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Bacterial contamination and semen quality

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Introduction

Monitoring bacteria in a boar stud is an important quality control step. Failure to control bacterial loads in the end product leads to reduced shelf life and agglutination problems in stored semen. This leads to increased returns and poor fertility. Although improper timing of insemination is the primary factor for vaginal discharges and uterine infections, contaminated semen can provide the opportunistic component.[1]

Antibiotic additives

Most semen extenders for swine include gentamicin sulfate as an additive to control bacteria. Other common antibiotics in use include linco-spectin, neomycin, and ceftiofur. It important to know what antibiotics are included in the extender. If bacteria are found in extended semen, a minimum inhibitory concentration (MIC) should be determined to see which antibiotic can be added to control those specific bacteria.

Culture routine

Boar studs should be routinely cultured. The most important item to culture is extended ejaculates. I recommend culturing extended ejaculate samples from each collection day. Many of the studs in our practice send in four doses from each collection day, which we streak out on 5% blood agar plates on day four after collection. Regular monitoring like this gives a good indication of the sanitation level of the stud and lab as well as continuous monitoring of the semen going out.

In addition to extended semen, certain areas of the lab should be cultured on a monthly basis. The areas to focus on include anything that touches semen or extender, and any area that is warm and/or moist. Lab equipment that touches semen or extender is important to monitor because contamination of such could result in contamination of the entire day's production. This list includes the following:

- Water system
- Pipette tips
- Extender
- Extender vats
- Extender bags
- Tubing used to transport semen or extender
- Semen dispensing equipment
- Thermometers

Areas of the lab that are warm and moist are high risk because a warm, moist environment promotes bacterial growth. Contamination in one of these results in explosive growth in a matter of hours and can be easily tracked all over the lab. Warm, moist areas of concern include the following:[2]

- Water baths
- Slide warmers
- Warming boxes

All of these areas should be cultured after cleaning to check the sanitation procedures. There should be no growth.

Raw ejaculates can also be cultured to see what kinds of bacteria are entering the lab with the ejaculates. Expect to see a wide variety of growth on these samples. The value of culturing raw ejaculates is to confirm the source of a certain isolate obtained in the lab or in extended semen.

A bacterial swab with Amies media works well when taking the sample at the stud. The swab can then be streaked on to 5% sheep blood agar later. I put four on one plate (each in its own quadrant) to save cost. If sampling water, day 0 semen, or day 1 semen, pre-incubation overnight in the Amies media is recommended to increase the sensitivity. Other samples can be cultured directly.

There should be no growth on extended semen samples or on any of the equipment listed above. If there is growth, sensitivity/MIC testing should be done to see which antibiotics could work. Also, sanitation procedures should be re-evaluated.

Table 1: Frequency of identifying bacterial contaminants.

Total number of cultures	1692
Cultures with growth (%)	13.9%
Positive cultures with pure isolate (%)	77.9%

Table 2: Bacteria isolated at Swine Vet Center from semen.

Bacteria Isolate	Frequency of isolation	Typical source of isolate
Alcaligenes xylosoxidans / *Alcaligenes* sp.	63	Water system
Pseudomonas sp. / *Pseudomonas fluorescens* / *Pseudomonas stutzeri*	50	Water system / environment
Bulkholderia cepacia / *Bulkholderia* sp.	30	Water system
Staphylcoccus sp.	29	Ejaculate
Corynebacterium sp.	20	Water baths / warming box
Strep sp.	19	Ejaculate
Ralstonia picketti	13	Water system
Bacillus sp.	12	Tubing/extending system
Enterococcus sp.	10	Ejaculate
Stenotrophomonas maltophilia / *Stenotrophomonas* sp.	10	Ejaculate
Flavimonas sp.	8	Ejaculate
Escherichia coli	4	Ejaculate
Micrococcus	4	Ejaculate
Flavobacterium sp.	3	Ejaculate
Acinitobacter sp.	3	Water baths / warming box
Other	14	-

Commonly found bacteria

When bacteria are cultured in extended samples, it is most often a single bacteria. It is important to forward the culture to a reference lab for identification because most of the isolates are not commonly identified in pigs. MIC testing should also be done. Cultures done at the Swine Vet Center on ten boar studs over the last seven years have yielded the results presented in **Table 1**.

Studies by Althouse[1] showed the most frequently isolated bacteria on 23 field investigations to be from enteric (*Enterobacter eloacae*, *Escherichia coli*, *Serratia marcescens*) and non-enteric (*Alcaligenes xylosoxydans*, *Burkholderia cepaceia*, *Stenotrophomonas* [*Xanthomonas*] *maltophilia*) groups. Routine cultures from ten different boar studs over the last seven years at Swine Vet Center have yielded the bacteria listed in **Table 2**.

Summary

Monitoring semen for bacterial contamination should be a routine part of a herd health program at a boar stud. Ensuring that the antibiotics in the extender are working will prevent potential losses at the sow farm due to reduced fertility.

References

1. Althouse GC, Kuster CE, Clark SG, Weisiger RM. Field Investigations of Bacterial Contaminants and their Effects on Extended Porcine Semen. *Theriogenology* 2000;53:1167-1176.
2. Reicks DL. Sanitation: Critical to good quality semen. *Minitube Spermnotes* Fall 1998.