Posters

Posters submitted for Student Competition

P1

Equine endometrial epithelial and stromal cells *in vitro* – preliminary results of morphofunctional characterization

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The aim of the present work was to investigate morphological and immunocytochemical charac-terristics of endometrial epithelial (EEC) and stromal (ESC) cells of mares *in vitro*. The cells were cultured in a serum-supplemented culture medium using different growth surfaces (culture inserts, cell culture flasks). They were examined cytomorphologically (haemalaun-eosin-, giemsa-, alcian blue-staining) and immuno-cytochemically (cytokeratin 8, 18, 19, vimentin, α-actin, desmin). The results were compared to the endometria *in situ* from which the cells were isolated. EEC *in vitro* revealed two morphologically different cell types and three growth patterns, whereas ESC showed a typical fibroblast-like morphology. Morphological disparity of EEC *in vitro* did not lead to apparent differences concerning immunolabelling. These cells exhibited charac-teristics comparable to uterine glandular epithelia *in situ* concerning cytokeratin 8, 18 and 19, while other immunocytochemical characteristics differed from the endometrium *in situ*. Both EEC and ESC *in vitro* partly showed immunolabelling for vimentin. Whereas numerous ESC were labelled for α-actin and desmin, not any EEC revealed expression of these cytoskeleton filaments. These results are the basis for a more detailed characterization of cultured equine EEC and ESC with special regard to the state of the mare's reproductive cycle and the degree of endometrosis.

P2

Effect of the number of inseminations on the fertility of sows inseminated with extended semen stored at 5°C or 17°C

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Optimizing artificial insemination (AI) in pigs has been a challenge for decades. However, the use of multiple inseminations per estrus is still a common practice in most swine farms. This is due to the long estrus duration and broad variations in the time of ovulation/estrus among breeds and individuals. In the present study we investigated the effect of the number of AIs on the fertility of sows inseminated with semen stored at 5°C or 17°C in a previously described container (Roner, 2006, Arq Bras Med Vet Zootec, 58, 78–86). A total of 70 primiparous and multiparous sows were distributed uniformly in two treatments: I-sows inseminated with semen diluted in BTS® and stored at 17°C; II-sows inseminated with semen diluted in glycine-egg yolk extender (Foote, 2002, Reprod Dom Anim, 37, 61–63) and stored at 5°C. The first insemination was performed 12 h after the onset of estrus and subsequently every 12 h, up to tree inseminations. If still in estrus, within 24 h from the third insemination, the sows also received a fourth insemination. The number of AIs was 3.44 ± 0.53 per female and did not differ ($p > 0.05$) between treatments. The pregnancy rate was influenced ($p < 0.05$) by the number of inseminations (3 AIs, 94.59% and 4 AIs, 78.13%), which means a decrease of 16.45% when four inseminations per female was used. These results demonstrated that fertility declines when the last of multiple AIs is performed during late estrus or metoestrus.

P3

Does Fructose in BTS Improve Quality Parameters in Boar Frozen-Thawed Sperm?

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Boar spermatozoa present constant expression of the fructose transporter GLUT5 and decreased expression of the glucose transporter GLUT3 along the cryopreservation process (Sancho, 2007, Reproduction, 134, 111–21). For that reason, we hypothesized that including fructose in the Beltsville Thawing Solution (BTS; Pursel, 1973, J Anim Sci, 37, 532–35) would improve the recovery of frozen-thawed boar spermatozoa. We tested the hypothesis on six frozen-thawed boar ejaculates from 4 different breeds (two from Large-White, two from Landrace, one from Duroc and one from Piétrain). A concentration of 1×10^9 spermatozoa per ejaculate was thawed in BTS 1:3 (v:v) and each ejaculate was split into three treatments: BTS with 102.5 mM glucose + 102.5 mM fructose 1:1 (v:v) (treatment A); BTS with 205 mM fructose (treatment B); BTS with 205 mM glucose (control, treatment C). Three sperm quality parameters (osmotic tolerance, membrane integrity and progressive motility) were assessed at 30 and 240 min after thawing. Results showed no significant differences in any parameter between treatments A, B and C. Therefore we concluded that the frozen-thawed boar sperm samples have the ability to obtain energy from both hexoses indistinctly.

P4

Cryopreservation of preantral follicles in ovarian cortical tissue from bitches

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Cryopreservation of ovarian tissue is a tool for preservation of genetic animal resources. Nevertheless few studies have been carried on canine tissue. The aim of this preliminary study was to evaluate the morphological damage and the viability rates induced by the cryopreservation process on preantral follicles in the bitch. Ovaries were harvested from bitches during routine ovariectomies (6–18 month, n = 12). Two permeating agents (dimethylsulfoxyde 2 M, DMSO or propylene glycol 2 M, PROH) supplemented with or without 0.2 M sucrose were used for equilibration before slow freezing process (freezing rate of 2°C/min). After thawing, no significant differences were observed between control (70%) and DMSO groups (67% with sucrose, 58% without sucrose) when considering the preservation rate of intact follicles. In contrast, there was a decrease of this preservation rate in the PROH-sucrose group (41%) compared with control, and DMSO-sucrose group ($p < 0.05$). The viability rate for PROH groups (23% without sucrose, 31% with sucrose) were lowers ($p < 0.0001$) than control (91%) and DMSO groups (78% without sucrose, 82% with sucrose). These results demonstrate that canine ovarian cortex can be successfully cryopreserved in the presence of DMSO with a morphology and viability preservation ratio of almost 90%. Moreover, even if the morphological evaluation of the follicles corroborates the viability test results, it should always be completed by a viability assessment or some other metabolic analysis methods to refine the observed results.

P5

Multifactorial analysis of preantral follicles during ovarian cortex cryopreservation in the bitch

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Numerous studies have been carried out on parameters affecting the cryopreservation process, but few of them investigated simultaneously the effects and possible interactions existing between them. The aim was to analyze the combined influence of four different freezing parameters on the viability of bitch preantral follicles during the cryopreservation process. To this end, 16 Beagle females (7–8 month) underwent elective ovariectomies. After dissection, ovarian cortical pieces were treated according to a fractional experimental design. The simultaneous effects of the dimethylsulfoxide (DMSO 1.5 M) vs. propylene glycol (PROH 1.5 M), sucrose vs. trehalose, the post-seeding freezing rate (0.3°C/min vs. 2°C/min) and the number of equilibration steps (1 step vs. 3 steps) on the follicle viability rate were evaluated. The cryoprotectant, the freezing rate and the number of equilibration steps highly influenced the viability rate of preantral follicles ($p < 0.0001$). The optimal combination was using DMSO, a freezing rate of 0.3°C/min and a single step equilibration, to allow the viability rate to reach 85%. No interactions were detected between the four factors. These results were consistent with the findings of our earlier study supporting DMSO as a good cryoprotectant for canine ovarian tissue. Furthermore, post thawing follicular morphology were assessed during this study but showed too much variability to be explored, indicating that viability assessment is currently a strong and safe indicator for freezing damage.

P6

Evaluation of acute phase proteins for the identification of systemic inflammatory response to endometritis in mares

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The purpose of this study was to investigate the usefulness of plasma concentrations of acute phase proteins (APP) serum amyloid A (SAA) and fibrinogen as parameters to identify the need for anti-inflammatory treatment in different uterine conditions. Samples were collected from clinically healthy standardbred mares ($n = 23$; age 5–19 year) during the normal oestrus cycle in Estonia (April and May 2009). Ovulation, endometrial oedema and presence of intraluminal fluid were recorded by rectal ultrasonography. For bacteriological and cytological examination the endometrium was sampled with a guarded culture swab. Blood samples were taken 48–96 h before and after ovulation to measure APP (SAA, fibrinogen). Described diagnostics identified the presence of a clean uterus in 12 (group 1), PMIE in five (group 2) and endometritis in six mares (group 3). Median (range) SAA concentrations (mg/l) 48–96 h before ovulation were 0.043 (0.02–0.553), 0.068 (0.02–0.124) and 0.159 (0.02–0.659; $p = 0.697$ – Kruskal-Wallis test) and 48–96 h after ovulation were 0.105 (0.027–0.336), 0.072 (0.02–0.416) and 0.088 (0.02–0.194; $p = 0.484$) in groups 1, 2 and 3 respectively. Median (range) fibrinogen concentration (g/l) 48–96 h before ovulation were 3.7 (2.1–4.3), 3.5 (3.4–3.6) and 3.3 (3.2–3.4; $p = 0.655$) and 48–96 h after ovulation were 3.3 (2.3–4.7), 3.3 (2.9–4.0) and 3.0 (2.3–3.5; $p = 0.172$) in groups 1, 2 and 3 respectively. In conclusion SAA and fibrinogen may not be useful markers to detect inflammatory conditions in mares' uterus. This study was supported by ETF Grant No 7539.

P7

Cytological diagnosis of endometritis in the mare: a comparative study

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Rapid diagnosis and adequate treatment of endometritis improves fertilization and embryonic development. Therefore, it is important to determine the most reliable diagnosis. Routine diagnosis is mainly based on presence of uterine fluid. However, these signs are not necessarily present. Other methods to assess endometritis include: (1) the cotton swab, (2) the cytology brush, (3) the small volume uterine flush, and (4) the endometrial biopsy, which is considered as the "gold standard". This study aims to compare these techniques in terms of ease of use and diagnostic accuracy. All diagnostic methods were performed on 10 cycles (5 mares): in dioestrus, oestrus, 24 h and 6 days after AI. Correlation between biopsy and other techniques was determined with *Pearson's* and *Least squares* methods (ANOVA). Sample recovery and legibility of slides were compared with *khi2* method. Proportion of granulocytes (No of granulocytes/Total No of cells) was compared between methods with ANOVA. Significance was set at 0.05. When combining technical failure for sample recovery and unreadable slides, cytospin after uterine lavage was the most difficult technique to perform ($p = 0.041$). Although a higher ($p = 0.002$) proportion of granulocytes was observed on uterine lavage cytospin slides, in comparison with slides from swabs or cytology brushes, a correlation ($r = 0.762$, $p = 0.01$) and a linear regression ($R^2 = 0.435$, $p = 0.007$) were observed between biopsy results and proportion of granulocytes on cytology brush slides. It was concluded that the brush swab was a promising diagnostic tool for use in field conditions.

P8

Sucrose and soy lecithin as an alternative to egg yolk in cryo-diluents for ram

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After attempting to define a chemical medium for freezing ram sperm, no synthetically produced component is better than egg yolk to provide a satisfactory protection when freezing sperm cells. In this study, effects of two alternatives to egg yolk, sucrose and soy lecithin, after freezing and incubating sperm for 6 h, are discussed. Plasma and acrosomal membrane integrity and mitochondrial functionality were measured by flow cytometry. All media contained 5% glycerol. We compared two sucrose-based media (repeated measures design, $n = 3$), the Swim-up modified (SwM) and Salamon's (S) medium, both containing 15% egg yolk, 5% palm or coconut oil. The addition of coconut or palm oil to S accounted for lower ($p < 0.01$) sperm quality values than S + egg yolk, which was also lower than S alone and all SwM variants. After 6 h of incubation, motility (subjectively assessed) in S + egg yolk samples scored the highest percentage ($p < 0.01$). When Lecithin (3.5%) was added to S, integral-membrane sperm increased (repeated measures design, $n = 3$, $p < 0.01$), with no differences for AnnexinV staining. However, mitochondrial membrane potential diminished. Motility assessment (by CASA) showed no differences between treatments. Our results indicate that sucrose based media provide a limited protection against cryoinjury but it makes cells "insensitive" to any other additive such as oils or egg yolk. Although lecithin provides an egg yolk-like protection, the specific evaluation of mitochondrial membrane potential reveals that this is a mitochondrial uncoupling compound. CICYT-FEDER AGL 2007-61229, 2008-01476.

P9

Could Western blot analysis be an alternative to radio-immunoassay for sheep pregnancy associated glycoproteins measurements?

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This study investigated whether Western blot analysis could be an alternative to radio-immunoassay (RIA) for sheep pregnancy diagnosis. Blood samples were taken from Boujaâd (n = 9) and Boujaâd x D'man (BD) cross breed (n = 12) ewes. The pregnancy associated glycoproeins (PAGs) were monitored in plasma collected weekly during gestation and the first month postpartum by two RIA systems (*El Amiri et al., 2007, Reprod Domest Anim 42:257–62*). The highly immunoreactive samples derived from PAG profiles were tested using Western blot based on the same antiserum as that used in RIA. Each week (from the 13th to the 19th week), two pools of plasma samples (4 ml each) were taken from single and multiple lambing ewes respectively. Samples were submitted to a protein extraction at neutral pH, followed by ammonium sulfate (A.S.) precipitation, dialysis, and lyophilization. The placental extract was used as a check. Results revealed that ewes with multiple lambs presented numerically higher PAG concentrations. In both RIA systems, the high concentrations were recoded around the 19th week of gestation and the maximal concentrations varied from 120 to 700 ng/ml. In Western blot, the placental extracts reacted positively while the plasma samples did not give any positive reaction. In conclusion, the RIA remains the only sensitive method to measure PAGs in plasma. The present study strengthens the need to develop an ELISA kit as an alternative to RIA systems.

P10

Fertilin immunolocalization in epididymal spermatozoa from fertile boars

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Fertilin is an integral membrane protein which participates in sperm-oocyte interaction. The aim of this study was to investigate the changes in fertilin localization during sperm epididymal maturation. Undiluted epididymal samples were obtained by cannulation of six epididymal regions of three adult fertile boars (proximal and distal parts of caput, corpus and cauda) and washed with PBS at 600 × g for 10 min. Sperm samples from each region were fixed (3% paraformaldehyde), washed and diluted at 10×10^6 spz/ml. Diluted samples were dropped onto slides and permeabilized (PBS 0.25% triton 100x) and blocked (PBS 0.25% Trition 100x 1% BSA). Incubations with primary anti-fertilin polyclonal antibody (1:100) and secondary antibody conjugated with Alexa fluor®488 (1:1000) were performed for 1 h. Fertilin localization patterns were obtained by counting under the fluorescence microscope, three replicates of 100 spermatozoa per boar. An intense labelling over the entire acrosomal region was the major pattern observed in spermatozoa from proximal (94.0 ± 2.0%) and distal caput (93.7 ± 0.6%) and proximal (92.3 ± 2.0%) and distal corpus (86.3 ± 1.5%), whereas spermatozoa from proximal (79.0 ± 2.6%) and distal cauda (88.0 ± 1.0%) exhibited an intense labelling on the acrosomal ridge. In conclusion, in boars the epididymal maturation of spermatozoa resulted in a change in localization of fertilin which migrated from the acrosomal region to the apical ridge. Fertilin migrated suddenly during the sperm transit though the proximal cauda.

P11

Validation of reference genes for RT-qPCR in *in vitro* cat oocytes and preimplantation embryos

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Quantitative real-time PCR (qPCR) is a commonly used approach for accurate assessment of gene transcription patterns in oocytes and early embryos. Comparing gene expression across different samples can lead to erroneous conclusions due to differences in amount of starting material, reverse-transcription yield or enzymatic efficiencies. An elegant way to control for these variables is normalisation by using the geometric mean of the most stable reference genes as an internal control. The stability of reference genes differs between species and tissue type. In this study, a set of reliable reference genes was selected for cat oocytes and early embryos. Oocytes were *in vitro* matured, fertilized, cultured and collected for RNA extraction and DNAse treatment, followed by conversion into cDNA. Transcription levels of 10 commonly used reference genes (ACTB, GAPDH, SDHA, RPS7, RPL17, HMBS, HPRT, GUSB, YWHAZ and B2M) were determined in single oocytes (n = 20) and embryos at different preimplantation stages (8-cell, compact morula, blastocyst, hatching blastocyst, n = 10/stage) using previously optimized qPCR programs. For each gene, qPCR was performed on all samples in duplex along with a dilution series in the same run. All data were interpreted using geNorm software (identifying YWHAZ, SDHA and GUSB as the most stable genes during early development, while RPS7, RPL30 and GAPDH appeared to be highly regulated (http://medgen.ugent.be/~jvdesomp/genorm), Based on these results, the geometric mean of YWHAZ, SDHA and GUSB is suggested for appropriate normalization of RT-qPCR studies of cat oocytes and early embryos.

P12

Generation of transgenic pigs by *Sleeping Beauty* transposition in pig zygotes

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Pigs have been widely used as models for cardiovascular disease, atherosclerosis, wound repair, cancer, diabetes and ophthalmological studies. Genetic engineering can expand the utility of pigs for modeling human diseases. However, at present porcine transgenesis is inefficient and tainted with low overall success rates. After pronuclear DNA-injection into porcine zygotes, typically only 1% of treated embryos develop into transgenic offspring. Similarly, somatic cell nuclear transfer in pigs is cumbered by low developmental potential of reconstructed embryos, and no more than 1–3% of reconstructed embryos develop to term. Only a fraction of transgenic offspring produced by these methods shows the expected expression patterns, most likely due to random DNA integration. Here, we assessed the *Sleeping Beauty* (*SB*) transposon system for enzyme-mediated transgene integration into the pig genome. A total of 141 *in vivo* zygotes were injected with a non-autonomous CAGGS-*Venus*-transposon and *SB*, and were transferred to four recipients, of which tree became pregnant. Two pregnancies were interrupted at day 28 p.c., the third recipient delivered 12 piglets at term. Molecular analysis revealed specific, transposase-mediated integration of 1–5 copies of the *Venus*-transposon in fetuses (n = 4) and vital piglets (n = 5). Phenotypically all organs appeared *Venus* positive. Together, the data show a highly efficient *SB*-mediated transgene transposition into the porcine genome; 57% of obtained fetuses and 42% of piglets were transgenic (in total this corresponds to 6.4% of treated zygotes).

P13

Plasminogen/plasmin system affects the *in vitro* fertilization results in porcine oocytes exposed to oviductal fluid

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Using *in vitro* matured oocytes, we reported previously the influence of the plasminogen/plasmin system on bovine and porcine *in vitro* fertilization (IVF) results. However, we also showed that the zona pellucida (ZP) of these species responds differently to sperm after the exposure to oviductal fluid than the ZP of *in vitro* matured oocytes (Coy, 2008, PNAS, 105, 15809–15814). The present experiments were designed to study the effect of the plasminogen/plasmin system on *in vitro* matured porcine oocytes exposed to oviductal fluid. Oocytes were *in vitro* matured in NCSU-37 medium and, 30 min before insemination, they were exposed to oviductal fluid collected from recently ovulated sows. Fresh ejaculated boar spermatozoa were selected by Percoll® gradient 45/90 and added to the insemination droplets at a concentration of 10^5 cells/ml. Plasminogen was added or not to the IVF medium at a concentration of 77 μg/ml 30 min after insemination. A paralell experiment with oocytes not exposed to oviductal fluid was conducted. The results showed that plasminogen keeps its effect of significantly ($p < 0.05$) reducing penetration and mean number of sperm per oocyte, and increasing monospermy when it is confronted to ''oviductal-like'' oocytes. However, its effect of reducing the number of sperm bound the ZP, observed with *in vitro* matured oocytes, is hidden when the ZP is exposed to oviductal fluid, probably because this fluid already decreases *per se* the sperm-ZP binding, as reported previously. Granted by MEC/FEDER, AGL2009-12512-C02-01.

P14

Variety in the quality of cooled-shipped stallion semen

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The quality of cooled-shipped stallion semen influences pregnancy rates in mares. In the present study we investigated shipment time, packaging, temperature, pH, volume as well as sperm concentration, motility and morphological defects of cooled-shipped semen ($n = 108$ doses) at arrival time on the centre for artificial insemination in Vienna. Values given are means ± SEM. The semen doses were collected from 20 different stallions (range 4–15 AI doses/stallion) and 15 AI centres (range 4–19 AI doses/centre). The shipment time was 25.5 ± 0.1 h. On arrival semen had a temperature of 9.5 ± 0.2°C (range 4–21°C) and a pH of 6.5 ± 0.0, this was neither affected by centre nor stallion (n.s.). Concentration of AI doses varied between 13.7 and 162.5×10^6/ml (mean $55.8 \pm 2.6 \times 10^6$/ml), total sperm count of semen dose between 0.2 and 2.5×10^9 ($0.8 \pm 0.03 \times 10^9$), total motility between 51 and 95% (84.3 ± 0.7%) and morphological semen defects between 16.6 and 86.5% (43.8 ± 1.2%). All parameters were significantly affected by stallion ($p < 0.001$) and insemination centre ($p < 0.001$). At time of insemination, 82 of 108 (76%) of semen doses fulfilled requirements of the World Breeding Association for Sport Horses and contained more than 300×10^6 progressivily motile spermatozoa. The mean pregnancy rate per cycle was 38%. The results demonstrate a wide variation in the quality of cooled-shipped stallion semen that is significantly influenced by sire and by the AI centre responsible for semen processing.

P15

Influence of inseminate components on the presence of leukocytes and spermatozoa in the porcine uterus 2 hours after artificial insemination (AI)

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Sows show a brief but intense post-breeding immune response characterised by the influx of leukocytes. The study aimed to determine whether the main impulse for the influx is derived from the seminal plasma (SP), the spermatozoa (S) or simply a volume effect, and in how far the number of immigrated leukocytes correlates with the number of spermatozoa found in the uterus. AI was performed on 47 synchronised German Landrace (GL) sows (6 months old) with one of six variants: S + SP, S + PBS, epididymal sperm (ES) + PBS, SP, PBS and a not inseminated negative control (NC). Fresh semen from one fertile GL boar was washed in PBS and extended to 3×10^9 sperm in 100 ml of the appropriate diluent. Sows were slaughtered 2 h after AI. One uterine horn was flushed with PBS, while a bacteriological swab was taken from the other (no positive results). Sperm and leukocytes were counted microscopically and data were analysed by ANOVA. Leukocyte influx significantly differed between groups with highest numbers after AI with ES + PBS ($4.5 \pm 1.4 \times 10^6$), followed by S + PBS ($4.2 \pm 0.7 \times 10^6$), SP ($3.1 \pm 1.4 \times 10^6$), PBS ($1.6 \pm 0.4 \times 10^6$), S + SP ($1.2 \pm 0.4 \times 10^6$) and the NC ($0.6 \pm 0.2 \times 10^6$). Sperm retrieval showed tendencies between groups ($p = 0.052$). Most sperm were retrieved after AI with S + SP ($3.9 \pm 1.1 \times 10^6$) followed by S + PBS ($2.4 \pm 1.0 \times 10^6$) and ES + PBS ($0.2 \pm 0.1 \times 10^6$). Although the lowest leukocyte influx coincides with highest number of retrieved sperm and vice versa, there was no correlation between leukocyte influx and retrieved sperm numbers ($r = -0.1$, $p = 0.66$). In conclusion, sperm and seminal plasma seem to cooperate in determining leukocyte influx.

