm not only makes the operator's work easier, but also improves the results.

The washing of cryopreserved suspension of sheep spermatozoa requires improvement of standard approaches to preparation of native sperm. The most widely used methods are the infusion of spermatozoa using a density gradient or the swim up method. (Olivares, 2017). A modified method of sperm infusion at a minimal density gradient is emerging as a more effective method of purification and selection of sperm for further growth (Marti, 2006).

The research was performed in the laboratory of biotechnology established by the Institute of Animal Breeding and Genetics. For the recovery of spermatozoa, cryopreserved ejaculated spermatozoa of the Sokil breed sheep were used (No. 80-4016, No. 55-06535, No. 044-05828). Granules of three specimens of sheep (No. 80-4016, No. 55-06535, No. 044-05828) were thawed and added to a minimum volume of density gradient (0.4 ml instead of the standard 1 ml). The concentration of spermatozoa was monitored in the Makler chamber at 400× magnification. The average sperm activity after defrosting became 41.7% ± 2.21 on average.

After preparation of cryopreserved suspension of spermatozoa from three different individuals of rams using a modified method of a minimal density gradient, we extracted the highest concentration of spermatozoa in ram no. 55-06535 (523 ± 6.6 million/ml), and the highest activity in ram No. 80-4016 – 20.7 ± 1.1%. After calculation of the active fraction of spermatozoa – the number of active spermatozoa in the sample we extracted is the highest in No 044-05828, and the lowest – in No. 80-4016 (13.5± 0.04 million /ml). On average, the volume of the loose fraction was 27 ± 0.1 million in 1 ml.

Spermatozoa of various types of animals, including ram, contain a variety of morphological spermatozoa, which in turn lead to changes in metabolic processes and the characteristics of the organism. This requires a thorough development of standard methods for preparing sperm suspensions for use in fertilization programs. Our results show that different individuals have individual characteristics of fluidity and sperm concentration. The method of soaking with a minimal strength gradient is a promising method for curing cryopreserved sheep sperm.

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Підбір цукрів для кріопротекторних середовищ на культурі клітин раку молочної залози MCF-7

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Selection of Sugars for Cryoprotective Media on MCF-7 Breast Cancer Cell Cultures

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Selection of the most appropriate compounds for cryoprotective media for long term storage of cell suspensions and tissues requires thorough exploration.

There was realized a series of experiments for comparing the efficiency of using sugars of different molecular weight in cryopreservation of MCF-7 breast cancer cell culture under rapid and slow freezing modes. The activity of antioxidant enzymes was analyzed. The survival and state of enzyme cell systems were measured using glutathione peroxidase, superoxide dismutase, glutathione S-transferase, and malondialdehyde. All these assays were obtained after procedures of cells thawing, passing and culturing for 48 hours. The study was performed with following sugars: galactose, xylose, lactose and maltose.

As a result, when using the rapid freezing procedure, the usage of cryoprotective media based on galactose, lactose and maltose solutions was found to be the most appropriate. The highest activity of the studied enzymes was observed in cryoprotective media with following sugars: glutathione peroxidase (891.16; 605.36 and 656.64 μmol/l × min, accordingly), superoxide dismutase (3.18; 2.7 and 4.35 %/min, accordingly), glutathione S-transferase (560.97; 483.07 та 438.59 μmol/ml × min, accordingly). Moreover, the lowest concentration of malonedialdehyde was observed in cryoprotective medium with lactose, that made 0.38 μmol. In cryoprotective media with either galactose or maltose, the concentration of malone dialdehyde was 0.43 μmol per each sample. In total, these data demonstrated the minimum damage to enzymatic redox processes in cells after thawing.

When using slow freezing procedure, the cryoprotective media containing xylose and galactose appeared to be the most efficient. The activity of enzymes were as follows: glutathione peroxidase (703.55 and 653.08 μmol/l*min, accordingly); glutathione S-transferase (695.26 and 462.66 μmol/ml × min, accordingly); superoxide dismutase (2.4 and 1.47 %/min, accordingly); malone dialdehyde (0.71 and 0.67 μmol).

The obtained data can by applied to improve protocols and procedures of cryopreservation of the cells of different origin taking into account the peculiarities of metabolic pathway of cancer cells.