



SANITARY QUALITY CONTROL OF THE BOAR SEMEN DOSES

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Bacterial contamination is a serious deterrent to profitable semen production. Contamination can result in reduced fertility and conception rates and reduced shelf life of semen doses. Minimizing the potential for bacterial contamination, can be done by hygienic semen collection and processing procedures; stringent laboratory sanitation procedures; and addition of antibiotics to the semen. With the concerns of antibiotic resistance and the potential limitation of the use of antibiotics in future animal production it is imperative to improve and maximize hygienic semen production procedures.

Throughout the swine industry, the presence of bacterial contamination in boar semen is observed when the semen is collected by the gloved-hand technique. Researchers found that 62.5% of raw ejaculates and 79% of extended semen doses contained bacterial contamination.¹ In a ten-year study, bacterial counts per milliliter (ml) of freshly collected boar semen normally ranged between 5,500 to 48,000 and averaged 27,000.² Therefore, when low conception rates and reproductive problems occur with artificial insemination, bacterial contamination of semen is an important subject to consider. Retrospective analysis of poor conception rates on several farms suggested that bacterial-related agglutination/clumping and sperm cell death within the first two days after processing as a possible explanation for the problem.^{3,4}

Contamination of insemination doses with microorganisms can cause problems with semen quality including reduction in motility and acrosome integrity,^{3,4,5,6} vulva discharges and endometritis,⁷ and sperm cell death associated with an isolate of Escherichia coli.^{2,3,4} Researchers have isolated gentamicin-resistant bacteria responsible for producing an acidic spermicidal environment that reduces the longevity of the extended semen.³ The most commonly found bacterial contaminants in porcine extended semen are: *Alcaligenes xylosoxidans*, *Burkholderia cepacia*, *Enterobacter cloacae*, *Echerichia coli*, *Serratia marcescens*, and *Stenotrophomonas Yxanthomonas maltophilia*. These six genera of bacteria accounted for 71% of all contaminated samples and were resistant to the aminoglycoside (gentamicin). This is significant because a majority of commercial extenders use this aminoglycoside to control bacteria.

Many more genera of bacteria have been isolated from raw semen ranging from 11 up to as many as 46 different microorganisms.^{1,2,3,6,8,9} However, the most important issue is which of the bacteria from those listed above are found in extended semen and which cause problems. The bacteria of the greatest concern are those not destroyed or held in check by the antibiotic in the semen extender. More than 11 genera were spermicidal in an acidic environment.³

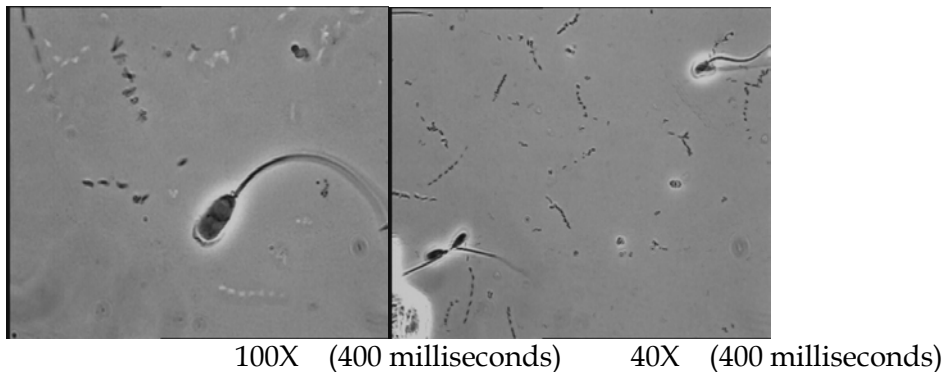


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Following collection, bacterial contamination can be viewed during initial evaluation of semen using a high-quality microscope with a phase contrast or differential interference-contrast objectives at 400x magnification. (Figure 1)



Figure 1. Photographs of motility tracks of semen with bacterial contamination.



Generally, when there is no antibiotic in the extender or the bacteria are resistant to the antibiotic, proliferation of the bacteria in extended semen increases as storage time increases. The addition of the appropriate antibiotic usually controls bacterial growth and may prolong viability and fertility of the sperm cells. The most common antibiotics used for this purpose are gentamicin, neomycin, a combination of penicillin G and streptomycin, amikacin, lincomycin and ceftiofur.¹⁰ Gentamicin and neomycin have been shown to preserve sperm motility and acrosomal morphology better than the other antibiotics when semen was stored up to five days in BTS extender.¹¹

Extended semen with bacterial contamination has been shown to contain high levels of sulphite-reductors and aerobic-mesophile isolates.¹² This provides evidence for the occurrence of environmental contamination during routine semen collection and processing.¹² Finding coliforms and *Streptococcus faecalis* suggests that fecal contamination from the boar is a likely source of bacterial contamination during semen collection. Because of poor sanitation procedures in housing boars, fecal material often contaminates the prepuce and its preputial fluids. These preputial fluids in turn contaminate semen during the collection process.^{12,13} This evidence is strong support for practicing good hygiene techniques, which minimize contamination.