P16

Knobbed sperm defect causing infertility in Finnish Yorkshire boars

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In this study we examined the incidence of this specific sperm defect in Finnish breeding boars. We focused on sperm morphology, fertility and testicular histology. Semen samples from 2048 (1097 Yorkshire, 951 Landrace) boars were collected in the period 1996–2005. None of the Landrace boars possessed the knobbed sperm (KA) defect. Of the Yorkshire boars, 0.8% was afflicted with the KA defect. Fertility data were available from two artificial insemination (AI) boars and six farm breeding boars affected with the KA defect. Boars possessing the KA defect showed a low fertility when the proportion of knobbed spermatozoa exceeded 25%. AI boars with 25–30% knobbed spermatozoa had a poor non-return rate (on average 47% compared to 85% for normal control boars) and produced small litters, on average 2.5 piglets less than other boars of the same breed. Breeding boars with 45–81% knobbed spermatozoa ($n = 6$) did not produce any litters. The boars with the KA defect had a smaller diameter of the seminiferous tubules ($p < 0.05$) and a lower number of Sertoli cells ($p < 0.05$) than controls. The average inbreeding coefficient for the boars afflicted with the KA defect was higher compared with the average inbreeding coefficients of the Yorkshire boar population. In the pedigrees of the boars with the KA defect two common boars were found. The mapping results of the KA defect in boars are very promising, but will be published later.

P17

Expression of functional Chemokines in the bovine teat

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Bacteria enter the mammary gland through the streak canal and the first tissues they encounter are those of the teat. On the basis of explant cultures we studied relevant genes that become activated in the distal region of the mammary gland during pathogen contact. The focus was laid on mRNA expression encoding for inflammatory and chemoattractive factors in lipopolysaccharide (LPS)-treated bovine teat explants. Explants were taken from the Fürstenberg's Rosette (FR) and teat cistern (TC) and were incubated for 3 h with and without LPS. The mRNA expression of CCL5, CCL20, CXCL8, IL-1β, TNF-α, S100A8, S100A9 and S100A12 were assessed with qRT-PCR. Gene expression showed a high heterogeneity between different teats but also between explants of the same teat. After LPS stimulation genes coding for CCL20, CCL5, CXCL8 and IL-1β were inducible whereas S100 proteins only showed an increased expression in TC but not in FR. However, in this region, we found the highest base line mRNA expression of S100 genes. To prove that explants also secrete active products, culture supernatants after 18 h *in vitro* were used in quantitative chemotaxis assays. Supernatants of stimulated explants significantly ($p < 0.001$) induced migration of neutrophils (transmigration rates 71% ± 3.3% vs. 1.9% ± 3.3%) compared with supernatants of unstimulated explants. Thus, the LPS stimulation led to production of functionally active chemokines in teat explants. The data support that explant cultures are valid to analyse the important sentinel function of the teat towards invading pathogens.

P18

Effects of prostaglandin E_2 and $F_{2\alpha}$ on progesterone production of equine corpus luteum cells

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The main function of the corpus luteum (CL) is production of progesterone (P4) which is essential for the establishment and maintenance of pregnancy. In a number of species, prostaglandin (PG) produced both within and outside the CL and ovary is involved in the mechanisms controlling the secretory function of the CL. PGE_2 may serve as local luteotropic factor. Oppositely, $PGF_{2\alpha}$ may act as a local luteolytic factor in several species. The aim of this study was to determine whether PGE_2 and $PGF_{2\alpha}$ influence P4 production, cell viability and 3-βHSD gene expression in cultured luteal cells of mares. Luteal cells were enzymatically isolated from the mid luteal phase CL ($n = 4$) and cultured in 24- or 96-well culture plates. After 8 h of preincubation and replacing the medium, the cells were exposed for 24 h to: 1) medium only (control), 2) PGE_2 (10^{-7} M), 3) $PGF_{2\alpha}$ (10^{-7} M) or 4) LH (10 ng/ml, positive control). Cell conditioned media were assayed for P4 by EIA. Relative quantification of 3β-hydroxysteroid dehydrogenase (3-βHSD) gene expression was accomplished by Real Time PCR ($\Delta\Delta$Ct method). Viability of the cells was analyzed using the MTT assay, a colorimetric assay measuring the reduction of dimethylthiazol-diphenyltetrazolium bromide to formazan in living cells. PGE_2 stimulated P4 production ($p < 0.05$), gene expression of 3-βHSD ($p < 0.01$) and viability ($p < 0.05$). $PGF_{2\alpha}$ reduced P4 production ($p < 0.05$) and gene expression of 3-βHSD ($p < 0.01$). These results suggest that PGE_2 may play a luteotropic role as an autoparacrine factor stimulating P4 production by the mare CL. On the other hand, $PGF_{2\alpha}$ seems to be an important luteolytic factor within equine CL. Supported by the Grant: Portugalia 78/2007.

P19

Effects of annual microclimatic conditions on quality of frozen bovine spermatozoa

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This study focused on the effect of natural microclimate of Thessaloniki area, Greece (40°41′1.04″, 22°51′56.81″) on post-thaw quality traits of frozen bovine spermatozoa. Hence, semen from three Brown Swiss, three Simmental and three Limousin bulls, housed in semi-open stalls and weekly ejaculating, was frozen. Semen samples of the first weeks of 12 consecutive months were analyzed using sperm chromatin structure assay. Plasma membrane integrity and acrosomal status were evaluated after propidium iodide and peanut agglutinin staining by means of flow cytometry. Average air temperature, relative humidity and dew point (ranging from 0,0 to 34,4°C, 25,4 to 92,6% and −13,6 to 19,6°C, respectively) were computed for the 6th, 7th and 8th week before ejaculation. Multilevel linear model analysis was conducted. DNA-fragmentation index and alpha-t parameter were affected by microclimate of 8th and 7th week ($p < 0.001$), respectively. The percentage of viable acrosome-intact spermatozoa was related to microclimatic factors of the 7th week ($p < 0.001$), while microclimate of the 6th week affected the percentage of acrosome-defected spermatozoa ($p < 0.001$) regardless of their membrane integrity. Thus, a delayed effect of microclimatic conditions on quality traits of frozen bovine semen is suggested.

P20

Brief exposure of *in vitro* matured porcine oocytes stained with Hoechst 33342 to ultraviolet irradiation impairs embryo development

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Previous results from our laboratory indicated that the exposure of Hoechst 33342 (H-42) stained mature porcine oocytes to UV irradiation for 30 s limited their developmental competence. This study was designed to evaluate the effect of a shorter UV irradiation (5 s) on *in vitro* fertilization (IVF) parameters and embryo development of porcine oocytes. Immature oocytes were collected from ovaries of prepubertal gilts, matured *in vitro* for 42 h and divided into four groups: (1) Oocytes stained with 5 μg/ml H-42 for 12 min; (2) Oocytes exposed to UV for 5 s; (3) Oocytes stained with H-42 and exposed to UV for 5 s; and (4) Control: untreated oocytes. After the treatments, the oocytes were incubated for 5 h with 1000 frozen-thawed spermatozoa (obtained from a single boar) per oocyte and cultured for 18 h ($N = 851$) to assess IVF parameters or for 7 d ($N = 1088$) to evaluate embryo development. Results analyzed by ANOVA showed no differences in IVF parameters and cleavage rates among groups. However, oocytes exposed to H-42 and UV for 5 s showed a lower ($p < 0.02$) rate of blastocyst formation (15.2 ± 4.5%) than oocytes from the other groups (range: 26.1 ± 4.5% −30.7 ± 4.5%). No differences were observed in total cell numbers of blastocysts among groups. These findings indicate that the exposure of mature oocytes previously stained with H-42 to UV irradiation for periods as short as 5 s decrease their developmental competence. Supported by SENECA (04543/GERM/07).

P21

Retinol improves *in vitro* Oocyte nuclear maturation under heat stress in dairy cows

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Heat stress is an important factor of infertility in dairy cows. Anomalous chromosomal segregation and cytoplasmic defects have been seen in oocyte maturation under high temperatures due to a rise in radical oxygen species. The objective of the present study was to evaluate the effect of retinol as an antioxidant agent (1.43 μg/ml) on bovine oocyte maturation under heat stress conditions (41.5°C, between 18–21 h of oocyte maturation). Both nuclear stage and distribution of cortical granules (CG) were simultaneously evaluated in each oocyte (n = 185) using Hoechst 33342 and FITC-LCA lectin staining, respectively. Chi-square test ($p < 0.05$) was used to evaluate possible effects of heat stress and retinol on CG distribution and MII morphology. Heat stress group (HS) showed a lower percentage of MII (40% HS vs. 96% control group; $p < 0.05$) and a larger proportion of the type of cortical granule distribution presumptive of exocytosis (48% HS vs. 0% control group; $p < 0.05$). The use of retinol in the maturation media can significantly stabilize nuclear maturation under heat stress conditions (72% MII retinol vs. 40% MII control; $p < 0.05$), but cytoplasmic maturation was largely modified. However, the proportion of cortical granule distribution presumptive of exocytosis was reduced (17% retinol vs. 48% control; $p < 0.010$). In conclusion, retinol proved to be valuable in heat stressed oocytes improving nuclear maturation.

P22

Mobile cells during estrous cycle and in bovine endometritis – a histopathological characterization

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Endometritis is one cause for infertility in dairy cows. Due to the lack of a standardized definition, this study defines the term endometritis histologically considering the degree and character of inflammation and setting a limit between normal cyclical cell infiltration and inflammation. Thus, mobile cells (mononuclear cells, neutrophils, eosinophils) detected during the cycle and in endometritis were characterized by immunohistochemistry (CD3, CD79A, MAC 387, mast cell tryptase/chymase)/special stains (Pappenheim's/May-Grünwald stain). The normal endometrial infiltration by mobile cells was evaluated in endometrial biopsies (EB) and cytological specimens (EC) from seven fertile cows (control group CG) on six different days of the cycle. The uteri and EC of 90 cows with endometritis mainly culled due to clinically symptomless (sub) infertility were examined. In the CG neutrophils peaked around estrus. The cows with endometritis showed a higher incidence of mobile cells ($p \leq 0.05$, Man–Withney–U-test) than the CG. ''Subclinical'' endometritis by EC but no histological inflammation (EB) as well as chronic nonpurulent endometritis (EB) but negative EC were found confirming the important role of EB as a diagnostic tool. The value of EC in the assessment of endometritis should be reconsidered as there is a high discrepancy between endometrial cytology and histology. Further, the state of the cycle shall be considered.

P23

Influence of sodium fluoride on the lifespan of sex sorted bovine spermatozoa

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Flow cytometric sperm sorting leads to lower fertility rates due to a reduced lifespan of sperm. Sexcess® is a modified processing and preservation protocol aiming to improve post-sort sperm quality. In this trial we evaluated the effects of adding sodium fluoride (NaF) to the Sexcess® protocol to induce temporary motility inhibition. Six ejaculates from each of three Holstein bulls were used. Two groups were sorted according to Sexcess®, treated either with or without NaF; there was also an unsorted control. Sperm quality was determined after thawing in a thermo-tolerance test evaluating motility (by CASA), viability (SYBR14/PI) and morphology. After 6 h at 37°C, NaF treated, sex-sorted, thawed sperm showed higher percentage of motile sperm ($p < 0.05$) in comparison to the group without NaF, in comparison to unsorted controls no significant difference could be found (34.2 ± 9.1% vs. 25.6 ± 10.1% vs. 38.9 ± 12.8% respectively). There were no differences in the percentage of intact acrosomes between sorted sperm with NaF or without NaF and unsorted controls (67.0 ± 8.3% vs. 64.3 ± 10.4% vs. 62.6 ± 8.2% respectively). Analysis of viability revealed no differences in the percentage of PI-negative sperm between the sorted groups with or without NaF but both groups showed a lower viability ($p < 0.001$) than unsorted controls (39.6 ± 8.0% vs. 36.7 ± 8.6% vs. 51.8 ± 6.2 respectively). These results show that sex-sorted sperm benefit from NaF supplementation, which is indicated by an increased motility.

P24

Clinical observations on inductions of abortions in mares between 3 and 7 months of pregnancy with cloprostenol

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Induction of abortion in mares allows to resolve unwanted or problematic pregnancies. After day 100, placental progestins maintain the gestation, but injections of natural prostaglandin (PG) F-2α or an analogue induce foetal expulsion. We tested the efficacy of daily im injections of 250 μg cloprostenol (Estrumate®) in 8 mares, at 96–215 days of pregnancy, and studied the consequences of the treatment. The injections were always done at 9 o'clock am. Transrectal uterine examinations were conducted twice a day. We obtained a 100% abortion rate, 43 to 102 h (67.7 ± 24.5 h) after the first injection, requiring 3–5 (3.9 ± 0.9) injections. The mares showed some abdominal discomfort and sweating during few hours after each injection. Transrectal ultrasonic examinations showed no early placental separation, even 5 h before the foetal expulsion. Neither premature lactation nor vulvar secretion was observed before the abortion. The foetuses were aborted alive inside intact foetal membranes. After abortion, no complications were noted and subsequent fertility was not altered. Our results confirm that daily injections of a luteolytic dose of a synthetic analogue of PGF2α with uterokinetic properties is an effective, simple and safe protocol to induce abortion in mares between 3 and 7 months of pregnancy. Same protocol using other PGF2α analogues with low uterokinetic properties were reported to fail in inducing abortion even after daily injections during 4 weeks. Further studies are necessary to understand how cloprostenol induces abortion after day 100 and to evaluate the possible role of endogenous prostaglandins (Madej et al. 1987, Journal of Reproduction and Fertility, suppl 35, 479–84).

P25

Heart rate and heart rate variability in pregnant warmblood and Shetland mares and their fetuses

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Heart rate (HR) is an important parameter of fetal well-being. In horses, HR and heart rate variability (HRV) can be determined by fetomaternal electrocardiography (ECG) from mid-pregnancy to foaling (Nagel et al. 2010, Theriogenology, e-pub ahad of print). Physiological values of full size breeds are often used as a reference for ponies. However, maternal HR increases with decreasing size of the animal. It is not known if fetal HR is affected by breed size and if values obtained in larger breeds are valid in pony fetuses. We determined fetomaternal HR and HRV in warmblood (n = 7) and Shetland pregnancies (n = 7) at d 280 and d 300 of gestation by ECG. Maternal HR was higher in pony than in warmblood mares (d 280: ponies 63 ± 3, warmblood 41 ± 2 beats/min, $p < 0.01$). On d 280 the HRV variable SDHR (standard deviation of HR) was higher in pony (3.6 ± 0.3) than in warmblood mares (2.1 ± 0.3 beats/min; $p < 0.05$) and RMSSD (root mean square of successive beat-to-beat interval differences) was lower in ponies ($p < 0.05$). In contrast, HR did not differ between warmblood and pony fetuses (d 280: warmblood 105 ± 4, pony 99 ± 2 beats/min) but SDHR was lower in pony (1.9 ± 0.2) than in warmblood fetuses (3.0 ± 0.3 beats/min, $p < 0.01$). In conclusion, although maternal HR is higher in pony than in warmblood mares, fetal HR is on the same level. Thus, fetal HR is at least in part independent from the size of the fetus and mare. To assess well-being of pony fetuses, HR reference values from larger breeds can be used.

P26

Estrus synchronization in pigs using estradiol dipropionate and prostaglandin $F_{2\alpha}$

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The aim of the study was to develop an alternative and simple protocol for estrus synchronization in pigs. The effects of the dose and timing of a single administration of estradiol dipropionate (EDP) on the establishment of pseudopregnancy and of prostaglandin (PG) $F_{2\alpha}$ treatment on the induction of estrus in pseudopregnant sows were investigated. Thirty-eight sows (200.9 ± 5.0 kg body weight) were divided in groups of 3–5 animals. In groups treated with 0, 10, 20 and 30 mg of EDP (Aska Pharmaceutical, Japan) intramuscularly once between 10 and 13 days after the onset of estrus, the efficiency of each dose on the induction of pseudopregnancy was 0%, 25%, 80% and 75%, respectively. To examine the optimum timing of EDP treatment for inducing pseudopregnancy, 20 mg of EDP was administered 5, 8, 11 or 13 days after ovulation. At 8 and 11 days after ovulation, more than 80% of the sows became pseudopregnant. Plasma estradiol-17β concentrations in pseudopregnant pigs increased rapidly following EDP treatment and remained high for 8 days. When pseudopregnant sows were treated with $PGF_{2\alpha}$ (15 mg dinoprost) twice at a 24-h interval between 24 and 28 days after EDP treatment, 80% of the sows were in estrus 4 or 5 days after $PGF_{2\alpha}$ treatment. The preovulatory LH surge and ovulation occurred on 6.8 ± 0.7 days and 8.2 ± 0.6 days after $PGF_{2\alpha}$ treatment, respectively. The number of ovulated follicles was 15.4 ± 1.1. These results indicate that a single treatment of EDP can induce pseudopregnancy in cyclic pigs. The combined use of EDP and $PGF_{2\alpha}$ may simplify the porcine estrus synchronization protocol.

P27

Cytokines and their receptors in the equine oviduct: a preliminary study

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The oviduct plays a critical role in providing the adequate environment for the establishment of pregnancy. Cytokines, such as tumour necrosis factor (TNF) α and interferon (IFN) γ, are potential mediators of many cellular functions acting through specific receptors. The aim of this study was to evaluate the expression of TNFα, and the receptors IFNγ-RI, TNFα-RI and TNFα-RII on the isthmus, ampulla and infundibulum of the oviduct in mares in the follicular, early and mid luteal phases. Blood and oviducts were collected *post mortem* from nine cyclic mares classified into: (1) follicular phase (n = 3), early luteal phase (n = 3); (2) or mid luteal phase (n = 3) based on plasma progesterone concentration and ovarian structures. Immunohistochemistry was used to study the localization of the cytokine TNFα and the receptors IFNγ-RI, TNFα-RI and TNFα-RII using Rabbit polyclonal antibodies. Positive reacting cells were randomly counted for each portion of the oviduct by light microscopy. The expression of TNFα was higher in the isthmus (88.93% ± 3.64) than in the ampulla (66.80% ± 7.22) and infundibulum (69.30% ± 3.64), during the mid luteal phase ($p < 0.05$). However, no difference was found concerning the expression of cytokine receptors TNFα-RI, TNFα-RII or IFNγ-RI. This study suggests that cytokines TNFα and IFNγ are present in the mare's oviduct and might play important roles in oviductal function. Increased TNFα expression in the istmus in the mid luteal phase might be related to a TNFα mediated immune function, when the embryo already left the oviduct and is no longer prone to the detrimental action of the cytokine.

P28

In vitro development of vitrified *in vivo* and *in vitro* fertilized pronuclear-stage rabbit embryos

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The improvement of embryo cryopreservation could play an important role in rabbit biotechnology. The aim of this study was to evaluate the Solid Surface Vitrification (SSV; Dinnyes, 2000, Biology of Reproduction, 63, 513–518) technique on post-warming developmental capacity of *in vivo* and *in vitro* produced pronuclear-stage rabbit embryos. The oocytes, sperm and the zygotes were collected from matured Hycole hybrid rabbits. The *in vivo* or *in vitro* (by IVF) produced zygotes were vitrified with SSV and were immediately warmed and rehydrated. Following 5 days of *in vitro* culture, blastocysts were stained with 5 μg/ml Hoechst 33,342 and cell nuclei were counted. At least three replicates were performed. The blastocyst rates of the non-vitrified zygotes were significantly ($p < 0.05$) higher (*in vivo* 47/60 (78%) vs. *in vitro* 38/50 (76%)) than that of vitrified ones (*in vivo* 45/141 (32%) vs. *in vitro* 12/33 (36%)). Although *in vivo* or *in vitro* produced blastocyst rates did not differ, blastocyst cell numbers of either fresh (121.6 ± 4.9) or vitrified (107.3 ± 16.8) *in vivo* zygotes were higher than that of the *in vitro* ones (fresh 105 ± 11.5 and vitrified 91.4 ± 9.6). In conclusion, the SSV technique was efficient to cryopreserve either *in vivo* or *in vitro* fertilized rabbit zygotes. Supported by "Plurabbit" (OMFB-00130/2010 ANR-NKTH), EU FP6 "Clonet" (MRTN-CT-2006-035468), Hungarian-Chinese Bilateral project (TÉT CN-56/2007).

P29

Insulin-like growth factor I and non esterified fatty acids plasma concentrations in the newborn calf

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In the adaptational process at birth, insulin-like growth factor I (IGF-I) is involved in gastro-intestinal tract development, while non esterified fatty acids (NEFA) are a source of alternative energy for the neonate. Thus, the present study aimed to evaluate IGF-I and NEFA plasma concentrations in newborn calves. The study was conducted on nine healthy Friesian calves born at term after spontaneous parturition. Blood samples were taken at 10, 20, 30 mins, 3, 6, 12, 24, 36 h and at day 2, 3, 4, 5, 6, 7, 10, 14, 21, 28 of age. IGF-I was evaluated by a modified radioimmunoassay method, while NEFA were analysed by an enzymatic-colorimetric method. There was a decrease in IGF-I plasma values from the first 10 (23 ± 11.7 ng/ml) −30 (25 ± 13 ng/ml) mins after birth to 6 days of age (9 ± 5.8 ng/ml) and beyond ($p < 0.05$). NEFA plasma values increased from 10 min (370 ± 114.06 μEq/l) to 20 (591 ± 259.21 μEq/l) and 30 min (871 ± 385.6 μEq/l) after birth, then decreased between 3 h of age (1197 ± 694.09 ?μEq/l) and afterwards (range: 796–382 μEq/l)($p < 0.05$). The results indicate that the initial rise in NEFA, as already reported (Haga, 2008, J Dairy Sci, 91, 3156–64), could be related to the need for prompt energy in the neonate, while high initial plasma IGF-I could mirror both maternal and/or neonatal production and are probably involved in the process of neonatal intestinal maturation (Blum, 2008, Adv Exp Med Biol, 606,397–422).

