Bacterial contamination by biofilm in a boar stud

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Summary

In boar studs, bacteria are introduced into the semen dose production line through boars, personnel, a poor semen extraction protocol, distilled water, etc.

The following is a case of a boar stud whose internal quality control system detects differences in terms of preservation between seminal doses from different packaging systems, seeing a drastic decrease in sperm motility in less than 96 hours.

After visits to the stud and the seminal analysis, contamination by *Serratia marcescens* is detected. It was in a biofilm formed in the packaging system of the semen doses. With the use of a special collection extender, the problem is solved, and the stud is able to continue working until the source of contamination is located and a series of effective hygienic measures are implemented.

Keywords: contamination, biofilm, *Serratia marcescens*, boar stud

Introduction

The problem appears in a commercial boar stud with 240 boars isolated from other animal nuclei for several kilometers. Through the internal quality control carried out by the stud, differences in terms of durability of the seminal doses are detected when using different packaging systems. In this boar stud, 3 different packaging systems are used: Bag No. 1, Bag No. 2 and sealing tube. Filling is done automatically using three packaging machines adapted to each system. The main symptom observed is a drastic decrease in the motility of seminal doses 96 hours after production. Sperm motility is an indicative of seminal quality and is used as a fertility reference point for boars to be used in artificial insemination.

Developed method

After receiving a request for help in diagnosing the problem, we made a visit to the stud facilities and took samples of an ejaculate that is manually packaged in the different systems: Bag No. 1, Bag No. 2 and sealing tube. These semen samples are duplicated, and half of them are checked in the stud laboratory and the other half in our laboratory. The results obtained by both laboratories in terms of motility on the fourth day of preservation do not show any alteration in any of the seminal packaging systems. The stud controls the problem by using reusable material and making changes in the cleaning system.

After 4 months, the problem relapses, and we visit the stud again to establish a new protocol for the analysis of seminal samples. We collect the ejaculate of 13 boars by making 3 replicas of each packaged in each of the 3 systems used (Bag No. 1, Bag No. 2 and sealing Tube), analyzing a total of 118 different seminal doses.

On this occasion, a large reduction in motility is confirmed on the 4th day of preservation in the packaging system in Bags No. 1 in all the doses analyzed (Figure No. 1).

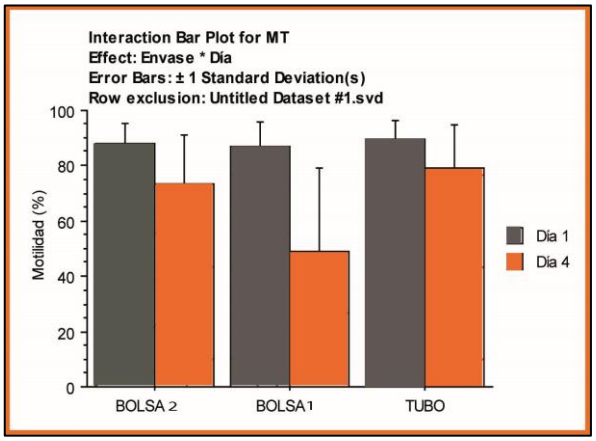

Figure 1. Motility differences between packagings (bag 2 , bag 1 and sealing tube) on the first day and the 4th day

When analyzing the samples in our laboratory using a CASA (Computer-assisted sperm analysis) system, signs of bacterial contamination (turbidity and agglutination in the ejaculate) can be seen in the group with the lowest sperm motility (Figure 3). Figure 2 shows a semen sample without bacterial contamination.

