OPTIMIZED PROTOCOL OF ANESTHESIA IN LARGE EXPERIMENTAL MODEL OF TRANSGENIC PIGS FOR TREATMENT OF CARTILAGE INJURIES WITH NEW GENERATION BIOMATERIALS AND CELL-BASED GRAFTS *IN VIVO*

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Introduction
The possibility of usage of transgenic pig for experimental treatment of cartilage injuries was investigated. Anesthesia and analgesia procedures in pigs are frequently required for surgically performed research treatments due to the nature of invasive procedures that are performed. Their use as preclinical models (translational research) frequently is connected with surgical implantation of dentures, serious invasive surgery and/or creation of disease conditions [1]. Selection of an appropriate protocol which considers the physiologic effects of the pharmacologic agents for anesthesia/analgesia is an important aspect of designing an experiment. Anesthesia takes into account the abolition of consciousness by inducing deep sleep and analgesia by general and local abolition of pain sensation [2]. Too deep anesthesia results in disturbances of the respiratory system, to collapse and cardiac arrest, and to a significant impairment of blood supply and oxygenation of tissues. It is very important to maintain physiological blood pressure and physiological oxygen saturation of hemoglobin and to reduce to a minimum ischemia time and keeping a proper oxygenation of tissues [3]. For the purpose of researching new generation biomaterials using a unique research model of transgenic pigs, an original model of swine inhalation anesthesia with minimal supply of anesthetic drugs and local anesthetic agents was developed. In order to maintain the proper oxygenation of tissues anesthesiological scheme was modified, which have been used so far.

Materials and Methods
In 9 transgenic pigs, three-fold complex anesthesia was performed. In the initial stage, the pigs were quenched pharmacologically by administering the administration of azaperone 2 mg / kg (Stresnil, Janssen). In the second stage, general anesthesia was induced by intravenous administration of a 1 - 2 mg / kg xylazine and 5 - 10 mg ketamine mixture. After the abolition of consciousness, animals were intubated and inhalation anesthesia was carried out. It was used inhalation anesthesia with gas mix isofluran, nitrous oxide, oxygen and air. It was used low pressure and low flow of anesthetic gases: isofluran 0,6 – 1%, nitrous oxide 20 – 25% and high pressure of oxygen 45 – 55% with positive end-expiratory pressure (PEEP) 5 mm H_2O. EKG with ST elevation, respiratory frequency, oxygen saturation (SpO_2), hyper- or hypocapnia ($PaCO_2$) and pressure in mixed anesthetic gases was constantly monitored. Low flow was used (1,5 – 2,5 L/ minute) for mix of anesthetic gases. With such a low anesthetic gas flows, anesthesia was supplemented with local anesthesia. For this purpose, the lateral side of the knee joint was injected with 8 ml of 1% lignocaine.

Results and Discussion
During the 45-minute operation, a cartilage defect was performed on the lateral femoral condyles of 6 mm in diameter and 3 - 5 mm in depth. During surgery was not monitored disturbances in EKG, respiratory frequencies was 12/min, SpO_2 was average 98% (95 – 100%), $PaCO_2$ was average 40mmHg (30 – 45%), all in physiological range. Local pain sensation was not observed.

Conclusion
The usage of presented protocol of anesthesia allows to maintenance optimal conditions of perfusioned organs and tissues with total elimination of pain sensation throughout the operation in large animal pig model *in vivo*.

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