P30

Pro/acrosin activity and western blot analysis in capacitated and non-capacitated boar spermatozoa

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Acrosin is an acrosomal serine proteinase involved in sperm binding and penetration through the zona pellucida. In ejaculated sperm, acrosin is present in zymogen form, pro-acrosin, which is activated during sperm capacitation. The aim of this experiment was to compare both pro-acrosin activity and protein expression between non-capacitated and capacitated sperm. Protein extracts from control and capacitated sperm samples from adult boars were analysed by SDS-PAGE, transferred to PVDF membrane and incubated with mouse monoclonal anti-acrosin (1:4000). Membranes were incubated with secondary antibody (1:5000) and developed using an enhanced chemiluminescence kit. Acrosin activity was determined spectrophotometrically after the separation of sperm from seminal plasma and their incubation with a detergent-substrate solution mixture. Acrosin activity was 145 ± 48.82 $\mu UI/10^6$ sperm in non-capacitated samples and 320 ± 115.46 $\mu UI/10^6$ sperm in those capacitated. Western blots showed the presence of α-acrosin (37 kDa) and *β*-acrosin (25 kDa) isoforms in both non-capacitated and capacitated samples, but γ-acrosin (15 kDa) isoform only in capacitated samples. Moreover, protein intensity of pro-, α- and *β*-acrosin isoforms was higher in capacitated samples. In conclusion, capacitation process increased sperm acrosin activity 2.25 fold as a result of pro-acrosin transformation into acrosin isoforms.

P31

Exposure of bovine spermatozoa to mild hydrogen peroxide enhances cleavage and blastocyst rates

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The objectives of this study were to elucidate changes in bovine sperm functional parameters, fertilization capacity and embryo developmental competence after exposure of sperm to low (100 μM), mild (200 μM) and high (500 μM) concentrations of H_2O_2 for 4 h prior to fertilization. A total of 1461 (3 replicates) oocytes were matured and fertilized *in vitro*. Sperm motility and velocity characteristics as assessed by Computer Assisteded Sperm Analysis were lower in H_2O_2-treated spermatozoa compared to nontreated control spermatozoa ($p < 0.05$). The membrane integrity (PI, Hoechst), acrosomal status (PSA), mitochondrial membrane potential (JC-1) and DNA integrity (TUNEL) of spermatozoa exposed to low H_2O_2 levels were not significantly different from the control group ($p > 0.05$). However, mild and high H_2O_2 levels negatively affected membrane integrity, mitochondrial membrane potential and DNA integrity ($p < 0.01$). Spermatozoa were subsequently used for IVF and embryos were cultured up to 7 days post insemination for cleavage and blastocyst formation. Higher percentages of cleavage and blastocyst development were observed in the mild group compared to the low and high groups ($p < 0.05$). Furthermore, lower percentages of apoptotic cells in blastocysts were evident in control, low and mild groups compared to high group. The results of the present study indicate that exposure of spermatozoa to mild H_2O_2 level prior to sperm-oocyte incubation may increase blastocyst development by suppressing apoptotic cascade.

P32

Endometrial prostaglandin synthases and oxytocin receptors in mares after oxytocin administration

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Oxytocin has been used to prolong luteal phase in the mare. However, the exact mechanism involved in this action is unknown. Therefore, the goal was to evaluate the effect of exogenous oxytocin administration on (1) luteal function and on the expression of (2) prostaglandin $F_2\alpha$ and E_2 synthases (PGFS and PGES); and (3) oxytocin receptors (OTR) in the mare's endometrium. Eleven cyclic mares were used. Control mares (n = 5; C group) received 6 ml of saline solution subcutaneously (sc), while oxytocin (60 IU/mare) was administered sc to another group (n = 6; OT group), every 12 h, from Day 7 to Day 14 after ovulation. Internal genitalia ultrasonography and blood samples were performed every other day. Plasma progesterone concentrations (P4) were determined by RIA. Endometrial biopsies were carried out on Day 10 post ovulation in C group, and on Day 24 in OT group. Further on, *PGES*, *PGFS*, and OTR transcription in the endometrium was accomplished by Real Time PCR. Luteolysis occurred 3 and 6 days after biopsy in C and OT groups, respectively. Prolonged luteal function occurred in 67% of OT mares. P4 in C group was higher (13.7 ± 1.5 ng/ml) than in OT group (8.33 ± 1.8 ng/ml) on biopsy day ($p = 0.05$). PGES gene transcription was reduced in OT group ($p < 0.05$), PGFS was similar between groups, while OTR was increased in OT mares ($p < 0.05$). These results suggest that a complex mechanism including *PGES*, *PGFS* and OTR transcription might be involved in oxytocin prolonged luteal function in the mare.

P33

Addition of insulin like growth factor-1 during maturation and culture enhances blastocyst rate and reduces apoptosis in bovine blastocysts derived from heat stressed oocytes

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Heat stress (HS) reduces bovine oocyte developmental competence. Previous research showed that the addition of insulin like growth factor-1 (IGF-1) during maturation reduced apoptosis in heat stressed oocytes with no apparent effect on blastocyst rates. We hypothesized that adding IGF-1 during maturation and culture may enhance blastocyst rate and decrease apoptosis in blastocysts derived from heat stressed oocytes. During the first 12 h of maturation, HS (40.5°C) was applied while IGF-1 (100 ng/ml) was added during maturation (M), during culture (C) or during both (MC). Two control groups were included (negative-control: no HS nor IGF-1 and HS-control: HS but no IGF-1). Blastocyst rates were 27.9 ± 2.1^{a}, 10.9 ± 1.2^{b}, 9.7 ± 1.2^{b}, 18.4 ± 2.4^{c}, and 15.3 ± 1.9^{bc} % in negative-control, HS-control, M, C and MC groups, respectively (^{a,b,c} $p < 0.05$). Apoptotic cell ratios (ACR) were 9.7 ± 1^{a}, 11.8 ± 1.2^{a}, 11.6 ± 2^{a}, 7.8 ± 0.8^{b} and 5.9 ± 0.7^{c} % in negative-control, HS-control, M, C and MC groups, respectively (^{a,b,c} $p < 0.05$). The addition of IGF-1 to the culture medium increased blastocyst rate in comparison to HS-control group. However, IGF-1 had more beneficial effects on ACR when added during both maturation and culture than during maturation or culture only. In conclusion, negative effects of HS on oocyte developmental competence can be reduced by adding IGF-1 during both maturation and culture.

P34

Boar spermatozoa from either the sperm-peak or the rest of the sperm-rich fraction equally survive simplified freezing in miniflatpacks (MFP)

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Boar spermatozoa present in the first 10 ml of the sperm-rich fraction, SRF (portion 1, P1, sperm-peak portion), had a higher cryosurvival than spermatozoa from the rest of the ejaculate (2nd portion of the SRF plus the post-spermatic fraction), even when using simplified freezing routines, due to the specific composition of seminal plasma in the P1 (glycoproteins, pH, and bicarbonate concentrations). Since P1-spermatozoa have not been compared to the rest of spermatozoa in the SRF (SRF-P1, usually 30–40 ml of the SRF), which are also routinely used for freezing, we have compared cryosurvival for P1 & SRF-P1, in terms of sperm kinematics (QualiSperm^{TM} system), membrane integrity (SYBR-14/PI), acrosome integrity (FITC PNA/PI), and sperm membrane stability (Annexin-V), explored using flow cytometry. The SRF portions were weekly collected from four mature boars (5–6 replicates per boar, sperm concentration: P1-1.8 ± 0.8, SRF-P1-$1.3 \pm 0.6 \times 10^{9}$ spz/ml) and simplified-frozen in MFP (Saravia, 2010, Anim Reprod Sci, 117, 279–287). The preliminary post-thaw results show a sperm motility reaching a mean of 50%, without differences between portions (ns), but with clear inter-boar variation ($p < 0.01$). Neither did plasma membrane or acrosome integrity differ (ns), but a tendency ($p < 0.052$) for an increased membrane instability was seen in SRF-P1. Studies are ongoing to disclose the proteome of these portions (Supported by SLF, Stockholm).

P35

Molecular mechanism of non-genomic progesterone (P4) effect on bovine myometrium: involvement of membrane progesterone receptor component 1 (PGRMC1) and serpine 1 binding protein (SERBP1)

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We found that P4 affects bovine myometrial cells genomically or non-genomically, but the latter mechanism is not fully understood. It is suggested that a complex of PGRMC1 with SERBP1 may be involved in non-genomic action of P4. The aim of the study was to investigate: (1) PGRMC1, SERBP1, nuclear P4 receptor (PR) mRNA expression by real time PCR and (2) cellular localization of proteins for PGRMC1, SERBP1 and PR in bovine myometrium, by immunohistochemistry, during the estrous cycle and early pregnancy. Uteri from cows on days 1–5, 6–10, 11–16, 17–21 of the estrous cycle and 3–5, 6–8, 9–12 week of pregnancy were used. We demonstrated, for the first time, the expression of PGRMC1 and SERBP1 mRNA in the bovine myometrium. We did not observe changes in PGRMC1 mRNA values during the estrous cycle. However, mRNA expression of SERBP1 and PR was lower after day 11 of the estrous cycle. The highest mRNA values of PGRMC1 and SERBP1 were found on 9–12 and 3–5 weeks of pregnancy, respectively. There were no changes in PR mRNA expression during pregnancy. The highest protein expression of PGRMC1, SERBP1, PR was found in endothelium of blood vessels in the myometrium. In conclusion, PGRMC1 and SERBP1 protein could be involved in non-genomic mechanism of P4 effect on the bovine myometrium and may take part in the regulation of estrous cycle and in the establishment of early pregnancy. Supported by grant NN 311348237.

P36

Can L-tyrosine modify estradiol concentrations and characteristics of heat in female dogs?

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The amino acid L-Tyrosine is considered to have a positive effect on fertility of female dogs. Several authors recommend feeding of 100 mg/kg L-Tyrosine during the follicular phase of the oestrous cycle to improve fertility and copulation behaviour. The aim of our study was to determine whether exogenous administration of the precursor L-Tyrosine would influence estradiol concentrations and the oestrous cycle in the bitch. Progesterone values were measured and the day of ovulation estimated once progesterone values exceeded 4 ng/ml. Sixty-two bitches were randomly allocated to one of two treatment groups in which each dog received 100 mg/kg/day of either tyrosine or milk sugar orally between day three and nine of heat. Every two to three days a gynaecological examination was performed and blood samples were taken to determine estradiol and progesterone concentrations. Copulation behaviour was observed, classified and documented on a standardized case report form by the owners. Results did not show marked modifications in copulation behaviour. No differences in volume and visual nature of vaginal discharge were observed. At the day of ovulation mean estradiol concentration in the treated group was 44.5 pg/ml and 44.2 pg/ml in the placebo group, respectively. The hypothesized increase in mean estradiol concentration therefore was not confirmed. As a conclusion treatment of female dogs with L-Tyrosine does not improve visual signs of heat or copulation behaviour and does not alter estradiol values.

P37

Detection of mRNA encoding ZP1 glycoprotein in domestic cat (*felis catus*) ovary

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The zona pellucida (ZP) is an extracellular matrix that surrounds mammalian oocytes. This coat plays a key role in gamete binding, induction of the acrosome reaction and block to polyspermy. It was generally believed that ZP was formed by three glycoproteins. However, it has been recently shown that ZP is formed by four glycoproteins in species like hamster, human, horse and rat. In domestic cat, previous studies have reported that ZP contains three glycoproteins: ZP2, ZP3 and ZP4. The aim of this study was to analyze the presence of ZP1 gene expression in the cat ovary. Thus, total cat ovarian RNA was isolated and cDNA was synthesized with oligo-dT as primer. The cDNA was used as template for polymerase chain amplifications by means of specific primers based on ZP1 sequences from the cat genome. A fragment of 792 bp was obtained. The amplified product was sequenced and revealed a high similarity with ZP1 of other mammalian species: 80%, 76%, 75% and 74% with horse, human, hamster and rat ZP1, respectively. In the present study, we demonstrated that ZP1 mRNA is expressed in the cat ovary and this indicates that the cat ZP could be composed by four glycoproteins: ZP1, ZP2, ZP3 and ZP4. Future analyses are necessary to amplify the complete open reading frame of the gene and to identify the ZP1 protein by proteomic approaches. This study was supported by a grant from Fundación Séneca de la Región de Murcia (0452/GERM/06).

P38

Ooplasmic influence on nucleolar remodelling in intergeneric somatic cell nuclear transfer (iSCNT) embryos during the first cell cycle

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The aim of present study was to investigate the immediate events of nucleolar remodelling in two types of intergeneric SCNT embryos: produced by SCNT of bovine fibroblast into porcine oocyte (bSCNT), and by SCNT of porcine fibroblast into bovine oocyte (pSCNT). As a developmental and maternal control, parthenogenetically activated bovine (bPA) and porcine (pPA) embryos were used. Embryos were produced according to Bjerregaard et al. 2007 (porcine oocyte donor) and Østrup et al. 2009 (bovine oocyte donor), and fixed at 4 and 12 h post activation (hpa). The reconstructed embryos (8–15 per group) were processed for immunofluorescence of nucleolar proteins; fibrillarin and upstream binding factor (UBF). UBF staining was lacking in all groups at 4 hpa and in all iSCNT embryos at 12 hpa. Both types of embryos derived from bovine oocytes showed at 4hpa localization to rounded structures of presumptive nucleolar precursors. Fibrillarin in all remaining groups was localized to ring-shaped structures. Only in bPA and pPA embryos at 12 hpa, UBF foci were co-localized with ring-shaped structures of fibrillarin. Based on our results, we can conclude that mammalian ooplasmic factors are not able to mediate the specific interactions required for focal localization of transcription factor, UBF in iSCNT, probably due to the species dependent sequence specificity.

P39

Culling due to infertility after embryo flushing in cattle

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We have previously shown that conception of donors after embryo flushing is fairly normal. The time interval from flushing to conception was on average 66.5 ± 57.0 d and 2.0 artificial inseminations (AI) were needed for pregnancy. Still the question remains whether the risk for culling due to infertility after an embryo recovery attempt is increased. The material was obtained from the Agricultural Data-Processing Centre Ltd, Finland, where milk recording and AI data are collected for all the cattle from Finland. All embryo recoveries during the years 1998–2003 were included and the data consisted of 2059 flushings. Subsequently, 1307 animals (405 cows/857 heifers) were inseminated again. Of these animals 97 (69/28) never calved again. Thirty-six (21/15) were culled due to infertility; in 24 (18/6) cases the reason was not reported. There were cases in which the fertility was compromised before the flushing (interval from calving to flushing >200 d, AIs before flushing), or after the flushing the interval from the 1st to the last insemination was less than 60 d and only 1–3 AIs were performed. After removing these cases 15 animals (6/9) were culled clearly due to infertility. Analysis of data (AIs, medical treatments, culling) regarding animals culled due to unknown reason revealed that 8 (6/2) were culled due to infertility. Thus, 1.8% (23/1307) of the flushed animals were culled due to infertility (2.7% of cows, 1.3 of heifers). Despite the descriptive nature of the study, it can be concluded that superovulation and embryo flushing procedures do not seem to increase the risk for culling due to infertility of donor animals.

P40

Ovarian histology in stimulated ewes submitted to follicular aspiration

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The aim of the present study was to evaluate presence of ovarian abnormalities caused by a single follicular aspiration in stimulated ewes. Ten Santa Ines ewes were divided in two groups: GA (aspirated group, n = 4) and GN (not aspirated, n = 6). Estrus was synchronized with vaginal sponges with 60 mg of MAP (Day 0) for 6 days. On Day 5, 37.5 μg of D-cloprostenol and 300 IU eCG were injected intramuscular route. On estrus day, ewes were stimulated with 80 mg of FSHp and 300 IU of eCG, in a single injection, 36 h before surgery. Aspirations were performed by video-laparoscopy under inhalant anesthesia. Seven days later, video-assisted ovariectomies were executed and the harvested ovaries were grossly evaluated and processed for histological analysis (Hematoxilin & Eosin - HH and Masson's Trichrome - MT). Inflammatory content and fibrous tissue formation were scored as absent (0), mild (1), moderate (2), and severe (3). Data were compared by the Wilcoxon Signed Rank test ($p < 0.05$). The mean ± standard deviation (SEM) of aspirated oocytes from the right and left ovary were 8 ± 0.8 and 7.5 ± 1.4, respectively. Gross and histological evaluation of the ovaries did not show different abnormalities between groups ($p = 0.12$), average ± SEM score was of 0.7 ± 0.5 (HH and MT). These few abnormalities corresponded to focal regions of fibrous tissue and mononuclear infiltrate, possibly due to previous trauma induced by aspirations. In summary, we concluded that a single-follicular aspiration session did not change the ovarian morphology in a significant manner. Financial support: FAPESP.

P41

Growth hormone and insulin growth factor-1 on nitric oxide production and angiogenic activity in the corpus luteum of the mare

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Growth hormone (GH) and insulin growth factor-1 (IGF) may participate in the regulation of luteal function in some species. The objective was to evaluate the role of GH and IGF-1 on nitric oxide (NO) and angiogenic factor(s) production that can modulate *in vitro* endothelial cell proliferation (BAEC) by the equine corpus luteum (CL) throughout the luteal phase. CLs were collected from early (Early-CL, n = 4), mid (Mid-CL; n = 5) and late luteal phases (Late-CL; n = 4). Luteal tissue was exposed for 24 h to: (1) media without hormones – negative control; or with (2) equine LH (positive control; 100 ng/ml); (3) equine GH (20 or 100 ng/ml); (4) IGF (30 or 50 ng/ml); (5) IGF + LH (30 + 100 ng/ml; 50 + 100 ng/ml); or (6) IGF + GH (30 + 20 ng/ml; 50 + 100 ng/ml). In the Mid-CL, NO production was higher in IGF + LH group (50 + 100 ng/ml) than in control and IGF (50 ng/ml) ($p < 0.05$). In the Late-CL, IGF + LH (50 + 100 ng/ml) increased NO production compared to IGF (30, 50 ng/ml), LH, GH (20 ng/ml) or IGF + GH (30 + 20 ng/ml) ($p < 0.05$). Also, with IGF + GH (50 + 100 ng/ml) treatment, NO was higher than in all groups ($p < 0.001$). In the Late-CL, IGF + LH (50 + 100 ng/ml) and IGF + GH (50 + 100 ng/ml) decreased BAEC proliferation when compared to LH stimulation ($p < 0.05$). This indicates that IGF and GH stimulate NO production and influence angiogenic activity in equine CLs. These peptides might contribute to vascular luteal involution possibly mediated by NO.

P42

Study on changes in metabolic hormone levels and association with the outcome of superovulation and embryo transfer in ewes

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The effects of metabolic hormones on the efficiency of superovulation and embryo transfer (ET) were studied in sheep. It has been well documented that several factors have influence on the efficacy of ET programs. However, there are limited data (mainly obtained in bovine) available on the role of metabolic hormones (MH) (IGF-1, insulin, T4) in response to superovulation and pregnancy rate of recipients after ET. Blood samples were taken three times from Merino ewes (n = 16) in either the breeding or non-breeding season (d0: at AI of donors or heat observation of recipients, d2: at beginning of fasting before surgery, d4: at embryo flushing/ET). The values of IGF-1, insulin, and T4 were determined. Preliminary results indicate significant differences between IGF-1 (203.00 vs. 153.02 nmol/l) and insulin (10.3 vs. 4.45 μIU/ml) values in breeding vs. non-breeding seasons. There was an association between the number of corpora lutea ($p < 0.005$), embryos flushed ($p < 0.0008$) and transferable embryos obtained ($p < 0.0009$), and IGF-1 blood values. T4 values were lower in the breeding season and in pregnant recipients ($p < 0.001$). In conclusion, MH (IGF-1, insulin, T4) may play an important role in folliculogenesis, steroidogenesis, oocyte maturation, and/or embryo development in sheep.

P43

Characterization of orai1 in porcine oocytes

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At fertilization, the oscillatory calcium signal is responsible for the activation of oocytes and subsequent embryonic development. In a number of cells, Orai-1 has been suggested to serve as the calcium influx channel in the store-operated calcium entry pathway. In this study, the presence and characteristics of Orai-1 were examined to better understand the mechanism underlying calcium signals during fertilization. Messenger RNA was isolated from *in vitro*-matured pig oocytes and used as a template for RT-PCR to clearly indicate the existence of Orai-1 transcripts in oocytes. The localization of endogenous Orai-1 in oocytes was determined by immunocytochemistry followed by laser-scanning confocal microscopy. We found Orai-1 protein localized primarily at the plasma membrane. Next, the complete Orai-1 open reading frame was cloned and tagged with the green fluorescence protein to generate a plasmid encoding the Orai-1-GFP fusion protein. The plasmid was transcribed *in vitro* and the resultant mRNA was microinjected into oocytes. Similar to the endogenous protein, GFP-tagged Orai-1 also localized in the cell cortex implying a role for Orai-1 as a membrane channel. Finally, we compared the abundance of Orai-1 transcripts between GV and MII stage oocytes using real-time PCR and western-blot analysis. Orai-1 expression at the MII stage was significantly lower compared to that in GV stage oocytes. These findings indicate that Orai-1 is a transmembrane protein in pig oocytes that may play an important role in the maintenance of the long-lasting calcium signal during fertilization.

P44

Effect of dextrose and lactose during the weaning-to-estrus interval on embryo development and uniformity in sows

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Dextrose and lactose in the pre-mating sow diet improves subsequent litter uniformity, possibly related to an insulin-stimulating effect. To unravel the mechanism, we studied effects of dextrose and lactose on pre-implantation embryo uniformity. To create large insulin contrasts, 32 multiparous sows were fed a dextrose- and lactose containing diet (DL; each 150 g/day) at 4 h intervals or an isocaloric control diet (CTRL; containing soybean oil) at 12 h intervals during the weaning-to-estrus interval (WEI). After ovulation, sows received a standard gestation diet at 12 h intervals. Statistical analyses used the GLM-procedure of SAS 9.1. Results are presented as means ± SD. Insulin parameters (basal, peak, AUC over 12 h) at day 2 and 3 after weaning were similar for both treatments, but the secretion pattern differed (6 vs. 2 peaks/day). Ovulation rate (CTRL: 24.3 ± 4.8; DL: 23.2 ± 3.1), pregnancy rate (CTRL: 93%; DL: 76%) and embryo survival rate (CTRL: 90% ± 8; DL: 88% ± 8) at day 10 of pregnancy did not differ between treatments. Embryo diameter tended to be higher in CTRL (7.1 ± 1.7 vs. 6.4 ± 2.2 mm; $p = 0.07$), but embryo protein and DNA values and embryo uniformity (SD; CV) did not differ between treatments. Insulin AUC was related to embryo diameter (intercept: 3.39; b: 0.15 mm/1000 μIU), but insulin parameters were not related to embryo uniformity. Whether insulin is a major factor in DL-effects on litter uniformity or whether these effects become apparent at a later stage of pregnancy needs further study.

