Review Article

Antimicrobial Peptides: Could it be an Alternative to Antibiotics in Boar Semen Extender?

Krittika Keeratikunakorn[1,2] Panida Chanapiwat[2] Kampon Kaeoket[2*]

Abstract

Since antimicrobial resistance is a major global issue, "One Health" approaches such as the use of antimicrobial peptides (AMPs) were developed as an alternative antimicrobial agent which might be used in pig farms to reduce or replace non-rational antibiotics usage. Although AMPs showed several effects on antimicrobial agents, including against bacteria, fungi, protozoa, and viruses, as well as effects on host immunity, the ability to eliminate bacteria while providing the minimum amount of damage to the host cell is a particularly outstanding characteristic. Two major mechanisms of action of AMPs include membrane-active and intracellular-active. During the past decade, many AMPs have been tested for a replacement of antibiotics in boar semen extenders. Many studies reported the potential for using AMPs, whether as single or cocktail AMPs; however, their antibacterial effectiveness depends on the concentration and incubation time. Using AMPs also showed promising results on boar sperm quality and fertility tests on the pig farm. This review article discusses the use of AMPs as antibacterial agents for supplementation in boar semen extenders and provides future perspectives on the development of AMPs and how to use them properly.

Keywords: Antimicrobial peptides, bacteria, boar, one health, semen extender

[1]*Department of Anatomy, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand*
[2]*Semen laboratory, Department of Clinical Sciences and Public Health, Faculty of Veterinary Science, Mahidol University, Nakhon Pathom 73170, Thailand*
****Correspondence****: kampon.kae@mahidol.edu* (K. Kaeoket)
Received October 11, 2024
Accepted December 17, 2024

Introduction

Antimicrobial resistance (AMR) has triggered concerns regarding the use of antibiotics in human as well as animal treatment, especially in the livestock industry. Antibiotic-resistant bacteria of animal origin have been detected in the ecosystems of livestock farms (Landers *et al*., 2012). Notably, these antibiotics belong to the critically important antibiotic group (CIA) (Lekagul *et al*., 2020). The most commonly used antibiotics on pig farms at all production stages by feed medication are amoxicillin and tiamulin, this may lead to the non-rational use of antibiotics (Landers *et al*., 2012; Lekagul *et al*., 2020).

In addition to directly supplying antibiotics to the pigs, the practice of adding antibiotics to liquid boar semen preservation aims to extend the shelf life of the semen storage and minimize spermatozoa damage, minimize endometritis or post-mating vaginal discharge caused by infection or inflammation in sows/gilts that results from bacterial contamination in the semen dose (Kaeoket *et al*., 2003; 2005; Maes *et al*., 2008; Schulze *et al*., 2016; Vickram *et al*., 2017). Antimicrobial resistance genes, such the *mcr-2* gene, have been detected in sow with vaginal discharge samples, while the use of antibiotics in boar semen extenders might similarly affect the bacteria flora in sow vagina, possibly resulting in resistance, as it had happened in boar semen (Kellerman *et al*., 2022; Keeratikunakorn *et al*., 2023; 2024[d]; Ngo *et al*., 2024). This might happen when using a commercial semen extender to dilute swine semen, which depends on the concentration of sperm in the original ejaculate rather than the number of bacteria present, resulting in decreased antibiotic concentrations and not enough to inhibit bacteria growth (Schulze *et al*., 2017). Boar semen with antibiotics is used annually in around 12.8 million liters worldwide (Contreras *et al*., 2022). Many techniques have been developed to manage bacterial growth in boar semen by minimizing the use of antibiotics (Santos and Silva, 2020). Strategies for controlling bacterial growth in semen doses encompass various approaches: (i) physically removing bacteria through methods such as single-layer centrifugation (Morrell *et al*., 2019; Ngo *et al*., 2024); (ii) utilizing antimicrobial peptides (AMPs) or short antimicrobial lipopeptides (Hensel *et al*., 2020); (iii) using other substances like lysozyme, kojic acid, and silver nanoparticles (AgNPs) (Schulze *et al*., 2019; Shaoyong *et al*., 2019; Pérez-Duran *et al*., 2020); and (iv) storing the semen dose at a low temperature (5°C) without antibiotic supplementation (Jäkel *et al*., 2021; Waberski and Luther, 2024). Currently, AMPs serve as a beneficial option for adding to semen extenders because of their ability to eliminate bacteria without detrimental effects on sperm cells (Andersson *et al*., 2016; Keeratikunakorn *et al*., 2024[b]). Lysozyme, the first AMP, was discovered by Alexander Fleming in the 1920s; in the 2000s, AMPs gained more attention and research (George *et al*., 2023). Studies have been performed on the practical application of AMP, which is often used as a feed additive in pig farms. One of those studies examined the use of lactoferricin to prevent *E. coli* infection (ETEC) in 21-day-old piglets and reduce antibiotics use on farm (Tang *et al*., 2011; Xu *et al*., 2020; Robles Ramirez *et al*., 2024). The purpose of the present review was to summarize previous research publications on preserving boar semen by applying AMPs as an alternative to antibiotics as One Health approach.

Bacteria contamination in boar semen

Artificial insemination (AI) with liquid boar semen is a common practice in the swine industry for the reason that AI can minimize disease transmission, improve genetics, and increase piglet production and quality (Pezo *et al*., 2019). Semen extenders are essential for maintaining the viability and quality of sperm because they prevent cold shock, control pH and osmotic pressure, and inhibit bacteria from developing (Pezo *et al*., 2019). For producing the AI dose, boar semen is diluted with a nutrient-rich semen extender and kept at a low temperature. As a result of nutrients in the extender that are good for bacterial growth and consequently impair semen quality, antibiotics are usually added to boar semen extenders for AI (Hensel *et al*., 2020; Schulze *et al*., 2020). The most common causes of bacterial contamination in boar include people, the barn, the preputial diverticulum, hair, skin, respiratory secretions, and boar feces, as well as contaminating the collector's hands or the instrument used for collecting containers (Althouse and Lu, 2005; Kuster and Althouse, 2016; Costinal *et al*., 2021). Despite being non-pathogenic, the bacterial contamination in the semen still negatively impacts spermatozoa and other semen quality parameters. This consequently causes a decrease in semen quality, which may affect the productivity of pig farms (Maes *et al*., 2008). Furthermore, the contaminated semen also resulted in embryonic or fetal death and endometritis in sows (Maes *et al*., 2008). In addition to the bacteria themselves, the secondary metabolites (such as endotoxins and exotoxins) also contributed to the adverse consequences of contamination (Althouse, 2024). This indicates that in addition to living bacteria, dead bacteria can also harm sperm by producing toxins such as glycoproteins and lipopolysaccharides (Farsimadan and Motamedifar, 2020).

