

## PATHWAYS TO ANTIBIOTIC-FREE PRESERVATION OF BOAR SPERMATOZOA

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With worldwide increased use of artificial insemination (AI) in swine reproduction, the contribution of antibiotics in semen extenders to the global bacterial resistance threat came into focus. Evolving multi drug resistances in AI centers caused by overuse of antibiotics together with institutional bans of the remaining efficient antibiotics are driving forces to search for alternative solutions. Risk associated with insufficient control of bacteria were identified in low fertility, transmission of diseases in sow farms, increase of antimicrobial resistances and loss of market position. On this basis, typically, the aim is complete eradication of bacteria in the extended semen portions. In the process of risk assessment, however, this goal should be reconsidered. An array of studies demonstrated that moderate amounts of bacteria (for most strains greater than 10<sup>6</sup> colony forming units/ml) do not harm sow fertility or health. Moreover, bacteria are now increasingly recognized as a natural cellular component of ejaculates which promotes fertility chances by immunogenic interaction with the female tract. Nonetheless, effective antimicrobial control is necessary to maintain microbial growth under the identified bacteria-specific thresholds for sperm damage during several days of semen storage. Alternatives to conventional antibiotics must not be toxic to sperm or to the environment, should have a high antimicrobial effectivity without evoking resistances, and should be easy and economical useable in AI centers. In this workshop contribution, alternatives to antibiotics in extended boar semen are reviewed, including novel evolving concepts with the use of antimicrobial effective semen extenders and low temperature storage. Strategies of antibiotic-free preservation of boar semen will be discussed in context of the principal steps of risk assessment process.

## INNOVATIVE SEMEN EXTENDER FORMULATIONS WITHOUT ANTIBIOTICS

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The demand for animal protein increases every year. For decades antibiotic substances have been used indiscriminately as growth promoters in different animal species; this, among other factors, places us today in front of the most critical global health problem, namely antibiotic resistance. The main objective of this work was to develop new semen extenders formulation containing alternative substances to antibiotics that are safe from the biological point of view and economically viable for industrial production.

The effects of biomolecules included in a formulation used to dilute boar semen were analyzed, the experimental input variables were planned under a D-optimal design experimental approach. Two substances coded as EL1 and EP1 applied at three concentration levels (-1, 0, and +1) were analyzed, and their effect on sperm quality and their ability to decrease the initial bacterial burden was evaluated. A negative correlation between progressive motility and total bacterial count at 0 h was observed. The tested formulations showed good quality in terms of motility (93%± 1.5) and progressive motility (86% ± 1.2) in the substance concentration groups of the experimental design. It was possible to identify a combination that obtained the best results in motility and acrosome integrity and decreased initial bacterial load. The formulation containing EP1 at lower concentration and EL1 at higher concentration provided values higher than 95% of motility with only 2% of damaged acrosomes and a reduction of the bacterial load even after three days. These values were statistically significant (p< 0.005) values compared to the control, namely semen extender without antibiotics.

We can conclude that using the proposed biomolecules as components of boar semen extender represents a suitable alternative to control the bacterial burden in boar semen doses for artificial insemination. This work offers the basis for a new product and generates new alternatives for an innovative concept of green production.