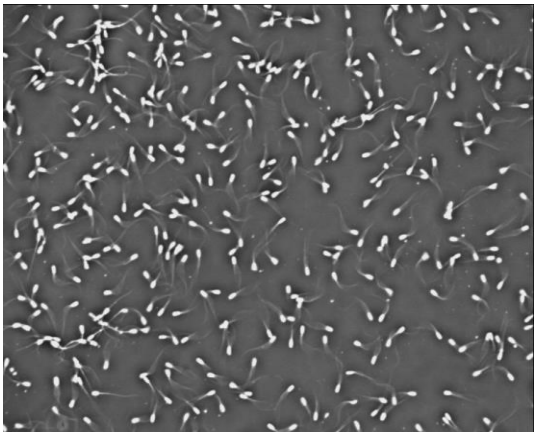

Figure 2: semen sample without contamination

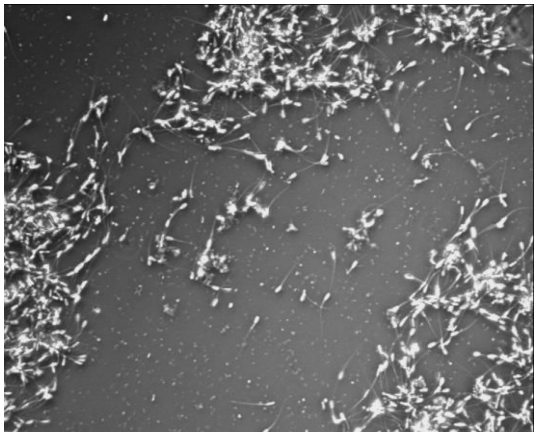

Figure 3: sample with contamination

Diagnosis

As the same ejaculate presents a different degree of contamination depending on the packaging system, the stud is visited again to find the origin of the problem. It is observed that the affected samples are those in which the packaging is carried out automatically, passing the diluted semen through the hoses of the packaging system. In unaffected samples the packaging is done manually and the seminal dose does not pass through the tube. This fact is what makes us suspect that the problem may be in the hose used for packaging, so it is decided to send samples for microbiological analysis. In addition, the containers (Bag No. 1, Bag No. 2 and sealing Tube) and the seminal doses are analyzed. Also it is performed an epidemiological study of contamination in the facilities that consisted of:

-Sampling with swabs of the surface of the bag packaging machine No. 1, bag packaging machine surface No. 2, surface of the heat sealer, heat sealer table, operator's hand, microscope plate, lock of the interior / exterior pre-laboratory window, boar saliva, surface of the extender preparation zone, surface of the ejaculate reception area, air conditioning device, foreskin of the boars, fixed and mobile seminal collection dummies, grills , etc

-Environment plates in the premises; laboratory, prelaboratory, changing rooms and flies.

- Tap water

Sample code	Reference of the client	Parameter	Result	units	Methodology
1206259	2	Aerobic mesophilic bacteria	>300x10^2	ufc/ ml	PT-MB-DS-01
1206260	4	Aerobic mesophilic bacteria	>300x10^2	ufc/ ml	PT-MB-DS-01
1206261	6	Aerobic mesophilic bacteria	>300x10^2	ufc/ ml	PT-MB-DS-01

Figure 4 . Contamination results in the seminal doses and in the hose for packaging

The contamination analysis in the containers (packaging) was negative and in the rest of the samples collected (swabs, plates of environment and water) the contamination was variable with isolates of ubiquitous agents that do not have greater relevance. However, in the doses sent, as well as in the hoses for packaging (Figure 4), bacterial contamination is confirmed by the isolation of multi-resistant Serratia marcescens (Figure 5). We consider contamination clinically relevant (hygienic or pathologic) with more that 300 CFU/ml.

Código muestra	Referencia Cliente	Parámetro	Resultado	Metodología	Valores referencia
1206259	2	Aislamiento + identificación	*Serratia marcescens*	PT-MB-DS-03	
		Antibiograma		PT-MB-DS-04	

1	2	3	4	5	6	7	8	9	10	11	12	13	14
R	R	R	R	R	R	R	R	I	R	R	R	R	R

Código muestra	Referencia Cliente	Parámetro	Resultado	Metodología	Valores referencia
1206260	4	Aislamiento + identificación	*Serratia marcescens*	PT-MB-DS-03	
		Antibiograma		PT-MB-DS-04	

1	2	3	4	5	6	7	8	9	10	11	12	13	14
I	R	R	R	R	R	R	R	I	R	R	R	R	R

Código muestra	Referencia Cliente	Parámetro	Resultado	Metodología	Valores referencia
1206261	6	Aislamiento + identificación	*Serratia marcescens*	PT-MB-DS-03	
		Antibiograma		PT-MB-DS-04	

1	2	3	4	5	6	7	8	9	10	11	12	13	14
R	R	R	R	R	R	R	R	I	R	R	R	R	R

Figure 5. Result of the microbiological isolations of the seminal doses and of the hose for packaging

Serratia marcescens is a gram negative bacillus belonging to the Enterobacteriaceae family (together with Klebsiella, Proteus or Escherichia) widely described in the literature and related to important infections of nosocomial origin in humans. Regarding swine semen, its has been observed its high capacity to create biofilm on wet surfaces and deteriorate the sperm cell to the point of causing sperm death within a few hours.

