PAPER

Boar semen bacterial contamination in Italy and antibiotic efficacy in a modified extender

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Abstract

The aims of the study were to identify microbial flora in boar semen under field conditions in northern Italy, to investigate antibiotic resistance and sensitivity of isolated bacteria, and to evaluate elimination of bacteria after storage in two types of extenders added with different antibiotics (amikacin *vs* gentamicin). A total of 60 boars were collected in 13 pig farms. Bacteriological and mycological investigations were performed immediately on raw semen samples, then at 48 and 120 h of storage on semen diluted randomly in a new short-term modified extender (ME-S) or in a commercial one (CRONOS™). Bacterial contamination was found in 63% of raw semen samples and different bacterial species were isolated: *E.coli*, *Serratia marcescens*, *Staphylococcus epidermidis* and *aureus*, *Proteus spp*., *Streptococcus spp.* and *Pseudomonas aeruginosa*. *E. coli* was the most isolated contaminant (53%); *Pseudomonas aeruginosa* was found only in one semen sample. The analysis of variance of factors affecting contamination levels was significant for the farm of origin (P<0.05) and not significant for the breed. Antibiotic resistance of these bacteria was assessed using different antibiotics. Significant differences (P<0.05) between observed and expected frequencies of bacterial isolates resistant or not to the antibiotics contained in the extenders were found. At 48 h of storage a reduction of aerobic contamination was found after ME-S dilution by 85.3% and after CRONOS™ by 63.8%. This paper proved the presence of pathogenic bacteria in semen. We thus believe it is highly advisable to perform periodic microbiological screening of boar semen in the swine industry to avoid the use of low sperm quality.

Introduction

Bacterial contamination is a serious and very real deterrent to profitable semen production in swine. It is routinely observed in raw and extended semen produced for artificial insemination (AI) when semen is collected by the gloved-hand technique. A study has reported that 62.5% of raw ejaculates and 79% of extended semen doses investigated yielded bacterial contamination (Tamuli *et al.*, 1984). Nonetheless, bacteriological surveillance is still seldom applied in swine management even if semen doses are stored at 15-20°C to avoid cold shock of boar spermatozoa (Paulenz *et al*., 2000; Pursel *et al*., 1973). Storage temperature favours the survival of bacterial contaminants. Bacterial contamination of porcine semen has been associated with deleterious effects on semen quality (Althouse *et al.*, 2008), affecting motility and structure of the sperm and causing sperm agglutination and shortening of viability (Monga and Roberts, 1994). This reduces fertility, conception rates, and litter size at birth (Engblom *et al.*, 2007) and shortens shelf life of semen doses (Althouse *et al.*, 2000). Contaminating microorganisms can come from different sources of animal or non-animal origin (Althouse *et al.*, 2005). Mammalian origin includes microorganisms from preputial fluids, urogenital system, skin, hair, respiratory secretions, feces, poor hygiene conditions, and personnel contamination (Althouse, 2008). Environmental contamination comes from contaminated water, feed, bedding, equipment, and air ventilation systems (Althouse and Lu, 2005; Althouse *et al.*, 1998). Many bacterial species have been isolated from semen, most frequently *Escherichia coli*, *Pseudomonas* spp., *Staphylococcus* spp., *Proteus* spp, *Serratia* spp, *Enterobacter* spp, *Klebsiella* spp, and occasionally also *Clostridium* spp. (Althouse and Lu, 2005; Arredondo *et al.*, 2001; Bussalleu *et al.*, 2013). For stallions, the environment in which they are housed has been reported to be the main cause of the presence of organisms on the external genitalia (Varner *et al.*, 1998); this may be true for boars as well. Hygienic semen collection and proper processing techniques with stringent laboratory procedures are the first and primary lines of defense to successfully reduce contamination. Also, antibiotics are added to semen extenders as a preventive measure to reduce bacterial contamination (Gadea, 2003; Johnson *et al*., 2000). New generation antibiotics such as apramycin and ceftiofur have been used, although conclusive results are not yet available (Althouse, 1997). Drug resistance has been observed among isolates from boar semen against antibiotics commonly used as preservative antimicrobials in commercial porcine semen extenders (Althouse and Lu, 2005). The aims of the present paper were to investigate the microbiological content of boar semen in northern Italy, to test the sensitivity and resistance of isolated bacteria to several antibiotics, and to evaluate elimination of bacteria after storage in two type of extenders added with different antibiotics (amikacin *vs* gentamicin), and if possible, to point out the importance of periodic microbiological screening of semen/doses performed in swine industry to avoid the use of semen with low sperm quality.

