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Використання поліетиленоксиду і гідроксіетилкрохмалю у якості замінника сироватки крові при кріоконсервуванні клітин інтерстицію сім'яника мишей

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Use of Polyethylene Oxide and Hydroxyethyl Starch as Serum Substitute for Cryopreservation of Mouse Testis Cells

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Cryopreserved testicular cells can be used to study testicular cell defects, to preserve endangered species, as well as in reproductive technology. Blood serum is one of the most common components of testis cryoprotective media [J. Tai, 1994; V. Keros, 2007]. It is a complex solution, comprising various components, the concentration of which may vary, but serum is able to inhibit the nucleation and crystal growth [P. Bruyère, 2013]. This action is mainly associated with presence of serum albumin, being a natural polymer [B. Zakharov, 2016]. Serum use is associated with the risk of contamination of biological material and variability of serum composition from batch to batch, which may affect the cryopreservation outcome. Thus, the substitution of synthetic polymers such as polyethylene oxide (PEO) and hydroxyethyl starch is of great importance for the development of cryopreservation techniques.

The research aim was to investigate the effect of polymers, namely PEO (400 kDa) and HES (200 kDa) in concentration of 10 mg/ml on the survival of mouse interstitial cells (ICs) during cryopreservation with 0.7; 1.4 and 2.1 M DMSO. The choice of the polymers and their concentration was stipulated with the successful use of these compounds as serum substitutes [Y. Petrenko, 2003; A. Stolzing, 2012]. Ham's/F12 solution without supplements or with 10, 20% fetal bovine serum (FBS) were used as controls. The samples were cooled with the rate of 1 deg/min to –80°C. Then they were plunged into liquid nitrogen. The ICs viability was assessed with trypan blue dye. We also examined preservation of Leydig cells by histochemical staining for β-hydroxysteroid dehydrogenase (HSD) activity. The data are represented as median with 25th and 75th percentiles. Nonparametric statistics were used to compare the data.

It was shown that the most optimal concentration of DMSO when cryopreserving ICs without supplements was 1.4 M. It allowed to preserve up to 17.4 (13.9; 24.3) % of viable cells and 8.3 (7.5; 11.7)% of Leydig cells. Addition of 10, 20% FBS or 10 mg/ml HES to the cryopreservation solution increased the total preservation of ICs and that of Leydig cells by more than 10 and 15%, respectively. Some studies have shown the toxic effect of DMSO after cryopreservation and during utilization of the cryopreserved material [P. F. Ferrucci, 2000; A. A. Gurtovenko, 2007], so it was of interest to investigate the possibility of DMSO concentration to be reduced for cryopreservation of ICs. The use of 10 mg/ml of hydroxyethyl starch in combination with 0.7 M DMSO was shown to have the best results in total ICs preservation, the one of viable and Leydig cells: 75.8 (53.3; 93.3), 55.6 (45.1; 69.4) and 57.1 (40.2;70.3)%, respectively. The use of polyethylene oxide was ineffective.

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Кріоконсервування сперматозоїдів людини при олігоастенотератозооспермії з полівінілпіролідоном

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Cryopreservation of Human Oligoasthenoteratozoospermic Spermatozoa with Polyvinylpyrrolidone

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Cryobiological methods are widely used in infertility treatment by assisted reproductive technologies (ART). Cryopreservation of normozoospermic spermatozoa has become a routine practice in ART. However the survival rate of frozen-warmed spermatozoa in the patients with oligoasthenoteratozoospermia (OAT) remains low. Therefore the development of effective cryopreservation methods for these cells is relevant. Polyvinylpyrrolidone (PVP) with 350,000 Da molecular weight belongs to artificial polymers and is used for the oocyte intracytoplasmic sperm injection. It is important to investigate this polymer cryoprotective properties to cryopreserve the spermatozoa of men with spermatogenesis abnormalities as this will allow to use the cells for fertilization immediately after warming and thereby to reduce a cell damage, occurring during centrifugation. The research aim was to evaluate the use of PVP to cryopreserve the spermatozoa of the men with OAT. The morphological and functional characteristics of spermatozoa were assessed.

The spermatozoa of men aged 20 to 40 years (n = 11) were studied using their written consent. Spermatozoa characteristics were determined using a CASA computer system. The motile sperm fraction was obtained by ejaculate centrifugation in a density gradient (Cook, USA) which was divided into 2 doses: sperm cryopreserved with two-stage method with glycerol (group 1) and 10% PVP (group 2). The samples were placed into cryotubes, which were kept for 10 min at 20 cm above the liquid nitrogen mirror and then quickly plunged into liquid nitrogen. Warming was carried out using water bath (40°C). Glycerol was removed by double centrifugation to counteract the cytotoxic effect. The sperm viability was evaluated by eosin-nigrosin staining (Magapor, Spain).

After cryoprotectant removal the number of motile cells in group 1 was (27.3 ± 4.8)%, in group 2 it made (41.4 ± 8.1)%. The sperm viability was (82.1 ± 8.6)% and (89.6 ± 8.6)% in group 1 and 2, respectively. The multiple abnormalities of head, middle piece and tail were significantly lower in the group 1 ((35.73 ± 3.59)%) compared with group 2 ((26.26 ± 2.61)%).

Two-stage cryopreservation with PVP was effective for ART because it enabled the obtaining of high morphological and functional characteristics of spermatozoa derived from OAT men and did not require the cryoprotectant removal stage after warming.