For the sample collection of seminal doses, and to confirm that the problem is in the filling hose of the packaging system that is used most in the stud, doses of 10 ejaculates of 30 ml, adding 3 ml of pure semen in each container, are manually packed using a sterile syringe in duplicate. Then, half of the seminal doses are checked in the boar stud. The other half is verified in our laboratory where motility is analyzed using CASA system after 24 and 96 hours, not being found motility differences between doses (Figure 6). In the boar stud there are no differences, either.

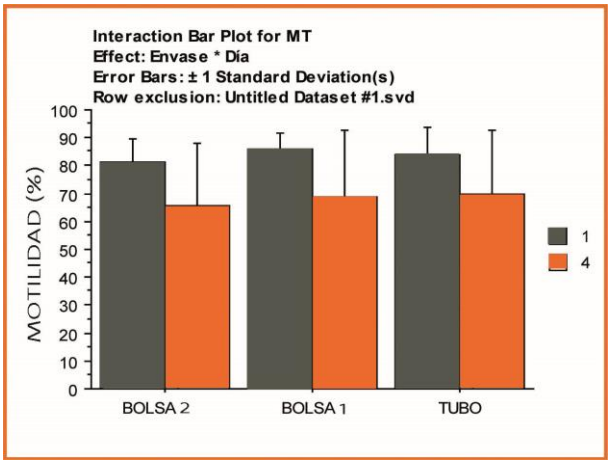

Figure 6. Motility on the day1 and 4 per kind of container (Bag 2, Bag1 and sealing tube)

Solution provided

During the search for the source of infection, a specific treatment was initiated for the boar stud to be able to continue working at a normal pace and producing quality doses. This treatment consisted of the use of a semen collection extender to sanitize the collected ejaculates (Dicol®), as well as the use of disposable material and the change of all hoses in the packaging devices.
Dicol® is used during the collection phase (150 ml in the collection cup). To sanitize the ejaculate, we use a registered mixture of non-spermicidal antibiotics. With this procedure we also monitor bacterial contamination, acting on a wide range of germs, including the most resistant (*Serratia spp*, *Achromobacter spp*, *Burkholderia cepacia*). This effect is achieved after 30 minutes in contact with the product. After this time, the final dilution with the usual extender and the preparation of the seminal doses can be made.

Once it was discovered that the problem was derived from improper cleaning of the hoses that are used for packaging, we proceeded to design cleaning and disinfection steps to combat bacterial contamination in the boar stud, including specific protocols in pens, dummies for seminal collection, laboratory and equipment used. Once all these measures were implemented, the motility during the preservation of the doses returned to normal values. What is more, the use of the specific collection extender that had been used while the seminal contamination problem existed could be eliminated from the protocol.

Conclusions

The hose in the packaging system was the focal point of contamination by Serratias in this case, which caused the decrease of durability in the ejaculates that passed through it during the packaging process.

Serratia marcescens is a primary pathogen for swine semen that, according to the authors, is present in 10.3% of pig semen isolates (Althouse & Lu, 2005) or in 12.55% (Luis et al., 2013). It is also very important due to its multi-resistance. The main source of Serratias seems to be the boar although we have not been able to isolate it either in foreskin or in pure semen. In addition, *Serratia* is an agent that easily contaminates the conduction systems and is resistant to the "usual" cleaning and disinfection steps performed routinely in the boar studs, thereby creating biofilms on wet surfaces that are difficult to access and can survive despite the cleaning measures that are set in the stud.

A strict cleaning and disinfection monitoring, as well as an internal quality control capable of detecting slight changes in the behaviour of the produced doses, are decisive to be able to detect the problem before the seminal doses are used in the farm.

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