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## Decreasing the use of antibiotics in the pig artificial insemination industry

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### 01. Rationale

The main objective of this project has been to obtain a method for the sanitisation of porcine semen that allows the collection, preservation and subsequent use of chilled semen for artificial insemination in an antibiotic-free environment.

Pig reproduction is currently carried out by artificial insemination. Since porcine semen can carry micro-organisms, it is essential that the cooling diluents include antimicrobial agents in order to control bacterial growth in semen and to avoid losses, both in the semen production centres and on the pig farms where they are used. Likewise, restrictions on the use of antibiotics have been increasing in order to prevent bacteria from developing resistance. Therefore, in this project, the substitution of the current preservation media for others that contain antimicrobial peptides rather than antibiotics is proposed.

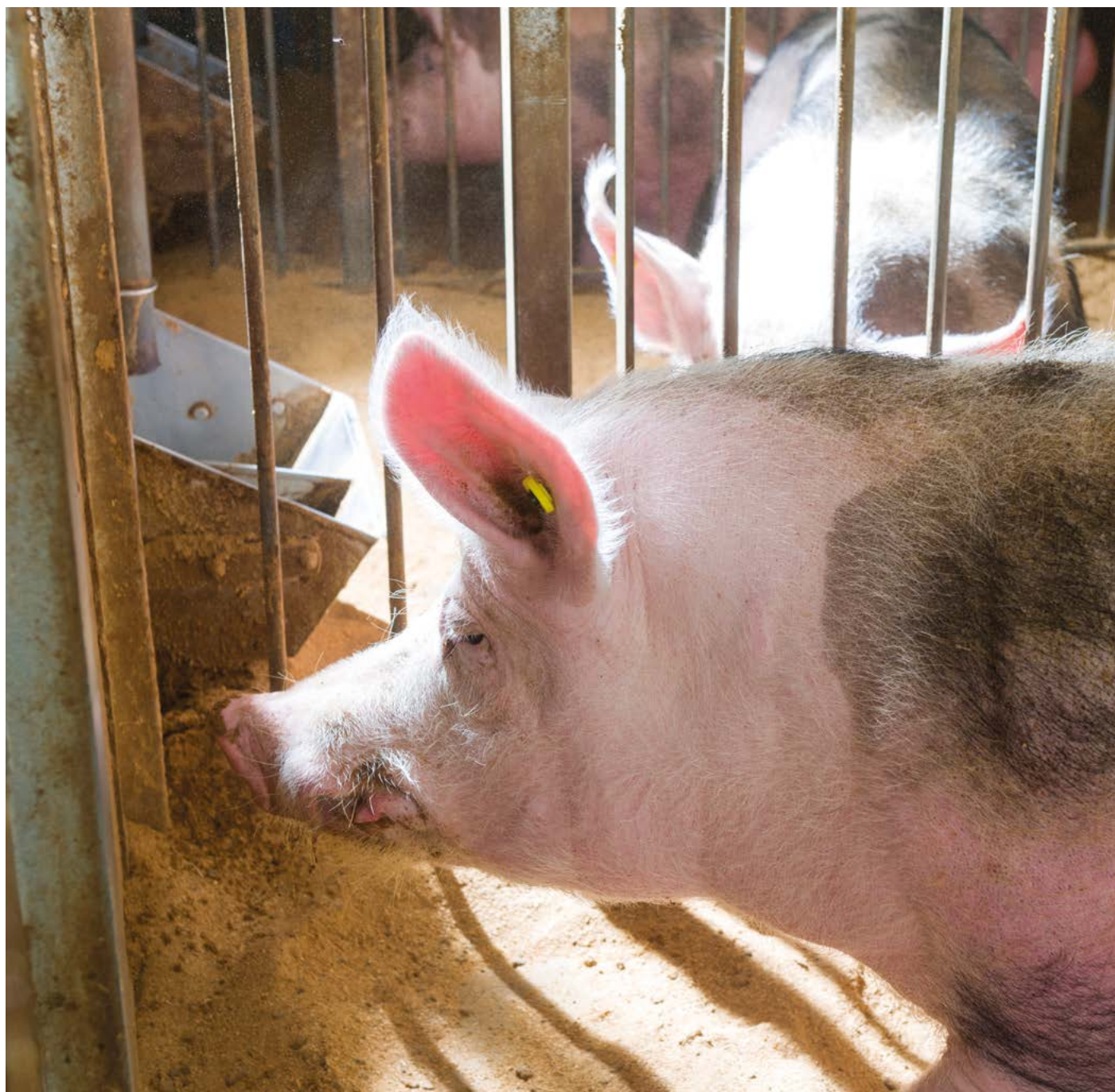


Photo: Operational Group.

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The specific technical objectives were as follows:

- Evaluate the effect of antimicrobial peptides on in vitro sperm quality.
- Validate the effectiveness of peptides in the production of semen doses.
- Evaluate the effectiveness of peptides in insemination centres with different environmental conditions.
- Determine the impact of antimicrobial peptides on the fertilising power of sperm.
- Draft a working protocol for the use of antimicrobial peptides in the preparation of semen doses.

## 02. Results and conclusions

Antimicrobial peptides have been shown to control the growth of aerobic and anaerobic bacteria at relatively low concentrations without detrimental effects on either sperm quality or fertility. Furthermore, the effectiveness of these peptides does not vary with environment or seasonality. Therefore, they can be safely used as a substitute for antibiotics in porcine semen preservation diluents at 17°C.

According to the results obtained, the LEAP2 and PMAP23 peptides were the most suitable ones, with the highest capacity to control the growth of anaerobic bacteria.