Further impacts for sanitation were shown in a trial that demonstrated the significant responses of maintaining sanitary boar housing and collection areas and using hygienic collection technique.¹⁴ In the first Treatment, animals and facilities were washed two days prior to semencollection with pens dry cleaned twice a day. In addition, hygienic collection procedures were used. In the second Treatment, animals and facilities were washed five to seven days prior to semencollection. Pens were dry cleaned twice a day. No care was taken to use hygienic collection techniques. After counting the colony forming units (CFU) by spread plate method, Treatment #1 had 490 ± 975 CFU/ml while Treatment #2 had $18,862 \pm 14,634$ CFU/ml



($p < .01$).¹⁴ This significant difference points out the need for creating an environment that reduces bacterial contamination. This would include managing air flow in the boar stud to help keep the floors dry. It is recommended to have the air flow traveling from the semen collection areas to the rest of the building. This prevents bacterial contamination in the collection area by air transmission. It is also important to establish a protocol for cleaning and sanitizing boars and pens using effective disinfectants for the identified bacteria.

Because preputial fluid has been implicated as a possible source of contamination,^{12,13,15} it is very important to practice sanitary and hygienic procedures during semen collection and processing. Several different methods to reduce bacterial contamination from the preputial fluids have been tried. Bacterial content was greatly reduced by extirpation of the preputial diverticulum (Table 1).¹⁵

Table 1.
Comparison of the Number of Bacteria in Semen Before and After Extirpation of the Diverticulum

Boar	Age (mo.)	Before operation		After operation	
		No. of samples	Av. No. bact./ml semen	No. of samples	Av. No. bact./ml semen
1	17	6	7,500	6	2,430
2	18	9	8,880	10	1,100
3	18	10	6,930	10	713
4	3	Operated when 3 months old		10	4,985
5	3	10	6,620	Control, not operated

Performing this surgical procedure on every boar is quite impractical. However, in a particular boar of extreme value, a procedure like this may be helpful in maintaining higher quality semen. The closed surgical procedure for resection of the preputial diverticulum is a simple procedure and has the advantage of eliminating contamination of the surgical site.¹⁶ Another method tested to help reduce contamination from the preputial fluid is to pour physiological saline over the penis and the gloved hand of the collector prior to grasping the penis for collection. Results showed that the average bacterial counts could be reduced from 27,000 to 611 utilizing this technique.² Field trials using only this technique to reduce bacterial contamination have shown variable success.

A study of the collection technique to fractionate ejaculates has shown that, on a per ml basis, bacterial concentration is greatest in the pre-sperm fraction of the collection.¹³ Typically, this



portion is voided onto the floor prior to collecting the sperm-rich ejaculate into the collection vessel. When collecting semen from a boar with known high bacterial contamination, try to avoid collecting the last few jets of semen that come with the final gel plug. This seminal fluid has a higher concentration of bacteria than the sperm-rich fraction, and it contains virtually no sperm cells even though it appears to have a milky appearance.¹³ 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, it is important to know when the boar is completing his ejaculation so that the collection vessel is not removed too soon in attempting to avoid collection of the final gel; and an excessive amount of semen is wasted. Bacterial contamination cannot be eliminated from a boar ejaculate through fractionation. However, fractionating the ejaculate does decrease the bacterial load when the pre-sperm and gel-fraction fluids are not included in the collection (Figures 2 & 3). The source of the bacteria did not appear to originate from the reproductive organs examined in this study.¹³

Figure 2.
Concentration of Bacteria - Trial 1

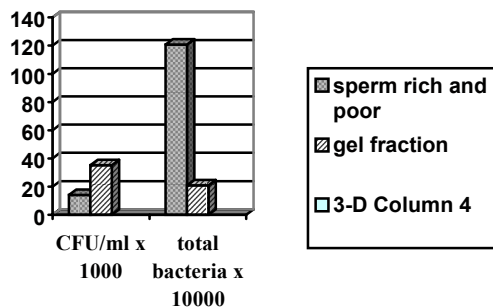
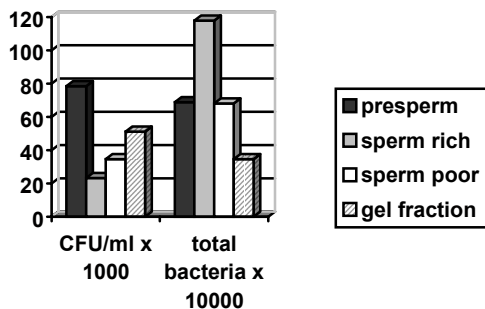


Figure 3.
Concentration of Bacteria - Trial 2



While extirpation of the preputial diverticulum and ejaculate fractionation help to reduce bacterial content, preserve semen quality, and improve storage condition and lengthen storage



time, there are other simple daily procedures and practices that maintain bacterial contamination at a minimum.