Materials and methods

Samples

During September and October 2012, 60 samples of raw semen from 60 different mature boars reared on 13 pig farms were collected. Thirty-five samples came from 2 farms specialized in semen dose production, processing, and delivery all over Italy; the other 25 were from 11 farms practicing integral pig production. The farms chosen were located in northern Italy (Provinces of Reggio Emilia, Modena, Mantova, Cremona, Brescia, and Verona), where more than 75% of Italian pigs

are housed (Moreno *et al.*, 2010). The boars included in this study belonged to different breeds (Large White, Landrace, Pietrain, Duroc) and synthetic populations (Goland, Hypor, Maxgrow, PIC, TopD) and were all housed in an individual box and were of proved fertility. All the semen presented good quality standards. Frequency of collection was 2/3 time a week by specialized farm personnel. Semen was collected by gloved-hand technique with a dummy into an insulated bottle (500 mL), with the opening covered with gauze to separate the gel particles; only the second and third phases were considered. All the boars used as semen donors were healthy.

Semen evaluation and dilution

Evaluation of semen characteristics (volume, pH, colour, smell, motility, concentration of the sperm) was performed immediately after collection. Only ejaculates with sperm motility higher than 75% immediately after collection and at least 90% of spermatozoa showing normal morphology were considered. After evaluation, raw semen aliquots were randomly diluted in one of two types of extender at 1:9 dilution (100 mL 3×10^6 sperm/mL): 35 samples were diluted in a new modified extender (ME-S) for short-term storage containing amikacin sulphate and fructose (Bresciani *et al.*, 2013) and 25 samples were diluted with a commercial extender CRONOS™ (Medinova™, Reggio Emilia, Italy). The composition of ME-S is reported in Table 1, while CRONOS™ contains gentamicin (0.33 g/L) and glucose, as declared by Medinova™. The sperm characteristics and motility during storage were evaluated *in vitro* as reported in a previous study (Bresciani *et al.*, 2013).

Bacteriological and mycological investigations

The raw samples were immediately sent to the laboratory and cultured for bacteriological and mycological investigations. The same investigations were performed on diluted semen after 48 and 120 h of storage at 15±1°C.

Culture swabs were plated on bovine blood agar medium (tryptose agar - Beckton Dickinson, Sparks, Maryland, USA- containing 5% bovine erythrocytes) (Quinn *et al.*, 1994; Carter *et al.*, 1995) and MacConkey agar (Beckton Dickinson, Sparks, Maryland, USA). For each specimen cultures were then incubated aerobically and anaerobically (AnaeroGen™, Oxoid, UK) for 24-48 h at 37°C. For mycological investigations, Sabouraud agar (Beckton Dickinson™, Sparks, MD, USA) plates were inoculated and incubated aerobically for 48 h at 35°C. Bacterial isolates were identified using

standard microbiological procedures, *i.e.* growth and colonial characteristics, Gram staining, cellular morphology, catalase and oxidase reactions, coagulase test and haemolysin production. Species identification was carried out using the API biochemical test systems (bioMérieux™, Marcy-l'Etoile, France) as well as conventional biochemical tests (Quinn *et al.*, 1994). Antibiotic sensitivity tests were performed with the standard Kirby-Bauer disk-diffusion susceptibility methods (Quinn *et al.*, 1994), evaluated on the basis of the criteria employed by the Clinical and Laboratory Standard Institute (CLSI), formerly the National Committee for Clinical and Laboratory Standards (NCCLS) (CLSI, 2008). In the present study 25 antibiotics were tested on 7 bacterial species isolated from fresh boar semen for a total of 43 strains (Table 2). We were specifically interested in amikacin and gentamicin activity because they are present in the two extenders compared in this investigation.

Data collection and analysis

Data were submitted to statistical analysis to assess the frequency of contaminated samples and the kind and degree of contamination. A x^2 test was performed to assess differences between observed and expected frequencies. An analysis of variance was then performed on data from raw and diluted samples to evaluate the effects of type of extender, breed of boar (nested within herd and type of extender), and kind of farm (nested within type of extender) on the degree of contamination.

