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The impact of bacteriospermia on boar sperm storage and reproductive performance

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ABSTRACT

Bacteriospermia is a documented risk to reproductive performance when using extended boar semen for artificial insemination. A substantial list of bacteria have been recovered from boar semen attributed to fecal, preputial, skin, and hair microorganisms, with these and other environmental bacteria from processing areas identified in doses prepared for artificial insemination. Gram-negative bacteria are most commonly recovered from extended doses, including both *Enterobacteriaceae* and environmental contaminants, such as those that inhabit water purification systems. The method of processing, distributing, and storing fresh liquid boar semen before insemination plays a role in bacterial growth dynamics and the degree to which the bacteria may damage the sperm or affect the sow. Not all bacterial isolates or contamination levels have the same impact on sperm, with multiple factors governing if and when storage longevity will be reduced through sperm-to-sperm agglutination, impaired motility, acrosome disruption, or loss of membrane viability. Suboptimal reproductive performance can occur because of reduced fertilizing capacity of the sperm or induction of a uterine environment hostile to sperm and/or embryonic survival. Effective bacterial control strategies are necessary to minimize the risk of bacteria contaminating extended semen doses, including monitoring programs designed for quick detection and intervention, should the need arise.

1. Introduction

Artificial insemination (AI) is a common assisted reproductive technology used in the swine industry whereby diluted semen containing viable spermatozoa is mechanically placed into the female reproductive tract. Unlike bovine AI where straws of frozen semen are often stored in liquid nitrogen, domestic swine AI systems are built almost exclusively on liquid storage at cool (15 °C–19 °C) temperatures, with doses often aging for multiple days between collection, distribution, and insemination. Inherent to the semen collection process is the risk of bacterial contamination of the ejaculate. Subsequent processing of the ejaculate into a format readily usable by sow farms can result in additional exposure to potential bacterial contaminants. Failure to adequately control these risks can have negative consequences, including decreased semen quality, reduced dose longevity, and impaired fertility. The purpose of this review is to summarize the current state of knowledge concerning bacterial contamination of boar semen and its reproductive consequences.

2. Bacterial contaminants

Although certain bacteria transmitted through semen are considered pathogenic in causing various clinical disease conditions in the sow, this review will focus exclusively on bacterial effects during sperm storage and subsequent reproductive performance [1]. Semen from healthy boars generally does not contain bacteria; however,

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the preputial diverticulum, skin, and hair of the boar do, as do the barn and collection environment which can contaminate the collector's hands or collection container [2,3]. Sterility is not a practical option for routine boar semen collection; consequently, a plethora of bacteria have been identified in raw boar semen by various investigators, as reviewed in Table 1 [4–6]. The overall percentage of extended semen samples positive for bacterial growth at the University of Pennsylvania Reference Andrology Laboratory (USA) ranged from 32% in 2002 to 2003 to 17% in 2005 and 26% in 2006 [7,8]. A slightly lower rate of 14.73% was reported for aerobic bacterial contamination in extended semen samples cultured at a quality control laboratory in Spain in 2012 [9]. The majority of contaminants recovered from extended semen cultures are gram-negative bacteria, with a large percentage from the family *Enterobacteriaceae* [7,9,10]. Resistance to the preservative antimicrobial(s) present in the semen extender is a common feature of bacteria recovered from production doses [8,10]. Bacteria commonly identified from extended semen doses are presented in Table 2.

3. Impact on sperm storage

As extended boar semen is essentially cell culture media, it is an ideal environment for the growth of contaminant bacteria resistant to the preservative antimicrobials meant for their control. The effects of that growth can differ. Sone [11] reported that the survival of boar sperm during storage at 15 °C was remarkably affected within 1 to 2 days when five species of enteric bacteria were present, including *Escherichia coli. Pseudomonas*, which was the most frequent bacteria isolated (80.4%) from the 46 samples tested, affected sperm survival to a lesser extent, and four species (*Alcaligenes* sp., *Actinomyces* sp., *Streptococcus* sp., and *Staphylococcus* sp.) had almost no negative influence on storage longevity despite bacterial levels of 10^{10} to 10^{12} CFU/mL causing a moderate reduction (6.3–6.5) in pH [11]. In a case report in which *Achromobacter xylosoxidans* was implicated in vulvar discharge and reduced reproductive performance, no decrease in sperm motility was apparent during storage [12], and in an *in vitro* study, no decrease in motility was apparent compared to controls for either *Achromobacter xylosoxidans* or *Ralstonia pickettii* in three different extenders over 14 days of storage when inoculated at 2.5×10^7 CFU/mL (*Achromobacter xylosoxidans*) or 2.0×10^6 CFU/mL (*Ralstonia pickettii*) [13].

