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## A new tool for control of bacterial contamination in boar semen.

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**Introduction:** Porcine semen contamination by bacteria is frequent and inevitable due to several factors such as the developed resistance by bacteria to many antibiotics used in semen extender. The use of antibiotics in semen extender to preserve seminal doses is not adequate and needs to be profoundly studied. Dicol® is a new medium containing a combination of 4 bactericides acting against a large spectrum of gram-negative and gram-positive bacteria. It was designed and developed based on a study which was carried out on a large number of samples of isolated bacteria from boar semen samples and a large list of antibiotics. It was especially designed for collection and preventive antibiotic treatment of porcine semen before final dilution with usual extender.

**Materials and Methods:** In this study the objective is to demonstrate the effect of Dicol® on control of *in vitro* semen contamination. Boar semen was collected with 150 ml of Dicol® and divided immediately in 10 aliquots, which were inoculated with 10<sup>6</sup> ufc/ml of final bacteria concentration of: *Klebsiella oxytoca*, *Proteus mirabilis*, *Serratia marcescens*, *Pseudomonas fluorescens*, *Morganella morganii*, *Providencia rettgeri*, *Achromobacter xylosoxidans*, *Escherichia coli*, *Delftia acidovorans* and *Pantoea spp.* All strains were isolated and identified from boar semen, purified and stored in a sample bank. Standard semen extender was used as control, and an extender free of antibiotics as negative control. All strains were checked for sensitivity to 29 antibiotics by antibiograms. Semen

samples were incubated at room temperature, and assessed for bacterial content by culture after 10, 20, 25, 30, 40, 50 min and 24h.

**Results:** There was a significant decrease in bacteria content in semen samples with Dicol® (p<0,0001) and significant differences between strains. The maximum decrease (<1ufc/ml) was reached at 10 min of incubation with *Klebsiella oxytoca*, *Serratia marcescens* and *Delftia acidovorans*; at 20 min of incubation with *Proteus mirabilis* and *Providencia rettgeri*; at 30 min of incubation with *Pseudomonas fluorescens* and at 40 min and 50 min of incubation with *Morganella morganii* and *Achromobacter xylosoxidans*, respectively. These last two strains showed multi-resistance to the tested antibiotics. After 24h, bacterial content was reduced drastically and reached <1ufc/ml in all samples with Dicol®. Moreover, control samples with standard extender exhibited a slight decrease of bacteria content and reached <1ufc/ml only after 24hours in case of *Klebsiella oxytoca* and *Pantoea spp.*

**Discussion and Conclusion:** This study was carried out with strains of frequent bacteria present in boar semen which showed multi-resistance to many antibiotics mainly used in semen extender. The synergetic antibiotic combination of Dicol® was able to reduce the bacteria content in semen to the minimum in a short time after infection with large concentration (10<sup>6</sup> ufc/ml). Hence, this finding showed evidence that Dicol® is a new tool, which offers an interesting control of common bacterial contamination in porcine semen.