Results and discussion

Bacterial species were present in 38 samples out of the 60 examined (63%). Thirty-three samples (86.85%) were positive for pure culture of a single bacterial species (16-*E. coli*, 6-*Serratia marcescens*, 5-*Staphylococcus epidermidis*, 1-*Streptococcus spp.*, 2-*Proteus spp.*; 2-*Staphylococcus aureus, 1-Pseudomonas spp.*). Five samples (13.16 %) were positive for co-infected cultures of two bacterial species (2-*E. coli* and *Proteus spp.*, 1-*E. coli* and *Staphylococcus epidermidis*, 1-*Staphylococcus epidermidis* and *Streptococcus* spp., 1-*E. coli* and *Serratia marcescens)*. A total of 43 isolates were detected: *E. coli* (n=20), *Staphylococcus epidermidis* (n=8), *Serratia marcescens* (n=6), *Proteus* spp. (n=4), *Staphylococcus aureus* (n=2), *Streptococcus* spp. (n=2), *Pseudomonas* spp. (n=1). *E. coli* was the most frequent contaminant bacterium (46.5%), followed by *Staphylococcus epidermidis* (18.6%), *Serratia*

Table 1. Composition (% w/w) of the modified extender used in the study.

Component	ME-S
Fructose°	78.60
Potassium chloride[#]	1.56
Sodium bicarbonate[#]	2.67
Na EDTA[#]	2.67
Sodium citrate[#]	12.00
Amikacin sulphate[#]	2.50

ME-S, Modified extender short-term; EDTA, Ethylenediaminetetraacetic acid. °Supplier: Acef SpA, Fiorenzuola D'Arda (PC), Italy; [#]supplier: Sigma-Aldrich, St. Louis, MO, USA.

marcescens (13.95 %), *Proteus* spp. (9.30 %), *Staphylococcus aureus* and *Streptococcus* spp. (4.65 % each), and *Pseudomonas* spp. (2.3 %) (Table 2). Each isolate was characterized as either obligate aerobic or facultative anaerobic. No strictly anaerobic isolate was detected. All tested samples of raw and diluted semen were negative for mycological investigation. The antibiotic resistance data regarding bacterial isolates are also shown in Table 2. Significant differences ($P<0.05$) between observed and expected frequencies of bacterial isolates resistant or not to the antibiotics contained in the extenders were found. Values of x^2 ranged from 5.67 to 200 (data not tabulated). In particular, *E. coli* isolates showed 50% resistance to amikacin and 70% resistance to gentamicin; *Staphylococcus epidermidis* was 12.5% resistant to amikacin and 50% to gentamicin; *Serratia marcescens* showed a resistance of 66.6% to amikacin and 50% to gentamicin; *Proteus* spp. isolates were 25% resistant to amikacin and 50% to gentamicin; *Streptococcus* spp. isolates were 0% resistant to amikacin and 50% to gentamicin, *Staphylococcus aureus* isolates were totally sensitive to amikacin (0% resistance) and 100% resistant to gentamicin. Some other antibiotics, like ceftiofur, enrofloxacin and marbofloxacin, proved to be effective in several cases and will be useful for further investigations. Table 3 shows the efficacy of the two extenders for the reduction of bacteria concentration. After 48 h of storage in ME-S extender, *E. coli* strains were reduced from 12 to 3 (75%) while in CRONOS™ group dropped from 8 to 4 (50%); at 120 h no changes were observed compared to 48 h. *Serratia marcescens* was inhibited in amikacin group at 48 h and in the gentamicin group it was reduced by 50%; *Proteus* spp. was present only in CRONOS™ extended sample and was reduced by 25% after 48 hours of storage. During storage, amikacin completely inhibited the growth of *Serratia marcescens, Pseudomonas* spp*., Staphylococcus*

aureus, Staphylococcus epidermidis, and *Streptococcus* spp. The analysis of variance of factors affecting contamination levels in raw and diluted sperm samples (Table 4) showed a significant effect of the farm of origin (P<0.05) and no significant effect of the breed of boar. The type of extender tended to be significant (P<0.10) for aerobic contamination of diluted semen. The least squares means of contamination degree (Table 5) showed a

reduction of aerobic contamination after ME-S dilution by 85.3% and after CRONOS™ by 63.8%. For anaerobic isolates the reduction was 100% and 91.8%, respectively. No difference in contamination degree was found between 48 and 120 h. Mean percentages of progressively motile sperm at 48 h and 120 h were not significantly different (P>0.05) between ME-S and CRONOS™ (70.0±5.2 *vs* 76.0±1.9 and 59.2±9.1 *vs* 55.0± 5.3).

