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Quantification of Bacteria in Fractionated Boar Ejaculates

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Introduction Bacterial contamination in boar semen collected by the gloved hand technique is well documented in the industry. Quantification of the bacteria strains and possible sources of these organisms will help develop strategies to control this contamination. The objective of this study was to quantify bacterial load via fractionation of the boar ejaculate.

Materials and Methods Trial 1: Semen from 67 boars was collected using a metal collection dummy by the standard gloved hand technique into new, clean and prewarmed styrofoam cups covered with filters. Each ejaculate was fractionated into two cups. Presperm flush was not collected. The combined sperm rich and sperm poor fractions were collected into the first labeled cup. The fel fracional fluid were collected into a second cup. Fraction volumes were determined by weight. Fractions were streaked onto 5% sheep blood agar plates using a calibrated loop (1μl). Plates were incubated at 38 degrees C for 24-48 hours until colonies could be easily visualized and counted. Trial 2: Over a two week period, semen was collected four times from each of five boars as in Trial 1 and isolated into parasperm, sperm rich, sperm poor and gel fractions during collection. Bacterial colonies were quantified as in Trial 1 for each of the fractions. After the final semen collection, each boar was humanely euthanized and the reproductive tract harvested. Swabs were obtained from the vesicular gland, prostrate, bulbourethral gland, testes, epidiymices and the penial urethra by sterile technique; paired glands had each gland swabbed. Tissue swabs were aerobically and anaerobically cultured on 5% sheep blood and MacConkey agar plates.

Results Bacterial species that were identified in culture were: Proteus sp., Corynebacterium spp., Pseudomonas spp., and Streptococcus spp. The reproductive organs were free of bacterial except for an infected vesicular gland of one boar which had Corynebacterium spp. and the prostate of another which had Streptococcus spp. Sperm concentrations($x10^9$) in the fractionated semen were different ($P<0.05$); Trial 1: combined 94.4±5.3, gel .018±0.02 and Trial 2: presperm 1.7±0.5, sperm rich 42.1±6.6, sperm poor 1.9±0.2, and gel 0.2±0.2. Trial 1: cfu/ml (colony forming units $x10^3$) in combined 14.0±2.7 and gel 35.2±3.8 ($P<0.01$). Total numbers of bacterial colonies ($x10^4$) was greater in combined 120.8 versus gel 20.9. Trial 2: cfu/ml ($x10^3$) was higher in presperm 78.5±6.2 ($P<0.05$) than in sperm rich 23.3±7.4, sperm poor 34.5±12.4 and similar to bacterial count in gel 51.1±12.4 fractions. Total bacterial count ($x10^4$) was highest again in sperm rich 118.0 versus presperm 68.8, sperm poor 68.0 and gel 34.5 fractions.

Conclusions Bacterial concentration per ml was greatest in the presperm fraction while totla number of bacteria was greatest in the sperm rich versus the remaining fractions. Boar reproductive tracts were relatively free of bacteria, with only a vesicular gland (boar 2) and a prostate (boar 5) showing gross microbial infection.

Implications We cannot eliminate bacterial contamination from a boar ejaculate through fractionation, but we can decrease the bacterial load by not collecting the presperm and gel fractions. Bacterial contamination in boar semen does not appear to originate from the reproductive organs examined in this study.