

The abstract of doctoral dissertation of mgr inż. Joanna Porankiewicz entitled:

**“The use of substitutes for conventional antibiotics in boar semen preservation”**

The doctoral dissertation carried out under the supervision of prof. dr hab. Zdzisław Smorąg  
in Department of Reproductive Biotechnology and Cryopreservation

The phenomenon of antimicrobial resistance forces the search for new biopreparations with antimicrobial activity. The aim of the research carried out in this study was to determine the possibility of using substitutes for antibiotics conventionally used in the preservation of boar semen, as well as to determine the composition of a new extender containing AMP (antimicrobial peptides). The subjects of the research were peptide compounds: daptomycin, polymyxin b, Pexiganan, Temporin A, Palm-KK-NH<sub>2</sub>, Camel and Thymosin β<sub>4</sub>. The effect of these peptides on boar semen was investigated and it was established that the addition of Camel and Temporin A peptides negatively influenced motility and survival of spermatozoa.

The analyzed samples were characterized by a large diversity of the composition of microbial contamination, therefore, in order to determine the antibacterial activity of the peptides, a susceptibility test of the selected strains of bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococcus suis*, *Salmonella enterica* and *Klebsiella oxytoca*) was determined by broth microdilution, as recommended by CLSI. All tested peptides, except Thymosin β, showed antibacterial activity against at least one tested strain of bacteria, but none inhibited the growth of all tested bacterial strains. Based on the results of the susceptibility assessment of the tested strains, the optimal composition of the peptide mixture in the extender containing Pexiganan at the concentration of 16 µg / ml and daptomycin at the concentration of 2 µg / ml was determined.

The next step of the study was to determine the effect of a new extender containing the composition of selected peptides on the semen. There were no statistically significant differences in motility and survival of spermatozoa during storage in the experimental extender compared to the reference extenders. The sperm motility analysis of individual boars revealed lower motility of semen from three of them, with an examined extender, compared to the control group, while the motility of semen from two males was unremarkable in all extenders. After 6 days of storage, a higher ( $p \leq 0.05$ ) percentage of spermatozoa with high mitochondrial membrane potential was found in Biosolwens Plus extender without antibiotics, as well as BTS Plus compared to the experimental extender. However, after 4 days of storage,

a higher ( $p \leq 0.05$ ) percentage of apoptotic spermatozoa was found in the experimental extender compared to Biosolwens Plus and Biosolwens Plus without gentamicin.

The study on the use of peptides with antibacterial properties in the preservation of boar semen at positive temperatures is the starting point for the development of detailed solutions aimed at replacing the standard antibiotics used for the antibacterial semen storage. The use of AMP is a part of the stream of research aimed at reducing the use of antibiotics. The obtained results also indicate the need for further research to confirm the effectiveness of AMP peptides and to define the specific conditions of their use.

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