



Battling the Bug | 2012 Edition

Strategies for keeping bacteria contamination at bay

Bacterial contamination is a serious and very real deterrent to profitable semen production and swine reproduction. It results in reduced fertility, lower conception rates, and shorter shelf life of semen doses. Hygienic semen collection and processing techniques and stringent laboratory procedures are the first and primary lines of defense in successfully managing contamination. Controlling bacterial growth in extended semen with antibiotics should be considered a secondary method of control.

Bacterial contamination in boar semen is routinely observed when semen is collected by the gloved hand technique. Studies show that 62.5% of raw ejaculates and 79% of extended semen doses contain bacterial contamination. In one 10-year study, bacterial counts per milliliter (ml) of freshly collected boar semen normally ranged between 5,500 to 48,000 colony forming units (CFUs) and averaged 27,000 CFUs. Therefore, when low conception rates and reproductive problems occur with artificial insemination (AI), bacterial contamination of semen is an important cause to consider.

Bacterial contamination of AI doses causes problems with semen quality by reducing sperm cell motility and acrosome integrity, and causing vaginal discharges, endometritis, and sperm cell death. The most commonly found bacterial contaminants in porcine extended semen are: *Alcaligenes xylosoxidans*, *Burkholderia cepacia*, *Enterobacter cloacae*, *Escherichia coli*, *Serratia marcescens* and *Stenotrophomonas maltophilia*. These 6 genera of bacteria account for 71% of all contaminated samples, and some strains of these bacteria are resistant to the aminoglycoside (gentamicin).

These gentamicin-resistant strains of bacteria are among those found responsible for producing an acidic spermicidal environment that reduces the shelf life of the extended semen. MOFA extenders contain Ampicillin and Apramycin as a first line of defense to control bacteria due to their ability to combat most of

the common bacteria found in porcine semen. MOFA offers consulting services to assist in bacterial assessment and to determine the best antibiotic combination to meet the specific needs and requirements special situations encountered in boar studs.

Studies have shown that, 46 different genera of bacteria have been isolated from raw semen samples. *Enterococcus faecalis* is one of the isolated strains causing most concern. This fecal-derived bacteria is labeled by many research organizations as "the resistant" bacteria. There are very few antibiotics that can effectively control this pathogen, and, unfortunately, most of them are not permitted for use in food-producing animals. Other identified genera, very similar in regard to their resistance are; *Klebsiella oxytoca*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.

The most important issues are which of these bacteria are found most often in extended semen and which ones cause problems because they are not held in check by the antibiotic in the semen extender. Bacterial contamination is not just a problem for production evidence of fertility suppression (~7%) caused by a single pathogen in semen held less than 48 hours. Therefore, production systems, and boar studs in particular, must use all the available technologies and resources to implement and monitor methods to reduce or eliminate bacterial contamination and to identify the emergence of antimicrobial resistant bacteria in their boar semen.

Offense begins with sanitary boar housing

Published studies show the importance of maintaining sanitary boar housing and collection areas and using hygienic collection techniques. In one study, boars were divided into 2 groups. In the first group, animals and facilities were washed 2 days prior to semen collection, and pens were dry-cleaned twice a day. In addition, hygienic collection procedures were used. In the second group, animals and facilities were washed 5 to 7 days

prior to semen collection, and pens were dry-cleaned twice a day. No care was taken to use hygienic collection techniques. Extended semen from the boars in each group was cultured for bacterial contamination using the spread-plate method and counting the CFUs.

Group #1 had 1490 ±975 CFU/ml while group #2 had 18,862 ±14,634 CFU/ml ($p < .01$). This significant difference clearly points out the need for creating and maintaining a sanitary housing and collection environment. This can be accomplished by establishing a protocol for cleaning and sanitizing boars and pens using disinfectants for the identified bacteria and by managing air flow in the boar stud to help keep the floors dry.

Study results of this method showed that the average bacterial counts were reduced from 27,000 to 611 CFUs. Other field trials using only this technique have shown variable success.