P45

Altrenogest treatment of early pregnant mares

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In cattle, progesterone supplementation in early pregnancy enhances embryonic growth and the embryonic signal for maternal recognition of pregnancy. So far, such effects have not been investigated in mares. Effects of altrenogest (ALT, 0.044 mg/kg once daily) or sunflower oil (C) treatment from day 6 to 11 of pregnancy were assessed in mares. Embryos (n = 10 per group) were flushed from the uterus on day 11, and endometrial biopsies were collected. Embryos were checked for size and osmolarity. In embryos and endometrium, mRNA expression of proteins potentially involved in embryo development and pregnancy recognition (receptors for progesterone, estrogens and growth hormone, aquaporin-3 and 5, prostaglandin-E synthase, P-450 aromatase, insulin-like-growth factor-1 and -2) were determined by quantitative real time PCR in relation to β-actin. The per cycle pregnancy rate of ALT (83.3%) and C mares (62.5%) did not differ ($p < 0.05$). ALT did not influence embryonic size (ALT 6.8 ± 0.8, C 6.5 ± 0.5 mm, n.s.) but decreased osmolarity of vesicle fluid (6 of 10 embryos floating in PBS in the ALT, 1 of 10 embryos in C mares; $p < 0.05$). Gene expression of aquaporin-3 tended to be higher in embryos from ALT (3.4 ± 1.2) than from C mares (1.2 ± 0.2, $p = 0.067$), but the expression of all other genes was not affected. Aquaporin-3 is a transmembraneous protein involved in water transport and blastocoel formation. ALT treatment in early pregnant mares was associated with a stronger expression of the aquaporin-3 gene and decreased osmolarity of day 11 embryos. Supported by the Mehl-Mülhens-Foundation.

P46

Comparison of three serum-free *in vitro* production methods for bovine embryos

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Serum in culture medium reduces the viability of blastocysts after freezing, involves a potential sanitary risk and causes large offspring syndrome. This study investigated the effects of three different serum-free *in vitro* production methods on blastocyst development. All oocytes (n = 1895) were matured serum-free in TCM-199 supplemented with 20 ng/ml epidermal growth factor (EGF) and 0.1% gentamycine. After fertilization, matured oocytes were at randomly assigned to one of the 3 serum-free culture methods or to a control medium for 7 days. The first group was cultivated in synthetic oviductal fluid (SOF) with 2% BSA; group 2 in SOF with 0.4% BSA, insulin-transferrin-selenium (ITS) and polyvinylpyrrolidone (PVP; George *et al*., 2008); and group 3 in SOF with 0.4% BSA and ITS. The control group was cultivated in SOF with 5% fetal calf serum (FCS). At 45 h, there were no differences in post fertilization cleavage rates (BSA: 75.1% ± 2.3; ITS/PVP: 76.5% ± 2.2; ITS: 75.4% ± 2.2; FCS: 72.7% ± 2.4). Blastocyst formation at 7 days post fertilization was lower in group 1 (BSA) and group 2 (ITS/PVP) in comparison with controls ($p < 0.01$). (BSA: 20.2% ± 2.16; ITS/PVP: 21.4% ± 2.10; ITS: 23.5% ± 2.13; FCS: 29.1% ± 2.42). Blastocyst rate in group 3 (ITS) was not different to the control group. Total cell number was counted by staining the blastocysts with bisbenzimide (Hoechst 33342) and there were no differences between groups (BSA: 113 ± 5.1; ITS/PVP: 121 ± 5.0; ITS 114 ± 4.8; FCS: 120 ± 4.8).

P47

Effects of Removal of Non-Cleaved and Degenerating Embryos and/or Refreshment of the Medium on Bovine Blastocyst Formation

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Presence of unfertilized oocytes in a droplet can decrease mouse embryo development (*Salahuddin et al., 1995: Hum Reprod 10, 2382–2385*). In this study, bovine putative zygotes were divided into four groups, including (1) undisturbed control group (C): continuous culture of all putative zygotes until 7 days post-insemination (dpi), (2) refreshment group (R): all putative zygotes were transferred to new synthetic oviduct fluid (SOF) medium droplets on 3 dpi, (3) removal and refreshment group (RF): removal of degenerating embryos and non-cleaved zygotes with subsequent regrouping of cleaved embryos in new SOF medium droplets on 3 dpi, and (4) removal and regrouping group (RG): removal of degenerating embryos and non-cleaved zygotes with subsequent regrouping of cleaved embryos in the same SOF medium droplets on 3 dpi. Cleavage rates (between 68.2 ± 2.7% and 75.6 ± 2.5%) at 3 dpi, and blastocyst rates (between 17.3 ± 1.7% and 20.5 ± 1.9%) at 7 dpi, did not differ between culture conditions. Total cell number (TCN) varied between 99.3 ± 5.5 for RG and 121.2 ± 4.5 for RF and was the only difference between these two groups ($p < 0.05$). Apoptotic cell ratio (ACR) was lower in C (4.3 ± 0.5%) in comparison with RG and R (7.2 ± 0.5% and 6.3 ± 0.5%, respectively; $p < 0.01$). In conclusion, different culture conditions in bovine *in vitro* production have more effect on TCN and ACR than on blastocyst formation rate.

Regular Posters Session

P48

Effectiveness of two different GnRH analogues with or without β-carotene and vitamin E supplementation in ovsynch protocol in Heifers

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It was aimed to investigate the effectiveness of Ovsynch protocol using two different GnRH analogues with or without β-carotene and Vitamin E on pregnancy rates (PR) and ovulation time in Holstein heifers (n = 80). Heifers were randomly divided into four groups (Group 1, 2, 3 and 4). Ovsynch protocol was applied to all animals. Busereline acetate was used as the GnRH agent in Groups 1 and 2; Lesireline acetate was used as the GnRH agent in the Groups 3 and 4 for Ovsynch protocol. Moreover, β-carotene + vitamin E injection was performed seven days before Ovsynch protocol to heifers in Groups 2 and 4. All animals were inseminated 20 h after the second GnRH administration. Blood samples were collected to measure β-carotene, vitamin E and progesterone levels. Pregnancy diagnosis was conducted on 30 and 60 days after artificial insemination by transrectal ultrasonography. Although PR was numerically higher in Group 4 (60%) compared to those in Group 1 (40%), 2 (50%) and 3 (50%), it did not differ significantly ($p > 0.05$). While β-carotene and vitamin E levels were different ($p < 0.05$), progesterone levels were not different ($p > 0.05$) in non pregnant and pregnant heifers at all groups. Most ovulations in Group 1, 2 and 3 were detected in 36–48 h after the second GnRH injection, in Group 4 they were observed in 20–36 h ($p < 0.05$). In conclusion, β-carotene and vitamin E injections prior to the Ovsynch protocol with long acting GnRH analogues could play a positive role on PRs and breeding maturity in heifers.

P49

Effects of two freeze-thaw cycles on motility parameters of brown bear (*Ursus arctos*) semen

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The development of artificial reproductive techniques for the brown bear (*Ursus arctos*) is mandatory, given the endangered status of some populations. Brown bear semen re-freezing may enable to reuse the samples. We have studied the effect of two freezing-thawing cycles on motility on brown bear semen (for refreezing thawed sperm samples). Ten brown bears were electroejaculated under general anaesthesia. Semen was diluted 1:1 with UL extender (TES-Tris, 4% fructose, 20% egg yolk, 6% glycerol, 2% EDTA, 1% Equex-Paste), cooled to 5°C, packed in 0.25 ml straws (10^8 ml^{-1}) and frozen in a programmable biofreezer (−20°C/min). Thawed samples (65°C/6 s) were re-frozen following the same protocol after 1 h at room temperature. Samples were analyzed by CASA before and after freezing, for total and progressive motility (TM, PM; %), velocity (VAP; μm/s), linearity (LIN; %) and amplitude of the lateral movement of the head (ALH; μm). Data analyses were performed by linear mixed-effects models (SAS^{TM}). TM (57.7 ± 4.3 vs. 33.5 ± 4.1), PM (21.5 ± 2.1 vs. 10.2 ± 1.5) and VAP (38.6 ± 2.7 vs. 33.4 ± 2.4) were lower after the second freeze-thaw cycle ($p < 0.001$), whereas LIN (32.8 ± 1.4 vs. 30.6 ± 0.5) and ALH (3.5 ± 0.1 vs. 3.3 ± 0.2) were not significantly different between cycles. The loss of motility in the second cycle shows that future studies should feature on how to prevent this detrimental effect. Supported by CICYT (CGL2007-63788/BOS) and Cantur SA.

P50

Are artificial insemination centres ready to replace egg yolk by low density lipoproteins (LDL) in extenders for bull semen cryopreservation? : recent results review

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Egg yolk is widely used by artificial insemination (AI) centers for bull semen cryopreservation. Laboratory studies revealed that yolk contains granules that inhibit the respiration of spermatozoa and reduce their motility. Also, they interfere with microscopic observations. Egg yolk could also be a source of microbial contaminations. Therefore, there have been many attempts to find out which components in the egg yolk provide the cell protection with the aim to replace egg yolk with it's cryoprotective fraction in order to prepare a clearly chemically defined extender without inconveniences. Many investigations showed that LDL is the cryoprotective fraction of egg yolk. However, prior to extending its use to AI centres, *in vivo* fertility studies were required. Semen was taken from three bulls and frozen-thawed in two extenders: the LDL extender and a standard tris-egg-yolk extender. The quality of the semen was assessed prior to AI: motility was assessed using Hamilton Thorne ceros 12, and the integrity of the plasma membrane was assessed using the hypo-osmotic swelling test. For the first time, pregnancies were obtained following the AI of cows in the field ($n = 193$) with semen that had been frozen-thawed in the LDL extender. No significant difference ($p > 0.05$) was detected between the success rates of AI between the semen that had been frozen-thawed in the LDL extender (59.2%) and the control extender, Tris- egg yolk (65.3%). In conclusion, the *in vivo* fertility of semen that has been frozen-thawed in the LDL extender is maintained since gestations are obtained following AI; the LDL extender is suitable for use by AI centres.

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Preliminary results of bull semen fertility after cryopreservation with low density lipoproteins (LDL) extender

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LDL was identified as the cryoprotective fraction of the yolk. An easy and rapid method for LDL extraction was developed in our laboratory, which could be applicable for an industrial scale. Bull semen motility was first evaluated by CASA. The results showed that semen motility was higher after freezing with LDL than after freezing with EY (54% vs. 30%, $p < 0.05$; 11 bulls, 2 ejaculates). *In vitro* and *in vivo* semen fertility was also evaluated, obtaining blastocysts and pregnancies respectively, finding no significant differences in fertility results between EY and LDL ($p > 0.05$). In order to reduce sperm number/straw, fifteen ejaculates were collected from 5 bulls. The semen was diluted with LDL and EY into 120, 60 and 20×10^6 sperm/ml, corresponding to 30, 15 and 5×10^6 spermatozoa/dose respectively before freezing. A loss of sperm motility at higher dilutions rates was observed in both extenders, but best motility results were obtained with LDL in comparison to EY: 53% vs. 46%; 61% vs. 32% and 29% vs. 17% respectively at 120, 60 and 20×10^6 sperm/ml ($p < 0.05$). The trials demonstrated that bull semen fertility was preserved after freezing in LDL extender. It offers a high number of functional spermatozoa available for AI and allows to reduce the number of spermatozoa in the straws and to increase the number of straws for sale. Therefore we can conclude that LDL extender is a clear and chemically defined medium, which could be used instead of EY extenders for bull semen cryopreservation.

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Development and embryo quality of the endangered piau pig breed

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The aim of this study was to describe the ovulation rate, recovery rate, quality and development stage of embryos from Piau swine breed raised in Brazil, which embryos will be used in the future to generate a gene bank from this breed. Ten Brazilian naturalized Piau gilts were used as embryo donors, 95.49 ± 14.49 kg body weight and 11.39 ± 2.65 months of age. Donors were submitted to embryo flushing 7.0 days after the first mating with Piau boars (day zero = standing estrus). Surgery was performed as described elsewhere (RD Cameron 1989, Australian Veterinary Journal, 66, 314–318). Embryos were graded within their developmental stage and quality (I: excellent, II: good, III: fair) in accordance with IETS recommendations (International Embryo Transfer Society, 1998). Data is presented as means ± standard deviation. The embryo recovery and ovulation rate was 60.98% ± 28.87 and 10.12 ± 3.16, respectively. One hundred embryos were recovered, out of them: 0% unfertilized, 4% degenerated, 3% 2 cell-embryos, 3% 4 cell-embryos, 10% morula (I), 5% morula (II), 29% blastocyst (I), 2% blastocyst (II), 1% blastocyst (III), 40% the expanded blastocyst (I), 2% expanded blastocyst (II), 1% hatched blastocyst (I). Developmental stages are not uniform, with 87% of embryos in morula and blastocyst stages, what corresponds chronologically to the time after mating, the other stages are asynchronies. Probably in addition to the lower

ovulation rate this asynchrony may be the only factor related to the inherent characteristic of low fertility in this breed.

P53

Influence of seminal plasma separation with different solutions on post-thaw sperm quality of angora male goat semen

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The aim of this study was to investigate the effect of different washing solutions on the freezability of Angora breed male goats' semen. Ejaculates of four Angora breed male goats, 2–4-years-old, were collected using the artificial vagina. Ejaculates were pooled and divided into three equal volumes. Two of the aliquots were separately diluted in TALP (T) or Ringer (R) washing solution (1:10) and submitted to centrifugation for the removal of the seminal plasma. The pellets as well as the not centrifuged third aliquot (C) were immediately extended with Tris-Egg yolk (20%)-Glycerol (7%) extender to a concentration of 50×10^6 spermatozoa for 0.25 ml straws. Then, the samples were maintained at 5°C for 2 h, after which, the straws were deposited in horizontal position for 15 min in liquid nitrogen vapor and then plugged and stored in liquid nitrogen. The straws were thawed at 40°C for 20 s. Percentage data from ten replicate trials were arc sine transformed before being analyzed with ANOVA. A Duncan multiple comparison test was used to locate differences. After thawing, the T group had significantly higher motility (34.5%) and membrane integrity (38.5%), compared with the C (25.5%; 30%) and R (22.5%; 25.4%) groups, respectively ($p < 0.05$). The T group had a lower percentage of dyed (54.7%) and abnormal (55.8%) spermatozoa compared with R (66.3%; 67.9%) and C (61.2%; 65.4%) groups, respectively ($p > 0.05$). In conclusion, not only the separation of seminal plasma but also the washing solution used for the separation may be important factors affecting freezability of semen from Angora breed male goats.

P54

Prolonged estrus and follicular cysts in a bitch after administration of a deslorelin implant

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Slow-release deslorelin implants provide an opportunity for effective contraception in male dogs. Some authors describe deslorelin as safe and effective means for the use in female dogs. It is well known that estrus can be induced if the implant is administered when plasma progesterone concentrations are lower than 5 ng/ml. A seven years old female Bernese mountain dog was presented to our clinic for suppression of reproductive function. One 4.7 mg deslorelin implant was injected sc. Three months later, the dog was re-examined because of prolonged estrus. Ultrasonography showed multiple thin-walled cysts on both ovaries. Several blood samples were taken and two treatments with 360 IU and 420 IU human choriongonadotropine (hCG) were conducted, respectively. Estradiol concentrations and vaginal smears corresponded with estrus whereas FSH and LH did not. The first treatment with hCG seemed to have induced a partial or complete degeneration or luteinization of the cysts but estradiol levels raised again within a few weeks. After the second treatment the bitch developed a pyometra. After successful conservative treatment of the inflammation with antibiotics and aglepristone the dog was submitted to ovariohysterectomy. This case demonstrates that the use of deslorelin implants in bitches may not be completely free of possible negative side effects and interactions with other hormones. The possible association between the deslorelin application and the occurrence of follicular cysts indicates further research.

P55

Induction of oestrus in akkaraman cross-bred ewes

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The efficiency of different progesterone and PGF2α treatments in the induction of ovarian activity and synchronization of oestrus was investigated in Akkaraman cross-bred ewes at the beginning of the transitional period. Ewes were divided into five groups containing 15 animals each. Vaginal sponges containing either 30 mg or 40 mg FGA were inserted into the vagina of the first (FGA30) and second (FGA40) group, respectively. The sponges were removed after 12 day. In the third group of ewes, ear implants containing 3 mg norgestomet (IMP) were inserted subcutaneously and removed after 9 day. In the fourth group, PGF2α was injected twice at an interval of 9 days. After the second PGF2α injection or the withdrawal of the sponges and implants, 600 IU PMSG was injected to all ewes. After the detection of oestrus, ewes were naturally mated. Oestrus response rates were significantly higher in the FGA30 (93%) and IMP groups (93%) than those in the PGF group (53%) and in the controls (27%). However, rates in the FGA40 group were only significantly higher (87%) than those in the control group. The pregnancy rates in the FGA30 (93%) and IMP groups (93%) were significantly higher than in the PGF group (53%). No significant difference was observed among the groups FGA30, FGA40, IMP and PGF in terms of lambing rates. In conclusion, a double dosed application of PGF2α was observed to be inefficient compared to different progesterone treatments in ewes at the early transitional period.

P56

Epidermal growth factor (EGF) and heparin binding-EGF (HB-EGF) mRNA expressions in cyclic and pregnant mare endometrium

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EGF and HB-EGF play crucial roles in embryonic development and peri-implantation. Aim was to characterize expression profiles of EGF and HB-EGF in mare endometrium during estrous cycle and early pregnancy. Biopsies were obtained from mares on day of ovulation (d0, n = 4), late diestrus (LD, n = 4, high progesterone [P_4]), and after luteolysis in the estrus phase (AL, n = 4, <1 ng/ml P_4) of the cycle. In pregnant groups, biopsies were taken on days 14 (P14, n = 4), 18 (P18, n = 4), 22 (P22; n = 4), and 60 (P60, n = 2). Relative mRNA expression levels of genes were quantified by using real-time RT-PCR in duplicates. A mixed model was fitted on the normalized data and least significant difference test was employed to determine significantly different groups. EGF expression was upregulated at LD compared to d0 while HB-EGF expression was not changed throughout the cycle. EGF expression was increased during early pregnancy with the highest expression level observed on P60. Similarly, HB-EGF mRNA level was increased on P60. Pregnancy induced EGF expression on P14 and P18 compared to LD and AL whereas expression of HB-EGF was significantly higher on P18 than that of AL. These results indicate that EGF expression is upregulated during the cycle at late diestrus when P_4 is high and is increased by pregnancy. HB-EGF expression is induced later in the pregnancy. In conclusion, both EGF and HB-EGF appear to involve in the events that happen in the mare endometrium during peri-implantation period. (Funded by TUBITAK).

P57

The effect of β-carotene and GnRH administration on luteal size and luteal blood flow after synchronization with prostaglandin $F_{2\alpha}$ in cows

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The aim of the present study was to determine the effect of administration of GnRH or GnRH + β-carotene combinations on Luteal Size (LS) and Luteal Blood Flow (LBF) in pregnant and non-pregnant cows. Estrus was synchronized with a single injection of 25 mg $PGF_{2\alpha}$ analogue (Enzaprost®) in 29 Holstein-Friesian cows. After artificial insemination (AI), cows were randomly allocated into two groups as β-carotene – (βC–; n = 15) and β-carotene + (βC+; n = 14). All animals received 20 μg GnRH (Receptal®) at the time of AI, on days 7 and 17 after AI. In the βC+ group β-carotene (Carofertin®) injections were administered on the day of synchronization and on days 7 and 17. LBF was determined with a computer program (Pixel Flux™) from the images taken by transrectal colour-Doppler sonography with a 10.0 MHz linear-array transducer (LOG-IQ BookXP, Germany) on days 7, 10, 17, 27 and 37 after AI. The pregnancy rate was higher in βC+ group (71.4%) than in βC– (53.3%). LS was higher in βC+ group on day 27 ($p < 0.001$). The mean LBF was also higher in βC+ group than βC– on days 7 (0.90 vs. 0.61 mm^2; $p < 0.001$) and 27 (1.15 vs 0.77 mm^2; $p < 0.05$). There was no statistical difference in mean LBF and LS between βC– and βC+ group in non-pregnant cows. In conclusion, GnRH + β-carotene injections may have a positive effect on LS and LBF during early and late luteal period in pregnant cows.

P58

Determination of sexual cycle stages of bitches by direct examination technique

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The objective of this study was to determine sexual cycle stages of the bitch by a direct examination technique and to assess the reliability of this new technique by comparing it with the classical staining techniques. Forty mixed breed bitches at different ages and sexually mature, were used in this study. A total of 120 vaginal smears were collected with cotton swab technique, three from each bitch. The collected samples were air dried and coded. One of the prepared samples was stained with May-Grünwald Giemsa and the second sample was stained with Papanicolaou. The third sample was not stained and used for direct examination. The stages of the sexual cycle were determined from all of the samples. Evaluation was made blindly and the results were compared after determination of the sexual cycle stages in all of the samples. The stages determined using the May-Grünwald Giemsa and Papanicolaou technique were completely consistent with each other. However, when the direct examination technique is compared with the classical staining techniques, there was a significant difference in proestrus, diestrus and anestrus of the cycle stages ($p < 0.05$) while there was not any significant difference in the estrus stage of the cycle. In conclusion, it was determined that the direct examination technique is not reliable for the proestrus, diestrus and anestrus stages of the cycle. Therefore, in order to determine the sexual cycle stages of the bitches by vaginal cytology, the classical staining techniques, such as May-Grünwald Giemsa and Papanicolaou, are more reliable.

