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Antimicrobial resistance in boar semen production

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What is antimicrobial resistance?

Antimicrobial resistance (AMR) is the ability of a bacterial strain to survive in the presence of a certain antibiotic. In medicine, antibiotics are used to treat bacterial infections, while in boar semen production, antibiotics are integrated in the boar semen extender to avoid bacterial growth in the diluted semen and prevent impairment of semen quality and transfer of diseases.

The World Health Organization declares AMRs to antibiotics as one of the biggest threats to global health, food security, and development today[1].

How does an AMR develop?

When bacteria are constantly exposed to antibiotics, they develop resistance mechanisms by mutation and selection in an evolutionary process. In boar semen production, this may be the case in the stable and in the laboratory, where careless use of antibiotics sometimes can be seen. If this is the case, germs are in frequent contact with antibiotics and to survive, they develop defense strategies against these antibiotics, which lead to AMRs (see Figure 1).

Once bacteria have developed resistance mechanisms against an antibiotic, they can continue to multiply in the presence of this antibiotic. AMR germs can also share their defense mechanisms with other germs, even across species, resulting in an amplified spread of multiple AMRs against a variety of antibiotics[2]. When germs acquire the right combination of resistance mechanisms, they become resistant to several classes of antibiotics, resulting in untreatable infections or uncontrollable contamination of boar semen doses.

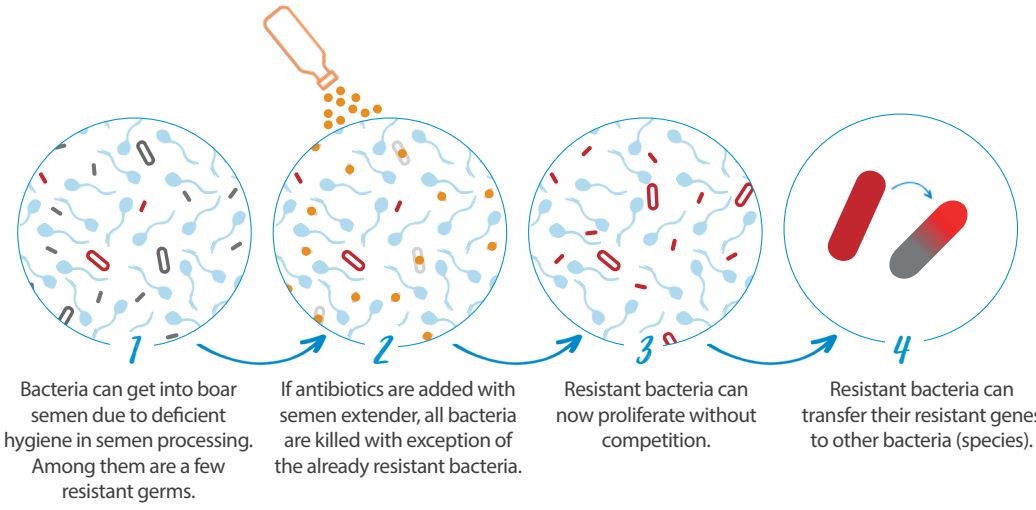

Figure 1: Development of antimicrobial resistance (AMR) during preservation of boar semen

Serratia marcescens are among the bacteria which often cause severe problems in boar sperm production if they develop single or multiple AMR. These gram-negative bacteria belong to the family of Yersiniaceae and are facultative anaerobe. They are often found in the urinary tract and prepuce of boars and especially at humid spots in the boar pen. Improper hygiene during semen collection allows Serratia to get into the raw semen and thus into the laboratory, where the germ meets semen extender containing antibiotics. Frequent contact allows Serratia to develop defense mechanisms against the antibiotics used in the laboratory and consequently, Serratia will grow in the diluted semen, although the semen extender includes antibiotics. If a Serratia contamination cannot be controlled by antibiotics, the sperm cells are damaged after 48 to 72 hours to an extent where fertility is strongly impaired.

This is a simplified example on how resistant bacteria can develop and make their way into boar semen doses, but unfortunately this can happen any time in boar studs with inadequate hygiene management and careless handling of antibiotics.

How to identify bacterial contaminations in semen doses?

In boar semen production, bacterial contamination due to AMRs is typically first noticed, when holding samples of diluted boar semen show a reduced motility and/or agglutinations. Depending on the degree of contamination and the strain of bacteria, the motility of contaminated holding samples may decrease slowly during storage or drop dramatically within only 24 hours. The reason for this finding is the exponential growth of resistant bacteria because antibiotics cannot control bacterial growth anymore.

Agglutinations of spermatozoa may also be caused by bacterial contamination. Figure 2 shows examples of boar semen samples with agglutinations, that severely impair semen quality.

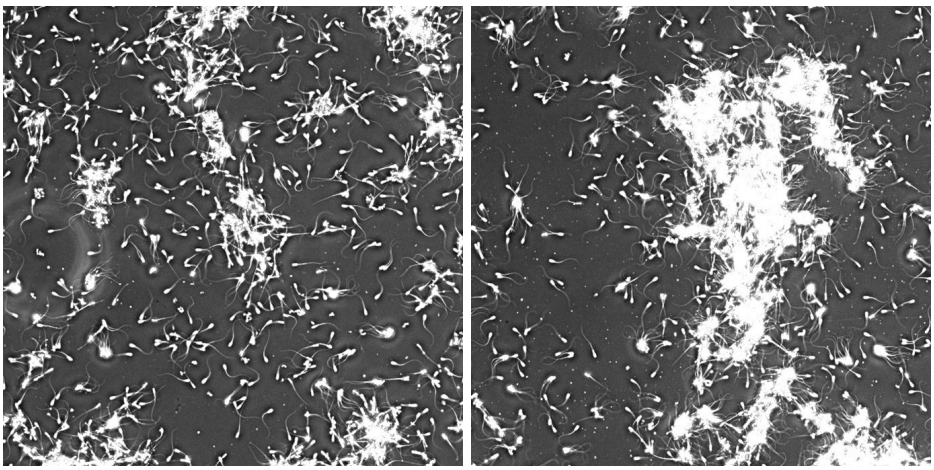

Figure 2: Two boar semen samples with semen agglutination caused by severe bacterial contamination at 100fold magnification.

