P 55 | Evaluation of sperm fertilising ability of porcine spermatozoa after voltage-dependent anion channel 2 (VDAC2)-blocking

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Sperm capacitation is a process which comprises changes in intracellular concentrations of calcium requiring the transport of this cation through ion channels located at plasma and mitochondrial membrane (Adeoya-Osiguwa and Fraser 2003, Mol Reprod Dev 65:228–236). Given that VDAC2 is implicated in in vitro capacitation in boar sperm (Martínez-Abad 2017, Reprod Dom Anim 52, Suppl 4:65–68), the aim of this study was to determine whether VDAC2 inhibition affects in vitro sperm fertilising ability. With this purpose, boar spermatozoa were selected with a double density gradient and incubated for 1 h with or without the presence of two VDAC2-inhibitors: erastin at 10 μM (E10) and olesoxime at 100 μM (O100). Subsequently, spermatozoa were cocultured for 1 h with in vitro matured oocytes and penetration rate and number of sperm per oocyte were evaluated at 18 h post-fertilisation. Exposure of spermatozoa to erastin did not affect sperm fertilising ability (sperm penetration rate; control: 76.49 ± 9.82 vs. E10: 89.42 ± 7.68 or the number of penetrated sperm per oocyte; control: 3.93 ± 0.22 vs. E10: 4.21 ± 0.25). Similarly, the addition of olesoxime did not show significant differences in the number of penetrated oocytes (control: 56.33 ± 20.69 vs. O100: 55.40 ± 18.93) and the spermatozoa able to fuse with the oocyte (control: 4.63 ± 3.41 vs. O100: 3.91 ± 2.70). Our results indicate that, in these conditions, VDAC-2 blocking does not affect sperm fertilising ability, although VDAC2 is involved in boar sperm capacitation. In conclusion, erastin and olesoxime may be considered useful for blocking capacitation without affecting the sperm ability to fertilise.

P 56 | Presence and function of kisspeptin/kisspeptin receptor system in corpora lutea of pseudopregnant rabbits (*Oryctolagus cuniculus*): in vitro studies

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The hypothalamic neuropeptide kisspeptin (KiSS) and its cognate receptors (KiSSR) have crucial function in mammalian reproduction, regulating GnRH production and release. The KiSS/KiSSR gene and protein are also expressed in several reproductive organs including the ovary. In the present study, we examined the expression of the KiSS/KiSSR system and its functional role in corpora lutea (CL) of pseudopregnant rabbits. CL were collected at early- (day 4), mid- (day 9), and late- (day 13) stages of pseudopregnancy following GnRH injection (day 0). KiSS immunoreactivity was localized in the nucleus and cytoplasm of all luteal cells; the density of immune reactive cells decreased from early- to late luteal stage. Immunosignals for KiSSR were detected in the cytoplasm of luteal cells only at early and mid-stage of pseudopregnancy, but not at late stage; the density of immune reactive cells decreased from early to mid-stage. In CL cultured in vitro, the agonist KiSS-10 increased progesterone and decreased prostaglandins $F_{2\alpha}$ and E2 secretion at early- and mid-stages of pseudopregnancy, whereas the antagonist KiSS-234 counteracted the effects of KiSS-10. The present study indicates that KiSS/KiSSR system is present in rabbit CL and that it affects the luteal endocrine activity with a luteotropic effect.

P 57 | Study of coconut water as chilled stallion sperm extender**

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Currently, there are numerous natural and commercial extenders to increase the equine sperm lifespan. However, more affordable and efficient alternatives are being studied. The aim of this study was to evaluate the effect of coconut water in combination to dimethylformamide (DMF) as chilled stallion sperm extender. Semen samples were obtained from the epididymis of 12 stallions. Sperm samples were centrifuged (1000 g/5 min) and resuspended in 4 experimental extenders: INRA 96® (control medium), coconut water (312 ± 3 mOsm/L), INRA 96® with 5% DMF, coconut water with 5% DMF. Motility parameters, osmotic response of the plasma membrane, viability and acrosome integrity were analyzed at 0 h, 24 h and 96 h after storage at 4°C. Data were analyzed by GLM test. Coconut water with or without DMF did not improve sperm quality parameters compared to INRA® at 0 h and 24 h. However, the experimental extender with DMF compared to the control medium significantly improved ($p < 0.05$) sperm motility parameters (56% vs. 41.36%) and acrosome integrity (89.63% vs. 85.38%) after 96 h of storage. In conclusion, coconut water with or without DMF is a suitable chill extender for epididymal equine sperm (Supported by DGA and Fondo Social Europeo (IA2).).

P 58 | Urethral obstruction caused by lithiasis in an Anglo-Nubian buck: a case report**

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Stranguria and dysuria in bucks and rams are very often associated to urolithiasis caused by a high grain and low roughage diets. High