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Вплив введення кріоекстракту спінальних гангліїв на скоротливу активність міометрію матки щурів пізнього репродуктивного віку

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Impact of Spinal Ganglia Cryoextract Introduction on Contractile Activity of Uterine Myometrium of Late Reproductive Age Rats

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Increasing age of prenatal women is a trend in modern society worldwide. Reducing the contractile uterine activity (CUA) in late reproductive age leads to discoordination of labor and delivery as well as the need for further operative one. On the other hand, the majority of sympathetic neurons with receptive fields in the uterus is known to express the receptors for the neurotrophins (NT) TrkA and p75. We have assumed that NT-dependent regulation may affect the CUA. As a source of NT we have used the cryoextract derived from spinal ganglia (CESG).

The research aim was to determine the effect of CESG on contractile activity of the uterus of late reproductive age rats.

CESG was prepared from the spinal ganglia of neonatal piglets by freezing in saline (S) three times down to –196°C and subsequent heating at room temperature, homogenization and centrifugation. The supernatant was collected, sterilized and used in the experiments. The experiments were performed in 14-month-old outbred white female rats, that corresponded to late reproductive age. Animals were divided into 2 groups: 1 – with the introduced CESG; 2 – with the introduced S (control). S and CESG were intraperitoneally administered daily for 9 days with 0.2 ml. The protein concentration in CESG was 0.3 mg/ml. Animals were sacrificed on days 28–29 after the start of substance administration. Isolated tissue strips (8 × 3 × 1 mm), obtained from the uterine horn, were placed in an organ bath (7 ml flow cell volume) and incubated in Krebs solution at 37°C for 60 min under isometric conditions with a 10 mN load. A Grass FT03C transducer connected to the OWON B41T^{+} multimeter adapter were used for registration. Induced CUA was determined by oxytocin at a final concentration of 4nM. The average maximum amplitude of contractions (MAC) and frequency of contractions (FC) were studied. The results are presented as Me (Q1; Q3) where Me – median, Q1 – 1st quartile; Q3 – 3rd quartile.

It was found, that FC increased significantly from 4 (3.3; 4) contr / 5 min in control animals to 5 (4.5; 5) contr / 5 min in animals with introduction of CESG (p = 0.001), and the MAC from 11.4 (10.7; 11.6) mN to 12.5 (11.9; 12.6) mN (p = 0.015).

Thus, the introduced CESG promotes enhancing contractile activity of the uterine myometrium in the rats of late reproductive age.

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Дослідження осмотичної реакції сперматозоїдів карася *Carassius auratus* (L., 1758) для розробки протоколу їх кріоконсервування

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Study of Osmotic Response of Crucian *Carassius auratus* (L., 1758) Spermatozoa to Develop Their Cryopreservation Protocol

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One of the ways to solve the task of preserving the biodiversity of animals is to use the technology of reproductive cells cryopreservation. Spermatozoa cryopreservation protocols are characterized with a high species specificity, actually, a unique method of their low-temperature storage should be created for each species [E. Cabrita *et al*.., 2010; E. Kopeika *et al.*, 2007].

To select the cryopreservation regimens and media, the parameters of spermatozoa, characterizing their osmotic resistance, *i. e.* the cells ability to remain viable due to changes in the osmotic pressure of the environment should be taken into account. To date, only a few results of the study of permeability of fish sperm membranes to water and cryoprotectants are known. The purpose of this research was to determine the parameters of permeability of spermatozoa membranes of crucian *Carassius auratus* (L., 1758) to water molecules and cryoprotectants as an important step to develop a protocol for their cryopreservation by vitrification.

Sperm was obtained using the injection with pituitary suspension adopted in fish farming assumed as 1 mg/kg body weight. Osmotic response of carp spermatozoa was studied using photoelectric colorimeter KF-77 (Poland), equipped with a magnetic mixer and thermostated cuvette compartment, according to our own technique [A. Puhovkin *et al.*, 2014]. To determine the permeability of crucian spermatozoa plasma membranes to cryoprotectant molecules, the spermatozoa were incubated in solutions of ethylene glycol (EG), 1,2-propanediol (1,2-PD), methanol (Met) of different concentrations, or a mixture of these cryoprotectants, prepared with isotonic to sperm plasma 0.12 M aqueous solution of NaCl. Permeability coefficients of spermatozoa plasma membranes for either water (Lp) or cryoprotectant (Kp) molecules were determined by fitting the experimental dependences of the relative cell volumes *versus* time and the solutions of theoretical model equations [A. Puhovkin *et al.*, 2016]. The activation energy (Ea) of substance transfer through cell membranes was calculated using he lnLp or ln Kp dependencies *versus* reciprocal temperature, the slope of those according to the Arrhenius equation was Ea/R, where R was the universal gas constant. The permeability of crucian spermatozoa membranes to water molecules at 20°C is 3.53 ± 0.18 × 10^{-14} m^3/N·s. Decrease in membrane permeability of crucian spermatozoa for DMSO, EG and 1,2-PD molecules within the range of 30...18°C was featured by the activation energy of 48 ± 4 kJ/mol for water, 82 ± 5 kJ/mol for EG, 99 ± 7 kJ/mol for 1,2-PD, and 84 ± 6 kJ/mol for mixture of EG, 1,2-PD and Met.

Thus, for the first time, the permeability coefficients of crucian spermatozoa membranes to water, ethylene glycol, methanol, and 1,2-propanediol molecules as well as the activation energy of these molecules transfer through membranes were determined.