Bacteriospermia was associated with fertility rate, according to the study of infertile men, the prevalence of bacteriospermia is 35.3%, while *Enterococcus faecalis* and coagulase-negative *Staphylococcus* are the major organisms of contamination (Vilvanathan *et al*., 2016). Bennemann *et al.* (2018) reported that 80% of the boar semen samples from southern Brazil were contaminated with bacteria and the major species of bacteria, including *Staphylococcus hyicus*, *E. coli,* and *Alcaligenes faecalis*. In addition to that, *P. aeruginosa*, *E. coli,* and *P. mirabilis* were among the Gram-negative bacteria that were discovered to be contaminated in fresh boar semen in particular experiments (Keeratikunakorn *et al*., 2023). Meanwhile, the extended semen was mostly *Staphylococcus* spp. and *E. coli* (Bennemann *et al*., 2018). In addition, it was demonstrated that 99% of boar semen samples found aerobic bacterial contamination, which caused a decrease in sperm motility, sperm agglutination, acrosome damage, and plasma membrane disruption as well as an increase in the total bacterial count after

storage for 3 days (Gaczarzewicz *et al*., 2016; Keeratikunakorn *et al*., 2024[b]). Sperm agglutination is often the primary effect and significantly influences other semen quality parameters (Althouse and Lu, 2005). The degree of negative effects may be related to the species and concentration of bacterial contamination (Althouse and Lu, 2005; Gaczarzewicz *et al*., 2016). In addition to pathogenic bacteria, it has also been found that pig semen contains probiotic bacteria, including *Lactobacillus* spp. (Ngo *et al*., 2023). Although some substances can be produced by *Lactobacillus* spp. that inhibit the growth of bacteria isolated from boar semen (Keeratikunakorn *et al*., 2023), which could enhance the quality of the semen.

Although many factors influence boar semen quality, including housing, feed, health management, and environmental conditions, efforts to enhance semen storage must also address bacterial contamination as a contributing factor (Rodriguez *et al*., 2017). To avoid the adverse effects of bacterial contamination, antibiotics are commonly added to semen extenders to restrict bacterial growth and reduce the negative effects of bacteriospermia and bacterial toxins, which depend on bacterial count (Althouse and Lu, 2005; Rodriguez *et al*., 2017). A variety of antibiotics are frequently used in boar semen extenders, either individually or in combination; subsequently, antibiotic resistance in fresh boar semen and bacteria isolated from pig vaginas and feces has been reported (Nguyet *et al*., 2022; 2023; 2024; Kaewchomphunuch *et al*., 2022). These bacteria have been reported for resistance to many antibiotics, including gentamicin, colistin, tiamulin, and penicillin, along with third-generation cephalosporins like ceftriaxone (Bresciani *et al*., 2014; Bennemann *et al*., 2018; Keeratikunakorn *et al*., 2023). A commonly utilized antibiotic used in boar semen extenders, gentamicin was found to be highly effective in limiting the growth of Gram-negative bacteria than Gram-positive bacteria (Gaczarzewicz *et al*., 2016).

Antimicrobial peptides (AMPs)

Antimicrobial peptides are amphipathic biological molecules composed of 10–50 amino acids with a small positive charge ranging from +2 to +13. They have a broad spectrum and dose-dependent activity against bacteria, fungi, protozoa, and viruses through direct killing (Xiao *et al*., 2015; Andersson *et al*., 2016; Mahlapuu *et al*., 2016; Wang *et al*., 2016; Zhang and Gallo, 2016; Kumar *et al*., 2018). The majority of AMPs are produced by animals, plants, bacteria, fungi, and archaea, respectively (Kumar *et al*., 2018).

AMPs usually interact directly and immediately with the outer cell wall of bacteria, binding rapidly to components such as lipopolysaccharide (LPS) in Gram-negative bacteria or teichoic acid in Gram-positive bacteria (Mahlapuu *et al*., 2016; Wang *et al*., 2016; Fazly Bazzaz *et al*., 2021). This specificity arises from distinctions in membrane composition between bacteria and animals (Figure 1). Consequently, AMPs primarily target bacterial membranes while sparing animal membranes (Mahlapuu *et al*., 2016). The positively charged AMPs strongly interact with the negatively charged lipopolysaccharides or teichoic acid on the outermost surface of the bacterial cell (Pushpanathan *et al*., 2013; Bechinger and Gorr, 2017; Fazly Bazzaz *et al*., 2021), but have weak interactions with the sperm membranes because the negatively charged are at the innermost, near the cytoplasm (Mahlapuu *et al*., 2016; Kumar *et al*., 2018) (Figure 1). In addition, the amphipathic property of AMPs can be used for folding into structures with both hydrophobic and hydrophilic surfaces (Andersson *et al*., 2016). A single AMP can interact via several mechanisms depending on the peptide's structure, peptide-lipid ratio, and the characteristics of the lipid membrane (Bechinger and Gorr, 2017). Generally, AMPs are generated by chemical methods rather than by bioreaction. Nevertheless, some AMPs such as beta-defensins have been produced in *E. coli* (Li *et al*., 2018). AMPs mechanisms are divided into two classes of action: membrane-active AMPs and intracellular-active AMPs (Xiao *et al*., 2015). Membrane-active AMPs are a primary mechanism for killing target cells by disrupting the cell membrane, resulting in membrane dysfunction, rupture, and lysis of bacterial cells, which can be proved through the investigations of bacterial DNA leakage and scanning electron micrographs as recently shown by a study of PA-13 (Figure 2A) (Keeratikunakorn *et al*., 2024[a]). The AMPs action can be divided into three models, including barrel-stave, toroidal, and carpet models (Figure 3) (Xiao *et al*., 2015; Wang *et al*., 2016). The barrel-stave model is a transmembrane pore model in which AMPs directly insert into the lipid bilayer of target cells by forming a peptide-lined transmembrane pore (Xiao *et al*., 2015; Mahlapuu *et al*., 2016; Kumar *et al*., 2018), which causes a leakage of cytoplasmic content, resulting in cell death (Guilhelmelli *et al*., 2013). A few AMPs, such as protegains and paradoxin, exhibit barrel-stave models (Kumar *et al*., 2018). The toroidal model, also known as the wormhole model, is considered one of the most cell membrane-active mechanisms in a transmembrane pore, like a barrel-stave model (Yeaman and Yount, 2003; Kumar *et al*., 2018). However, this particular model inserts AMPs into the target cell membrane by inducing membrane curvature and forming membrane pores (Xiao *et al*., 2015; Kumar *et al*., 2018). Nevertheless, the end outcome is similar to the barrel-stave model (Guilhelmelli *et al*., 2013). The AMPs have been shown to have toroidal models such as magainin 2, lacticin Q, and melittin (Kumar *et al*., 2018). The carpet model is non-specific membrane permeabilization which degenerates the target cell by covering the cell membrane surface like a "carpet" without forming a membrane pore (Xiao *et al*., 2015; Wang *et al*., 2016; Kumar *et al*., 2018). Changes in membrane fluidity or reductions in membrane barrier properties cause the loss of membrane integrity (Yeaman and Yount, 2003), which in turn produces a detergent-like effect and the formation of micelles (Mahlapuu *et al*., 2016; Kumar *et al*., 2018). AMPs, including cecropin, indolicidin, aurein 1.2, and LL-37, exhibit carpet models in their action (Kumar *et al*., 2018). For a not-complete carpet model, the AMPs will remove the bacterial membrane by partial micellization (Figure 3) (Bechinger and Gorr, 2017).

