Minimising microbial growth during liquid storage of stallion spermatozoa

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Introduction
A synthetic medium has been developed which allows stallion spermatozoa to be stored at room temperature (RT) for up to 7 days. However, prevention of microbial growth in this medium has been difficult. In the porcine industry, nanoparticle coated storage bags Bactibags™ have been developed to assist in the elimination of bacteria during storage for 5-7 days. The aim of these experiments was to identify whether Bactibags™ and EquiPure™ centrifugation could effectively prevent bacterial growth during the liquid storage of stallion spermatozoa at RT.

Materials and Methods
_UoN Media:_ UoN extender (patent pending) was supplemented with 0.25 mg/mL gentamicin, 50 U/mL penicillin and 50 µg/mL streptomycin.

**Experiment 1:** Three ejaculates from each of four stallions were extended 1:2 with EquiPure™ and centrifuged at 350 x g for 15 min. Sperm pellets were resuspended to 50 x 10⁶/mL and divided into three different treatments. T1: Equiplus™ in semen safe syringes at 4 °C (commercial control), T2: UoN media in syringes at RT and T3: in UoN media in Bactibags™ for at RT.

**Experiment 2:** Ejaculates (N=12) were divided into four aliquots and diluted 1:2 with either INRA 96™ or Equiplus™ for the treatments described below. T1: Initial dilution in INRA 96™ followed by centrifugation at 350 x g for 15 min, T2: Initial dilution in INRA 96™ layered over an EquiPure™ gradient and centrifuged at 400 x g for 20 min, T3: Initial dilution in Equiplus™ followed by centrifugation at 350 x g for 15 min or T4: Initial dilution in Equiplus™ layered over an EquiPure™ gradient and centrifuged at 400 x g for 20 min. Sperm pellets were re-suspended in UoN media supplemented with 100 U/mL Nystatin to a sperm concentration of 50 x 10⁶/mL and stored at RT. Motility (CASA) was assessed and aliquots cultured on blood agar at 3, 7 and 10 days.

Results
**Experiment 1:** Total motility was different (P ≤ 0.05) between all groups at days 0 and 3 of storage (Day 0: 56.3 ± 6.4%, 38.3 ± 6.0% and 80.3 ± 5.1%, Day 3: 29.0 ± 5.6%, 7.5 ± 3.4% and 57.5 ± 6.5% for T1, T2 and T3 respectively). All T1 replicates demonstrated bacterial growth at 10 days. Bacterial growth was not eliminated by storage in Bactibags™, and there was a significant yeast/fungal growth in all RT groups.

**Experiment 2:** EquiPure™ centrifugation eliminated bacterial growth at day 7 (0% vs 60% for pooled T2/T4 and T1/T3 respectively; Chi-square, P ≤ 0.05). Nystatin effectively prevented fungal growth in all samples at day 7 and all but one of each of T3 and T4 (Equiplus™) at day 10. Total motility was improved by EquiPure™ treatment (96.9 ± 0.8% vs 92.7 ± 0.8% and 84.5 ± 1.6% vs 74.2 ± 2.0% for pooled T2/T4 and T1/T3 at 0 and 7 days of storage respectively; P ≤ 0.05).

Relevance to Australian clinical equine practice
Purification through gradient centrifugation with EquiPure™ and the addition of Nystatin to the UoN media appeared to reduce Bacterial and Yeast/Fungal contamination in the sample following 7 and 10 day incubation at RT. The future use of this medium will greatly simplify sperm transport logistics and artificial insemination regimens for Equine Veterinarians.