

specificity for the criterion value of SI at $180 \times 10^6/\text{ml}$, while LR's indicate that absorbances lower than or equal to $A_{610} = 0.209$ were at least 11.3 times as likely to be found in good semen samples than in poor ones. Other criterion values did not provide both sensitivity and specificity over 90% at selected optimal cut off values. Discrimination of good versus poor semen samples according to SI of $180 \times 10^6/\text{ml}$ is a valuable method of assessing the quality of boar spermatozoa, which could be of benefit to livestock producers and veterinary practitioners.

Abstract P142

Fusarium Mycotoxins affect Proliferation of Pig Granulosa Cells *In Vitro*

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Fusarium mycotoxins, like trichothecenes and zearalenone (ZEA), are common grain and foodstuffs contaminants. Some of these like deoxynivalenol (DON) and T2-toxin (T2) can negatively impact pregnancy success in swine, but evidence for direct ovarian effects of DON and T2 is meager. To evaluate the effects of three mycotoxins, ZEA, DON and T2 on porcine granulosa cell (GC) proliferation, pig GC from small follicles (1–5 mm) were cultured for 2 days in 5% fetal bovine serum and 5% porcine serum-containing medium followed by 2 days in serum-free medium containing control (no mycotoxins) or mycotoxins (at various doses/combinations). Each mycotoxin exhibited a biphasic effect on GC growth: ZEA enhanced serum- and IGF-I-stimulated cell growth at concentrations $< 0.10 \mu\text{M}$; DON at $< 0.1 \mu\text{M}$; T2 at $< 0.001 \mu\text{M}$, and estradiol mimicked the stimulatory effect of ZEA. Whereas ZEA, DON and T2 doses of $> 9.4 \mu\text{M}$, $1 \mu\text{M}$ and $0.002 \mu\text{M}$, respectively, significantly inhibited IGF-I-induced cell proliferation. Complete block of the IGF-I-induced cell proliferation was observed at $> 3.3 \mu\text{M}$ for DON and at $> 0.05 \mu\text{M}$ for T2. The highest dose of ZEA tested (i.e., $31 \mu\text{M}$) only inhibited cell proliferation by 31%. T2 also significantly inhibited basal cell numbers. Neither ZEA nor DON altered the inhibitory effects of T2 on cell proliferation. In conclusion, mycotoxins have direct dose-dependent effects on granulosa cell proliferation.

Abstract P143

Immunolocalization of Heat Shock Protein 70 (Hsp70) in Boar Spermatozoa

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Hsp70 has been identified in human (Miller et al., Hum Reprod 7:637, 1992) and bovine (Kamaruddin et al., Andrologia 36:327, 2004) ejaculates as well as in extracts from boar sperm (Huang et al., Anim Reprod Sci 63:231, 2000). The aim of this study was to determine Hsp 70 presence and distribution in ejaculated, capacitated and acrosome-reacted boar spermatozoa; the fate of this protein after fertilization was also studied. In fixed-permeabilized fresh semen $92.3 \pm 1.8\%$ of cells showed a triangular-shaped immunoreactivity in the equatorial segment of the head. The localization of fluorescent signal underwent several rearrangements after capacitation, becoming particularly evident in the equatorial line in $72.6 \pm 5.3\%$ of cells, and in a sub-equatorial band in $88.9 \pm 2.3\%$ of sperm after induced acrosome reaction (AR). The proportion of unfixed cells showing the fluorescent signal significantly ($p < 0.05$) increased after AR. No differences in Hsp70 amount between fresh, capacitated and reacted sperm were observed by Western blot. A loss of Hsp immunoreactivity in demembrated spermatozoa was recorded. Perivitelline sperm cells after IVF showed the same pattern as AR-induced cells; after sperm incorporation into the oocyte Hsp positivity became fuzzy and disappeared during sperm decondensation. Hsp 70 seems to undergo redistribution along the sperm membrane and it is probably translocated from the inner to the outer leaflet after capacitation and acrosome reaction.