Intracellularly active AMPs have the ability to kill a target cell by limiting the synthesis of proteins, DNA,

RNA, and cell walls without rupturing the target cell membrane (Xiao *et al*., 2015; Kumar *et al*., 2018). Kumar *et al*. (2018) suggested that these mechanisms are similar to penicillin. However, most of the action of AMPs is membrane-active (Bechinger and Gorr, 2017). According to Yeaman and Yount (2003), the interactions of charge, conformation, polar angle, hydrophobicity, and amphipathicity determined how the AMP structure affected action (Figure 4).

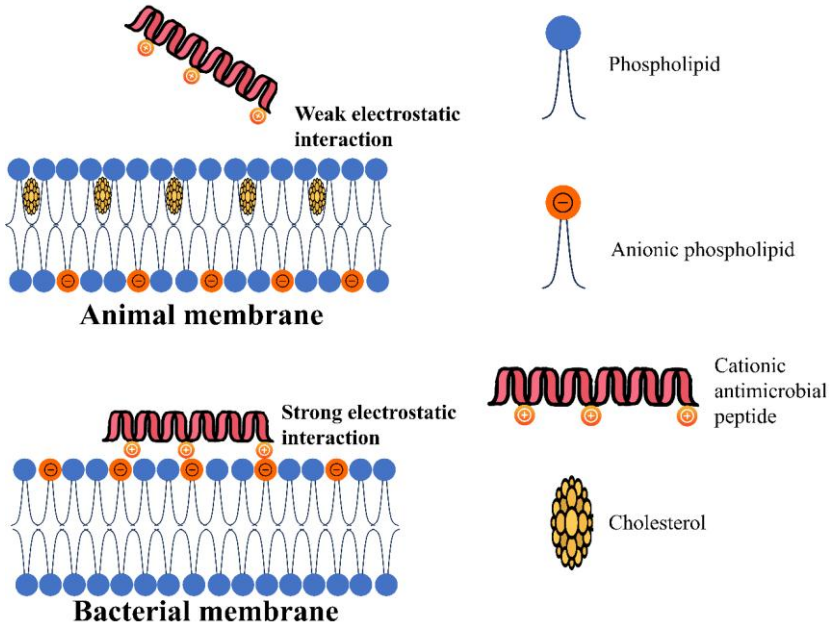

Figure 1 The selective mechanism of antimicrobial peptide between animal and bacterial membrane. On the bacterial cell's outermost surface, the negatively charged lipopolysaccharides or teichoic acid interact strongly with the positively charged AMPs. (adapted from Mahlapuu *et al*., 2016; Kumar *et al*., 2018).

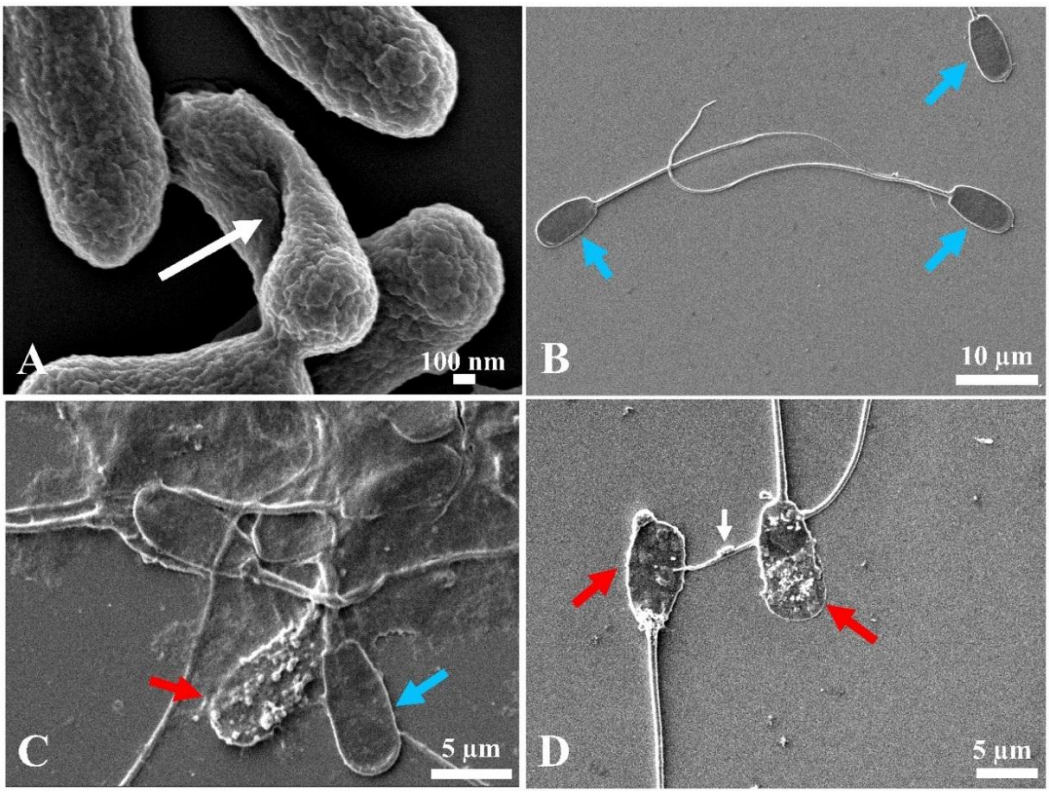

Figure 2 The scanning electron micrograph of AMPs on *E. coli* (A) and boar sperm morphology (B-C). *E. coli* with ruptured cell surface after incubation with PA-13 at 15.625 µg/mL (white arrow) (A). The abnormal (red arrow) and normal (blue arrow) boar sperm were stored in a semen extender supplemented with BiF2_5K7K (C). The non-antibiotic semen extender incubation showed anomalies on the sperm's head (red arrow) and attracted bacteria (white arrow) (D). The antibiotic-treated semen extender presented normal boar sperm morphology (blue arrow) (B).