Practice sanitation during collection

The bacterial count in semen is not the same for all fractions of the collection. Study shows that the bacteria per ml are greatest in the pre-sperm fraction. Usually, this portion is voided onto the floor prior to collecting the sperm-rich ejaculate into the collection vessel. The last few jets of semen that come with the gel plug at the end of the ejaculate also have a higher concentration of bacteria than the sperm-rich fraction; and, while they appear to have

Boar	Age (mo.)	Before Surgery		After Surgery	
		No. of Samples	Avg. No. Bact./ml Semen	No. of Samples	Avg. No. Bact./ml Semen
1	17	6	7,500	6	2430
2	18	9	8,880	10	1,100
3	18	10	6,930	10	713

Table 1. Comparison of the number of bacteria in semen before and after extirpation of the diverticulum

Protect the boar from contamination

Studies show that extended semen can have high levels of sulphitereductors and aerobic-mesophile isolates (*Enterococcus faecalis* coliforms and *Streptococcus faecalis*). This is strong evidence that the contamination originated in the collection environment and occurred during routine semen collection and processing.

Because of poor sanitation procedures in boar housing and infrequently washed boars, fecal material often contaminates the prepuce and the preputial fluids. These preputial fluids, in turn, contaminate the semen during the collection process. One way to reduce contamination of preputial fluid is to extirpate or remove the preputial diverticulum. This closed surgical procedure for resection of the preputial diverticulum is simple and has the advantage of eliminating contamination of the surgical site.

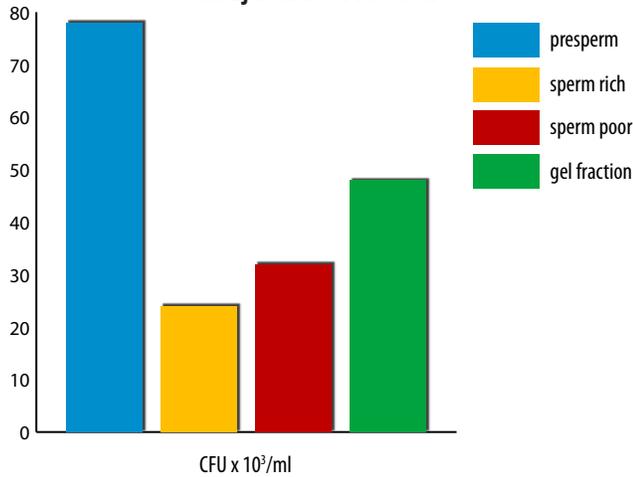
Table 1 shows the reduction in bacteria per ml of semen after surgery was performed. While performing this surgical procedure on every boar is quite impractical, it may be justified for a high-value boar in order to maintain higher-quality semen. Another and easier method to reduce contamination from the preputial fluid is pouring physiological saline over the penis and the collector's gloved hand before grasping the penis for collection.

a milky appearance, they contain virtually no sperm cells. It is advisable to avoid collecting this fraction, especially when collecting semen from a boar with known high-bacterial contamination. It is, however, important to collect seminal plasma with the sperm-rich fraction, because it has a role in sperm transport and fertility.

When using the fractionation technique, care must be taken not to waste semen by removing the collection vessel too soon in an attempt to avoid collection of the final gel. Bacterial contamination cannot be eliminated from a boar ejaculate through fractionation. However, when the pre-sperm and gel-fraction fluids are not included in the collection, the level of bacterial contamination is decreased. Figure 1 shows the difference in bacterial concentration among fractions. The source of the bacteria did not appear to originate from the reproductive organs.

To decrease the chance of bacterial contamination during collection it is recommended to replace the gloved hand method with an automated collection process. In a study comparing the AutoMate® with the gloved hand method it was found that most bacteria included surface contaminants found on the glove, AC, or in the neat semen sample. Although no difference in

Figure 1: Concentration of Bacteria in Ejaculate Fraction



bacterial contamination was observed between the collection systems themselves the neat semen (Fig2) from the AutoMate® collection had fewer numbers of colonies compared to the gloved-hand system. The data reflects an advantage of using an AC to minimize direct exposure of semen to the collection material contaminated by preputial and pre-sperm fluid and or the barn environment during ejaculation.