Basic practices to minimize bacterial contamination during semen collection

- House boars in a facility separated from the collection area (not always practical in older facilities).
- Create an airflow pattern that moves air from the collection area to the boar barn.
- Periodically trim hair from around the preputial opening.
- Manually evacuate preputial fluids prior to grasping the penis for semen collection.
- Evacuate preputial fluids in a pre-prep pen (if possible) prior to the boar entering the collection pen.
- Have the semen collector wear disposable vinyl gloves and wash hands with soap and thoroughly rinse with water between collecting different boars (soap is spermicidal).
- Use a double-glove technique to prevent any contamination of the actual collection glove.
- Clean the preputial opening and surrounding area with a clean disposable paper towel.
- Clean and dry the glans end of the penis.
- Hold the penis to minimize the chance of preputial fluid running down shaft of the penis into the semen collection vessel.

Or

Block any flow of fluid down the shaft with a folded strip of paper towel or clean gauze.

- Do not touch the collection vessel with the collection hand during the collection process.
- Allow 1 to 2 cm of the penis to extend beyond the gloved hand when grasping the penis or open the last finger to allow a free flow of semen into the vessel.
- Divert initial jets of the ejaculate (pre-sperm fraction containing urethral flushings/urine) from the semen collection vessel onto the floor.
- Dispose of the rubber band and filter/gauze or top portion of the US BAG™ before passing the ejaculate to the processing laboratory.
- Collection pens and dummies should be washed daily and sanitized a minimum of once each week.
- Do not cover collection dummies with carpet.

Sanitation and bacterial monitoring procedures

Bacterial contamination is not only a problem in the boar housing and collection areas, but the laboratory has also been identified as a source of antibiotic-resistant bacteria causing harmful effects on fertility.³ Sanitizing or keeping the laboratory free of bacteria is essential. Sanitation



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involves more than cleaning. It is taking the measures necessary to prevent bacterial contamination, sterilize equipment, and minimize bacterial growth where it cannot be eliminated.

Laboratory counter top surfaces: All laboratory surfaces must be cleaned before the start of each day, again after semen processing, and finally during lab clean up at the end of the day. Periodic cleaning throughout the day should also be performed as necessary. Counter top surfaces should be thoroughly cleaned using a spray-on application of an antibacterial cleaning solution. They should be wiped clean with a clean synthetic sponge and allowed to air dry prior to use. The sponge should always be allowed to air dry between uses and discarded at the end of each week. Surfaces that come into direct contact with semen, whether neat or diluted, should not be cleaned in this manner.

Laboratory equipment: It is imperative to use a detergent such as 409[®] or Contrad 70[®] that thoroughly cleans *and* completely rinses from the equipment surfaces to avoid a spermicidal residue. Select an effective detergent that is not corrosive to the laboratory equipment. If a commercial laboratory dishwasher is used, specific detergents are available that also meet these criteria. For equipment that must be hand washed, use very hot water and wear rubber gloves to protect the skin from detergent irritation and excessively hot water. First 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 periodically replaced (i.e. monthly) to prevent bacterial buildup on the brush. After thorough washing, the equipment should be triple rinsed with hot tap water followed by triple rinsing with distilled or deionized (DI) water. Because equipment cleaning is time consuming and labor intensive, many labs choose to use disposable products when economically feasible. Disposable products also play a significant role in reducing and preventing bacterial contamination.

Sanitize equipment by one of three methods: exposure to dry heat at 200°C for 30 minutes, moist heat (autoclave), or rinsing/soaking in 70% ethyl alcohol. The equipment's ability to withstand high temperatures and moisture should be taken into consideration when selecting a sanitation technique. Some items, such as peristaltic tubing, may not withstand either of the high temperature sanitation methods. If 70% ethyl alcohol is used, all surfaces should be exposed for 30 minutes. The items should then be triple rinsed with distilled or DI water and completely dried prior to the next use. Freshly-washed equipment used with extenders can be immediately used by rinsing with a small quantity of extender. This extender must then be discarded.