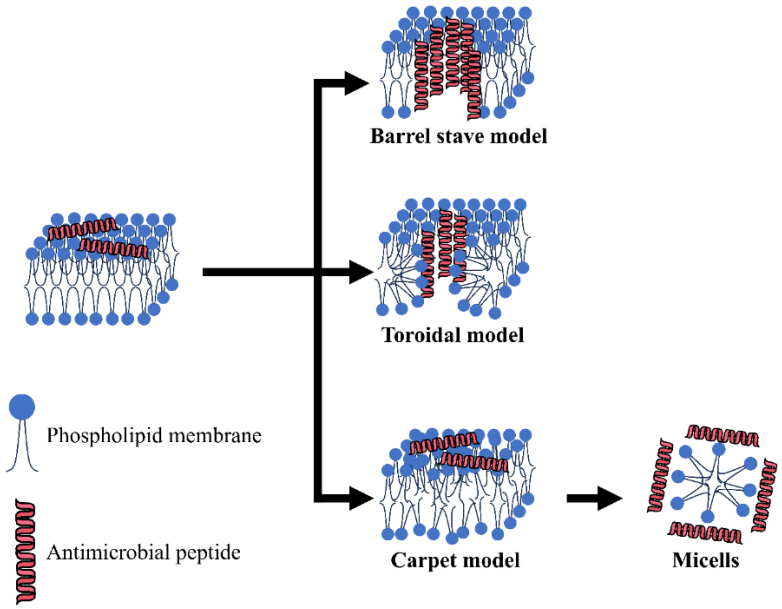

Figure 3 The action of membrane-active AMPs, including barrel-stave, toroidal, and carpet models (adapted from Pushpanathan *et al*., 2013; Mahlapuu *et al*., 2016; Kumar *et al*., 2018).

One of the most important ideas in selecting the most effective AMPs is the structure-activity relationship (Yeaman and Yount, 2003). An example of the relationship between the charge and conformation of antimicrobial peptides is shown in Figure 5. AMPs that have a positive electrical charge and are localized on the same side of the helical wheel exhibit varying levels of bactericidal performance (Klubthawee *et al*., 2020).

Generally, it is understood that using AMPs would not cause widespread resistance due to their mode of action. Compared to antibiotics, the development of antimicrobial resistance is less probable (Assoni *et al*., 2020). However, there remains a potential for bacterial resistance, as indicated by Bechinger and Gorr (2017). The development of bacterial resistance may be impeded by the capability of individual AMPs to interact with multiple targets or multiple AMPs to interact with a single target (Bechinger and Gorr, 2017). A primary mechanism enabling bacterial survival involves the modification of the bacterial surface charge (Andersson *et al*., 2016). Presently, the development of AMPs-resistant pathogens cannot be definitely predicted (Schulze *et al*., 2016). In order to design AMPs that have the opportunity to be effective antimicrobial agents, it was important to understand the possibility of AMP resistance mechanisms (Guilhelmelli *et al*., 2013).

Application AMPs in boar semen preservation

Boar semen can be contaminated by bacteria, which are usually found in the boar reproductive tract or during semen collection (Pezo *et al*., 2019). To prevent the previously mentioned problem, semen extenders can be supplemented with antibiotics or other antimicrobial agents, including AMPs. The antimicrobial additive must show characteristics as follows: broad-spectrum activity, non-cytotoxicity (dose- and time-dependent toxic), no effect on fertility, high stability, ease of use, and low cost, and decreasing antimicrobial resistance by target cells (Figure 6) (Schulze *et al*., 2015; 2016; Li *et al*., 2018; De Mandal *et al*., 2021). Additionally, because of these characteristics, it is important to perform an extended study to identify the optimal concentration of each kind of AMP for use in practice in the AI unit. Consequently, AMP use is not yet widespread (Keeratikunakorn *et al*., 2023[c]). However, occasionally, the quality of semen wasn't impacted by low concentrations of bacteria in boar semen. However, it's additionally critical to consider the possibility of endometritis from using boar semen contaminated with bacteria (Constinar *et al*., 2021). For the most effective outcomes, using AMPs to minimize the number of bacteria should be performed in conjunction with extremely thorough hygiene management (Ros-Santaella *et al*., 2024). According to the examination of three AMPs (A-11, AP19, and PA-13) on bacteria that were isolated from fresh boar semen. The growth of Gram-negative bacteria, which include *E. coli*, *P. aeruginosa*, and *P. mirabilis*, was inhibited by these three AMPs (Keeratikunakorn *et al*., 2024[a,c]). Especially PA-13, this AMP can inhibit *E. coli* carrying antibiotic-resistant genes (*mcr-3* and *int1*) (Keeratikunakorn *et al*., 2024[a]). However, the efficacy of PA-13 was concentration-dependent; an excessive concentration may decrease PA-13's ability to kill bacteria due to the electrostatic repulsion, which leads the antimicrobial peptides to attach to the bacterial membrane less effectively (López Cascales *et al*., 2018; Keeratikunakorn *et al*., 2024[a]). Similar to gentamicin, both AMPs (A-11 or AP-19) were able to limit bacterial growth in boar semen for AI for 24 hours after storage at 18°C when combined with a boar semen extender, but the use of gentamicin can control the growth of bacteria during 3-4 days of storage (Keeratikunakorn *et al*., 2024[b,c]). Although AMPs are an effective alternative

to antibiotics for boar semen extenders, not all AMPs are spermatozoa safe. For example, Nisin is a cationic AMP that shows spermicidal activities and immobilizes spermatozoa. Therefore, Nisin is tested for developing contraceptives in humans (Aranha *et al*., 2003). Sancho *et al*. (2017) investigated the effect of antimicrobial peptide protegrine 1 (PG 1) on boar semen and found that PG 1 can reduce the bacterial count in boar semen stored at 17°C. Unfortunately, the PG 1 treated group had lower sperm viability as compared to the control group. In contrast, the boar sperm quality remained maintained following incubation with 15.625–62.50 µg/mL of BiF2_5K7K at 18°C, especially in terms of total and progressive motility during 3 days of incubation. Furthermore, it was discovered that BiF2_5K7K protects against bacterial damage to the morphology of the boar sperm (Figure 2B-D) (Keeratikunakorn *et al*., 2024[b]). Similarly to the effects of A-11 and AP19, the optimal concentrations for boar sperm quality during storage at 18°C for 3 days were 15.625-62.5 and 15.625-31.25 µg/mL, respectively (Keeratikunakorn *et al*., 2024[c]).