P59

Effect of post partum uterine disorders and milk production for pregnancy rate and embryonic loss in a holstein-friesian herd

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Post partum uterine disorders are a serious problem in high yielding dairy herds. In parallel with increased milk production the rate of cows affected by uterine disorders have been dramatically increased. The aim of this study was to determine the relationships among the numbers of uterine treatments (UT), pregnancy rates (PR), late embryonic loss (LEL) and milk production (MP). Data were collected from a dairy farm with high milk production (average 10450 kg/cow/year) in Hungary between 2003 and 2009. Early pregnancy determination was carried out 30–36 days post partum (PP) from blood sera by Biopryn (PSPB) ELISA test and rectal palpation 60 days PP served for confirming LEL. Data of 8121 AI's were recorded and 5444 (67.0%) cows were found pregnant, while 1231 (22.6%) cases of LEL were confirmed. In 4076 (50.2%) cases UT was applied post partum. In uterine treated cows PR was lower than in non treated (63.6% vs. 70.5%) and LEL was higher than in non-treated animals (24.3% vs. 21.1. %). MP, PR, LEL data of cows were analyzed by logistic ANOVA. A significant negative effect of number of UT and MP for PR was found ($p < 0.05$). Similar results were confirmed for LEL too: increased number of UT caused a higher rate of LEL ($p < 0.05$) and higher milk production was also associated with increased number of LEL ($p < 0.05$). In summary, post partum uterine diseases had a negative effect on PR and LEL in a high producing HF herd.

P60

Comparison of histoprep continuous gradient and swim up on isolation of buffalo (*Bubalus bubalis*) epididymal sperm

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Purification of motile epididymal sperm is an important step for successful IVF programs, because different types of somatic cells (e.g., blood, immune and epithelial cells) are released during epididymal sperm isolation. Different techniques (e.g., swim up and Percoll gradient centrifugation) are used for motile sperm isolation. The Histoprep (BAG Health care, Germany; 5.6% (w/v) Ficoll and Natriumdiazitroat) is used to separate different cell blood types. The aim of the present study was to compare Histoprep and swim up with regard to isolation of buffalo epididymal motile sperm. Buffalo testes (n = 6) were collected and transported to the lab. The caudae epididymides were dissected and cut in Sperm TL medium for 15 min. The medium was centrifuged (1200 g for 5) and the samples from the precipitated pellet analyzed (pre-analysis). Each remained sample was divided to two equal volumes (250 μl). One part was subjected to swim up in 4 ml sperm TL for 1 h at 39°C, 5% CO2 and 95% humidity. The other part was extended to 4 ml with sperm TL, layered on 4 ml Histoprep and centrifuged at 500 g for 15 min. Samples from the two methods were analyzed for sperm concentration and progressive motility. The GLM procedure of SAS was used for analysis. The results showed a higher sperm motility in swim up (77%) than Histoprep (70%: $p < 0.05$) and pre-analysis (65%; $p < 0.05$). Total sperm concentration (sperm/ml) was 3.6×10^6, 76×10^6 and 152×10^6 for swim up, Histoprep and pre analysis, respectively. In conclusion, more studies are required for adjusting the best protocol based on the Histoprep gradient for sperm purification.

P61

Influence of sperm-oocyte coincubation period on porcine *in vitro* fertilization (IVF) efficiency

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A major obstacle for successful *in vitro* production of porcine embryos is the polyspermic fertilization. One possibility to reduce polyspermic penetration is decreasing the number of spermatozoa added to the fertilization medium. Unfortunately, the lower rate of polyspermy is accompanied by a reduced penetration rate. A short gamete coincubation period of 10 min has been described to obtain fertilization rates similar to 6 h of coincubation and may improve IVF efficiency (number of monospermic fertilized oocytes/total number inseminated) depending on sperm-oocyte ratio (Gil, 2007, Theriogenology, 67(3), 620–626). Here we demonstrate that the optimal coincubation period in our IVF conditions is between 10 min and 6 h. *In vitro* matured oocytes (n = 600) were inseminated with frozen-thawed epididymal semen with 600 spermatozoa per oocyte and coincubated for 2, 4 and 6 h. At 2 and 4 h post insemination (hpi), oocytes were vortexed and transferred to fertilization medium without spermatozoa. At 6 hpi, presumed zygotes of all groups were washed three times in culture medium and cultured. At 22 hpi, zygotes were fixed overnight and stained with Hoechst 33,342 for the assessment of fertilization and polyspermy. The IVF efficiency was higher for the 4 h group (40 ± 5%) than the 2 and 6 h group (19 ± 8% and 17 ± 5%). Between 4 and 6 h of gamete coincubation, the increase in the number of polyspermic oocytes was relatively higher than the increase in penetration rate (+39% vs. +15%), resulting in a decline in efficiency. (This study was supported by Research Foundation-Flanders).

P62

Comparison of three semen extenders: tris egg yolk, EQUEX® and LDL (low density lipoproteins) in canine sperm cryopreservation

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Chicken egg yolk is held as an excellent cryoprotective agent for freezing canine semen. Recent advances enabled the extraction of low density lipoproteins from egg yolk, which are responsible for the cryoprotective abilities of the latter. The aim of the study was to compare three semen extenders for freezing canine semen: two containing egg yolk (Tris egg yolk and Equex STAMP) and one containing 6% LDL. After freezing and thawing 20 ejaculates from five different dogs, the 6% LDL extender produced 50% mobile spermatozoa, compared with 48% with the Equex® extender and 27.7% with the extender containing Egg Yolk alone (EY). *In vitro* functional tests demonstrated that plasma membrane integrity (hypoosmotic swelling test) was maintained in 65–66% of spermatozoa as a function of the extender; DNA integrity was maintained in more than 97% of the spermatozoa. The Equex® extender provided superior acrosome integrity (FITC/PSA test): 68.4% compared with 55.1% with LDL and 53.3% with egg yolk. However, the 6% LDL extender resulted in fewer spermatozoal anomalies (Spermac® test), with 54.6% normal spermatozoa compared to 53.6% for Equex® and 53.3% with the egg yolk. All six of the bitches inseminated artificially via the intrauterine route (Scandinavian technique) using semen frozen in the 6% LDL extender became pregnant. The LDL extender resulted in percentages of mobile spermatozoa and movement characteristics that were as good if not better than those obtained with the reference extenders following thawing. The 6% LDL extender appears to have the same cryoprotective qualities as the reference diluent, Equex® STAMP.

P63

The effect of egg yolk on the survival of sperm from an ai dose after thawing

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Extenders with egg yolk are regularly used for the preservation of bull ejaculate for protection of sperm. Egg yolk influences the stability of the extender to a certain degree. The use of egg yolk as an ingredient of extenders is restricted mainly for hygienic reasons. The sperm activity of an AI dose after thawing by a heat test (38°C, 120 min) was monitored. Two extenders without egg yolk (Andromed, Bioxcell) and two extenders with egg yolk (Triladyl, Optidyl) were used for the prodution of the AI doses. A total of four selected sires used in the system of insemination of dams were evaluated. The bulls were of the same age and had the same quantity and quality characteristics of semen. The results were evaluated by the SAS GLM procedure. Higher activity of sperm after thawing (48.4–51.6%, $p < 0.05$–0.001) was detected in extenders with egg yolk. Sperm activity declined unevenly during the survival test. Sperm activity was significantly higher ($p < 0.01$–0.001) in egg yolk extenders (26.7–31.4%) compared to ones without egg yolk (22.2–25.9%) after 120 min of the survival test. Significant differences in sperm survival among individual sires were determined. Funded by MSMT 6046070901 and QI91A061.

P64

Urachal calculi in a dalmatian bitch

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Various urachal abnormalities has been documented in several animal species (Laverty P.H., Salisbury S.K.: Surgical management of true patent urachus in a cat. (Journal of Small Animal Practice, 2002, 43, 227–229). One of the developmental abnormalities of the puppy bladder is a persistent urachus. In the fetus, the urachus is a tube-like structure, which, by exiting through the umbilical (navel) area, connects the puppy's bladder to the placental tissues. The most frequent type of urachal disordes are: bladder urachal diverticulum, urachal sinus, urachal ligament, urachal cysts, patent urachus and urachal calculi. A dalmatian bitch 6 years old weighing 10 kg was presented for ovaryohysterectomy. Physical examination of the bitch did not reveal any abnormalities in the navel area. Blood samples were collected from cephalic vein for standard pre-surgery haematology analysis. Surgery was performed to remove the uterus and ovaries by standard method. After premedication with atropine sulphate (Atropina solfato, Ati, Ozzano Emila, Bologna Italy) 0.05 mg/kg, the anaesthesia was induced using a mixture of ketamine (Imalgene100, Merial, Italia) 5 m/Kg and medetomidine (Domitor, Pfizer, Roma, Iyaly) 40 mcg/kg i.m. The anaesthesia was maintained with isoflurane (2%) and oxygen was supplied by a cuffed endotracheal tube. Cephalexin (Mylan, Milano Italy) 15 mg/kg was administered at the time of induction. The bitch was in dorsal recumbency and the abdominal region was prepared for standard surgical procedure. One incision caudally to the umbilicus was performed to remove genital appatatus using a standard technique. The postoperative treatment applied was amoxicillin and clavulanic acid (Synulox Pfizer, Roma, Italy) 10 mg/kg and 2.5 mg/kg respectively every 12 h for 7 days. At the opening of abdominal cavity the apex of the bladder appeared adjoined to the umbilicus by means of a short tubular structure. Inside this structure there was a calculus of 4 mm in diameter. The application of light pressure to the bladder not resulted in urine appearing in the umbilical zone and no umbilical stoma was showed. The bladder was normally developed; the lateral ligament and umbilical arteries were in normal condition. After identification of the ureters, the urachus was isolated with a circumferential ligature

with two metric poliglactin 910 sutures (Vicryl, Ethicon) and cut cranial to the ligature.

P65

The effectiveness of osaterone acetate treatment on prostatic regression in dogs with benign prostatic hyperplasia (BPH)

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Orchidectomy is the most effective treatment of benign prostatic hypertrophy (BPH). The alternative to surgery is the pharmacological treatment. The aim of our study was to evaluate the effects of an anti-androgen agent - osaterone acetate (OSA) – treatment on prostatic regression and its safety in terms of side effects. Materials and methods. OSA was administered orally at doses of 0,25 mg/kg/day for 7 days to 40 dogs which were examined before and after the treatment with one-month intervals. Prostatic size was measured by ultrasonography. The semen quality, hematological parameters, liver and renal enzyme activity and hormone concentration were measured. Results. In 39 out of 40 treated dogs clinical signs of BPH disappeared within few days after the start of the treatment. The fast regression of the size of prostate gland without effects on hematological parameters and enzymes level was observed. A decrease of testosterone and estradiol-17β levels compared to the pretreatment values was revealed at first post-treatment examination. Changes in semen quality were observed rarely and were transient. Prostatic size returned to pretreatment values within 6 months.Conclusions. This study shows that OSA is clinically effective therapeutic agent for treatment of canine BPH. Administration of OSA does not exclude dogs from the breeding program and does not induce remarkable side effects.

P66

Reproductive performance of sows returned to estrus after a DUI insemination

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Deep intrauterine insemination (DUI) is a reliable procedure for artificial insemination (AI) in pig farms, providing good fertility rates with low number of spermatozoa, including frozen-thawed or sex-sorted semen. However, there has been speculation about the potential damage that the DUI catheter may cause to the endometrium in its progress along the uterine lumen, compromising the later reproductive performance of the inseminated sows. In order to elucidate this, we evaluated the later reproductive performance of sows that return to estrus after a DUI insemination. A total of 237 sows (parity 2–8) returning to estrus after two DUI-inseminations (1.5×10^9 frozen-thawed spermatozoa per AI-dose) on three commercial farms (DUI sows) were conventionally inseminated (two cervical inseminations with 3×10^9 liquid-stored spermatozoa per AI-dose) in the next regular estrus (20–23 days after DUI-insemination). The reproductive performance of 148 sows derived from the same farms at the same time, which returned to estrus after conventional AI and were subsequently inseminated as descrined above, was used as control (control sows). There were no significant differences between DUI and control sows in the percentages of sows returning again to estrus (21.5% vs. 26.3%) and farrowing (66.2% vs. 63.5%), and in the number of piglets born per litter (10.9 ± 0.2 vs. 11.2 ± 0.2). These results show that the sows returning to estrus after DUI insemination have a normal reproductive performance, which is indicative that deep intrauterine is a safe and suitable procedure to inseminate sows. *Supported by Seneca foundation (04543/07, Murcia, Spain).*

P67

Maceration and complete retention of fetal bones during a singleton pregnancy in a mare

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A 21 year old pluriparous Haflinger mare was presented to our clinic. Case history reported that the mare was diagnosed pregnant but 2 months before the term mammary glands enlarged and then regressed without delivery. The owner referred the animal to us because a vulvar purulent discharge was present since two months. Transrectal ultrasonography showed a great amount of echogenic uterine fluid and ovaries with the presence of a corpus luteum and ovarian activity. The uterus was flushed with 3 l of Ringer solution with added iodopovidone and hydrogen peroxide 1%. Systemic antibiotic therapy and $PGF_{2\alpha}$ were administered IM. After 3 days ultrasonography revealed linear hyperechoic structures in the uterine lumen. The cervix was manually dilated to allow passage of a gloved lubricated hand. A nearly complete fetal skeleton was removed along with copious amounts of purulent fluid. Uterine flushing was repeated for two more times and the clean uterine mucosa treated with an intrauterine foam containing rifaximin (Fatroximin® Fatro-Italy). Radiographic evaluation of the teeth established that fetal age was about 275 days. Ultrasound examination two weeks later showed a uterus in good condition and the uterine swab was sterile. The mare was induced in oestrus, artificially bred and then diagnosed pregnant. Maceration of a single fetus is rare in mares and may arise when fetal problems prevent foaling. In conclusion, this case shows that fetal maceration can be treated and may result in a complete recovery with subsequent pregnancy of the mare.

P68

Changes of luteal blood flow, progesterone and prostaglandin $F_{2\alpha}$ secretion after oxytocin infusion in cows

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The possibility of influencing the blood flow in the corpus luteum (CL) after an increased dose of oxytocin infusion, during the early luteal phase of the estrous cycle in cows was assessed. For this purpose on day 7 of the estrous cycle (D7) cows (n = 7) received 100 IU of oxytocin or a placebo. Infusion lasted 60 (±3 min) min. Size and blood flow (LBF) of the CL were examined with Power Doppler ultrasonography just before and 72 h after the infusion. Luteal size and LBF continued to increase in both groups, with no differences between them ($p > 0.05$). Plasma estrogen and prostaglandin were not different either within the groups across time or between the groups at any time point ($p > 0.05$). The progesterone of the oxytocin group differed significantly from the placebo group 360 min after the infusion ($p = 0.01$) and as a tendency at the time points 450 min, 48 h and 72 h (all $p = 0.08$). Results from the present study support the hypothesis that oxytocin is not directly involved in the mechanism(s) governing the blood flow of the CL. Further investigation is needed to elucidate the role of oxytocin in CL blood flow during early and late luteal phase.

P69

Transperitoneal or transuterine sperm migration in the pig?

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Deep intrauterine insemination allows sperm deposition only into one uterine horn, however, bilateral fertilization of oocytes occurs. How the sperm reach the contralateral oviduct remains unclear. The aim was to study transperitoneal and/or transuterine sperm migration ways. Follicle growth and ovulation were induced in 24 peripubertal gilts with eCG and hCG 72 h after eCG. Endoscopic intrauterine insemination (IUI) was performed 32 h after hCG with 20 ml of extended semen (60×10^6 spermatozoa) as followes: control group – IUI into the right horn, the left horn served as non-treated control; ligature group – IUI into the right horn, the left horn was closed by endoscopic double ligature close to the bifurcation; intraperitoneal group (IP) – IUI into the right uterine horn, the left horn was closed by double ligature and semen was deposited intraperitoneally at the surface of the left ovary. Genital tracts were removed 65 h after hCG, the oviducts flushed and oocytes were analyzed for fertilization and cleavage. Furthermore, accessory spermatozoa (AS) count/oocyte was graded as 0: without, 1: <5, 2: 5–50, 3: 50–100 and 4: >100 spermatozoa. Results indicate: low dose IUI into one horn provides a lower grade of AS in the contra-lateral side (1.57 vs. 2.75), and cleavage to ≥4-cell-embryo is delayed (7.3 vs. 16.6%). The grade of AS was limited ($p < 0.05$) after ligation of the uterine horn (0.12 vs. 2.50) and IP sperm deposition (0.29 vs. 3.17), and fertilization was reduced (14 vs. 75%). Transuterine but not transperitoneal sperm migration and fertilization seems to be possible after deep intrauterine deposition of low dose semen in the pig.

P70

Study on stimulatory activity of synthetic lamprey GNRH-III on gonadotropin release in gilts

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Based on the supposition that lamprey GnRH-III has FSH releasing activity in swine, Maprelin® × P10 (Veyx-Pharma) was used in cyclic gilts. The secretion of FSH, LH, estradiol and progesterone was analyzed, and follicle growth and ovulation recorded. Altogether 24 German Landrace gilts were treated after an 18-day long oestrus synchronization with Regumate® as follows: 48 h after the last Regumate® feeding follicle growth was stimulated either with 150 µg Maprelin® × P10 (n = 6), 50 µg Gonavet (GnRH, n = 6), 850 IE Pregmagon® (PMSG, n = 6) or saline (Control, n = 6). Additionally, in eight gilts the secretion of FSH and LH was analyzed after application of 150 µg Maprelin® XP10 (n = 3), 50 µg Gonavet (n = 3) or saline (n = 2) at mid-cycle (d10 of oestrus cycle). Blood samples were collected frequently via jugular vein catheters. Ovarian features were judged endoscopically at the end of the Regumate® feeding and on days d5 and d6 post injection. Maprelin® XP10 had no selective effect on FSH release in gilts; neither at the preovulatory period nor at mid-cycle. Differently, GnRH stimulates FSH secretion both during the follicular phase and mid-cycle, but less compared to LH. Due to direct action on the ovary, PMSG did not stimulate the release of FSH and LH. Increased estradiol concentrations during d2 and d5 after Regumate® in all groups of gilts indicate preovulatory follicle growth independent from the treatment. PMSG stimulated a significantly higher number of ovulatory follicles compared to Maprelin, GnRH and Control groups. Regardless of treatment 83–100% of gilts had ovulated by d6 post injection.

P71

Expression of oxytocin and vasopressin receptor transcripts in pregnant and cyclic mares between days 12 and 16 post ovulation

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Endometrial biopsies were taken from pregnant and non pregnant mares at day 12 (n = 7), 14 (n = 10) and 16 (n = 8) post ovulation (ovulation = day 0) to investigate the expression pattern of equine oxytocin and vasopressin V1a and V2 receptors. Immediately after collection the biopsies were shock frozen and stored in liquid nitrogen. For the preparation of DNA free total RNA, the samples were homogenized in TriReagent, RNA was extracted and DNAse I (Fermentas) treated to remove putative traces of genomic DNA. Real time RT-PCR was performed using specific primers and probes (5′Fam – 3′Tamra) for equine oxytocin, vasopressin V1a and V2 receptor using equine beta actin (β-act) and GAPDH as reference genes. No significant changes in the expression of the oxytocin receptor in early-pregnant mares could be observed at any time point (mRNA expression in relation to β–act: day 12 0.9 ± 0.6, d14 0.6 ± 0.2, day 16 0.9 ± 0.1, n.s.). The expression was not different from cyclic mares (mRNA expression in relation to β–act: day 12 0.7 ± 0.1, d14 0.8 ± 0.2, day 16 0.6 ± 0.3, n.s.). V1a receptor expression varied in a wide range and V2 receptor mRNA was absent or expressed at a very low level. Our results suggest that down regulation of the endometrial oxytocin receptor is not involved in inhibition of luteolysis in pregnant mares. The mechanism of of maternal recognition in this species is therefore suggested to be different from that described in ruminant species.

P72

Use of a rapid lateral flow device to detect bovine luteinizing hormone

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A new in-clinic lateral flow device (Witness LH, Synbiotics) was recently designed to detect luteinizing hormone (LH) in dogs and cats. A preliminary study was conducted to evaluate the suitability of this rapid lateral flow device for bovine serum samples. Eight cyclic, non-lactating cows were synchronized with a double injection of prostaglandin-F2 alpha ($PgF_{2\alpha}$, Estrumate 3 ml i.m., Schering-Plough) 11 days apart and checked for estrous behavior by visual observation for 5 days starting the day after the second injection of $PgF_{2\alpha}$. Cows were considered to be in estrus if they stood when being mounted by a herdmate. The ovaries of each animal were scanned daily using a real-time ultrasonography with a 7.5 MHz transrectal linear probe to follow ovarian activity. Blood samples were collected every 6 h following the second injection of $PgF_{2\alpha}$. LH concentrations were measured by ELISA and determined semi quantitatively by Witness LH (as negative, intermediate and positive results). The overall correlation coefficient between LH concentration and Witness LH result was $\rho = 0.738$ (Spearman's rank coefficient). These results confirm that Witness LH can be used for rapid detection of LH peaks in bovine serum samples and may be used as an additional tool to improve heat detection before AI.

P73

Predisposition to repeat breeding in commercial UK cattle and the subsequent success of AI or AI plus ET

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Cattle that returned to oestrus after three inseminations were defined as Repeat Breeders (RBs). This study aimed to: (1) establish if there is a sub-population of RBs within the dairy cattle that is less fertile (e.g., because of milk yield, prior periparturient conditions etc); and, (2) to estimate the efficacy of AI (artificial insemination) or AI + ET (embryo transfer) to reduce repeat breeding. The study was carried out in two main parts: the first one involved 85 RBs that received AI alone and relevant controls. In the second part, 128 RBs were inseminated and received an embryo 7 days later; controls were subjected to ET alone on Day 7. The calving to pregnancy interval was 80 days longer in RBs ($p = 0.01$), irrespective of prior fertility treatment (mainly targeting the ovaries). The incidence of dystocia was similar in RBs and controls, but the incidence of moderate uterine infection was higher in RBs ($p = 0.04$). In RB cows, pregnancy rates following AI alone were 30% after the fourth AI (vs. 45 to 64% after 1–3 AI in controls) compared with 53% after a fourth AI + ET (controls ET alone: 49%). Pregnancies following AI + ET produced a 6.3% incidence of twins. In conclusion, greater attention to eliminate uterine contamination in potential RBs is recommended, and if that fails, the use of AI + ET is a viable option.