Abstract P144

Effect of Preservation Temperature before Centrifugation on Sperm Survival in Catalanian Donkey Semen

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30 ejaculates of 7 Catalanian donkeys were collected. Gel-free semen was diluted 1:5 with Kenney extender and allowed in 4 air-free 50 ml Corning tubes. One tube was centrifuged using a temperature programmable centrifuge at 20°C immediately after semen dilution. The other tubes were maintained at 20°C, 15°C, 5°C during 2 hours and then were centrifuged at 20°C, 15°C and 5°C respectively. Percentage of viable spermatozoa using the eosin-nigrosin stain was evaluated after semen collection, before centrifugation for each temperature (20°C, 15°C, 5°C) and after centrifugation in all cases. Data were processed by the SAS Statistical Package (2000). Mean sperm survival in fresh semen was $69.4\% \pm 22.05$. Significant differences were observed between ejaculates. No evidence of variability was observed on sperm survival after 2 h at 20°C (70.7 ± 20.28) and 5°C (69.3 ± 20.84). Nevertheless, the storage at 15°C affected significantly the spermatozoa (66.8 ± 20.25). Centrifugation immediately after collection had no significant effect on the viability (68.5 ± 22.72). On the other hand, centrifugation after storage induced a decrease of survival at 5°C (61.5 ± 22.88) and 15°C (61.9 ± 22.49), but no effect was observed at 20°C (68.0 ± 23.56). These results indicate that it is possible to store semen from Catalan Donkeys at 20°C during 2 hours before centrifugation in a freeze program.

Abstract P145

Comparison of Texture Descriptors in Automatic Classification of Boar Semen

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Automatic classification of spermatozoa by means of cytoplasmic densities can be achieved by finding groups of cells with the same texture pattern. This work comprises spermatozoid head segmentation, definition of a model for the training sets and obtaining of results with testing sets. Spermatozoid heads are isolated from gray-level images of boar sperm samples by a segmentation process. Then, training sets are created: spermatozoids with a clear density distribution according to the post nucleus cap, the intermediate area and the acrosome are considered as "good". If some abnormalities are detected, heads are labelled as "bad". For each set, two statistical texture methods are applied: co-occurrence matrix and auto-covariance coefficients. Haralick features vector derived from the co-occurrence matrix has been used for distances of 4, 5, 6, 7 and 8 pixels and four orientations (0°, 45°, 90° and 135°). Both classes of heads, "good" and "bad", are modelled averaging each feature obtained for each image belonging to the training set. For each head, a 10×10 auto-covariance matrix of coefficients is also computed. Models of both classes of heads are defined by a texture feature vector of the means of each coefficient for every set. Experiments have been done with a testing set of 200 images of sperm heads and employing the k-nearest neighbour classifier. Results show a right classification rate of 62.63% for descriptors derived from co-occurrence matrix and 62.38% for auto-covariance features.

Abstract P146

Characterization of the Different Sialic Acids Contained in the Bovine Zona Pellucida

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Carbohydrates play a key role in the sperm-zona pellucida (ZP) interaction. We have demonstrated that sperm binding to bovine ZP is

strongly inhibited by the treatment of the ZP with neuraminidase as well as by lectins specific for sialic acid residues. The aim of this study is to identify the different types of sialic acid residues contained in the bovine ZP by means of chemical and cytochemical analysis. For chemical analysis, sialic acid were released by treatment of the isolated ZP with trifluoro acetic acid and analyzed by HPLC. For ultra-structural cytochemistry, ovarian oocytes were fixed in glutaraldehyde and embedded in LR White. The results obtained using the HPLC analysis demonstrated that the bovine ZP contained two types of sialic acid: Neu5Ac (85%) and Neu5Gc (15%). A strong cytochemical labelling was obtained with the lectins LFA, MAA and SNA. No labelling was observed with SBA and sialyl-Lewis antibodies. However, SBA showed a strong affinity for the ZP after neuraminidase digestion. These results indicated that Neu5Ac is the most abundant type of sialic acid. Moreover, the following carbohydrate sequences Neu5Ac/Neu5Gc(α 2-3)Gal(β 1-4)GlcNAc, Neu5Ac/Neu5Gc(α 2-6)Gal/GalNAc and Neu5Ac/Neu5Gc-GalNAc are demonstrated using MAA, SNA and Neuraminidase-SBA respectively. Furthermore, bovine ZP was shown not to contain the sialyl-Lewis tetrasaccharide (Supported by AGL2003-03144 and BFU2004-05568).

Abstract P147

Early Post-partum Stimulation of Ovarian Activity in Lactating Rabbit Does: Study of Reproductive and Endocrine Parameters

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This study investigated the effect of eCG treatment and of two different intervals of doe-litter separation methods to stimulate ovarian activity in lactating rabbits on day 4 post-partum. Rabbit does (n = 137) were inseminated at day 4 post-partum and were weaned at 25 days. Experimental groups were: BIO48 (a separation mother-young 48 hours before AI), BIO24 (24 hours separation), eCG (25 IU 48 hours before AI) and CONTROL. Plasma samples were taken on day 2, 3 and 4 post-partum at 9:00 a.m. Oestradiol was determined by EIA. High conception rates (79.3%) were obtained in primiparous does of both the eCG and BIO24 group. BIO24 and BIO48 methods offered similar results than eCG-treatments from third parturition. Prolificacy was higher (8.56) in BIO24 and eCG groups compared to control (7.55, $p < 0.05$), but the highest number of pups born dead was observed in the eCG-group. Regarding hormones, oestradiol levels were similar on day 2 and 3 post-partum (113.3 ± 13.7 and 142.1 ± 13.6 pg/mL, respectively). On day 4 post-partum, eCG, BIO24 and BIO48 groups had higher mean oestradiol levels than values observed on day 3 post-partum ($p < 0.001$). It was concluded that doe-litter separation could be an alternative method to eCG treatment (Supported by MCYT, AGL02-310).

