placenta (n = 60); 38.36% of the analyzed placentas showed slight non-inflammatory changes (n = 60); 16.64% of the placentas presented inflammatory cells (n = 60). Progesterone and cortisol levels were not correlated with histopathological lesions, whereas estrone sulphate levels rise in mares with pathological changes in their placentas.These results strongly suggest that estrone sulphate measurement may be used as a diagnostic tool for early detection of placental illness in mares.

Abstract P151

Protein Expression of Cortisol Metabolising Enzymes in the Boar Reproductive Tract

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The stress hormone cortisol is implicated in a wide range of reproductive processes including semen quality. Cortisol is interconverted with cortisone by isoforms of the enzyme 11beta hydroxysteroid dehydrogenase (11βHSD). Investigations in our laboratory have demonstrated that the boar reproductive tract secretes modulators of 11βHSD activity. We hypothesise that modulation of 11βHSD in the boar tract alters the environment in which the sperm mature, thus modifying sperm quality and the ability to withstand cold temperature storage. The aims of this study were to establish whether 11βHSD1 and 11βHSD2 proteins are expressed in the boar reproductive tract. Boar testis, epididymis, vas deferens, bulbourethral glands and penis were obtained from slaughterhouse material from 7 animals. Tissues were homogenised in a Tris-glycerol-SDS lysis buffer containing a protease inhibitor cocktail. Proteins were resolved by SDS-PAGE and 11βHSD protein expression was assessed using Western blotting. The immunopurified 11βHSD1 antibody recognised a protein band of 32 kDa in porcine liver (positive control) and in boar testis, epididymis and vas deferens, but not in porcine kidney (negative control), bulbourethral glands or penis. Alternatively, the immunopurified 11βHSD2 antibody recognised a protein band of 57 kDa in all reproductive tissues assessed. In conclusion, 11βHSD protein was expressed at several sites in the boar reproductive tract. Cortisol generation by 11βHSD is known to influence both sodium concentration and pH, thus modulation of this protein may result in changes to the luminal environment ultimately affecting sperm quality.

Abstract P152

Concentration of Carnosine, Anserine, L-Histidine and 3 Methylhistidine in Boar Spermatozoa by a Modified HPLC Method

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This study detected histidine dipeptides as a part of the non-enzymatic antioxidant defence mechanism in fresh boar semen. The concentrations of Carnosine, Anserine, l-Histidine and 3-methyl-Histidine were measured by HPLC in spermatozoa from fresh semen obtained from 22 fertile boars. The samples, after extraction, were processed by HPLC with fluorimetric detection by o-phthalaldehyde post column derivatization at constant temperature of 55°C that increases sensibility of 20–35 %. All histidine dipeptides studied, Carnosine, Anserine, l-Histidine and 3-methyl-Histidine were present in boar spermatozoa. Intracellular concentrations (mean ± S.E. ng/10^9 cells) of Carnosine (218.51 ± 39.16) and Anserine (218.72 ± 51.96) were significantly lower than l-Histidine (8145.91 ± 864.12) and 3-methyl-Histidine (14216.87 ± 1405.35) levels. Positive correlations were found between Carnosine and Anserine contents ($r = 0.78$; $p < 0.01$) and between l-Histidine and 3-methyl-Histidine ($r = 0.69$; $p < 0.01$). The antioxidant and buffering activity of these molecules could be decisive in the protection of spermatozoa against membrane lipid peroxidation and consequently useful to improve semen quality during cool storage.

Abstract P153

Quantitative Determination of Imidazole Dipeptides in Stallion Spermatozoa and Seminal Plasma

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The aim of this study was to detect in stallion spermatozoa and seminal plasma the presence of imidazole dipeptides as a part of the non-enzymatic antioxidant defence mechanism. Concentrations of Carnosine, Anserine and l-Histidine were measured in both spermatozoa and seminal plasma from 12 fertile stallions, using HPLC with fluorimetric detection by o-phthalaldehyde post column derivatization. Intracellular concentration (mean ± S.E. ng/10^6 cells) of Carnosine (18.59 ± 2.39) was significantly lower than l-Histidine (316.33 ± 34.50), and both data were significantly correlated ($r = 0.72$, $p = 0.01$). No measurable Anserine content was instead detect in the spermatozoa. In the seminal plasma, Carnosine concentration (2.35 ± 0.43 μg/ml) was lower than Anserine (4.44 ± 1.11) and l-Histidine (11.32 ± 2.03). Positive correlations were observed between Carnosine seminal plasma concentrations *vs* Anserine ($r = 0.76$, $p < 0.01$) and l-Histidine, ($R = 0.57$; $p < 0.01$) respectively. Carnosine intracellular content was significantly related ($p < 0.05$) to motility measure as total ($r = 0.59$) and progressive motility ($r = 0.58$), progressive velocity (VSL) ($r = 0.73$) and path velocity (VAP) ($r = 0.65$). The antioxidant and buffering activity of these molecules, could be decisive in the protection of stallion spermatozoa against membrane lipid peroxidation and useful during cool storage.

Abstract P154

Bovine in Vitro Embryo Production (IVP) and Pregnancy Outcome after Inhibition of Meiosis Resumption

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This study aimed to produce high quality bovine oocytes to increase IVP in Parthenaise (P) and Villars de Lans (VL) breeds by using a 2 steps *in vitro* maturation (IVM) procedure. IVP after 2 steps IVM was first tested with slaughterhouse oocytes. Oocytes were either IV matured (M199) for 22 h (controls) or incubated for 5 h in inhibition medium (M199 + Roscovitin 12.5 μM + Butyrolactone I 6.25 μM), then matured for 22 h before fertilisation and culture. IVP following 2 steps IVM (respectively of 36% and 37.9% with fresh or frozen inhibition media) was not different from the controls (37.6%; n = 194). In subsequent field experiments, females were stimulated with FSH (OPU performed 6 h after last FSH injection) and were collected twice (1st session, control IVM ; 2nd session, 2 steps IVM). Percentages of oocytes developing into blastocysts were respectively of 31.2% vs 43.9% (VL) and 34.6% vs 37.5% (P) for control and inhibited oocytes (NS). The number of embryo produced per session was higher for VL after 2 steps IVM but this was due to a higher number of oocytes collected at 2nd session. After transfer of fresh embryos to recipients, pregnancy rates were not different with control embryos (5/8) or embryos issued from 2 steps IVM (7/15). These results show no increase in development rates after inhibition of meiosis resumption. However, IVP and pregnancy rates may allow to use this 2 step maturation procedure to facilitate organisation of on farm OPU.