P74

Caprine sperm responsiveness to *in vitro* capacitation and acrosome reaction in relation to different inter-male sperm quality

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This study was carried out to assess the responsiveness of spermatozoa from two groups of males with different semen quality to *in vitro* capacitation and acrosome reaction. Semen was collected from 8 Alpine bucks, 18 months of age, housed and reared together; semen quality was previously monitored throughout 4 months. The criterion to classify the males in ''good'' and ''bad'' semen producers was based mainly on the percentage of sperm abnormalities. After semen collection, seminal plasma was removed and sperm were resuspended in TALP medium. The final sperm concentration was 100×10^6/ml. In a preliminary test (stage 1) diluted sperm were incubated for 6 h at 38°C in 5% CO_2 to verify the time sperm need to capacitate. Motility, capacitation status and acrosome integrity were assessed at 0, 4 and 6 h. Percentages of non-capacitated, capacitated and acrosome-intact sperm were similar after 4 and 6 h of incubation. Thus, for the actual trials (stage 2) incubation was for 4 h only. There were no differences between males regarding the decrease in proportion of non-capacitated or the increase in proportion of capacitated sperm from 0 to 4 h of incubation ($p > 0.05$). Also, there was no difference between males regarding the decrease in proportion of acrosome-intact sperm from 0 to 4 h of incubation ($p > 0.05$). However, sperm respons-iveness to *in vitro* capacitation and acrosome reaction, considering all assessed variables, showed slight inter-male variation. Sperm from males producing ''good'' or ''bad quality'' semen do not respond differently to *in vitro* functional tests.

P75

Successful introduction of genetic material by fixed-time artificial insemination with deep frozen semen in a Mangalica sow in Switzerland

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The Mangalica breed had nearly disappeared in the 1970s. Different Mangalica types have survived in Hungary, Germany, Austria, Serbia and Switzerland. The main population of the Swallow-Belly Mangalica (SBM) breed are kept in Switzerland. The aim of this project was to introduce new genetic material via artificial insemination of frozen semen (AI) into the Swiss SBM population. It was decided to synchronise the recipient to improve the success rate of AI. The sow was treated daily with 11 mg altrenogest for 15 days. Altrenogest was administered orally at the same time every day. The animal received 1000 UI PMSG to stimulate follicle growth 24 h after the last altrenogest treatment and ovulation was induced with 500 IU hCG 80 h after PMSG. Insemination was carried out twice – 24 h and 40 h after the hCG injection. AI was conducted with deep frozen semen. The semen was thawed in a 50°C water bath for 45 sec. After thawing the semen was diluted in 100 ml extender (Modena) which was warmed up to 20°C before use. The semen was deposited intracervically immediately after final dilution. Three piglets were born alive after 116 days of gestation. Usually, the mean litter size of Mangalica is 5.0 piglets (Brüssow, 2005, Pig News and Information, 26 (I), 23–28). In consideration of this, the above-mentioned procedure is successful and effective in introducing new genetic material into the SBM population.

P76

GnRH receptor immunolocalization and *in vitro* GnRH effects in leydig cells of adult Alpaca (*Lama pacos*) testis

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The main objective of study was to examine the modulatory *in vitro* effects of GnRH on isolated Leydig cells of adult alpaca (*Lama pacos*) testis. We first evaluated for the presence of GnRH receptor (GnRHR) and of cyclooxygenase 1 (COX1) and COX2 in alpaca testis. We then studied the *in vitro* effects of buserelin (GnRH analogue), antide (GnRH antagonist), and buserelin plus antide or inhibitor of phospholipase C (compound 48/80) and COXs (acetylsalicylic acid) on the production of testosterone, PGE_2, and $PGF_{2\alpha}$ and on the enzymatic activities of COX1 and -2. Immunoreactivity for GnRHR was detected in the cytoplasm of Leydig cells and in the acrosomal region of spermatids. COX1 and -2 immunosignals were noted in the cytoplasm of spermatogonia, spermatocytes, spermatids, Leydig cells, and Sertoli cells. The *in vitro* experiments showed that buserelin alone increased ($p < 0.01$) and antide and buserelin plus acetylsalicylic acid decreased ($p < 0.01$) testosterone and $PGF_{2\alpha}$ production and COX1 activity, while antide and compound 48/80 counteracted buserelin effects. Prostaglandin E_2 production and COX2 activity were not affected by buserelin or antide. These data suggest that GnRH up-regulates directly testosterone production in Leydig cells of adult alpaca testis with a postreceptorial mechanism that involves PLC, COX1 and $PGF_{2\alpha}$.

P77

Development of bovine blastocysts after *in vitro* fertilisation of oocytes derived from ovum pick up with sex-sorted sperm

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Sex preselection using X or Y sperm is a method that has been recognized to be more efficient than embryo sex determination. Using sexed sperm for *in vitro* fertilisation (IVF) in conjunction with ovum pick up (OPU) facilitates the production of gender preselected embryos from living and known donors. Our objective was to investigate the effects of flow cytometrical sperm sorting processed with Sexcess® on blastocyst development of *in vitro* fertilized OPU derived oocytes. In total 43 OPU sessions were performed in 15 cyclic, dry and non-stimulated cows. Oocytes were matured and fertilized *in vitro* with frozen sex sorted sperm (sorted group) or frozen unsorted sperm (unsorted group) from the same ejaculates. The presumptive zygotes were cultured *in vitro* in synthetic oviductal fluid. Cleavage rate, evaluated 48 h post inseminations, was higher in the unsorted group than in the sorted group (69.09% vs. 52.4%, respectively, $p < 0.05$). However, blastocyst rate from total oocytes and also from cleaved embryos, evaluated at days 7, 8 and 9 post insemination did not show differences between groups (21.82%, 31.59% in the unsorted group vs. 15.53%, 29.63% in the sorted group). We conclude that IVF with sex sorted sperm and OPU derived oocytes results in lower cleavage rates than with unsorted sperm, but the blastocyst rates do not differ. Therefore, sorted sperm processed with Sexcess® are an efficient means to obtain sexed embryos from OPU derived oocytes.

P78

A new sevice for aspiration of follicular fluid for acid-base balance analysis in cattle

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We evaluated a new device for ultrasound guided transvaginal aspiration (TVFA) of follicular fluid for acid-base balance analysis (ABR) in cattle. An aspiration syringe was placed in the front part of the new tool handle next to the transducer to enable a direct collection of sample into the syringe. To obtain a sufficient amount of tested fluid we first used a reservoir of urine (rubber balloon) for aspiration in laboratory conditions ($n = 15$) and subsequently we performed TVFA in cows bearing ovarian cysts with a diameter of at least 3 cm ($n = 5$). Three samples (two aspirations/sample) were collected from each of the follwing structures: the new device (ND) and a sequence of two samples with a modified ovum pick-up equipment (OPUe) – first aerobic collection (AE) with air present in aspiration needle and tubing (total volume of 0.9 ml) and then anaerobic collection (AN) immediately after AE. In the *ex vivo* trials using urine we found significantly higher pH (7.703), lower pCO_2 (10.30) and higher pO_2 (8.67) in AE samples in comparison to ND and AN samples (pH 7.685, 7.692; pCO_2 11.13, 10.85 and O_2 8.67, 7.02). In the TVFA trials aspirating cystic fluid we found significantly higher pH in AE samples in comparison to AN samples (7.357 vs. 7.348), lower pCO_2 (6.85) and higher O_2 (14.12) in AE samples in comparison to ND and AN samples (pCO_2 7.36, 7.30; O_2 9.95, 10.63). We proved that ABR assays are affected by air after AE in comparison to AN and ND. The new device facilitates anaerobic aspiration of follicular fluid when collection of two samples in sequence using OPUe is impossible due to insufficient follicle volume. (Supported by grant MSM Czech Rep. no. 6215712403)

P79

Effects of nutrition during the luteal and follicular phase in gilts

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Plane of nutrition during the luteal phase and equally, during the follicular phase is known to influence the number of selected follicles and ovulation rate in gilts. The present study aimed to measure carry-over effects of nutrition from the pre-follicular (luteal) phase to the follicular phase, specifically questioning whether malnutrition during the pre-follicular phase can be compensated for during the follicular phase. Follicle development was monitored longitudinally by using transcutaneous ultrasound. Twenty F2 gilts were subjected to a 2×2 design. Just after their second estrus, with a BW of 117 ± 2 kg, gilts were allowed a low (L: $1 \times$ Maintenance) or high (H: Maintenance + 1.5 kg) feed level during the pre-follicular (luteal) phase. Subsequently, during the follicular phase, 50% of the gilts were switched to a L or H feed level. Start of the follicular phase (d0) was induced by giving a PGF2α injection at 12 days after the second ovulation. Ovulation rate (post mortem) was 14.5 ± 1.0, 13.8 ± 0.5, 13.4 ± 1.5, 12.4 ± 0.5, for HH, HL, LH and LL gilts (N.S.). A H feed level during the luteal phase increased average diameter of the largest follicles on d2 of the follicle phase (4.5 ± 0.2 vs. 3.9 ± 0.2 mm; $p < 0.05$). A H feed level during the follicle phase increased follicle diameter on d5 (7.8 ± 0.2 vs. 6.8 ± 0.2 mm; $p < 0.05$) and at ovulation (8.5 ± 0.2 vs. 7.8 ± 0.1 mm). These preliminary data indicate that nutrition during both luteal and follicular phase impact follicle growth and ovulation rate and that the effects on ovulation rate seem additive.

P80

Effect of Leukemia-Inhibitory Factor (LIF) on proliferation of pig granulosa cells *in vitro*

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During *in vitro* culture granulosa cells (GC) quickly reach the luteal phenotype characterised by enhanced progesterone synthesis and decreased proliferative potential. It was demonstrated that luteinizing human GC can be maintened in culture over prolonged periods of time in the presence of LIF. The aim of the present study was to elucidate the effect of LIF on proliferation of pig GC derived from small (1–2 mm) and large folicles (5–7 mm). Granulosa cells were isolated from healthy small (SF-GC) and large (LF-GC) follicles and cultured for 48 and 72 h in DMEM/F12 medium with supplements. pFSH was added to stimulate the cells. LIF was used at a concentration of 1000 IU/ml. To determine GC proliferation potential the newly synthesized DNA in cell cultures was measured by incorporation of ^{3}H-thymidine using the technique of TCA precipitation and liquid scintillation counting. Data were analysed by ANOVA. LIF did not influence the proliferation of SF-GC after 48 h of culture in basal as well as in FSH stimulated conditions. Elevated but not significantly increased level of ^{3}H-thymidine incorporation was measured in SF-GC cultured for 72 h in the presence of LIF in basal conditions. The proliferative potential of LF-GC was higher ($p < 0.05$) in comparision with control after 48 and 72 h of culture in basal conditions. No effect of LIF on LF-GC proliferation was observed in FSH stimulated conditions. The results of the study indicate that LIF may increase survivability and developmental potential of pig GC *in vitro*.

P81

Effect of SOD (superoxide dismutase) on chilled epididymal cat spermatozoa

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Preservation of gametes is an important tool in assisted reproduction programmes. New approaches are being developed to find new methods that ensure a longer storage of cooled semen. It is known that high concentrations of reactive oxygen species (ROS) cause sperm pathology. The metalloprotein superoxide dismutase (SOD) is responsible for H_2O_2 and O_2 production. The aim of this study was to assess the quality of chilled cat semen processed with extenders containing SOD as antioxidant additive. Epididymides were collected from 20 domestic cats during routine neutering procedures. The cauda epididymis was finely minced to release spermatozoa. Each sample was divided in two aliquots: spermatozoa diluted in Tris extender without (1) or with SOD (2). Each sample was analyzed for motility, viability and acrosome status, immediately after semen preparation (T0) and after storage at 5°C for 24 h, 48 h and 72 h (T1, T2 and T3 respectively). The acrosome integrity was evaluated by PNA-FITC conjugated staining. A proteomic approach of ERK quantification was also evaluated as an indicator of oxidative stress. Quality parameters of sperm were significantly higher in aliquots added with SOD. ERK phosphorylation was statistically higher in the aliquots without SOD. In conclusion, SOD addition in semen extenders improved the quality of chilled cat semen and reduced ERK activation.

P82

The epidermal growth factor stimulates ram sperm capacitation and protein tyrosine phosphorylation

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The presence of epidermal growth factor receptor (EGFR), a specific tyrosine kinase receptor, in ram spermatozoa was determined by incubating ram sperm with Alexa488-conjugated EGF. Results are shown as mean ± SEM of the number of samples indicated in each case. ANOVA test was performed, and post hoc comparisons were made using the Student-Newman-Keuls Multiple Comparisons Test. After 2 h of incubation in capacitating conditions, the proportion of sperm binding EGF increased up to 39.3 ± 7.4%, 57.1 ± 6.0% and 52.0 ± 4.5% with 25, 50 and 100 nM EGF, respectively ($n = 6$). Western-blot analysis of the presence of EGFR in ram sperm lysates revealed a band of approximately 170 kDa (predicted molecular weight for this receptor). Having identified EGFR in ram spermatozoa, we investigated the effect of the inclusion of EGF during incubation on capacitation. Chlorotetracycline staining showed that 100 nM EGF increased the proportion of capacitated sperm from 36.2 ± 1.8% in control conditions to 49.9 ± 2.0% after 3 h of incubation ($p < 0.001$). An increase in protein tyrosine phosphorylation of 14.5 ± 0.6% ($p < 0.01$) was concomitantly achieved ($n = 3$). The addition of tyrphostin AG555, a specific inhibitor of EGFR kinase activity, accounted for a significant increase ($p < 0.001$) in the percentage of non capacitated sperm (32.0 ± 2.3% vs. 59.1 ± 4.5%, 56.9 ± 3.8% or 62.0 ± 3.4% when 25, 50 or 100 μM tyrphostin ($n = 4$). However, protein tyrosine phosphorylation did not change. Although tyrphostin acts as a stress signal activating the MAP kinase pathway, it is not clear if the described effects are the result of inhibiting EGFR or another upstream tyrosine kinase.

P83

Effects of PGF_{2A} administration at the onset or the end of a short-term progestagen treatment in serrana goats

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In order to assess the reproductive effects of Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) administration at the onset or the end of a progestagen treatment in Portuguese Serrana goats, an initial group of 44 females (five were later rejected for several reasons) aged between 3 and 7 years was used. In May (beginning of the breeding season), all goats were treated with an intravaginal sponge impregnated with 20 mg of fluorogestone acetate (FGA) for 5 days and injected (i.m.) with 300 UI of eCG at the time of sponge removal. Half goats received an injection (i.m.) of 100 μg of cloprostenol at sponge insertion (SI) and the other half at sponge removal time (SR). Blood samples were taken for progesterone determination and four intact bucks with harness marker were used to identify oestrus. Transrectal ultrasound scanning was performed for pregnancy diagnosis 41 days after eCG administration. $PGF_{2\alpha}$ injection at the onset of the FGA treatment had a positive effect in oestrus (SI – 100.0% vs. SR – 90.9%; $r^2 = 9.424$; $p < 0.01$), ovulation (SI – 100.0% vs. SR – 95.5%; $r^2 = 4.082$; $p < 0.01$), pregnancy (SI – 100.0% vs. SR – 90.9%; $r^2 = 9.424$; $p < 0.01$) and fertility (SI – 100.0% vs. SR – 72.7%; $r^2 = 31.214$; $p < 0.001$) rates. Time of $PGF_{2\alpha}$ injection had no significant effect in prolificacy (SI – 2.1 ± 0.8 vs. SR – 2.2 ± 0.8; $p > 0.05$). In conclusion, data indicate that $PGF_{2\alpha}$ should be administrated at the onset of the FGA treatment.

P84

Efficacy of tuohy needle in oocytes collection from excised mare ovaries

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Oocyte aspiration from equine follicles gives a low recovery rate and yields oocytes largely denuded of cumulus cells. Follicle scraping is labour intensive and increases the time required for collection, extending the holding time of oocytes that delays their maturation. The aim of this work was to develop an effective method for collecting equine oocytes combining the feature of aspiration (fastness) with that of scraping (high recovery rate of cumulus-intact oocytes). Furthermore, we examined differences in cumulus morphology and maturation rates, comparing this technique to aspiration and scraping, with or without tunica albuginea removal. Collection by vacuum pump aspiration was performed using a 16 g needle while the combination of aspiration and scraping was performed using a Tuohy needle (16 g) that is usually employed for inserting an epidural catheter and its tip shape is similar to a small curette. In unpeeled ovaries, the recovery rates by the Tuohy needle was higher ($p < 0.05$) than in the 16 g needle aspiration and in the scraped ovaries (57% vs. 36% and 47%) while the rate of cumulus-intact oocytes was higher than aspiration (46.9% vs. 39.36%) but lower than scraping (46.97%) ($p < 0.001$). In unpeeled ovaries there was no difference in maturation rate of oocytes recovered by Tuohy needle in respect to scraping in peeled ovaries (58.54% vs. 58.24% respectively; $p < 0.05$). In conclusion, combination of aspiration and scraping by Tuohy needle allows a faster and reliable collection of oocytes suitable for horse IVM.

P85

Sperm α-D-mannosidase and α-L-fucosidase effect on porcine IVF

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Sperm-egg interaction leading to fertilization is a species-specific carbohydrate mediated event. The aim of this study was to analyze sperm α-D-mannosidase and sperm α-L-fucosidase effect on porcine IVF. Fresh boar spermatozoa were capacitated through Percoll gradient. Porcine oocytes were *in vitro* matured using NCSU-37. Capacitated sperm and matured oocytes were coincubated 3 h in TALP with Swainsonine (SWA α-D-mannosidase specific inhibitor) and Deoxyfuconojirimycin hydrochloride (DFM a specific inhibitor of α-L-fucosidase). Oocytes were washed, transferred in fresh TALP and kept for 18 h. After incubation time, the putative zygotes were fixed and Hoechst stained. The data were analysed by ANOVA ($p < 0.05$). Sperm ZP-binding, penetration and monospermy rates were evaluated in control, SWA, DFM and SWA + DFM groups (n = 137, 148, 135 and 123). Results showed that sperm ZP-binding was significantly lower in SWA, DFM and SWA + DFM groups (7.24 ± 0.25^{a}, 9.82 ± 0.37^{b}, 7.43 ± 0.27^{a}) than control group (14.44 ± 0.40^{c}); penetration rate was lower in SWA group (58.11 ± 4.1^{a}) than DFM (71.11 ± 3.9^{b}), SWA + DFM (76.42 ± 3.8^{b}) and control (73.99 ± 3.8^{b}) groups. Monospermy rate improved in DFM (66.67 ± 4.8^{a}) and SWA + DFM (67.02 ± 4.8^{a}) compared with SWA (52.33 ± 5.4^{ab}) and control (43.00 ± 4.9^{b}). In conclusion, these results provided direct evidence indicating an important role of sperm α-L-Fucosidase during porcine IVF. Granted by MEC-FEDER AGL2006-03495, AGL2009-12512-C02-01, Séneca 0452/GERM/06 and Vitrogen P.

P86

Effect of the presence of glycosidase inhibitors on porcine embryo development *in vitro*

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In preliminary experiments, we demonstrated that presence of glycosidase inhibitors on porcine IVF, improved monospermy rates (unpublished data). The aim of the study was to analyze the effect of sperm α-D-mannosidase and sperm α-L-fucosidase inhibition on porcine embryo development. Fresh boar spermatozoa were capacitated through Percoll. Porcine oocytes were *in vitro* matured using NCSU-37. Capacitated sperm and matured oocytes were coincubated for 3 h in TALP with 500 μM Swainsonine (SWA α-D-mannosidase specific inhibitor) and 500 μM Deoxyfuconojirimycin hydrochloride (DFM α-L-fucosidase specific inhibitor). Oocytes were transferred and kept for 18 h in fresh TALP. The zygotes were cultured in NCSU-23 for 7 days. After culture, they were fixed and Hoechst stained. The data were analysed by ANOVA ($p < 0.05$). Cleavage rate (48 h), blastocyst formation rate and cell numbers/blastocyst were evaluated in control, SWA, DFM and SWA + DFM groups (n = 147, 177, 201, 206) Results did not show any statistical differences in cleavage rate (control: 55.78 ± 4.1, SWA: 64.97 ± 3.5, DFM: 59.70 ± 3.4, SWA + DFM: 60.68 ± 3.4), blastocyst rate (11.11 ± 3.5, 13.91 ± 3.2, 10.0 ± 2.7, 13.60 ± 3.0) and cell numbers/blastocyst (28.66 ± 1.9, 29.50 ± 1.0, 29.83 ± 1.5, 28.70 ± 1.4), respectively. In conclusion, the presence of glycosidase inhibitors does not affect the porcine *in vitro* embryo development. Granted by MEC-FEDER AGL2006-03495, AGL2009-12512-C02-01, Séneca 0452/GERM/06 and Vitrogen P.

P87

Post-partum cervicitis in dairy cows: incidence and impact on fertility

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On contrast with endometritis now evidenced by cytological examination, impact of endocervical inflammation on reproductive performance has been poorly investigated. Transrectal palpation, vaginal mucus examination and endocervical smear using a cytobrush were performed, in that order, on 196 Holstein cows, between 21 and 60 days in milk (DIM), in three herds. Parity, calving history, body condition score and peri-partum disorders, if any, were recorded. Neutrophils (NC) represented 89% of the total leukocyte count in endocervical smears. The proportion of NC was higher when free or intra-cellular bacteria were found in the samples (0.5% vs. 4.5%, $p < 0.01$). For cows between 21 and 34 days postpartum at examination (n = 103), based on Akaike's information criterion, the cut-off point for impaired fertility was set at 5% NC. Indeed, the pregnancy rate at 150 DIM was 51% for cows with < 5% NC and only 25% for cows with ≥5% in their smears (*Plogrank* < 0.01). For cows 35 DIM or more at examination (n = 93), the cut-off point for impaired fertility was set at 4% NC. The pregnancy rate at 150 DIM was 56% in cows with < 4% NC and only 33% in cows with 4% or more NC in their smears (*Plogrank* < 0.01). Among the cows with positive endocervical smear before 35 DIM (n = 40) and after 35 DIM (n = 21), 7 (11%) presented an enlarged cervix (≥7.5 cm), 18 (29%) presented pus in the vagina and 14 (23%) presented both. This study demonstrates the impact of endocervical inflammation on further reproductive performance in the cow.