If no immediate action is taken by the boar stud, the bacterial contamination will significantly reduce the amount of fertile sperm cells in a semen dose and can even, depending on the type of bacteria, cause infections in the inseminated sows.

How to prevent and avoid contaminations of semen doses with resistant bacteria?

When a boar stud is confronted with bacterial contamination of semen doses due to AMRs, immediate action is required to be able to continue producing semen doses. Even more importantly, sustainable measures need to be taken to solve the problem in the long term and avoid the development of further AMRs, which would make it impossible to control bacterial contamination at some point.

Immediate action to prevent bacterial contamination of semen doses

This usually requires in a first step the application of an antibiotic additive to the semen extender, which can still control the problematic bacteria. This antibiotic must be identified with the help of an antibiogram, where the bacterial strain is isolated and tested for its resistance and sensitivity against different antibiotics in a specialized microbiology laboratory. The "emergency" additive must be an antibiotic that is still effective against the problematic strain, and which is not toxic to sperm cells. Of course, this requires that the antibiotics in the semen extender and the antibiotic additive are disclosed by the supplier and known to the user. Other than Minitube, not all suppliers of boar semen extenders provide that information, which is crucial to take immediately effective action against resistant bacteria.

This emergency measure is a fast and effective measure to maintain semen production, but it must be seen as a temporary solution only. The real cause and source of the resistant bacteria must be identified and eliminated. The following step is of utmost importance and inevitable:

Identify the source of the contamination and eradicate it!

Typical sources of bacterial contamination in a boar stud are those spots in the laboratory that are difficult to clean and provide a warm and humid environment, suitable for bacterial growth. These spots and critical items include:

- Pneumatic delivery system
- Water baths, sinks, and drains
- Water treatment system and tubing
- Extender vats, especially inside the lid and grommets
- Extender and extender transfer tubing
- Semen staining solutions
- Any item with contact to extended semen

After a general cleaning of the laboratory using an effective disinfectant with special focus on above mentioned areas of concern, it is advisable to take swabs from several spots and check them for bacterial growth. This can be performed by the laboratory staff with kits, that enable bacteriological culture, e.g., EasyCult. If one or more of these locations show bacterial contamination, a second thorough cleaning is imperative until the source is completely eradicated.

The most effective measure for the prevention and avoidance of resistant contaminant bacteria in a boar stud and laboratory is the rigorous application of hygiene and sanitation protocols[3,4].

These include:

- Caretaking of boars (shortening of the preputial hair, general cleanliness of the boar pen and the boar)
- Hygienic semen collection using triple gloving and an automated collection system (BoarMatic) with closed sperm flow (artificial cervix)
- Avoiding the collection of any preseminal fluid, especially preputial fluid
- Strict separation of barn and laboratory (staff, material, air)
- General laboratory and personal hygiene, frequent disinfection of laboratory surfaces and hands
- Strict instructions for disinfection of all objects that come into contact with antibiotic-containing extender (e.g., pump tubing and sinker, extender vats, beakers)
- Appropriate instructions for the discarding of liquids containing antibiotics
- Regular hygiene and sanitation training of laboratory staff
- Regular monitoring and auditing of processes
- Regular contamination controls of laboratory surfaces and semen doses
- Appropriate sanitation of the semen collection area

Future perspectives

Hygiene in the boar semen production is surely the most important mean to prevent the development of bacteria with AMRs. In the modern AI industry, a couple of further possibilities have been developed to prevent AMR by limiting or even avoiding the contact between bacteria and antibiotics.

Accurate dosage of antibiotics (ADA)

ADA is a novel protocol in the process of diluting native boar ejaculates, where the addition of antibiotics is decoupled from the dilution of the ejaculate[5]. In conventional dilution protocols, the antibiotic is included in the semen extender and hence the antibiotic concentration is reduced, when the extender is added to the raw ejaculate. In contrast, adding the antibiotics separately in form of a concentrate to an antibiotic-free extender allows accurate dosage in every batch of diluted semen, providing precisely controlled amounts of antibiotics in the final product. To ensure dust-free preparation of the antibiotic concentrate, water-soluble packaging was developed, which protects the laboratory environment and personnel from constant exposure to antibiotics and thus reduces the risk of development of AMRs. ADA also enables a higher flexibility and specificity in the addition of antibiotics and eliminates the need to discard left-over extender with antibiotics at the end of a production day.

5°C storage of boar semen in specialized semen extenders

Recent extender developments allow the storage of boar semen doses at +5°C[6]. At this temperature, there is only a very limited proliferation of bacteria, which enables completely antibiotic-free storage of boar semen and thus entirely prevents the development of AMRs in bacteria. In case of Minitube's Androstar Plus and Androstar Premium extender, an organic bactericidal substance (OBS) is included in the extender as well[7]. OBS shows bactericidal action but is not an antibiotic in the classical sense, building up AMR against OBS is therefore not possible.

Conclusion

The establishment and the continuous development of a consistent and effective hygiene concept from boar husbandry, semen collection and semen processing to general laboratory hygiene is a prerequisite for the sustainable and effective operation of a boar stud. A hygiene concept and the application of modern processing protocols prevent the building up of AMRs, which is highly desirable in boar semen production, as well as for global health, food security and future development.

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