Worldwide contamination of boar semen levels requires the use of extenders that can guarantee effective protection against several bacteria species isolated in this potential contaminated vehicle. In addition, by using the same small number of antibiotics over the years, bacteria may develop resistance. Since, as the literature reports, the presence of bacteria in semen doses during storage leads to deleterious effects on semen motility and con-

Table 2. Bacterial contaminants and antibiotic resistance in raw semen.

Antibiotic	*E. coli* n=20	*Staph. epidermidis* n=8	*Serratia marcescens* n=6	*Proteus mirabilis* n=4	*Streptococcus* spp. n=2	*Staph. aureus* n=2	*Pseudomonas* spp. n=1
Amikacin (30 µg)	10 (50%)	1 (12.5%)	4 (66.6%)	1(25%)	0 (0%)	0 (0%)	0 (0%)
Amox. + Clav. acid (30 µg)	11 (55%)	2 (25%)	4 (66.6%)	2 (50%)	0 (0%)	1 (50%)	1 (100%)
Ampicillin (25 µg)	15 (75%)	3 (37.5%)	5 (83.3%)	3(75%)	0 (0%)	1 (50%)	1 (100%)
Aztreonam (30 µg)	11 (55%)	0 (0%)	3 (50%)	1(25%)	0 (0%)	1 (50%)	1 (100%)
Aminosidine (60 µg)	nd	3 (37.5%)	4 (66.6%)	nd	nd	1 (50%)	nd
Cefapirin (30 µg)	nd	2 (25%)	nd	nd	nd	nd	0(0%)
Cefazolin (30 µg)	11 (55%)	3 (37.5%)	5 (83.3%)	3(75%)	0 (0%)	1 (50%)	1 (100%)
Cefoperazone (30 µg)	13 (65%)	1 (12.5%)	5 (83.3%)	2 (50%)	0 (0%)	1 (50%)	N.D.
Ceftiofur (30 µg)	4 (20%)	1 (12.5%)	4 (66.6%)	0 (0%)	0 (0%)	1 (50%)	1 (100%)
Ceftriaxone (30 µg)	nd	0 (0%)	nd	nd	0 (0%)	nd	nd
Cefquinome (30 µg)	7 (35%)	3 (37.5%)	5 (83.3%)	3(75%)	0 (0%)	0 (0%)	0(0%)
Colistin (10 µg)	19 (95%)	7 (87.5%)	6 (100%)	4(100%)	1 (50%)	2 (100%)	1 (100%)
Doxiciclina (20 µg)	17 (85%)	3 (37.5%)	5 (83.3%)	1(25%)	0 (0%)	1 (50%)	1 (100%)
Danofloxacin (5 µg)	17 (85%)	3 (37.5%)	5 (83.3%)	4(100%)	0 (0%)	1 (50%)	1 (100%)
Enrofloxacin (5 µg)	1 (5%)	0 (0%)	6 (60%)	1(25%)	0 (0%)	0 (0%)	1 (100%)
Florfenicol (30 µg)	13 (65%)	2 (25%)	3 (50%)	3(75%)	0 (0%)	0 (0%)	1 (100%)
Flumequine (30 µg)	12 (60%)	2 (25%)	5 (83.3%)	3(75%)	0 (0%)	1 (50%)	1 (100%)
Gentamicin (10 µg)	14 (70%)	4 (50%)	3 (50%)	2 (50%)	1 (50%	2 (100%)	0 (0%)
Marbofloxacin (5 µg)	0 (0%)	0 (0%)	0 (0%)	1(25%)	0 (0%)	0 (0%)	0 (0%)
Oxitetracyclin (30 µg)	17 (85%)	3 (37.5%)	5 (83.3%)	4(100%)	0 (0%)	1 (50%)	1 (100%)
Penicillin G (10 µg)	17 (85%)	3 (37.5%)	5 (83.3%)	4(100%)	0 (0%)	1 (50%)	1 (100%)
Rifaximin (40 µg)	14 (70%)	3 (37.5%)	5 (83.3%)	1(25%)	0 (0%)	1 (50%)	1 (100%)
Streptomycin (10 µg)	17 (85%)	3 (37.5%)	5 (83.3%)	3(75%)	0 (0%)	1 (50%)	1 (100%)
Tiamulin (30 µg)	20 (100%)	7 (87.5%)	6 (100%)	4(100%)	1 (50%)	2 (100%)	1 (100%)
Tylosin (30 µg)	20 (100%)	7 (87.5%)	6 (100%)	4(100%)	2 (100%)	2 (100%)	1 (100%)

nd, not detected.