Abstract P148

Effect of Growth Hormone and Inhibitors on the *In Vitro* Maturation of Equine Oocytes

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This study evaluated GH addition to maturation medium to increase oocyte maturation rates in equine oocytes. Aspiration and scraping (ovarian follicles < 30 mm in diameter) were used to recover oocytes (COC) which were cultured in TCM199+BSA+antibiotics, and placed as: a) control (no additives); b) 400 ng/mL eGH. Inhibitors for adenylate cyclase (DDA) and to PK-A pathway (H-89) were added in each treatment. After 30-h of culture COCs were denuded, fixed and stained to evaluate stage of maturation. A limited number of COCs

were processed to describe the cytoplasmic cortical granule (CG) distribution based upon fluorescein isothiocyanate-labeled *lens culinaris* agglutinin (LCA) by laser confocal microscopy. 131 ovaries were processed, and 146 oocytes were cultured. Higher maturation results were observed in oocytes cultured with GH (15/31, 48.3%) compared to controls (7/30, 23.3%, $p < 0.05$). In addition, use of inhibitors DDA and H-89 reduced the number of oocytes reaching maturation compared to GH-only treatment (5/24, 20.8%; 5/22, 22.7%; $p < 0.05$, respectively). As maturation proceeded, progressive centripetal migration of CG to the oocyte cortex occurred only in GH-matured COCs. GH has a positive oocyte maturation effect and this is mediated by adenylate cyclase and PK-A pathways (Supported by UCM-UCD Del Amo Programme).

Abstract P149

Relationship between Resumption of Ovarian Cyclicity and Oestrous Expression in Postpartum Dairy Cows

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This study examined the effect of delayed resumption of ovarian cyclicity on the oestrous expression. Two university experimental herds (A and B) and one commercial herd (C) were used. Milk samples were collected 2 to 3 times a week until 70 to 80 days postpartum for progesterone analysis using EIA. Visual observation of standing oestrus was carried out at 4 h (herd A), 8 h (herd B) intervals or twice a day. (herd C). Ovarian cyclicity was classified as normal, prolonged luteal phase, delayed first ovulation and other abnormalities. Heat detection rate (HDR) after the 2nd ovulation was calculated by number of cows detected in estrous divided by number of cows ovulating based on milk progesterone profile. Percentage of cows with normal, prolonged luteal phase, delayed first ovulation and other abnormalities were 52, 12, 24 and 12% in herd A, 26, 26, 37 and 11% in herd B and 50, 30, 15 and 5% in herd C, respectively. HDR in cows with normal ovarian cyclicity in herds A and B was 36% and 18%. In cows with delayed first ovulation, HDR (33% and 30%) was similar to normal groups. HDR in cows with prolonged luteal phase (50% and 42%) was higher than in normal groups. Percentage of cows showing standing oestrus at the 2nd to 4th ovulations were 37, 33 and 67% in herd A and 32, 43 and 33% in herd B. It is indicated that oestrous expression was not adversely affected in cows with delayed first ovulation, while the oestrous expression was enhanced in cows after prolonged luteal phase.

Abstract P150

Prenatal Diagnosis of Placental Pathology in Mares: Relationship between Hormonal Levels and Histological Lesions

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Placentitis is the most common cause of abortions, stillbirths and perinatal deaths in mares. Early detection of this pathology is complex and the impossibility of invasive techniques successfully used in the interior of the pregnant uterus limits the diagnostic techniques. There remains an urgent need to identify the hormone changes associated with compromised pregnancies so that novel therapies can be developed to promote the health and well-being of the late gestation mare and the foetus. The aim of this report is to evaluate hormonal levels that correlate with histopathological lesions of the placenta. Sixty PRE breed mares were monitored during their last month of pregnancy measuring the levels of progesterone, estrone sulphate and cortisol in blood. After parturition allantochorion samples were subjected to histological evaluation. Mares were classified into three groups: (1) normal placenta, (2) slight non-inflammatory changes, (3) presence of inflammatory cells; 45% of the mares presented a normal