With regard to the analysis of the impact of the presence of LEAP2 and PMAP23 peptides on the response of spermatozoa to in vitro capacitation and the progesterone-induced acrosome reaction, the highest concentrations of both peptides were ruled out, since both have a negative impact on these phenomena.

Thus, from the in vitro experimentation it was observed that, at the lowest concentration, the LEAP2 peptide does not decrease the fertilising capacity of the spermatozoa, and from the in vivo results it was concluded that the use of the LEAP2 peptide, at this con-

centration, and in a medium without antibiotics, makes it possible to control aerobic and anaerobic microbial growth and has no detrimental effects on either sperm quality or fertilising capacity. Therefore, this peptide, whose effectiveness does not vary with either environment or seasonality, could be safely used as a substitute for antibiotics in porcine semen preservation diluents at 17°C.

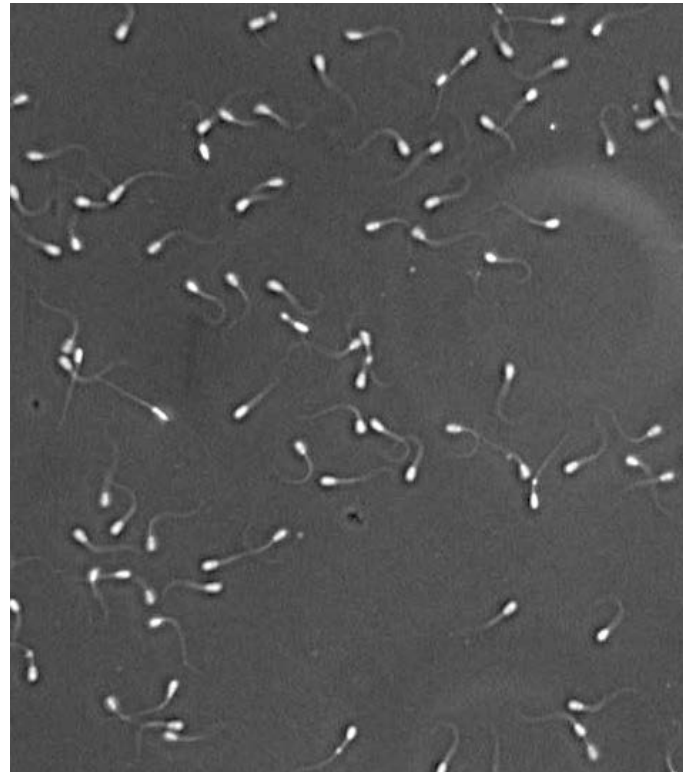
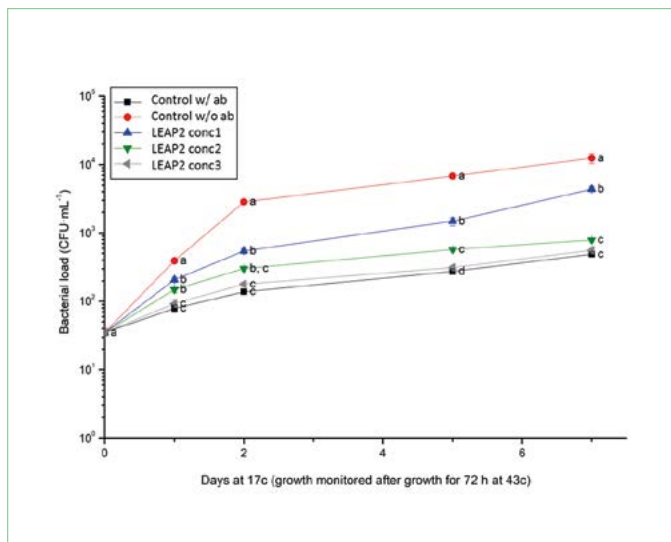
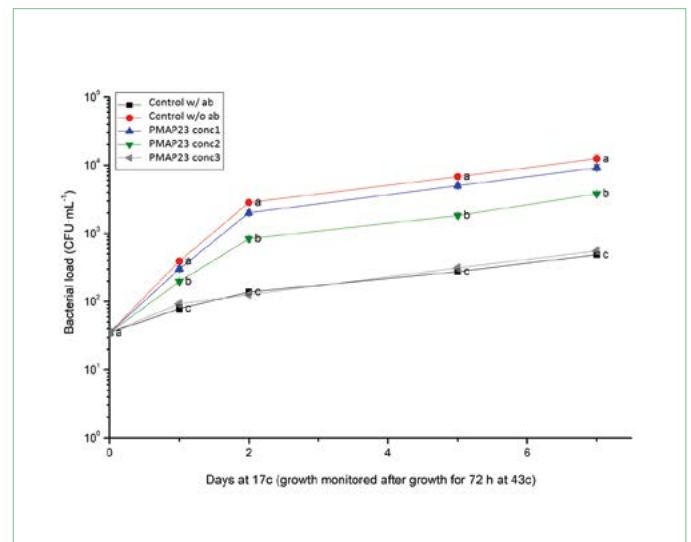


Photo: Operational Group.



**Figure 1.** Growth results of anaerobic bacteria (mean ± SEM) after culture for 72 hours at 43°C and in the presence of LEAP2 peptide. The different letters represent significant differences ( $P < 0.05$ ) between treatments on the same day of storage. Source: Operational Group.



**Figure 2.** Growth results of anaerobic bacteria (mean ± SEM) after culture for 72 hours at 43°C and in the presence of PMAP23 peptide. The different letters represent significant differences ( $P < 0.05$ ) between treatments on the same day of storage. Source: Operational Group.