Table 3. Comparison of bacterial contamination in extenders containing amikacin (ME-S) and gentamicin (CRONOSTM) during storage at 15±1°C.

	Survival of bacteria isolated in diluted semen					
Bacterial isolates	Semen samples diluted		Semen storage 48 h		Semen storage 120 h	
	ME-S (n=35)	CRONOSTM (n=25)	ME-S (n=35)	CRONOSTM (n=25)	ME-S (n=35)	CRONOSTM (n=25)
Escherichia coli	12	8	3 (25%)	4 (50%)	3 (25%)	4 (50%)
Staphylococcus epidermidis	5	3	0	0	0	0
Serratia marcescens	2	4	0	2 (50%)	0	2 (50%)
Proteus spp.	0	4	0	1 (25%)	0	1 (25%)
Streptococcus spp.	2	0	0	0	0	0
Pseudomonas spp.	1	0	0	0	0	0
Staphylococcus aureus	1	1	0	1 (100%)	0	1 (100%)
Total strains	26	17	4	7	4	7

ME-S, Modified extender short-term.

Table 4. Analysis of variance (P values) of factors affecting boar semen contamination.

	Type of extender	Breed of boar	Herd	RSE	R^2
Raw semen					
Aerobic	ns	ns	0.035	0.544	0.503
Anaerobic°	ns	ns	0.028	0.474	0.373
Diluted semen, 48 h					
Aerobic	0.083	ns	0.021	0.311	0.556
Anaerobic°	ns	ns	0.004	0.114	0.492
Diluted semen, 120 h					
Aerobic	0.083	ns	0.021	0.311	0.556
Anaerobic°	ns	ns	0.004	0.114	0.492

ns, not significant; RSE, relative selection efficiency. °Facultative anaerobic.

Table 5. Least squares means of degree of contamination as affected by type of extender.

	Raw semen		Diluted semen°	
Group	ME-S	CRONOSTM	ME-S	CRONOSTM
Aerobic	0.641±0.099	0.784±0.118	0.094±0.057[a]	0.284±0.068[b]
Anaerobic[#]	0.318±0.087	0.353±0.103	0.000±0.021	0.029±0.025

ME-S, Modified extender short-term. Degree of contamination refers to the following parameters: 0=sterile, 1=contaminated by one bacteria species, 2=contaminated by two bacteria species. °After 48 and 120 h; [#]facultative anaerobic. [a,b]Means in the same raw with different superscript are significantly different at 0.10<P>0.05.

