Abstract of doctoral dissertation:

“The effect of modified *in vitro* oocyte maturation conditions and quality and preparation methods of boar semen on *in vitro* fertilization in pigs”

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In vitro production of pig embryos (IVP) is still the object of multiple studies, because of the fact that a number of issues related to this method have not been resolved yet. The major problems with IVP are: the quality of *in vitro* matured oocytes, the quality of semen after capacitation, polyspermy, and the quality of *in vitro* obtained embryos. In spite of a certain progress, the efficiency and repetitiveness of the IVP method, especially with pigs, is not satisfactory.

The aim of this study was to modify the *in vitro* culture conditions of immature pig oocytes and to establish the quality parameters of boar semen after capacitation that might have an influence on *in vitro* fertilization in pigs. To achieve the main goal of the study the following were undertaken: 1. studying the effect of thymosin, hyaluronic acid and high hydrostatic pressure on *in vitro* maturation of pig oocytes and *in vitro* fertilization; 2. studying the susceptibility of ejaculated and epididymal boar semen, as well as semen without plasma, to capacitation; 3. studying the evaluation of *in vitro* and *in vivo* pig embryos obtained after *in vitro* fertilization.

The research materials for this study were immature pig oocytes obtained from ovaries after slaughter, ejaculated semen from boars of different breeds and epididymal semen obtained from boar epididymis after slaughter. The immature oocytes were cultured *in vitro* to the metaphase II stage in a medium supplemented with thymosin (TYM) or hyaluronic acid (HA). Some of the oocytes were exposed to high hydrostatic pressure (HHP) before or after maturation. The control group consisted of immature oocytes, cultured in a standard medium with no exposure to HHP. Both the ejaculated and epididymal semen were evaluated under the microscope as well as with the computer-assisted analysis method (SCA). Some ejaculates were centrifuged in order to remove the plasma. Selected ejaculated and epididymal semen, as well as semen without plasma, underwent capacitation. Semen with best parameters after capacitation was used for *in vitro* fertilization. The obtained potential zygotes were cultured *in vitro* up to the expanding blastocyst stage. The culture period was 6-8 days. The obtained

blastocysts were evaluated in terms of quality, where the criteria were: the number of cells and the level of nuclear DNA fragmentation. A part of the potential zygotes and 2-4 cells embryos were evaluated *in vivo* after transferring them to synchronized recipients. The transfer effectiveness was evaluated based on ultrasound imaging in the 30th and 45th day after the transfer.

The study has shown that the modification in the *in vitro* maturation conditions of oocytes by supplementing the culture medium with thymosin, results in obtaining a high rate of competent oocytes (97%) that are susceptible to fertilization and development to the blastocyst stage. At the same time, the addition of thymosin to the pig oocytes maturation medium had a positive effect on the quality of obtained blastocysts, by limiting the occurrence of apoptosis. It has been established, that the supplementation of pig oocytes maturation medium with hyaluronic acid has not resulted in the enhancement of their developmental competences and ability to fertilize *in vitro*. A positive effect of HHP on pig oocytes maturation and *in vitro* fertilization has also not been observed. After *in vitro* capacitation of boar semen a similar susceptibility to this process was observed in ejaculated and epididymal semen as well as semen without plasma. The method of extracting the plasma from boar semen did not have a positive effect on its ability to be capacitated and on its *in vitro* fertilization competences. The highest effectiveness of *in vitro* fertilization, measured by the percentage of obtained blastocysts, was observed in ejaculated semen (26%) and epididymal semen (30%). Pig embryos obtained after *in vitro* mature oocyte fertilization in the presence of thymosin were able to undergo full *in vivo* development after their transfer to synchronized recipients.

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