P88

Intrauterine insemination in rabbits

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In the present study the effectiveness of a human intrauterine insemination catheter in rabbits was investigated. Seventy one female New Zealand White Rabbits were used. Bucks were mated with different does in group 1 (n = 17), shortly after the first mating, bucks were mated again with different does (group 2, n = 14). In third and fourth groups does were inseminated with human intrauterine insemination catheter with the first (group 3, n = 10) and second ejaculates (group 4, n = 10) which were collected via artificial vagina respectively. In the fifth (n = 10) and sixth (n = 10) groups, the first and second ejaculates were deposited in the vagina of does respectively. The pregnancy rates and litter size parameters were compared by chi square test and sex ratio results were analyzed with the Kruskal-Wallis test by SPSS 13.0 program which works under windows. Pregnancy rates, litter sizes and sex ratios (male ratio (%)) were 88.2, 64.3, 70.0, 70.0, 50.0, 40.0 ($p > 0{,}05$), 7.4 ± 0.53, 6.7 ± 0.58, 6.9 ± 1.44, 7.6 ± 1.29, 7.2 ± 0.86, 6.0 ± 1.48 ($p > 0{,}05$) and 52.3, 50.0, 37.5, 49.1, 44.4, 41.7 ($p > 0{,}05$) in group 1, 2, 3, 4, 5 and 6, respectively. In conclusion, insemination using a human intrauterine insemination catheter achieved acceptable fertility results via transcervical inseminations in rabbits. Nonetheless we believe that it could be possible to obtain statistically significant results by the further studies using more animals.

P89

Intrauterine treatment of clinical endometritis in cows using hydrogen peroxide – preliminary results

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In the trial we used 3% hydrogen peroxide as an intrauterine treatment of clinical endometritis in cows. First we examined 12 involuted uteri from slaughtered cows after application of 50, 80, and 100 ml of the solution and we determined 80 ml to be the maximum volume for intrauterine administration. Subsequently we performed intrauterine treatment of clinical endometritis using 80 ml of 3% hydrogen peroxide in 23 cows 22–28 days post partum. The cows were either treated for the first time (E1, n = 13) or had been treated previously for retained placenta or puerperal metritis (E2, n = 10). Cows without symptoms of the disease represented the control group (C, n = 18). Clinical (vaginal and rectal) and bacteriological (eight cows in E1 and E2 and four cows in C) examinations of uterus were performed before treatment and 7 days later. In addition first service pregnancy rate, services per conception, calving to first service interval, and pregnancy by day 100 and 150 pp were compared among the groups. Clinical symptoms of the disease disappeared in 85% and 70% cows ($p < 0.05$) 7 days after treatment in groups E1 and E2, respectively. Similarly, general infection as well as infection with A. pyogenes in treated cows decreased by approximately 50% but differences were not significant. Reproductive parameters in groups E1, E2 and C were not different. The results suggest that 3% hydrogen peroxide could be used for clinical endometritis in cows. Supported by the grant MSM Czech Rep. No. 6215712403.

P90

Effect of chitosan on quality and fertility of frozen bull semen

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It is known that some high molecular weight compounds have a protective effect against freeze-thaw damage to sperm. The goal of this study was to evaluate the protective effect of water-soluble derivate of chitosan (N-deacetylated chitin) during freezing of bull semen. In experiment 1 the ejaculates (n = 16) of Holstein bull (n = 4) were split and diluted 1:9 with Tris- extender contained different concentrations (0.12–3.0 mg/ml) of chitosan (CH) or without CH (control). Samples were packed in 0.5 ml French straws at 37°C, cooled to 5°C over 2 h, and frozen in vapour of liquid nitrogen. After thawing in a water bath (37°C, 30 s), samples were evaluated for progressive sperm motility (CASA), survival time at 37°C and acrosome morphology. In experiment 2 the effect of CH at 1% concentration on the results of insemination (80) of cows with frozen-thawed semen (10 million motile sperm/dose) was studied. Statistical analysis was performed using SAS system for Windows. The progressive sperm motility immediately after thawing in groups frozen with 0.12; 0.25; 0.5; 1.0; 20 and 3.0 mg/ml CH were 41; 41; 42; 48; 44 and 44%, respectively, vs. 43% in control. The survival time of thawed sperm at 37°C in the group with 1% of CH was significantly increased by 18%, vs. control ($p < 0.01$). Acrosome integrity was similar for all groups after thawing and 3 h incubation at 37°C. There was no statistical significance difference in conception rate of cows inseminated using semen frozen in 1% CH (45%), vs. controls (46.6%). These data suggest that CH at 1% concentration has moderate cryoprotective action on the bull sperm, but did not improve their fertility.

P91

Attractiveness of vaginal flora to stud dogs (*Canis familiaris*) during oestrus in bitches

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The objective of the study was to assess the effect of presence of vaginal bacterial flora on mating behaviour in dogs. A total of 20, 2–5 years old German Shepherd bitches, and one experienced 5-year old German Shepherd stud dog were used. Ten healthy bitches and 10 bitches treated during oestrus with antibiotics administered intravaginally were used. Bitches were exposed to the male when progesterone concentration reached 6–10 ng/ml. The interaction of the male dog when placed individually with each of the females from the two groups was recorded according to following criteria: Reaction type I if the male was immediately interested in the bitch and after a short contact mounted the female within 3 min. Reaction type II if the male showed a little interest in the bitch, smelled the vulva, but there was no mating within 3 min. Sexual attractiveness was analysed by Mann-Whitney U test. Females not treated with antibiotics were significantly more attractive than females treated with antibiotics ($p < 0.05$). The reaction of the male when in contact with eight females of group A was classified as reaction type I, while only two type I reactions were detected in group B. The results of experiments showed that the reduction of vaginal flora had a significant impact on bitch attractiveness for a male.

P92

Protective effect of monomethyl ester of glycerol under deep freezing of bull semen

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Several compounds have been examined for their protective effect during deep-freezing of bull spermatozoa, but none has proved better than glycerol. The goal of this study was to evaluate protective effects of monomethyl ester of glycerol (MME) under deep-freezing of bull spermatozoa. The ejaculates (n = 18) of Holstein bulls (n = 3) after assessment of semen quality were split and diluted 1:9 with egg yolk (20%) – lactose (11.5%) extender contained the different concentration of MME (1–6%) or 6% of glycerol (control). A one-step dilution at 30°C was performed. Diluted semen was placed into 0.25 ml straws and equilibrated pre-freezing for 2 h, and frozen in nitrogen vapour at −120°C. The straws were thawed in a water bath (37°C, 30 s). Post-thaw progressive sperm motility, survival time at 37°C and acrosome morphology were evaluated. Statistical analysis was performed using SAS system for Windows. The progressive sperm motility immediately after thawing in groups frozen with 1%; 2%; 3%; 4%; 5% and 6% of MME were 46%; 45%; 45%; 41%; 41% and 40%, respectively, vs. 44% in control. The survival time of thawed spermatozoa at 37°C in groups with 1% and 2% of MME were significantly increased by 25% and 21%, respectively, vs. control ($p < 0.01$). MME at the concentration 5% and 6% significantly increased the sperm acrosome abnormalities (12 and 14%) and decreased their survival time (27% and 32%) in comparison with control ($p < 0.05$). It is concluded that monomethyl ester of glycerol is beneficial at low concentration (1%) for deep-freezing of bull sperm.

P93

First results of an ultrasonographic evaluation and characterization of mammary neoplasia in bitches using b-mode and color Doppler mode

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The use of ultrasonography as tool to evaluate mammary tumors in bitches is limited and has low sensitivity. To our knowledge no studies have so far demonstrated a correlation of ultrasound images and characterization of malignancy in dogs. Therefore as in humans, color Doppler ultrasound could be a useful tool to detect neovascularization. Therefore, the aim of our study is to diagnose with ultrasonography mammary cancer in female dogs by means of B-mode and color Doppler mode, which will be supplemented with physical examination, histopathology, measurement of plasma progesterone, estrogen and prolactin levels and estimation of growth factor (VEGF) in the affected tissue. First results: Five animals were evaluated so far. It was found that turbulent masses presenting neovascularization and increased vascular indices in Doppler are coincident with the histopathological findings of malignancy and with invasive characteristics (e.g. carcinoma complex solid invasion – turbulent vascularization – speed 41.2 cm/s in the vascular supply of neoplasia). From this first results it can be concluded that the evaluation with Doppler ultrasonography might be sensitive enough to diagnose and charactarize mammary neoplasms in bitches.

P94

Doppler ultrasound evaluation of a canine fetal umbilicus – a case report

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Doppler ultrasound of umbilical and placental vascularization in human medicine, are of great importance in the evaluation and detection of pathological processes in the region of the placenta and umbilical cord abnormalities for mother and foetus. The main measures used are the index of vascular resistance (IR) and peak systolic velocity (PI). In veterinary medicine there have been no such vascular studies in pregnant bitches. The purpose of this case report was to compare the values obtained by Doppler of the vascular region of placentation and the umbilical cord of a canine fetus with normal values in human medicine. A Yorkshire terrier bitch was evaluated by ultrasonography using a SONOSCAPE scanner with a 7.5 MHz transducer. Through the conventional examination, the fetuses exhibited normal development and the gestational age was estimated 43 days. In colour Doppler, we evaluated the umbilical and placental circulation. Measurements obtained were heart rate (169/min), IR (0.75HZ) and PI (1.29HZ). By comparing with normal values in human medicine (IR 0.67–0.87HZ and PI 1.09–1.79HZ) with the data acquired from the examination of the canine fetuses, they appeared to be within normal limits. Further studies on vascular doppler ultrasonography of canine fetuses should be made, since this approach to monitor pregnancy has potential application in veterinary medicine.

P95

Reproductive performance of Norwegian red heifers

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In cattle, limited data are available regarding the pattern of ovulations between ovaries during subsequent oestrous periods. The objective of the study was to record which ovary had ovulation during a number of subsequent heats. The ovaries were palpated per rectum during two to six cycles. Sixty-three heifers were followed in a total number of 222 heats. In 117 (52.7%) of them the heifers ovulated in the right ovary (RO), in 103 (46.4%) in the left ovary (LO) and in two heats (0.9%) it was one ovulation in each ovary. After an ovulation in LO 37.8% of the heifers had a new ovulation in LO while 62.2% ovulated in RO. The distribution was not significantly different. After an ovulation in RO, 54.5% of the heifers ovulated in LO and 45.5% in RO. For 151 following heats there were 35 (23.2%) ovulations in RO and 28 (18.5%) ovulations in LO both times, while ovulations alternated from RO to LO in 42 (27.8%) heats and from LO to RO in 46 (30.5%) heats. Four heifers ovulated in the same ovary for more than two subsequent heats, three of them had three ovulations in the same ovary, two of them in LO, and one in RO. These heifers were recorded for only these three ovulations. One heifer had ovulations in the same ovary for four following heats, in her left ovary. The fifth ovulation was in her right ovary. Six weeks after insemination 58 heifers were diagnosed as pregnant using rectal palpation. Among them 35 (60.3%) had a corpus luteum graviditatis (CL_{grav}) in right ovary, and 22 (37.9%) had a CL_{grav} in left ovary, all having their embryo in the ipsilaterale uterine horn. There was one heifer (1.7%) with a CL_{grav} in each ovary, but only one embryo.

P96

Canine prostatic diseases: a retrospective a study on 481 cases

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Epidemiological data about the incidence of prostatic diseases in the dog is limited and sometimes contradictory (1, 2). Our aim was to investigate the incidence of prostatic diseases and to enlighten their epidemiological features. All clinical cases in our database from December 2002 to September 2009 were recorded. Benign prostatic hyperplasia (BPH), prostatitis, chronic prostatitis, cysts, prostatic abscesses, prostatic neoplasia and squamous metaplasia of the prostate were considered and their clinical and epidemiological features were determined. Incidence of dogs suffering from prostatic disease was evaluated at 0.7% (481/72300 male dogs). Mean age was 8.6 ± 3.2 years. BPH was suspected in 45.9% cases (192/424) and prostatitis in 35.9% (150/424). When confirmed diagnosis was obtained, BPH represented 48.2% (95/192) and prostatitis 16.2% (32/192). 33.3% of dogs suffering from prostatic neoplasia were neutered ($p < 0.05$). Fifty-eight dogs suffering from prostatic disease did not exhibit any clinical signs. These asymptomatic animals were 6 ± 2.5 years, whereas symptomatic ones were 9.1 ± 3.2 years ($p < 0.05$). These results tend to show that prostatic diseases may not be uncommon, but many dogs become symptomatic nearly 3 years after the occurrence of the disease. Therefore, preventive screening of male dogs may be beneficial after the age of 6 years.

P97

Bovine oocytes exposed to bovine *herpesvirus* type 5 inhibit some pathways of apoptosis in *in vitro* – produced embryos

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Apoptosis is an active physiological process which naturally affects *in vitro* -produced bovine embryos and oocytes. The aim of this study was to verify the influence of apoptotic pro-markers (Annexin-V, caspase-2 and -3) and anti-apoptotic (Bcl-2) on bovine embryos. Oocytes were selected and randomly assigned into two groups: I (unexposed; n = 179) and II (BoHV-5-exposed, $10^{-2}TCID_{50}$/ml/1 h; n = 185). Oocyte maturation was in TCM 199 (GIBCO, Grand Island, USA) for 24 h at 39°C and 5% CO_2-air. Following the fertilization period (18 h), presumptive zygotes were cultured in SOF medium. Embryos at day 7 after FIV were fixed on slides and evaluated by TUNEL assay (Invitrogen, São Paulo, Brazil). Indirect

immunofluorescence was used for other apoptosis markers. Data were analyzed using Kruskal-Wallis and Dun tests with $p < 0.05$. The TUNEL assay showed moderate and light immuno-labeling signals for embryos in groups I (60%) and II (80%), respectively. The results for Annexin-V were similar to those obtained for TUNEL assay. However, Bcl-2 was only detected for embryos in group II (70%). Light signals of caspase-2 and -3 expression were observed for embryos in group II (60 and 80%, respectively). In conclusion, BoHV-5 suppressed some apoptosis pathways in *in-vitro* derived bovine embryos under these conditions, suggesting that the virus stimulates Bcl-2 protein which, in turn, inhibits the release of cytochrome *c* and, therefore, prevents the activation of some types of caspases. (Supported by FAPESP-07/57774-7)

P98

Influence of serum supplementation on lipid content in *in vitro* matured bovine oocytes and embryos

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The effect of various components of the culture medium on cellular characteristics related to cytoplasmic maturation of oocytes are largely unknown. Oocytes matured in FCS supplemented medium contained more triglyceride than oocytes matured in PVA supplemented medium. A semi quantitative method to evaluate the lipid content (present in the lipid droplets) in single oocytes is the staining with Nile red, a fluorescent dye specific for intracellular lipid droplets. The aim of the study was to evaluate the lipid content in oocytes depending on serum supplementation (ECS or FCS) during IVM and subsequent development. Cumulus-oocyte complexes were matured, fixed and stained with Nile red, or fertilized *in vitro* and cultured. The fluorescence of the whole oocyte was visualized by fluorescence microscopy and quantified with a photometer and photomultiplier connected to the microscope. Based on measurement of the cytoplasmic fluorescence intensity oocytes matured with ECS or FCS had similar fluorescence intensities (in μA/oocyte) [740.7 ± 13.7 (n = 297) or 716.6 ± 15.0 (n = 230)], but were significantly higher than before maturation [498.0 ± 16.8 (n = 150)]. During embryonic development the lipid content increased significantly from day 2 to day 8 using both sera. The highest intensity was measured with ECS on day 8 [799.6 ± 18.3 (n = 115) vs. 760.1 ± 22.2 (n = 89)]. The number of nuclei was significantly increased in blastocysts matured and cultured with ECS [103.0 ± 4.5 (n = 115) vs. 88.6 ± 4.0 (n = 89)]. The results showed that lipid concentration increased during IVM and IVC, and ECS may be preferred over FCS.

P99

Effect of sire for post partum production and reproduction of holstein-friesian cows

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Data of eight sires (at least 200 inseminations – AI – of their progenies) were analyzed in two high producing Hungarian dairy herds (mean milk production 9650 and 10,450 kg per cow per year, respectively). Early pregnancy determination was carried out 30–36 days post AI from blood sera by Biopryn (PSPB) ELISA test and rectal palpation at 60 days post AI served for confirming the late embryonic loss (LEL). Totally data of 2917 AI's (representing 839 cows) were collected, the rate of the pregnant samples (PR), LEL, daily milk production (DMP), number of the post partum uterine treatments (UT), open days (OD) and daily minimum, maximum and average ambient temperatures (T) were recorded. Linear mixed effects model and logistic regression with random factor were applied for analysis of the results. Based on the findings it looks that daughters of two sires have significantly lower ($p < 0.05$) OD. Higher age affected higher OD, while higher milk production (MP) associated with lower number of OD ($p < 0.001$). Logistic regression not surprisingly showed negative effect of T for PR ($p < 0.001$), while no significant effect of sires for PR was found. Higher age of cows associated with higher LEL ($p < 0.001$) and higher number of post partum UT's. Sire effect was confirmed for number of UT as well ($p < 0.001$). Cows with less UT have higher DMP, so indirect sire effect had been confirmed. In summary, sire effect for number of UT, OD and DMP were stated, while – although high differences were detected among sires – no significant effect for PR and LEL was found.

P100

Maternal recognition of pregnancy in the mare: an *in vitro* mode

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In the mare, it is believed that embryo movement throughout the uterine horns before implantation is essential for the establishment of maternal recognition, which mainly depends on a conceptus induced reduction of the luteolytic PGF2α release. However, the mechanisms triggering this response needs to be understood. Therefore, a previously described *in vitro* model was used to mimic embryo presence and movement in the mare's uterus. Thus, the main goal of the study was to investigate if mechanical action alone could influence endometrial prostaglandin E2 and F2 alpha synthases activity (PGES and PGFS). Uterine horns were obtained post mortem from 10 cyclic mares in the early luteal phase. Uterine horns were clamped and filled with culture medium alone (Control group; n = 5), or with a 15 mm diameter, 3.0 g of weight glass ball (GB group; n = 5) and incubated under slow agitation, in culture medium at 37°C for 24 h. PGES and PGFS transcription on endometrium was carried out by Real Time PCR. Both gene transcription was reduced in GB uterine horns ($p < 0.05$). These results suggest that the mechanical effect of embryo might involve a reduction in PGES and PGFS transcription during maternal recognition of pregnancy.

P101

Cystic ovaries and low milk production as risk indicators of decreased fertility in high producing dairy herds

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The present study was conducted to examine possible association between production, reproductive disorders and the fertility of high producing dairy herds by using annual and monthly records. Data were derived from a total of 8649 lactations on four high producing dairy herds over a 4 year study. High monthly conception rate was defined as the percentage of pregnant cows of the total AI per month higher than the mean conception rate of the four herds (29%). Logistic regression analysis indicated no significant effects of herd, lactation number, twinning, days in milk, relation of protein/fat, uterine disorders, anovulatory follicles, season and year on high monthly fertility. Based on the odds ratio, high monthly fertility was 0.14 and 0.55 times less likely for mean daily milk production per month < 30 kg and 30–35 kg, respectively, than for values > 35 kg, used as reference. The likelihood of high monthly fertility decreased for each one unit % increase in the monthly incidence of ovarian cysts by an odds ratio of 0.62. An annual rising trend of milk production was observed besides a reduced incidence of uterine disorders and increased conception and anovulatory follicle rates. In conclusion, our results indicate that monthly summary records can provide useful risk markers of fertility variations. Monthly values of decreased milk production and increased incidence of ovarian cysts were associated with a decreased fertility.

P102

Improvement of sperm quality parameters in the Holstein bulls by dietary addition of n-3 fatty acids

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Polyunsaturated fatty acids of the n-3 family are important for sperm membrane integrity, sperm motility and viability. Altering the PUFA sources in the diet resulted in concomitant changes in the n-6 and n-3 composition of sperm (e.g. boar, cockerel). The aim of the current study was to investigate the effect of feeding a source of n-3 fatty acids on the quality of bull semen. Samples were obtained from 19 Holstein bulls at Semen Production Center, Karaj, Iran. Control group were fed a standard concentrate feed while treatment group had this feed top dressed with 100 g of a commercially available n-3 enriched nutriceutical (Optomega 50, Optivide International Limited, Nottinghamshire, UK). Semen quality was assessed on ejaculates collected after 10 weeks of supplementation. Computer-assisted assessment of sperm motility (Hamilton Thorne Biosciences, Beverly, MA, USA), viability (eosin-nigrosin) and hypo-osmotic swelling test (HOST) were conducted. The treatment significantly increased total motility, progressive motility, HOST, average path velocity and straightness in the fresh semen of bulls ($p < 0.05$). However it didn't significantly affect viability, straight-line velocity and curvilinear velocity, but there was an increasing tendency in the viability and sperm velocities evaluated (VSL and VCL). Therefore, it can be concluded that dietary supplementation of n-3 fatty acids result in improvement of fresh semen quality parameters in Holstein bulls.

P103

Preliminary study for a new thawing semen extender in boar semen

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The aim of the work was to validate a new thawing semen extender, DL12, containing antioxidants and antibiotics.
Nine replicates were made and heterospermic samples from two tested boars were frozen by standard methods with LEY-LEYGO extender. Two frozen samples were thawed at 37°C/21 s. Both were resuspended in a two thawing extender: BTS as control, and DL12. BTS composition was: glucose, EDTA, CO_3HNa, citrate Na, ClK. DL12 composition was: glucose, citrate Na, ClK, EDTA, tris, cystein and antibiotic combination. Each sample was divided into two aliquots, and was incubated at 15°C or 37°C. All of them were tested at 3 and 5 h for motility (SCA®), viability (eosin- nigrosin), acrosome and membrane integrity (HOST). Immediately after thawing, no significant differences appeared between extenders in any parameter. For BTS, there were no significant differences in temperatures and incubation time. For DL12, significant differences ($p < 0.005$) were observed after 5 h incubation with a better motility at 15°C than at 37°C: 57.2 ± 21.9% vs. 73.8 ± 17.7%, respectively. When comparing the extenders, significant differences ($p < 0.05$) were detected only for acrosome integrity at 3 h of incubation and 15°C, increasing for BTS (53.0 ± 12.0%). We can conclude that DL12 is a valid thawing extender for boar semen under different incubation temperatures. Further studies are needed.