sequently on livestock reproductive efficiency (Althouse, 2008), there is constant demand for innovative boar semen extenders to improve the viability of semen doses (Bresciani *et al.*, 2013). The present study reported that bacterial contaminant species in boar semen are also commonly isolated (63%) in Italy though the species isolated are partly different from those reported in the literature. In our study we isolated 7 species (*E. coli; Serratia marcescens*, *Staphylococcus epidermidis*, *Streptococcus spp.*, *Proteus spp.*; *Staphylococcus aureus, Pseudomonas spp.*) while other authors have counted an additional 18 bacterial species in North America, Brazil, Cuba, Korea, and Thailand (Althouse *et al.*, 2000; Bussalleu *et al.*, 2013; Maroto Martín *et al.*, 2010; Park *et al.*, 2008; Suwimonteeraburt *et al.*, 2011;). The majority of the contaminants identified in extended semen are Gram-negative bacteria, almost all belonging to the *Enterobacteriaceae* family. It has been scientifically proven that the presence of contaminants damages the viability and fertility of the semen (Althouse, 2008). As reported in humans by Monga and Roberts (1994), *E. coli* induces an agglutinating effect on 40%-75% of motile sperm, reported even in pigs (Althouse, 2008; Bussalleu *et al.*, 2011). Bacterial contamination overgrowth yields worse semen quality and longevity (Aurich and Spergser, 2007, So *et al.*, 2011) in a concentration-dependent manner (Althouse and Lu, 2005). If such doses are used in AI programs can increase returns to oestrus, *post*-insemination vulvar discharges, and reduced herd reproductive performances (Sone *et al.*, 1989). The substitution of amikacin in place of gentamicin in our new modified extender has shown to improve the fertility of the semen. In fact, for all the 7 different bacterial species from 43 isolates amikacin had good antibacterial activity, and in a recent field trial the fertility was increased, with a significant rise in the number of piglets (Bresciani *et al.*, 2013). *E. coli* was the most frequently isolated contaminant bacteria [20/38 (prevalence 53%) of semen samples]; a potentially pathogenic agent like *Ps. aeruginosa* (Pasing *et al.*, 2013) was found only in one semen sample. A previous study reported that microbiological controls in boar farms that complained of reproductive management problems (*i.e.* sperm agglutination, decreased sperm longevity, increased regular returns to estrus, or even vaginal discharges across parity) showed the presence of bacteria in 66% of semen doses. All contaminant bacteria were found to be resistant to gentamicin and *E.coli* exhibited spermicidal activity even in the absence of acid environment (Althouse *et al.*, 2000). The World Organisation for Animal Health (OIE; Office International des Épizooties) published the standard of $5.0×10^3$ cfu/mL as a limit for the bacterial contamination of bovine semen samples (OIE, 2001) and Maroto Martín *et al.* (2010) reported that when boar semen used for AI is contaminated with *E. coli* above a threshold value of $3.5×10^3$ cfu/mL, there is a significant reduction in litter size. According to Bussalleu *et al.* (2011), significant adverse effects on porcine sperm quality were observed from 10^3 cfu/mL *E. coli* in the experiment performed at 37°C. In our study, pH was maintained neutral in both extenders. Some authors advised a minimum contamination technique (MCT) protocol to standardize hygiene and sanitation since in field conditions bacteria come from multiple origins but almost all relating to boar preparation, semen processing, and laboratory sanitation (Althouse, 2008; Althouse *et al.*, 2000). Likewise, extenders are very rich media that contain enough nutrients to support bacterial growth (Maroto Martín *et al.*, 2010). Amikacin showed a good activity against isolated bacteria, in our opinion improving semen quality by reducing bacterial contamination. In a previous study, the ME-S amikacin concentration did not show any deleterious effect on sperm viability, and reproductive fertility increased in an AI field trial (Bresciani *et al.*, 2013). In our samples we did not identify *Clostridium* species, which has been reported in swine industry (Baker *et al.*, 2010; Maroto Martín *et al.*, 2010) and in frozen equine semen (Corona and Cherchi, 2009). Varner *et al.* (1998) demonstrated that amikacin in extender for equine semen was more effective in eliminating bacterial growth than was polimixin B, which shown detrimental effects on sperm motility, while amikacin appeared to provide the best conditions for maintenance of sperm motility. Moreover, Aurich and Spergser (2007) demonstrated that gentamicin was not effective in reducing bacteria detrimental effects in equine semen samples. The presence of *Ureaplasma diversum, Histophilus somni, Chlamydophila abortus, Escherichia coli,* and *Sthaphylococcus spp.*, has been reported during reproductive tract infection of the cows and *Pseudomonas,* are opportunistic microorganisms of the bull's genital tract (Schlafer and Miller, 2007). Recently, Kilburn *et al.* (2013)

reported the presence of Gram-negative bacteria *Stenotrophomonas* spp. *Sphingomonas* and *Pseudomonas,* sensitive to gentamicin in bovine semen.

Greater scientific understanding of the transfer of antibiotic resistance, especially in pathogenic bacteria, is needed and concerns over the routine use of antibiotics as a substitute for good animal husbandry practices within intensive farming must be addressed (Kilburn *et al*., 2013).

Conclusions

The results obtained in the present study showed that raw boar semen in northern Italy has a consistent microbiological content. Almost all microorganisms came from collecting procedures (herd environment) or from health conditions of the boar. The germs are different from those isolated by authors working in other countries. Amikacin showed a higher microbicidal activity compared to gentamicin decreasing germ viability and concentration and thus is a useful component of boar semen extenders. Obviously, contamination control of semen by adding an adequate antibiotic in the medium must be accompanied by optimal hygiene measures during semen collection. In our opinion, performing periodic microbiological screening of boar semen in the swine industry is highly advisable so as to avoid the use of low sperm quality doses.

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