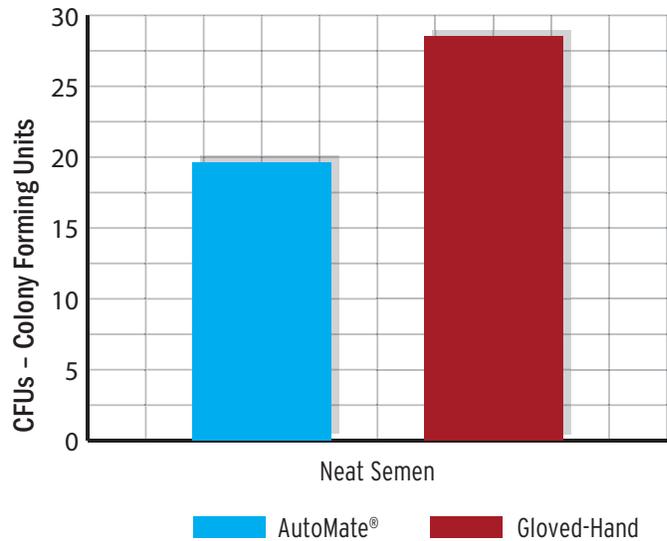
Good sanitation of collection dummies is also important. It is recommended to routinely wash the dummy with soap and hot water after collection to eliminate bacteria and prevent contamination.

Laboratory countertop surfaces

Sanitize the laboratory. Bacterial contamination is not only a problem in the boar housing and collection areas. The laboratory has also been identified as a source of antibiotic-resistant bacteria. Here, sanitation involves more than cleaning. It is taking the measures necessary to prevent bacterial contamination.

All laboratory surfaces must be cleaned before the start of each day, again after semen processing, at the end of the day, and throughout the day as necessary. Countertop surfaces should be thoroughly cleaned by spraying with an antibacterial cleaning solution and wiping completely dry.

Figure 2: Collection Hygiene



Laboratory equipment

Laboratory equipment must be cleaned with a noncorrosive detergent such as Contrad 70® that thoroughly cleans and completely rinses from the equipment surfaces to avoid a spermicidal residue. If a commercial laboratory dishwasher is used, specific detergents are available that meet these criteria. For equipment that must be hand washed, wear rubber gloves and use very hot water. Thoroughly rinse equipment in hot tap water, and then wash with a laboratory cleaning brush in a solution of hot water and detergent. Brushes should be replaced monthly to prevent bacterial buildup on the brush. Washed equipment should be triple rinsed with hot tap water followed by triple rinsing with distilled or deionized (DI) water. Using disposable products as often as possible saves time and will aid in preventing bacterial contamination.

Use 1 of 3 methods to sanitize equipment

- 1) Place equipment in dry heat at 200°C for 30 minutes,
- 2) Autoclave (moist heat and pressure) for 20 minutes, or
- 3) Rinse/soak in 70% isopropyl alcohol for 30 minutes, then triple rinse with DI water and dry before use. Consider the equipment's ability to withstand high temperatures and moisture when selecting the sanitation technique. Some items, such as peristaltic tubing, may not withstand either of the high temperature sanitation methods. Freshly washed equipment used with extenders can be immediately used by rinsing with a small quantity of extender, which must then be discarded.

Laboratory & Personnel

Prior to handling any laboratory equipment or processing semen, the technicians must thoroughly wash their hands with an antibacterial soap, rinse with hot tap water, and dry with a fresh, clean disposable towel. Clothing and boots from the barn environment should never enter the laboratory. Eating and drinking should be prohibited in the laboratory. Equip the lab with positive air pressure to create an air flow through the pass through window from the lab to the barn. If needed, repackage bulk products such as extenders into smaller working quantities immediately after opening to avoid repeated entry and contamination. Screen all guests, instruct them in biosecurity, and require them to follow all biosecurity procedures while in the lab or on the premises.

Prevent bacterial problems through surveillance. Routinely performing bacterial culture tests on laboratory surfaces is an excellent way to monitor the effectiveness of cleaning procedures and the laboratory environment. If bacterial contamination is detected, and a cleaning protocol does not clear up the problem, consult an expert to isolate and identify the genera of the particular bacteria so that the proper disinfectants and antibiotics can be used.

Contact MOFA Global for more information or to schedule an on-site technical consultation to address bacterial contamination issues.

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