YSC 11 | **In vitro evaluation of antimicrobial effect of ozone against bacteria responsible for equine endometritis**

G. Donato[1]; T. Nervo[1]; M. Stella[1]; P. Nebbia[1]; G. Romano[2]; D. Necchi[3]; L. Bergamini[4]; M. Poletto[1]; D. Scalas[1]; P. Robino[1]

[1]*University of Turin, Grugliasco, Italy;* [2]*Centro Equino Arcadia, Villafranca Piemonte, Italy;* [3]*Keros Embryo Transfer Center, Passendale, Belgium;* [4]*Allevamento le Fontanette, Vigone, Italy*

Infectious endometritis is observed in up to 60% of barren mares and requires intrauterine or systemic antibiotic therapy. The goal of the present study was to characterize the in vitro antimicrobial properties of two different ozone preparations (O_3) in respect of the most common bacteria linked to endometritis. Accordingly, we tested strains of *Streptococcus equi subsp. zooepidemicus*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* isolated from the uterus of mares diagnosed with infectious endometritis. Briefly, bacterial suspensions were prepared and adjusted to 0.5 McFarland turbidity in sterile saline solution and inoculated onto Tryptic Soy Agar or Blood Agar plates. The cultures were divided in different groups: untreated positive control, distilled water, continuous gas flow of O_3 at a concentration of 15 μg/mL and 40 μg/mL for three time points (1, 3 and 5 min) and distilled water ozonated at 40 μg/mL for 10 min. Gaseous ozone drastically reduced the growth of bacteria in all concentrations and times tested, resulting in a minimal residual growth (complete inhibition or maximum 10 CFU/plate) after 5 min of continuous gas flow at 40 μg/mL. On the contrary, ozonated water did not reduce bacterial growth since bacterial density was similar to the one observed in the control groups. In conclusion, gaseous O_3 represents a promising therapy for infectious endometritis and could prevent or scale down the use of antibiotics, which are routinely used in broodmares, while water ozonated at the concentration and time used in this study did not seem to be effective.

YSC 12 | **Fluorescent dyes to assess sperm competition in female north African houbara bustard (*Chlamydotis undulata*): Impact on sperm motility and viability**

L. Meunier[1]; C. Silva Vieira[1]; C. Warret Rodrigues[1]; S. Sadiq Shiek[1]; Y. Hingrat[1]; M. Saint Jalme[1]; G. Sorci[2]; F. Lacroix[1]; J. Torres Carreira[1]

[1]*Reneco International Wildlife Consultants, Abu Dhabi, UAE;* [2]*Biogéosciences, UMR 6282 CNRS, Université de Bourgogne Franche-Comté, Dijon, France*

Female birds can store sperm for weeks in their oviduct. In polyandrous species, last male precedence (LMP) allows the last male copulating to sire the offspring. Little is known about mechanisms leading to LMP. Finding suitable staining molecules to visualize different males' sperm in storage sites is challenging, it must permit good microscopic observation without impairing sperm function, and efficacies can be species dependent. We compared the effects of two fluorescent dyes on sperm motility and viability over time using beta regressions, to further investigate sperm competition and sperm storage in the oviduct of a wild-polyandrous bird, Chlamydotis undulata. Twenty semen samples were stained with 2 fluorescent dyes, Hoechst 33,342, NUCLEAR-ID® Red DNA (NR), plus a blank control. Motility (4°C) and viability (4°C & 40°C) were evaluated after 20 min, 24 h, 48 h, and 72 h after staining. Fluorescence signal was checked by microscopy for both dyes at each interval. Both dyes gave good results for microscopic observations of fluorescent signal up to 72 h. Hoechst dye did not impact sperm motility ($z = 0.90$, $p = .37$) or viability over time (4°C: $z = 0.98$, $p = .32$; 40°C: $z = -2.17$, $p = .08$), but the NR stain impaired sperm motility after 48 h ($z = -2.89$, $p < .01$) and viability after 24 h (4°C: $z = -2.29$, $p = .02$, 40°C: $z = -6.21$, $p < .01$). Hoechst preserved key sperm characteristics for artificial insemination (AI) and so, can be tested in vivo. NR stain, however, may only be used for shorter time incubation experiments. In vivo studies are necessary to verify fluorescent signals from sperm cells in the female tissue after AI.

YSC 13 | **Alterations of Sertoli cells in dogs with chronic asymptomatic orchitis**

P. Rehder, E. Packeiser, H. Körber, S. Goericke-Pesch

Unit for Reproductive Medicine, Clinic for Small Animals, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany

Chronic asymptomatic orchitis (CAO) is the most common finding in male dogs with acquired infertility and is associated with severe histopathological changes including a spermatogenic arrest and immune cell infiltration. Its aetiology is unknown due to the lack of clinical signs, of abnormal bacterial or endocrine findings. As Sertoli cells play an important role for spermatogenesis and the testicular micromilieu, alterations in Sertoli cell number and function might be involved in disruption of spermatogenesis in CAO. Results were obtained from bilateral testicular samples of 15 CAO dogs (biopsies for diagnostic purpose) and compared to those of 15 normospermic healthy controls (CG) (castrated on owners request). Number of Vimentin-positive Sertoli cells were counted in 20 seminiferous tubules per sample at 40× magnification. Results were statistically compared using GraphPadPrism. Significantly less Sertoli cells were found in CAO compared to CG ($p < .0001$). Besides, immunohistochemistry was performed using antibodies against GDNF (Glial cell line-derived neurotrophic factor) and bFGF (basic fibroblast growth factor) to study Sertoli cell function. Sertoli cells stained GDNF and bFGF positive in both groups, as did the blood vessels. Besides, some peritubular cells stained GDNF immunopositive. Despite the significant reduction in number, Sertoli cells in CAO still express GDNF and bFGF indicating (at least partial) functionality of Sertoli cells for stem cell replication and maintenance. Further studies should focus on additional markers to better characterize Sertoli cell maturation status and function, also for a possible spermatogonial stem cell