

Assessment of bacteriospermia using flow cytometry

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Monitoring the bacteria load of ejaculates and Artificial Insemination (AI) doses is a crucial part of boar station's hygiene program. For this purpose, samples are incubated using Blood Agar Plates (BAP) at 36°C for 48 h prior to determining the number of Colony Forming Units (CFU/mL). In spite of this, BAPs underestimate the number of bacteria because the volume of samples is small, bacteria aggregates form CFU, and only viable-culturable bacteria may be counted. Flow cytometry can count total bacteria individually, detecting smaller rises in bacteria number, without the need for incubation. In this work, ejaculates from 72 boars in the Netherlands were checked for the total number of bacteria using a flow cytometer (Guava® EasyCyte, Luminex Corporation, Inc.; EasyKit Bacteria Concentration; IMV Technologies). After final dilution, AI doses from these ejaculates were analyzed for the total number of bacteria (n=72) and sperm motility assessed with the IVOS CASA (Computer Assisted Sperm Analysis) system (Hamilton Thorne Inc., Beverly, MA) after three days of storage at 17°C. A viable sperm:bacteria ratio ($\frac{\text{viable sperm cells per ml}}{\text{detected bacteria cells per ml}}$) was calculated for all AI doses. The average number of total bacteria in raw semen was 122.9 billion, and for AI Doses was 0.83 billion/dose, with an average viable sperm:bacteria ratio of 5.53. Least Square Means (LSMeans) for motility and motility drop at day 3 were calculated for viable sperm:bacteria ratio and then categorized as slow, medium, or high (Low: n = 21 ejaculates, 0.1 to 4.8 sperm: bacteria; Medium: n=32 ejaculates, 5.0 to 7.1 sperm:bacteria, and High: n = 19 ejaculates, 7.6 to 15.8 sperm:bacteria). Motility (Low: 88%, Medium: 86% and High: 87%, P=0.42) and motility drop (Low: 3.3%, Medium: 4.3% and High: 5.2%, P=0.74) were not significantly different between categories. This shows that even a ratio of viable sperm:bacteria in AI doses of 3.2 does not necessarily lead to motility loss during three-day storage. To our knowledge, this is the first time a flow cytometer has been evaluated for routine monitoring of bacteria number in boar semen.