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Liquid storage of boar semen: Current and future perspectives on the use of cationic antimicrobial peptides to replace antibiotics in semen extenders

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ABSTRACT

Antibiotics are of great importance in boar semen extenders to ensure long shelf life of spermatozoa and to reduce transmission of pathogens into the female tract. However, the use of antibiotics carries a risk of developing resistant bacterial strains in artificial insemination laboratories and their spread *via* artificial insemination. Development of multiresistant bacteria is a major concern if mixtures of antibiotics are used in semen extenders. Minimal contamination prevention techniques and surveillance of critical hygiene control points proved to be efficient in reducing bacterial load and preventing development of antibiotic resistance. Nevertheless, novel antimicrobial concepts are necessary for efficient bacterial control in extended boar semen with a minimum risk of evoking antibiotic resistance. Enhanced efforts have been made in recent years in the design and use of antimicrobial peptides (AMPs) as alternatives to conventional antibiotics. The male genital tract harbors a series of endogenic substances with antimicrobial activity and additional functions relevant to the fertilization process. However, exogenic AMPs often exert dose- and time-dependent toxic effects on mammalian spermatozoa. Therefore, it is important that potential newly designed AMPs have only minor impacts on eukaryotic cells. Recently, synthetic magainin derivatives and cyclic hexapeptides were tested for their application in boar semen preservation. Bacterial selectivity, proteolytic stability, thermodynamic resistance, and potential synergistic interaction with conventional antibiotics propel predominantly cyclic hexapeptides into highly promising, leading candidates for further development in semen preservation. The time scale for the development of resistant pathogens cannot be predicted at this moment.

1. Introduction

Antimicrobial agents are of extraordinary importance for the control of bacterial growth in liquid-preserved ejaculates from farm animals. To counteract increasing bacterial resistance to conventional antibiotics, novel agents with different active mechanisms have to be developed. Increasing efforts have been made over the past few decades to develop antimicrobial peptides (AMPs) as agents for clinical implementation [1]. So far, most success has been achieved by the topical application of such agents. To date, in reproductive medicine, the development of AMPs has focused on their applications in the area of contraception [2]. The implementation of AMPs as extender

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additives represents a novelty in artificial insemination (AI) management. For semen preservation, antimicrobial additives ideally satisfy the following requirements: (1) broad spectrum of antimicrobial action [3], (2) absence of sperm toxicity [4], (3) no interference with fertility [4], (4) high stability [4], (5) high activity at common semen storage temperatures [5], (6) low potential to evoke resistance [3], (7) ease of application, and (8) economic feasibility. As primary goals, the examination of sperm compatibility and the examination of antimicrobial activity are crucial to the development of novel additives for boar semen preservation. The present article reviews current developments and future perspectives for the use of AMPs in liquid boar semen preservation.

2. Sources and consequences of bacterial contamination in preserved boar semen

Virtually ejaculates collected from healthy donors contain bacteria stemming from natural occurrence within the male reproductive tract. Bacterial load in raw semen commonly ranges between 10^4 and 10^6 cfu/mL [6]. In addition to animal origins, microbials from the environment may contaminate semen during collection or processing in the laboratory [7]. Boar spermatozoa are sensitive to chilling injury and therefore are usually stored in the liquid state for up to 7 days at 16 °C to 18 °C. Nutrient-rich extenders guarantee favorable growth conditions for bacteria by buffering oscillations in osmolarity and maintaining pH values between 6.8 and 7.2. Besides the initial type and amount of germs in native semen [8], potential risk factors for increased germ content in diluted ejaculates include delayed cool-down rate of freshly processed semen samples [9], elevated temperature during transport and storage [10], extended duration of storage [11], and the composition of the extenders [12].

The majority of bacterial contaminants are gram negative and attributable to the family of enterobacteriaceae [13]. Species of the family pseudomonadacea also appear on a regular basis. Besides bacteria of animal origin, contaminants of nonanimal origin are attracting more and more attention [7]. A recent study revealed that 26% of 344 semen doses collected in 24 European AI centers during 2010 to 2011 were positively tested for bacterial contamination. Twenty-one different, mostly gram-negative bacteria species were identified, and 18 of them showed multiresistancy against common antibiotics [6]. Most of these bacteria are opportunistic pathogens commonly related to nosocomial infections in humans and animals clearly pointing to the risk of spreading multiresistant bacteria into the environment [14]. Noteworthy, in only 4.5% of contaminated AI doses, the respective bacteria were also isolated from the raw ejaculate, indicating that the majority of contaminations occurred within the laboratory surroundings [6]. European AI companies organized in the Association for Bioeconomy Research (FBF e.V.) together with the two spermatology reference laboratories at IFN Schönow and Veterinary University Hannover therefore have established an effective, strict hygiene control concept for minimizing bacteria load in semen doses [15].

Consequences of bacterial contamination in the setting of AI practice predominantly reside in loss of sperm motility, and induction of sperm agglutination and membrane damage, resulting in poor fertility and high economic losses in sow herds [16,17]. Transmission of a few diseases *via* AI by specific pathogenic bacteria, such as *Brucella suis*, *Chlamydia* sp., and *Leptospira*, is of concern. These organisms are subjects of strict and efficient diagnostic vigilance and biosecurity measures in boar studs [18,19]. More recently, development of resistant bacterial strains in AI laboratories [6,20] and their transfer into sow herds has come into focus. Causative agents of hospital-acquired infection, known in human medicine, such as *Burkholderia cepacia*, *Serratia marcescens*, and *Stenotrophomonas maltophilia*, have repeatedly been isolated from extended boar semen [6,7]. Given that semen backflow after insemination of sows is up to 70% of the insemination dosage [21], such bacteria together with small amounts of antibiotics can be transmitted into the environment. Although bacterial load can be effectively reduced by general sanitary protocols [16] and strict hygiene control concepts during semen processing [6], bacterial control remains mandatory in liquid-preserved boar ejaculates.

Supporting and alternative strategies to conventional antibiotics for minimizing the risk of developing bacterial multiresistance are under development. They include automated semen collection [22], hypothermic semen storage below 15 °C [9,23], physical removal of bacteria by colloid centrifugation [20], and the use of alternative antimicrobials [3,4] reviewed in the present article.

3. Use of conventional antibiotics and bacterial resistance in boar semen preservation

The addition of antibiotics to semen extenders is regulated by national authorities. In the European Union, these regulations are outlined in the Council Directive 90/429/EEC and prescribe an effective combination of antibiotics, in particular against leptospires and mycoplasmas. Since the 1980s, penicillin–streptomycin combinations of antibiotics have had to be abandoned because of microbial resistance problems [24].

A 2005 retrospective study in the United States found evidence of bacteriospermia in one-third of AI doses. Most of these isolates were resistant to the antibiotics generally used in applied settings, such as amoxicillin, gentamicin, lincomycin, tylosin, and spectinomycin [7]. Between 2010 and 2011, 16 of 24 AI boar centers that were evaluated in Germany and Austria revealed gentamicin-resistant bacteria isolated from extended semen. After identification of critical hygiene control points and implementation of strict sanitary guidelines, a clear reduction of the numbers of semen doses with resistant bacteria was confirmed at repeated stud audits [6].

Continuous exposure of semen and fluids (water, extender) to subinhibitory concentration of antibiotics in sinks, tubes and surfaces in AI laboratories favor the development of resistant microbial strains [3]. With a lack of antimicrobial efficiency, “there is a tendency to use a cocktail of broad spectrum, highly potent antibacterials,