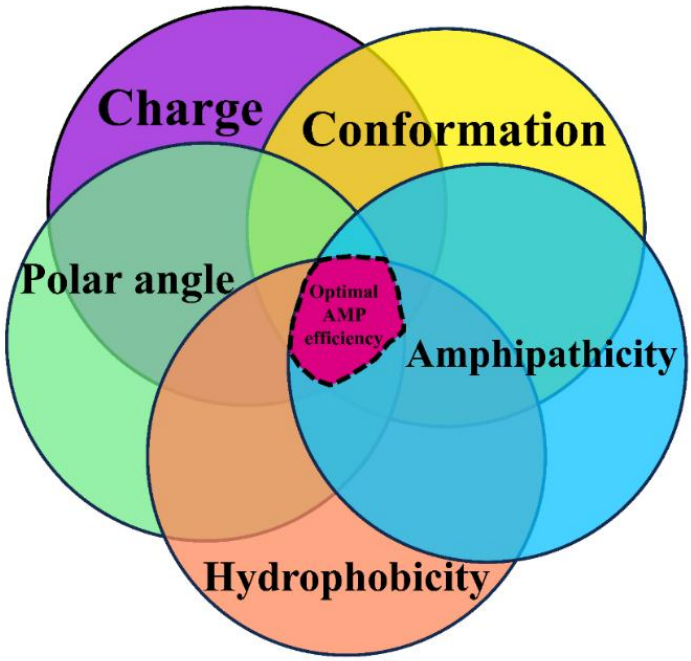

Figure 4 The relationship between charge, conformation, polar angle, hydrophobicity, and amphipathicity for the AMP structure. The ideal AMP efficacy is shown as a pink circle (adapted from Yeaman and Yount, 2003).

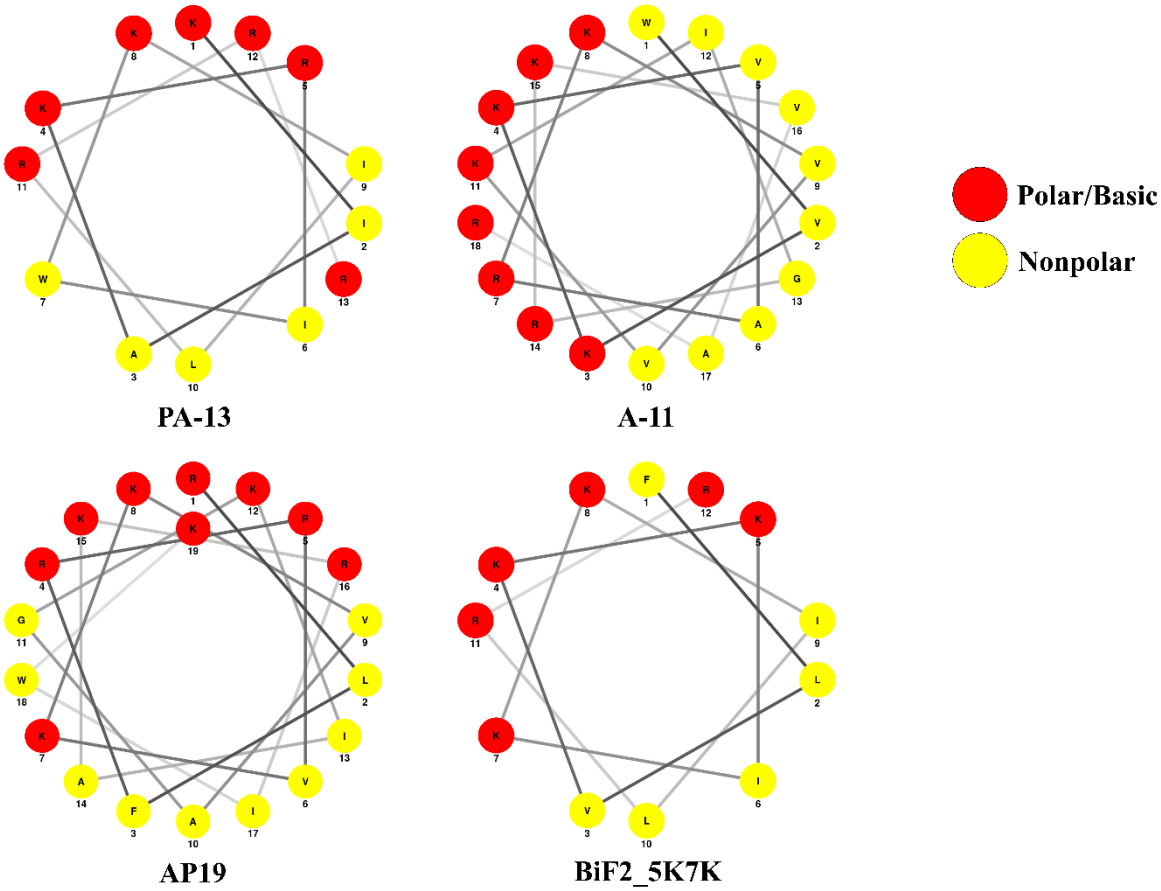

Figure 5 The graphic demonstrates the relationship between the charge and conformation of four AMPs, including PA-13, A-11, AP19, and BiF2_5K7K, with the helical wheel projections. The structure and order of amino acids affect the distribution of electric charges, causing variations in antibacterial efficiency, as demonstrated by a helical-wheel diagram of AMPs.

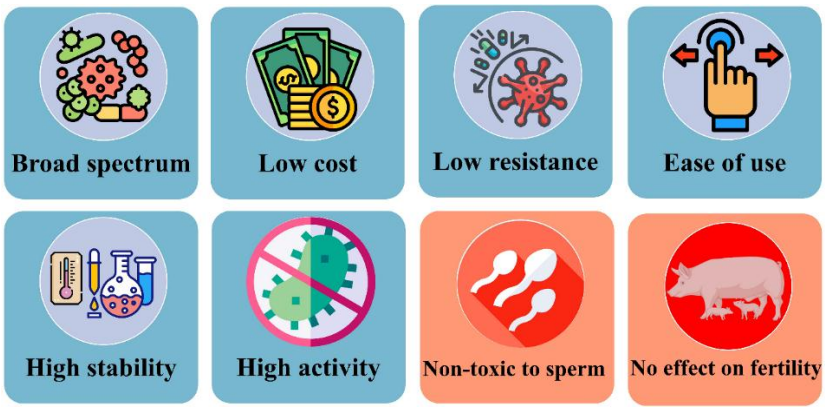

Figure 6 The optimal characteristic of AMPs for boar semen extender supplementation.

The synthetic cyclic hexapeptides, c-WWW and c-WFW, are cationic AMPs that can inhibit two Gram-positive and eleven Gram-negative bacterial growth in liquid boar semen preservation, such as *E. coli* and *Staphylococcus aureus*, but not for *Proteus* spp. (Speck *et al*., 2014; Schulze *et al*., 2016). According to previous reports, semen extenders supplemented with cationic AMPs, showing antibacterial activity without damaging boar spermatozoa, can be used for AI after being stored at 16°C (Schulze *et al*., 2014). Furthermore, the use of cationic AMPs has the potential to lower the concentration of antibiotics in semen extenders

(Schulze *et al*., 2014). At its optimal concentration of 4 µM, c-WFW can be combined with 16 µg/mL of gentamicin. This combination effect is similar to gentamicin at a concentration of 250 µg/mL (Schulze *et al*., 2014). It is worth noting that AMPs and antibiotics may be combined to minimize the adverse effects of an excessively high AMP concentration on boar sperm and its high cost compared with antibiotics. It has been shown in liquid-stored boar semen that a combination of 0.16 g/L epsilon-polylysine (ε-PL) and 0.125 g/L gentamicin resulted in sperm qualities the same as when adding 0.25 g/L gentamicin alone (Shaoyong *et al*., 2019). Additionally, it has been reported that combining more than two types of AMPs could effectively inhibit the growth of multiple drug-resistant bacteria (Santos *et al*., 2022).

