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Bacterial contamination of boar semen affects the litter size

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ABSTRACT

One hundred and fifteen semen samples were collected from 115 different boars from two farms in Cuba. The boars belonged to five different breeds. Evaluation of the semen sample characteristics (volume, pH, colour, smell, motility of sperm cells) revealed that they meet international standards. The samples were also tested for the presence of agglutinated sperm cells and for bacterial contamination. Seventy five percent of the ejaculates were contaminated with at least one type of bacteria and *E. coli* was by far the major contaminant, being present in 79% of the contaminated semen samples ($n = 68$). Other contaminating bacteria belonged to the genera *Proteus* ($n = 31$), *Serratia* ($n = 31$), *Enterobacter* ($n = 24$), *Klebsiella* ($n = 12$), *Staphylococcus* ($n = 10$), *Streptococcus* ($n = 8$) and *Pseudomonas* ($n = 7$). Only in one sample anaerobic bacteria were detected. Pearson's analysis of the data revealed that there is a positive correlation between the presence of *E. coli* and sperm agglutination, and a negative correlation between sperm agglutination and litter size. One-way ANOVA and post hoc Tukey analysis of 378 litters showed that the litter size is significantly reduced when semen is used that is contaminated with spermagglutinating *E. coli* above a threshold value of 3.5×10^3 CFU/ml.

1. Introduction

Microorganisms are important contaminants of many body fluids, including semen of animals and humans. Consequently, the extent of microbial contamination is an important parameter to consider in the quality control of semen that is used for artificial insemination or direct mating. Semen is an ideal medium for the establishment and growth of many microorganisms including bacteria and fungi. In boar semen for example, bacteria belonging to at least 25 different genera have been detected as contaminants, the most frequently occurring ones being *Escherichia coli*, *Pseudomonas*, *Staphylococcus* and *Proteus* spp. (Tamuli et al., 1984; Dagnall, 1986; Danowski, 1989; Sone et al., 1989; Arredondo et al., 2001; Althouse and Lu, 2005).

Microorganisms have a deleterious effect on sperm function, both directly by altering the structure of the sperm, by affecting its motility (Diemer et al., 1996; Depuydt et al., 1998), or by provoking a premature acrosome reaction (Kohn et al., 1998) and indirectly by stimulating the production of antibodies that can be directed against the sperm glycocalyx complex (Auroux et al., 1991; Kurpisz and Alexander, 1995).

There are two main sources of contamination, i.e. those of animal and those of non-animal origin. The first source includes, among others, contaminations originating from feces, preputial fluids, respiratory secretions, skin and hair. The second source might be contaminated water, non-sterilized glassware, equipment, poor hygienic conditions, and human contamination (Althouse et al., 1998; Althouse and Lu, 2005).

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In male mammals, the urethra is a common part of the urinary and the genital system. Consequently, microbial contamination of semen can originate from both systems. Many urinary tract infections are caused by typical uropathogenic bacteria, but often, because of the proximity of the intestinal tract, they are infections provoked by strains that belong to the normal intestinal flora (see Bergsten et al., 2005, for a review). Bacteria infecting the urinary tract express virulence factors that allow colonization, invasion and tissue damage, and provoke diseases such as asymptomatic bacteriuria, cystitis or pyelonephritis (Johnson, 1991; Blanco et al., 1995, 1997).

Bacteria can also be recovered from the reproductive tract of males as well as of females. Some microorganisms directly affect this system, e.g. *Chlamydia trachomatis*, which is a major organism that causes sexually transmitted diseases (Eggert-Kruse et al., 1989).

In this paper, we describe the results of investigations on the contamination of boar semen by bacteria and the correlation between the presence of *Escherichia coli*, sperm-agglutination and litter size.

2. Materials and methods

2.1. Origin of semen samples

Semen samples from two different pig farms were collected in Cuba from 115 boars, i.e. 80 samples originated from a farm practicing integral pig production, and 35 samples were from a farm specialized in semen production (the first farm has an average of 55 boars and the second one has 18 boars). In an integral pig production farm all categories of pigs are present from birth till slaughter, i.e. pigs for reproduction (boars, gilts and sows), weaning and post weaning piglets, and pigs that are fattening. The boars included in this study belong to different breeds, i.e. Landrace, L-35 Landrace subline, Yorkshire, Duroc-Jersey and F1 (Pietrain × Hampshire hybrids). Semen collection frequency is two times a week. Boars are kept in individual boxes with no contact between them. Before each semen collection, the animals are thoroughly cleaned prior to conducting them to the properly cleaned collection room.