Sanitation rules for the technicians and lab area

Prior to handling any laboratory equipment or processing semen, the technician's hands should be thoroughly washed with an antibacterial soap, rinsed with hot tap water and dried



with a fresh, clean disposable towel. Clothing and boots from the barn environment should never enter the laboratory.

With a pass through window between the barn and lab, it is important to make sure that there is positive air pressure and airflow from the lab into the barn area to prevent contamination in the laboratory by incoming air. Provide employees with a suitable area for breaks and lunch time. Smoking and eating should be prohibited in the laboratory. Guests should not be allowed into the lab or stud without proper screening and instruction in biosecurity procedures. Guests should be required to follow all biosecurity protocols and procedures while they are on the stud premises. Bulk products such as extenders should be repackaged into smaller working quantities immediately after opening. This prevents multiple entry into products like extenders, which could easily become contaminated.

Bacterial monitoring procedures

Routinely performing bacterial culture tests on laboratory surfaces is an excellent way to monitor the effectiveness of cleaning procedures and the laboratory environment. More labs are implementing these types of procedures to assure that effective sanitation and lab-cleaning protocols are in place. If a bacterial contamination problem is found and a cleaning protocol does not clear up the problem, an expert should isolate and identify the genera of the particular bacteria so that the proper disinfectants and antibiotics can be utilized. Items necessary to perform a simple culture test for bacteria are listed below. Items that should routinely be swabbed and checked for bacteria are also listed. A basic procedure for performing the bacterial culture test is outlined. Other protocols can also be used.

Equipment required to perform cultures:

- 1) Incubator (38°C)
- 2) Sterile culture swabs
- 3) Sterile culture loops
- 4) Agar plates (Trypticase[®] Soy with 5% Sheep Blood or equivalent)
- 5) Record sheets

Items to be swabbed and cultured each week:

- 1) Lab counters
- 2) Balance surfaces
- 3) Draining racks
- 4) DI water
- 5) Sinkers
- 6) Peristaltic pump tubing (inside lumen, middle of the tube)
- 7) Extender
- 8) Extended semen
- 9) Neat semen



- 10) Water bath surfaces
- 11) Vat lids
- 12) Pitchers (extending equipment)
- 13) Slide warmer surface
- 14) Microscope stage surface

Procedure for monitoring bacterial growth in the lab:

- 1) Preheat the incubator to 38°C.
- 2) For each item to be checked for bacteria, remove an agar plate from the refrigerator and allow it to warm to room temperature (23°C).
- 3) Identify and label each agar plate with a permanent marker and record the corresponding label information (i.e. number or letter) on a record sheet along with the item to be swabbed.
- 4) Swab dry equipment after standard cleaning and sanitation procedures have been performed.
- 5) Remove a sterile swab from its package. Do not contaminate the sterile tip. Wipe the sterile tip over the surface being swabbed, turning it as it is wiped to expose most of the tip to the item's surface.
- 6) Open an agar plate and streak the swab down the center of the agar media from the top of the dish to the bottom. With the same swab, start near the edge of the plate on one side of the center line and streak back and forth across the line to cover the entire distance from one end of the line to the other in a zig-zag pattern.
- 7) Liquids can be swabbed with a sterile loop dipped into the liquid and streaked across an agar plate in the same manner as described.
- 8) Once streaked, the plates should be placed in the incubator at 38°C for 24 hours.

Reading results

- 1) Following 24 hours of incubation at 38°C, remove the plates from the incubator and count and record the number of bacterial colony forming units (CFU). Return the culture plates to the incubator for an additional 24 hours of incubation.
- 2) Following 48 hours of incubation (from initial start time), remove the plates from the incubator and repeat the colony count and record process.
- 3) Plates should be sterilized by autoclave or chemical sterilization before being discarded.
- 4) The number of colonies counted represents the relative amount of bacterial contamination. Colonies growing in areas separate from the swab marks on the agar surface should not be counted. They represent air contamination that occurred during streaking. High numbers of colonies indicate inadequate cleaning procedures or poor-quality cleaning solution, both of which require immediate attention.
- 5) Colonies can be identified by a trained veterinarian or technician. However, the types of bacteria present are not usually as important as the relative number of colonies. Any bacterial contamination is indicative of poor cleaning procedures.



- 6) Identification of colonies from extended semen may indicate strains resistant to the antibiotic used in the semen extender.

The boar has a natural ability to compensate for the adverse side effects of bacterial contamination with an overabundance of sperm. By producing the maximum number of doses with the minimum number of cells, the artificial insemination process eliminates this natural ability of the boar. Consequently, artificial insemination requires established quality control procedures for sanitation, collection, and proper antibiotic use to minimize the risk of reduced fertility resulting from bacterial contamination of the semen.

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