Porcine myeloid antimicrobial peptide 37 (PMAP-37) at a concentration of 3 µM was one interesting choice for replacing the common antibiotic in boar semen extenders (Bussalleu *et al*., 2017). Comparing this AMP to kanamycin supplemented in BTS semen extender, PMAP-37 can inhibit bacterial growth and showed less impact on sperm motility and viability after 10 days of storage at 17°C than PMAP-36, the porcine myeloid antimicrobial peptide and PR-39, the proline-arginine-rich antimicrobial peptide, (Bussalleu *et al*., 2017). The latter PR-39 can be isolated from the porcine small intestine and also from the blood of pigs (Yu *et al*., 2010).

Beta-defensins are a large family of AMPs that have antimicrobial activity (Kalita, 2015; Avila, 2017). Some beta-defensins are highly expressed in the male reproductive tissue of vertebrates and localized in sperm cells (Avila, 2017). It has been reported that porcine beta-defensins-1 (PBD1) and-2 (PBD2) can be used at a concentration of 3 µM in the antibiotic-free extender in order to control bacterial growth and have no negative effect on sperm quality (Puig-Timonet *et al*., 2018); however, they did not study the effect on gilt/sow reproductive performance (Puig-Timonet *et al*., 2018). Recently, Keeratikunakorn *et al*. (2024[b]) reported that the BiF2_5K7K at a concentration of 31.25 µg/mL inhibited the growth of bacteria in diluted boar semen and stored at 18 °C without adding antibiotic. Moreover, it was also found that there was no effect on semen quality and reproductive performance of sows after AI in a commercial pig farm. More research is necessary to determine the mechanism by which the specific antimicrobial peptides may affect the sow's reproductive performance (Keeratikunakorn *et al*., 2024[b]). Generally, it is understood that using AMPs on farms, however, requires careful investigation, especially in terms of the possible impact on the environment and the development of bacteria resistant to AMPs (Naiel *et al*., 2023).

Conclusion

AMP is a novel antimicrobial agent that can either reduce or replace the use of antibiotics to solve antimicrobial resistance in pig farms. The supplementation of AMPs in boar semen extenders has been studied, and its effect of controlling bacterial growth has no negative effect on spermatozoa. It also has a promising fertility test in pig farms. In order to reduce non-rational antibiotic use in pig farms, future research on AMPs should focus on selecting the best AMP and whether to use it as a single or cocktail AMP.

Acknowledgments

The authors would like to thank the Faculty of Veterinary Science, Mahidol University, for all the support that made this article successful.

Author Contributions: Kr.K. conducted the first draft of the manuscript. Kr.K., P.C., and K.K. performed the writing and revision of the manuscript. All authors contributed to this article and approved the final version of the manuscript.

References

Althouse GC 2024. Contaminant toxicity of concern for boars and semen used in assisted reproduction programs. Anim Reprod Sci. 269: 107519.

Althouse GC and Lu KG 2005. Bacteriospermia in extended porcine semen. Theriogenology. 63(2): 573-84.

Andersson DI, Hughes D and Kubicek-Sutherland JZ 2016. Mechanisms and consequences of bacterial resistance to antimicrobial peptides. Drug Resist Updat. 26: 43-57.

Aranha C, Gupta S and Reddy KVR 2024. Contraceptive efficacy of antimicrobial peptide Nisin: *in vitro* and *in vivo* studies. Contraception. 69(4): 333-8.

Assoni L, Milani B, Carvalho MR, Nepomuceno LN. Waz NT, Guerra MES, Converso TR and Darrieux M 2020. Resistance mechanisms to antimicrobial peptides in Gram-positive bacteria. Front. Microbiol. 11: 593215.

Avila EE 2017. Functions of antimicrobial peptides in vertebrates. Curr Protein Pept Sci. 18(11): 1098-119.

Bechinger B and Gorr SU 2017. Antimicrobial Peptides: Mechanisms of action and resistance. Dent Res. 96(3): 254-60.

Bennemann PE, Machado SA, Girardini LK, Sonalio K and Tonin AA 2018. Bacterial contaminants and antimicrobial susceptibility profile of boar semen in Southern Brazil studs. Rev. MVZ Cordoba. 23(2): 6637-48.

Bresciani C, Cabassi CS, Morini G, Taddei S, Bettini R, Bigliardi E, Di Ianni F, Sabbioni A and Parmigiani I 2014. Boar semen bacterial contamination in Italy and antibiotic efficacy in a modified extender. Ital J Anim Sci. 13: 83-7.

Bussalleu E, Sancho S, Briz MD, Yeste M and Bonet S 2017. Do antimicrobial peptides PR-39, PMAP-36 and PMAP-37 have any effect on bacterial growth and quality of liquid-stored boar semen? Theriogenology. 89: 235-43.

Contreras MJ, Núñez-Montero K, Bruna P, García M, Leal K, Barrientos, L and Weber H 2022. Bacteria and boar semen storage: progress and challenges. Antibiotics (Basel). 11(12): 1796.

Costinar L, Herman V, Pitoiu E, Iancu I, Degi J, Hulea A and Pascu C 2021. Boar Semen contamination: Identification of Gram-negative bacteria and

antimicrobial resistance profile. Animals (Basel). 12(1): 43.

De Mandal S, Panda AK, Murugan C, Xu X, Senthil Kumar N and Jin F 2021. Antimicrobial peptides: novel source and biological function with a special focus on entomopathogenic nematode/bacterium symbiotic complex. Front Microbiol. 12: 555022.

Hensel B, Jakop U, Scheinpflug K, Muhldorfer K, Schroter F, Schafer J, Greber K, Jung M and Schulze M 2020. Low temperature preservation of porcine semen: influence of short antimicrobial lipopeptides on sperm quality and bacterial load. Sci Rep. 10(1): 13225.

Farsimadan M and Motamedifar M 2020. Bacterial infection of the male reproductive system causing infertility. J Reprod Immunol. 142: 103183.

Fazly Bazzaz BS, Seyedi S, Hoseini Goki N and Khameneh B 2021. Human antimicrobial peptides: spectrum, mode of action and resistance mechanisms. Int J Pept Res. 27: 801-16.