2.2. Sample collection

All semen samples were collected using the gloved-hand methodology, as described by Larsen (1986) and in the Pig Breeding Manual (Manual de Crianza Porcina, Cuba) of the Instituto de Investigaciones Porcinas (2001). Only phases two and three of the ejaculations were collected because they are the sperm-rich fractions.

Five millilitres of each semen sample were used for the general evaluation and microbiological analysis. The remainder was used for the preparation of the services (doses) for insemination.

2.3. Semen evaluation

For each sample, the volume and pH were measured and the colour (clear, cloudy, presence of blood, turbid or other uncommon colour) and smell (typical, no smell, urine-smell, other) were evaluated. The pH of each sample was measured directly after collection using a Consort pH-meter. To avoid contamination, the electrode was thoroughly washed before and after each measurement with 0.1 M PBS, pH 7.0. Colour was evaluated by observing the semen in a transparent flask. Smell was evaluated directly on the top of the flask. Immediately after semen collection, the concentration of sperm cells and their motility was evaluated. The concentration of sperm cells was determined using the standard Neubauer haemocytometer and observations were made with a light microscope.

The motility was determined by microscopic observation of diluted semen samples. In short, a small drop of diluted spermatozoa (the same dilution for all evaluations) was placed on a warmed microscope slide and overlaid with a cover slip. The samples were diluted so that individual sperm cells could be observed at a magnification of 400. Results are expressed as percentage of cells displaying forward motility (the average of five different fields).

Morphology was evaluated with a phase contrast microscope (400× or 1000×) using semen that was diluted (1:10) with semen extender. Semen extender consisted of 6% (w/v) glucose, 0.37% (w/v) EDTA, 0.35% (w/v) sodium citrate, 0.03% (w/v) sodium hydroxide, supplemented with penicillin and di-hydro-streptomycin. Sperm cells with head or tail deformations, or showing the presence of cytoplasmic droplets were considered as abnormal.

The agglutination of sperm cells in the semen samples was observed using a light microscope and the extent of agglutination was scored as 0 (no agglutination), 1 (5–10% of cells agglutinated), 2 (10–15% agglutinated) and 3 (15–23% agglutinated). Clumping of non-moving sperm cells was not considered as a sign of sperm agglutination.

2.4. Bacteriological evaluation

2.4.1. Culturing conditions

All 115 semen samples were analyzed for the presence of bacterial contamination by plating them on blood agar and on brilliant green agar (Oxoid). These media allow the initial differentiation of many *enterobacteriaceae* including *E. coli*, *Serratia* spp., *Enterobacter* spp., *Proteus* spp., and *Klebsiella* spp. Blood agar was also used for the isolation of *Staphylococcus* spp. and *Streptococcus* spp. because of the ability of most strains to produce haemolysis. For bacterial growth, the plates were incubated at 37 °C for 18 h.

For the isolation of anaerobic microorganisms, plates containing blood agar were cultured at 43 °C in a 5% CO_2 atmosphere. Readings were made 24, 48 and 72 h after incubation.

McConkey agar and Endo agar plates were used for the specific isolation of *E. coli* from those samples that were positive on brilliant green agar. McConkey and Endo agar were prepared according to the instructions of the manufacturer (Oxoid).

Luria-Bertani (LB) medium (1%, w/v, bactotryptone; 0.5%, w/v, yeast extract; 1%, w/v, NaCl; pH 7.3) and MINCA liquid medium (0.45%, w/v, KH_2PO_4; 0.57%, w/v, K_2HPO_4; 0.2%, w/v, NH_4Cl; 0.02%, w/v, $MgSO_4{\cdot}7H_2O$; 0.0005%, w/v, $FeSO_4{\cdot}7H_2O$; 0.0005%, w/v, citric acid; 0.0011%, w/v, $CaCl_2$; 2.5 ml 1.0 M glucose per liter; pH 7.3) were used for