A series of controlled *in vitro* studies involving *E coli, Clostridium perfringens*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa* reported detrimental effects of bactetriospermia as bacterial concentrations increased, with sperm motility and viability most consistently affected [14–17]. The same studies noted that sperm morphology changes were not induced by bacterial inoculation, but other parameters such as acrosome integrity, agglutination, osmotic resistance, or pH may be affected by certain bacteria at sufficient concentrations [14–17].

An early case report from two boar studs during the rapid adoption phase of AI in the United States described severe sperm agglutination and motility reduction from 0% to 25% within 36 to 48 hours after collection and extension because of growth of *Serratia marcescens* resistant to the preservative antibiotic gentamicin in the semen extender [18]. Acidic sample pH and compromised acrosome integrity were also features of the stored samples. A comprehensive case study on bacteriospermia involving 23 North American field investigations undertaken over a 3-year time period was published in 2000 [10]. In addition to reporting the condition of extended semen samples submitted to the andrology laboratory for analysis, pure cultures of the six most frequently identified bacteria (*Enterobacter cloacae*, *E coli*, *Serratia marcescens*, *Alcaligenes xylosoxidans*, *Burkholderia cepacia*, and *Stenotrophomonas maltophilia*) were inoculated into freshly extended semen under controlled conditions, with the same findings as the

Table 1
Common bacterial flora isolated from the neat boar ejaculate.

Tamuli et al. [4]	Dagnall [5]	Dagnall (cont'd)	Sone et al. [6]
Escherichia coli	*Citrobacter* spp.	*Bacillus* spp.	*Pseudomonas* spp.
Pseudomonas spp.	*Pseudomonas* spp.	*Actinobacillus* spp.	*Micrococcus* spp.
Bacillus spp.	*Corynebacterium* spp.	*Staphylococcus* spp.	*Staphylococcus* spp.
Staphylococcus spp.	*Streptococcus* spp.	*Flavobacterium* spp.	*Klebsiella* spp.
Klebsiella spp.	*E coli*	*Klebsiella* spp.	*E coli*
Proteus spp.	*Actinomyces* spp.	*Micrococcus* spp.	*Citrobacter* spp.
Enterobacter spp.	*Bacteroides* spp.	*Proteus* spp.	*Proteus* spp.
Pasteurella spp.	*Lactobacillus* spp.		*Actinomyces* spp.
Citrobacter spp.	*Acinetobacter* spp.		*Serratia* spp.
			Enterobacter spp.
			Bacillus spp.
			Streptococcus spp.

Adapted from Althouse and Lu [7].

Table 2
Common bacterial flora[a] isolated from extended semen doses submitted for culture.

2002/03	2005	2006
Enterococcus spp.	*Enterococcus* spp.	*Ach (Alc) xylosoxidans*
Stenotrophomonas maltophilia	*Escherichia coli*	*Acinetobacter* spp.
A xylosoxidans	*Proteus mirabilis*	*Bacillus* spp.
Serratia marcescens	*Pseudomonas* spp.	*CDC EF4*
Acinetobacter lwoffii	*Serratia marcescens*	*Corynebacterium* spp.
E coli	*Stenotrophomonas maltophilia*	*Klebsiella oxytoca*
Pseudomonas spp.		*Providencia* spp.
Comamonas testosterone		*Pseudomonas* spp.
Klebsiella spp.		*Ralstonia picketti*
Providencia rettgeri		*Serratia liquefactions*
Burkholderia cepacia		*Serratia marcescens*
		Staphylococcus spp.
Enterobacter cloacae		*Stenotrophomonas maltophilia*

[a] Only fully identified isolates recovered from two or more samples were included.
Adapted from Althouse and Lu [7] and Althouse et al. [8]