Gaczarzewicz D, Udala J, Piasecka M, Blaszczyk B and Stankiewicz T 2016. Bacterial contamination of boar semen and its relationship to sperm quality preserved in commercial extender containing gentamicin sulfate. Pol J Vet Sci. 19(3): 451-9.

George B, Pooja S, Suchithra TV and Sebastian D 2023. Historical developments of antimicrobial peptide research. in: Antimicrobial peptides. K Ajesh and K Sreejith (ed). Academic Press. 1-16.

Guilhelmelli F, Vilela N, Albuquerque P, Derengowski LD, Silva-Pereira I and Kyaw CM 2014. Antibiotic development challenges: the various mechanisms of action of antimicrobial peptides and of bacterial resistance. Front Microbiol. 4: 353.

Jäkel H, Scheinpflug K, Mühldorfer K, Gianluppi, R, Lucca MS, Mellagi APG, Bortolozzo FP and Waberski D 2021. *In vitro* performance and *in vivo* fertility of antibiotic-free preserved boar semen stored at 5 °C. J Anim Sci Biotechnol. 12: 9.

Kaeoket K, Persson E, Dalin AM 2003. Influence of pre-ovulatory insemination and early pregnancy on the distribution of CD2, CD4, CD8 and MHC class II expressing cells in the sow endometrium. Anim Reprod Sci. 76(3-4):231-44.

Kaeoket K, Tantasuparuk W, Kunavongkrit A 2005. The effect of post-ovulatory insemination on the subsequent embryonic loss, oestrous cycle length and vaginal discharge in sows. Reprod Domest Anim. 40(5):492-4.

Kaewchomphunuch T, Charoenpichitnunt T, Thongbaiyai V, Ngamwongsatit N and Kaeoket K 2022. Cell-free culture supernatants of *Lactobacillus* spp. and *Pediococcus* spp. inhibit growth of pathogenic *Escherichia coli* isolated from pigs in Thailand. BMC Vet Res. 18(1): 60.

Kalita DJ 2015. Antimicrobial Peptides: The next generation therapeutic agents. Int J Curr Microbiol App Sci. 4(6): 731-6.

Keeratikunakorn K, Aunpad R, Ngamwongsatit N and Kaeoket K 2024[a]. The effect of antimicrobial peptide (PA-13) on *Escherichia coli* carrying antibiotic-resistant genes isolated from boar semen. Antibiotics (Basel). 13(2): 138.

Keeratikunakorn K, Chanapiwat P, Aunpad R, Ngamwongsatit N and Kaeoket K 2024[b]. Effect of Antimicrobial Peptide BiF2_5K7K on Contaminated bacteria isolated from boar semen and semen qualities during preservation and subsequent fertility test on pig farm. Antibiotics (Basel). 3(7): 579.

Keeratikunakorn K, Chanapiwat P, Aunpad R, Ngamwongsatit N and Kaeoket K 2024[c]. The Effects of different antimicrobial peptides (A-11 and AP19) on isolated bacteria from fresh boar semen and semen quality during storage at 18 °C. Antibiotics (Basel). 13(6): 489.

Keeratikunakorn K, Kaewchomphunuch T, Kaeoket K and Ngamwongsatit N 2023. Antimicrobial activity of cell free supernatants from probiotics inhibits against pathogenic bacteria isolated from fresh boar semen. Sci Rep. 13(1): 5995.

Keeratikunakorn K, Kaeoket K, Ounjai P, Wannigama DL, Chatsuwan T and Ngamwongsatit N 2024[d]. First detection of multidrug-resistant and toxigenic *Pasteurella aerogenes* in sow vaginal discharge: a novel threat to swine health in Thailand. Sci Rep. 14(1): 25510.

Kellerman C, Malaluang P, Hansson I, Eliasson Selling L and Morrell JM 2022. Antibiotic resistance patterns in cervical microbes of gilts and sows. Animals (Basel). 12(1): 117.

Klubthawee N, Adisakwattana P, Hanpithakpong W, Somsri S and Aunpad R 2020. A novel, rationally designed, hybrid antimicrobial peptide, inspired by cathelicidin and aurein, exhibits membrane-active mechanisms against *Pseudomonas aeruginosa*. Sci Rep. (1): 9117.

Kumar P, Kizhakkedathu JN and Straus SK 2018. Antimicrobial peptides: Diversity, mechanism of action and strategies to improve the activity and biocompatibility *in vivo*. Biomolecules. 8(1): 4.

Kuster CE and Aithouse GC 2016. The impact of bacteriospermia on boar sperm storage and reproductive performance. Theriogenology. 85(1): 21-6.

Landers TF, Cohen B, Wittum TE and Larson EL 2012. A review of antibiotic use in food animals: Perspective, policy, and potential. Public Health Rep. 127(1): 4-22.

Lekagul A, Tangcharoensathien V, Mills A, Rushton J and Yeung S 2020. How antibiotics are used in pig farming: a mixed-methods study of pig farmers, feed mills and veterinarians in Thailand. BMJ Glob Health. 5(2): e001918.

Li Z, Hu YH, Yang YY, Lu ZQ and Wang YZ 2018. Antimicrobial resistance in livestock: Antimicrobial peptides provide a new solution for a growing challenge. Anim Front. 8(2): 21-9.

López Cascales JJ, Zenak S, García de la Torre J, Lezama OG, Garro A and Enriz RD 2018. Small cationic peptides: Influence of charge on their antimicrobial activity. ACS Omega. 3(5): 5390-8.

Maes D, Nauwynck H, Rijsselaere T, Mateusen B, Vyt P, de Kruif A and Van Soom A 2008. Diseases in swine transmitted by artificial insemination: an overview. Theriogenology. 70(8): 1337-45.

Mahlapuu M, Hakansson J, Ringstad L and Bjorn C 2016. Antimicrobial peptides: An emerging category of therapeutic agents. Front Cell Infect Microbiol. 6: 194.

Morrell JM, Nunez-Gonzalez A, Crespo-Felez I, Martinez-Martinez S, Alborcia MJM, Fernandez-Alegre E, Dominguez JC, Gutiérrez-Martín CB and Martínez-Pastor F 2019. Removal of bacteria from boar semen using a low-density colloid. Theriogenology. 126: 272-8.

Naiel MAE, Ghazanfar S, Negm SS, Shukry M and Abdel-Latif HMR 2023. Applications of antimicrobial peptides (AMPs) as an alternative to antibiotic use in aquaculture – A mini-review. Ann Anim Sci. 23(3): 691-701.

Ngo CB, Suwimonteerabutr J, Morrell JM and Tummaruk P 2024. Sow reproductive performance following artificial insemination with semen doses processed using Single Layer Centrifugation without antibiotics in the tropics. Theriogenology. 226: 194-201.

Ngo CB, Suwimonteerabutr J, Prapasarakul N, Morrell JM and Tummaruk P 2023. Bacteriospermia and its antimicrobial resistance in relation to boar sperm quality during short-term storage with or without antibiotics in a tropical environment. Porc Health Manag. 9(1): 21.

Nguyet LTY, Keeratikunakorn K, Kaeoket K and Ngamwongsatit N 2022. Antibiotic resistant *Escherichia coli* from diarrheic piglets from pig farms in Thailand that harbor colistin-resistant *mcr* genes. Sci Rep. 12: 9083.

Nguyet LTY, Ounjai P, Kaeoket K and Ngamwongsatit N 2023. Feasibility of crude F4 fimbriae extract as a vaccine candidate for preventing *Escherichia coli*-induced diarrhea in piglets. Vet World. 16(10):2063-70.

Nguyet LTY, Ounjai P, Ngamwongsatit N and Kaeoket K 2024. The immune response of pregnant sow after vaccination with crude fimbriae (F4) extracts vaccine and immunoprotection of nursery pig against pathogenic *E. coli* ($F4^+$ETEC). Acta Trop. 254: 107173.

Pezo F, Romero F, Zambrano F and Sanchez RS 2019. Preservation of boar semen: An update. Reprod Domest Anim. 54(3): 423-34.

Pérez-Duran F, Acosta-Torres LS, Serrano-Díaz PN, Toscano-Torres IA, Olivo-Zepeda IB, García-Caxin E and Nuñez-Anita RE 2020. Toxicity and antimicrobial effect of silver nanoparticles in swine sperms. Syst Biol Reprod Med. 66(4): 281–9.

Pushpanathan M, Gunasekaran P and Rajendhran J 2013. Antimicrobial peptides: Versatile biological properties. Int J Pept. 2013:675391.

Puig-Timonet A, Castillo-Martin M, Pereira BA, Pinart E, Bonet S and Yeste M. 2018. Evaluation of porcine beta defensins-1 and-2 as antimicrobial peptides for liquid-stored boar semen: Effects on bacterial growth and sperm quality. Theriogenology. 111: 9-18.

Robles Ramirez O, Osuna G, Plisson F and Barrientos-Salcedo C 2024. Antimicrobial peptides in livestock: a review with a one health approach. Front Cell Infect Microbiol. 14: 1339285.

Rodriguez AL, Van Soom A, Arsenakis I and Maes D 2017. Boar management and semen handling factors affect the quality of boar extended semen. Porc Health Manag. 3: 15.

Ros-Santaella JL, Nový P, Scaringi M and Pintus E 2024. Antimicrobial peptides and proteins as alternative antibiotics for porcine semen preservation. BMC Vet Res. 20: 257.

Sancho S, Briz M, Yeste M, Bonet S and Bussalleu E 2017. Effects of the antimicrobial peptide protegrine 1 on sperm viability and bacterial load of boar seminal doses. Reprod Domest Anim. 52: 69-71.

Santos CS and Silva AR 2020. Current and alternative trends in antibacterial agents used in mammalian semen technology. Anim Reprod. 17(1): e20190111.

Schulze M, Dathe M, Waberski D and Muller K 2016[a]. Liquid storage of boar semen: Current and future perspectives on the use of cationic antimicrobial peptides to replace antibiotics in semen extenders. Theriogenology. 85(1): 39-46.

Schulze M, Grobbel M, Muller K, Junkes C, Dathe M, Rudiger K and Jung M 2015. Challenges and limits using antimicrobial peptides in boar semen preservation. Reprod Domest Anim. 50: 5-10.

Schulze M, Grobbel M, Riesenbeck A, Brüning S, Schaefer J, Jung M and Jung M 2017. Dose rates of antimicrobial substances in boar semen preservation-time to establish new protocols. Reprod Domest Anim. 52(3): 397-402.

Schulze M, Junkes C, Mueller P, Speck S, Ruediger K, Dathe M and Mueller K 2014. Effects of cationic antimicrobial peptides on liquid-preserved boar spermatozoa. Plos One. 9(6): e100490.

Schulze M, Nitsche-Melkus E, Hensel B, Jung M and Jakop U 2020. Antibiotics and their alternatives in artificial breeding in livestock. Anim Reprod Sci. 220: 106284.

Shaoyong WK, Li Q, Ren ZQ, Xiao JY, Diao ZX, Yang GS and Pang W 2019. Effects of kojic acid on boar sperm quality and antibacterial activity during liquid preservation at 17 C. Theriogenology. 140: 124-35.

Speck S, Courtiol A, Junkes C, Dathe M, Muller K and Schulze M 2014. Cationic synthetic peptides: assessment of their antimicrobial potency in liquid preserved boar semen. Plos One. 9(8): e105949.

Tang ZR. Sun ZH, Tang XS, Feng ZM, Zhou D, Xiao DF, Zhang B and Li LL 2011. Dietary bovine lactoferricin improved gut microflora, benefited intestinal mucosal morphology and inhibited circular cytokines delivery in 21-day weaned piglets. J Appl Anim Res. 39(2): 153–57.

Vickram A, Dhama K, Archana K, Parameswari R, Rameshpathy M, Iqbal H and Sridharant B 2017. Antimicrobial peptides in semen extenders: a valuable replacement option for antibiotics in cryopreservation- A prospective review. J Exp Biol Agric. 5(5): 578-88.

Vilvanathan S, Kandasamy B, Jayachandran AL, Sathiyanarayanan S, Singaravelu VT, Krishnamurthy V and Elangovan V 2016. Bacteriospermia and its impact on basic semen parameters among infertile men. Interdiscip Perspect Infect Dis. 2016: 2614692.

Waberski D and Luther AM 2024. Boar semen storage at 5 °C for the reduction of antibiotic use in pig insemination: Pathways from science into practice. Anim Reprod Sci. 269: 107486. Wang S, Zeng XF,

Yang Q and Qiao SY 2016. Antimicrobial peptides as potential alternatives to antibiotics in food animal industry. Int J Mol Sci. 17(5).

Xiao H, Shao FY, Wu MM, Ren WK, Xiong X, Tan B and Yin Y 2015. The application of antimicrobial peptides as growth and health promoters for swine. J Anim Sci Biotechnol. 6(1): 19.

Xu BC, Fu J, Zhu LY, Li Z, Wang YZ and Jin ML 2020. Overall assessment of antimicrobial peptides in piglets: a set of meta-analyses. Animal. 14(12): 2463-2471.

Yeaman MR and Yount NY 2003. Mechanisms of antimicrobial peptide action and resistance. Pharmacol Rev. 55(1): 27-55.

Yu PL, van der Linden DS, Sugiarto H and Anderson RC 2010. Antimicrobial peptides isolated from the blood of farm animals. Anim Prod Sci. 50(7): 660-9.

Zhang LJ and Gallo RL 2016. Antimicrobial peptides. Curr Biol. 26(1): R14-9.