P104

Estrus and pregnancy rates in transilvanian merino ewes, treated with two cloprostenol doses at different intervals

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In this study, we aimed to establish the effect of a double treatment of cloprostenol, given at different intervals on estrus and pregnancy rates after the artificial insemination in Transilvanian Merino ewes. Two injections of 100 μg of cloprostenol were given to the ewes in group A, B and C (n = 125 ewes for every group), at an interval of 7, 11 or 14 days, respectively. Ewes in group D (n = 125) served as control and were not treated with cloprostenol, but they received a placebo injection at the same time periods as the treated groups. Ewes from group A, B, and C were teased by rams twice daily during 5 days after the second treatment to detect estrus; ewes from group D were detected teased for a 21-day period. Ewes that exhibited estrus in group A, B, C and D were 86.87%, 88.67%, 100.00% and 80.51%, respectively. Estrous ewes were inseminated using 0.1 ml of fresh semen (225×10^7 spermatozoa/ml with an average motility of 80%). Pregnancy rates were calculated on the basis of lambing records and these parameters in group A, B, C and D were 73.60%, 75.89%, 83.51% and 68.43%. In conclusion we appreciate that the 14 days intervals between injections with cloprostenol provide the best pregnancy rate.

P105

Effect of simulated stress on *in vitro* production of pig embryos

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Stress may affect reproduction, having negative consequences on oocyte competence and early embryo development. We assessed the ability of oocytes to undergo fertilisation and early development after exposure to blood plasma from sows which had experienced simulated stress through repeated injections of ACTH before ovulation (known concentrations of cortisol and reproductive hormones). Pig oocytes were exposed to 10% plasma from ACTH-treated sows (A group), non-treated control sows (C group) or media with BSA (B group) for 46 h during *in vitro* maturation. Afterwards, oocytes were stained to monitor progression to metaphase II (n = 716) or were co-incubated with spermatozoa and cultured *in vitro* to assess development (n = 1275). Exposure to plasma from A group sows did not affect the proportion of oocytes that reached MII compared to controls (mean ± SD; A: 63.4 ± 11.1; B: 64.2 ± 17.6; C: 63.8 ± 6.4%; $p > 0.05$). Cleavage (A: 46.0 ± 8.4; B: 36.3 ± 19.7; C: 47.5 ± 13.0%) and blastocyst rates (A: 21.2 ± 17.3; B: 19.8 ± 18.9 and C: 18 ± 9.5%) did not differ between treatments ($p > 0.05$). Blastocyst quality, considered as the total number of nuclei, was not affected by the treatment (A: 37.9 ± 16.8; B: 29.9 ± 14.9 and C: 37.3 ± 22.1; $p > 0.05$). Oocytes might be able to overcome possible deleterious effects of physiological levels of stress hormones after *in vitro* maturation in media with semi-defined plasma from sows subjected to simulated acute stress before ovulation. The ability to generate offspring from these blastocysts should be explored. (Funded by Formas)

P106

Conception rate after timed artificial insemination protocols using by $PGF_{2\alpha}$ and GnRH in dairy heifers

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This study aimed to test two different TAI protocols in dairy heifers using prostaglandin $F_{2\alpha}$ (PGF) and GnRH. The Heifers (n = 290) used in this study were aged between 13 and 26 month. Heifers were synchronized with two PGF administrations in a 14 days intervals. After the second PGF, heifers were randomly assigned into two groups. In Group 1 (n = 156), GnRH was given at 56 h following 2nd PGF and TAI was performed at 16–18 h after GnRH. In Group 2 (n = 134), GnRH was injected at 72 h after 2nd PGF at the time of TAI. Ultrasonography was performed at the times of PGF administration to determine cyclicity, at the time of TAI to measure ovulatory

follicle size, seven days after TAI to determine ovulation, 31 and 62 days post-AI to determine pregnancy. Maximum follicle size at the time of TAI did not differ between groups (12.8 ± 1.6 mm in Group 1 vs. 13.6 ± 1.8 mm in Group 2). Synchronization rate (85.9%; 134/156 and 91.0%; 122/134) and conception rate (CR) (50.6%; 79/156 and 48.5%; 65/134) were not different between Group 1 and 2; respectively. There were no differences, when CR and maximum follicle size were evaluated according to cold and hot seasons. In conclusion, as CR and synchronization rate did not differ between groups, GnRH administration at the time of TAI was found to be more useful to reduce handling of heifers in large dairy farms.

P107

Effects of clinical mastitis on reproductive performance in Holstein cows

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The objective of this study was to determine the effects of clinical mastitis on reproductive performance in 135 early lactation cows. The animals were divided into two groups according to the occurrence of mastitis as follows: group I ($n = 45$), clinical mastitis prior to the first artificial insemination breeding; group II ($n = 45$), clinical mastitis after artificial insemination and being diagnosed pregnant. Forty-five cows without any mastitis served as control group. Calving to first service intervals were significantly longer ($p < 0.05$) in cows with clinical mastitis before first service (group I; 95.2 ± 5.4 days) than in cows with clinical mastitis after first service (group II; 77.4 ± 8.2 days) and in control cows (75.9 ± 6.3 days). Calving to conception interval with clinical mastitis before first service (119.1 ± 10.6 days) and in cows with clinical mastitis after first service (141.7 ± 14.0 days) was significantly longer ($p < 0.05$) than in control (94.1 ± 10.3 days) cows. On the other hand, the number of services per conception was larger ($p < 0.05$) in cows with clinical mastitis after first service (3.4 ± 0.9) than in cows with clinical mastitis before first service (2.1 ± 0.9) and in cows with no clinical mastitis (1.8 ± 0.8). This study indicated that clinical mastitis during early lactation in Holstein cows had a negative impact on their reproductive performance.

P108

Macromorphological irregularity of the mammary gland in the bitch

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The aim of this study was to evaluate and determine the degree of irregularity in the macromorphology of the bitch mammary gland. In the bitch the mammary glands are typically arranged in two bilaterally symmetrical rows extending from the ventral thoracic region to the inguinal region. The number of mammary gland complexes in the bitch is typically ten, but may vary between eight and twelve. This study was carried out on 416 pure bred and 34 mongrel bitches. The bitches were divided into groups comprising small (up to 9 kg), medium (10–25 kg), large (26–40 kg) and giant (>40 kg) breeds. Accessory teats were found in 4.4% of individuals. In the majority (98.8%) accessory teats were positioned caudolaterally to the inguinal complexes with predominance of occurrence being in the Labrador Retriever, Belgian Shepherd Dog, German Shepherd Dog and Alaskan Malamute breeds. Mammary gland complexes were absent in 25.8% of individuals. The mean numbers of missing complexes within each breed group were 1.2, 1.5, 2.1 and 1.7 for small, medium, large and giant breeds, respectively. The most frequently absent complexes within these individuals were the first thoracic (95.0%).

P109

The testing and evaluation of ram semen preservation methods

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The aim of the study was to test commercial extenders used for short-term and long-term sperm preservation. Semen was collected in the reproduction season from June to December. The ejaculates were obtained from single services and the analysis of the semen was performed immediately after the collection. The examination included semen volume, colour and texture, sperm concentration and motility, ejaculate turbulence and percentage of sperm with abnormal morphology. The semen was diluted with an extender in the ratio of 1:4. Ejaculates diluted with Ovipro, Optidyl, Triladyl and Andromed CSS gave very good results of viability (81.23–83.41%) after 24 h of storage. After 48 h, Ovipro, Andromed, Optidyl and Triladyl gave values above 75. After 72 h, high viability values were obtained with Triladyl, Andromed, Andromed CSS. For Andromed and Andromed CSS, sperm viability exceeded 40%. Statistically significant differences were found between Triladyl-Ovipro and Triladyl-Biladyl ($p < 0.01$), and between Triladyl and Biociphos ($p < 0.05$). The Triladyl extender proved to be a good stabilizing agent, showing consistent results in a long-term preservation. Other preservation media did not show any improving or worsening effects. The extender Ovipro showed a high viability effect in the first 48 h only, and hence it appears to be the best solution for the short-term preservation. Supported by project MEYS CR MSM 2678846201, LA 09031, NAZV QH81324.

P110

Immunohistochemical detection of glucocorticoid receptors and 11β-hydroxysteroid dehydrogenase type 1 in equine testicular and epididymal tissue

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In males stress may interfere with reproductive functions by direct action of glucocorticoids. However, these effects can be modulated by the two known isoforms of 11β-hydroxysteroid dehydrogenase (11β-HSD)-1 and -2 which convert active cortisol into inactive cortisone and vice-versa. The aim of the present study was to determine the presence of glucocorticoid receptors (GCR) and 11β-HSD-1 in equine testicular and epididymal tissue by immunohistochemistry. Tissue samples (caput, corpus, cauda epididymidis and testes) were collected at the time of routine castration, fixed in Bouin's solution and embedded in paraffin. Immunohistochemistry was performed using primary monoclonal and polyclonal antibodies raised specifically against GCR (AB2768, Abcam®, dilution 1:50) and 11β-HSD-1 (AB39364, Abcam®, dilution 1:100) respectively. Antigen retrieval was realized with Tris/EDTA at pH 9.0 and a biotinylated anti-goat antibody was used as second antibody. A positive intranuclear immunoreaction for GCR was seen in Leydig cells and epithelial cells of caput, corpus and cauda epididymidis. 11β-HSD-1 was localized in Leydig cells and in the cytoplasm of epithelial cells of the caput epididymidis, only. No positive staining for GCR or 11β-HSD-1 could be detected in the tubuli seminiferi. Therefore, in the stallion, glucocorticoids may interfere with testicular steroidogenesis and epididymal function but do not seem to have direct effects on spermatogenesis. Metabolisation of glucocorticoids in these tissues by 11β-HSD-1 is possible.

P111

Reproductive disorders in the swiss eringer "fighting" cow – a retrospective analysis of clinic patients

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In the years 2005–2009, 69 Eringer cows were referred to our clinic due to fertility problems. Mean (min/max) age was 6.6 years (3.1/11.3), with last calving 17 months (2/44) ago. Routine examination consisted of cytological and bacteriological assessment of an uterine swab, histological assessment of an uterine biopsy, and patency testing of the oviducts. n = 11 cows were slaughtered without consecutive insemination because of poor fertility prognosis based on tests performed. Of the remaining 58 cows, microbial culture of uterine swabs was positive in 20 cows, inflammatory cells were seen on smears of 27 cows, uterine fibrosis and/or inflammation of uterine tissue was found in 24 biopsies. Of the 58 animals inseminated at the clinic, 17 were confirmed pregnant, including four cows which subsequently aborted. Of the 41 cows not pregnant when leaving our clinic 5 became pregnant at a later date (between 3 and 80 weeks later). Knockout criteria for future fertility of the cows referred to our clinic were positive culture of *A. pyogenes* from uterine swabs and missing patency of the oviducts (use as embryo recipient still possible). Only one cow (of n = 11) suffering from distinct perivascular and periglandular fibrosis became pregnant again. In the described population, mean days open for all cows calving (n = 18) was 886 (448/1438) days compared with 146 (13/842) days in the total Eringer population. According to our data, only 45% of the cows had a grave (knockout) fertility problem, all other cows had fertility problems associated with management. Overall, the pregnancy rate within the 5 year period was 31%.

P112

Treatment of ovarian cysts and reproductive performance in dairy cows

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In a retrospective study, the effect of routine treatments of ovarian cysts (OC) with special respect to the time of diagnosis and treatment on reproductive performance in dairy cows was evaluated. Data collected over a period of 15 years from 16 dairy herds, which participated in a herd health program, were analysed. OC were defined as follicular structures on one or both ovaries with a diameter of ≥2.5 cm in the absence of a functional corpus luteum. Diagnosis was made via transrectal palpation and ultrasonography. 740 cows with OC were identified of which 329 were treated (n = 155 GnRH; n = 99 $PGF2_{\alpha}$; n = 47 progesterone device; n = 3 ovsynch; n = 25 manual rupture). 411 cows with OC were left untreated. All treated and untreated cows were inseminated and 556 became pregnant. When OC were first diagnosed ≤42 days postpartum (pp), fertility measures were better in treated than in untreated cows: first service conception rate (FCR) 45.2% vs. 42.9%; days open (DO) 116.4 ± 53.7 days vs. 121.5 ± 58.1; interval from first insemination to conception (FIC) 27.6 ± 45.7 days vs. 32.9 ± 46.8 days, pregnancy index (PI): 1.9 ± 1.0 vs. 2.0 ± 1.5 ($p < 0.05$). In contrast, when OC were first diagnosed >42 days pp, reproductive performance was diminished in treated compared with untreated cows: FCR 27.2% vs. 32.7%; DO 176 ± 87.6 days vs. 160 ± 84.6 days; FIC 72.6 ± 78.9 days vs. 58.9 + 76.0 days, calving interval 449 ± 85.0 days vs. 428.4 ± 69.1 days; PI 2.9 ± 1.6 vs. 2.5 ± 1.2 ($p < 0.05$). The results suggest that early diagnosis and treatment of ovarian cysts might be important in order to ensure reasonable reproductive performance in cows with OC.

P113

Isolation of mesenchymal stem cells from fetal fluid, fetal membranes but not endometrium in the domestic cat

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Domestic cats are preferred models for normal physiology and several human diseases. Many researchers used feline bone marrow MSCs isolated antemortem or at necroscopy (Douglas et al., 2002 Experimental hematology, 30: 879–886). We evaluated feline fetal fluids and membranes, and the endometrium as possible sources of stem cells. Samples were recovered from five pregnant queens after ovariohysterectomy. Gestational sacs were separated from uterine wall. According to the time of pregnancy, vitelline fluid (VF), allantoic fluid (ALF) and amniotic fluid (AF) were aspirated, diluted 1:1 in PBS and centrifuged for 15 min at $300 \times g$. Pellet was re-suspended in 5 ml of culture medium: DMEM/TCM-199 (1:1) plus FBS 10%, and antibiotics. Choriovitelline membrane (CVM), chorioallantoic membrane (CAM), amniotic membrane (AM) and endometrium (E) were incubated with collagenasis, then washed with culture medium. All cell lineages were seeded into 25 cm^2 flasks and cultured in a 5% CO_2 incubator at 38.5°C. Medium was first refreshed after 48 h, then twice a week. At passage 2, chondrogenic and osteogenic differentiation ability of all cell lineages was evaluated by culturing cell monolayers in differentiating media for 21 days. In all the samples, adherent fibroblastoid spindle-shaped cells were observed. Chondrogenic and osteogenic differentiation were confirmed by positive Alcian Blue and von Kossa stainings, respectively, for all lineages except E. Based on the results obtained, VF, ALF, AF, CVM, CAM and AM, but not E, represent alternative sources of stem cells in the cat.

P114

Influence of immunization against GnRH on semen quality and testicular function in the adult boar

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The objective of this study was to investigate the influence of a GnRH vaccine (Improvac™, Pfizer Animal Health) on gonadal function in the adult boar. The experiment was performed using a total of 12 AI-boars aged between 2 and 3 years with good semen quality. Two animals served as control. Ten animals were vaccinated twice with 2 ml (400 μg GnRH-protein conjugate) Improvac™ 4 weeks apart and observed for a total of 8 weeks. Ejaculates were collected weekly from all boars and semen quality evaluated (volume, concentration, total sperm, sperm motility, normal sperm). In addition, blood samples were taken for determination of serum testosterone concentration and GnRH-antibody titer. At slaughter, 4 weeks after the booster injection, testes were weighed and slices of testicular tissue fixed for histological examination. Cross-sections of the seminiferous tubules were evaluated to assess the spermatogenic activity and the diameter of the seminiferous tubules. Results show that vaccination individually influenced all parameters examined. Two weeks after the booster injection a clear drop (>30%) in sperm motility and normal sperm was observed in seven and eight vaccinated boars, respectively. Paired testis weight ranged between 443 and 1021 g in vaccinated and between 745 and 1235 g in control animals. The mean diameter of the seminiferous tubules ranged between 173 and 264 μm in vaccinated and between 217 and 221 μm in control boars. From our results it is concluded that adult boars can still be used for breeding until 2 week after the second vaccination with Improvac®.

P115

ROS-Production by frozen-thawed stallion spermatozoa can be used as an indicator of potential fertility in artificial insemination

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Evaluating stallion sperm quality in frozen-thawed semen samples is problematic since artificial insemination (AI) with apparently good quality samples may not result in pregnancies. The objective was to investigate whether reactive oxygen species (ROS)-production can be used as an indicator of fertility in frozen-thawed stallion semen. Straws of frozen semen were available from 10 stallions at a commercial stud (up to 4 per stallion). After thawing in a water bath at 37°C for 30 s, aliquots of semen were analysed for motility (Qualisperm^{TM} Motility Analyser), viability (SYBR14/PI staining and flow cytometric [FC] analysis) and production of ROS (hydroethidine and 2′,7′-dichlorodihydrofluorescein staining and FC analysis), at 0 h, 1 h and 2 h after thawing. There was considerable variation between the stallions: progressive motility 14–65%, viability 15–51%, ROS-negative 17–39%, ROS-positive 10–34%. There were correlations between progressive motility and viability at 0 h ($r = 0.75$, $p < 0.001$), and also between viability and the proportion of viable spermatozoa that were ROS-producing at 0 h ($r = -0.48$; $p < 0.01$). Frozen-thawed semen from one of the high ROS-producing stallions was used for AI in 10 fertile mares: no pregnancies resulted. In conclusion, ROS-production may be useful as an indicator of the potential fertility of cryopreserved stallion spermatozoa, either alone or combined with other tests of sperm quality. Funded by the Swedish Foundation for Equine Research, Stockholm, Sweden.

P116

Effectiveness of two different intrauterine and a conventional PGF2alpha treatments on creatine kinase/aspartate aminotransferase levels and reproductive performance in cows with chronic endometritis

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The aim of the study was to investigate the effect of three different treatments on serum Creatine Kinase (CK)/Aspartate Aminotransferase (AST) levels as screening parameters for treatment period and reproductive performance. Forty five multiparous Holstein-Friesian cows with chronic endometritis, aged between 3 and 5 years, were allocated in three groups. Group 1 (G1; $n = 17$) was treated with an intrauterine infusion of 200 ml 0.2% peroxyacetic acid (Uterofertil®; Hektaş, Istanbul), group 2 (G2; $n = 17$) with 150 ml 4% metacresolsulfonic acid (Lotagen®; Essex Tierarznei, München) and group 3 (G3; $n = 11$) with an intramuscular injection of PGF2 alpha (Enzaprost®; Ceva-Dif, Istanbul). Treatments were done during routine pp examinations while endometritis was diagnosed. Blood samples were collected from jugular vein on day 0 (pre-treatment), 3 and 6 (post-treatment). Pregnancy was diagnosed on day 30 after artificial insemination. The pregnancy rates after first insemination (PR-FI) were statistically different ($p < 0.05$) between G1 (41.2%) and G3 (29.4%). Serum CK levels were lower in G1 than in G3 ($p < 0.05$) on day 3. Besides, serum AST levels were lower in G2 than in G3 ($p < 0.05$) on day 0. There was no difference between day 3 and 6 in G1, G2 and G3 with respect to AST levels. The results of this preliminary study indicate that; treatment with an intrauterine infusion of peroxyacetic acid can improve reproductive performance without affecting CK and AST levels in cows with chronic endometritis.

P117

Improvement of the boar sperm quality by including of spirulina platensis in the diet

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The microalgae *Spirulina platensis* is an interesting food supplement mainly due to its rich content of compounds important to vitality: protein (60–70%), vitamins, β-carotene, minerals and phytochemicals. The effect of the full biomass of Spirulina on quantitative and qualitative characteristics of boars' sperm was studied. The experiment was conducted with six sexual mature boars from the Danube white breed. During the control period the animals received a basal diet. In the experimental period (40 days) 1.4 mg/per head *Spirulina platensis* was added. The sperm were collected by artificial vagina once per week from each boar. The sperm volume, concentration, motility and thermal resistance of spermatozoa to 39°C and to 15°C were investigated. The total dehydrogenase (DH) activity of spermatozoids was measured using the methylene-blue reduction method and lactate dehydrogenase (LDH) in the water and triton extracts were estimated by spectrophotometry. The evaluation of the ability of spermatozoa to produce reactive oxygen species (ROS) was performed using the nitroblue tetrazolium (NBT) staining test. Data were developed by the computer program Statistica (Stat Soft Inc., Ver.6.0). An enhancement ($p < 0.05$) in semen volume (up to 11%), sperm motility (up to 5%) and activity of DH and LDH in the post treatment period was established. Also the thermal resistance of the spermatozoa to 39°C and to 15°C was higher. The NTB test pointed out a lower ROS production in spermatozoa from post treatment period. Enriching of the main diet with Spirulina significantly improves the boar sperm quality.

P118

Isolation and characterization of type-a spermatogonia in buffalo (*Bubalus bubalis*)

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Spermatogonial stem cells represent a small percentage of cells in the testes of animals. Presently, there is no specific (morphological, biochemical or antigenic) characteristics to identify the buffalo spermatogonial stem cells. Thus, our study was carried out to develop a method to purify and enrich the pure populations of buffalo type-A spermatogonia. For this, spermatogonial cells were isolated from testes of 3–7 month old buffalo calves. Testes cells were disaggregated by double enzymatic digestion. Mixed population of cells were then plated on Datura stramonium agglutinin coated dishes for attachment of somatic and sertoli cells and desired cells were obtained from suspension. Spermatogenic cells were then loaded on discontinuous density gradient using percoll (20–65%) for obtaining different types of spermatogonia at interface of each layer and cultured on sertoli cell feeder layer. The expression of pluripotency genes were studied by either immunoflorescence or RT-PCR. The isolated spermatogonial cells have spherical outline and two or three ecentrically placed nucleoli, created a colony after proliferation during first week or immediately after passage. The resulted colonies showed expression of the spermatogonial specific genes like Plzf and VASA; and pluripotency related markers viz. alkaline phosphtase, CD9, CD90, SSEA-4, OCT-4, NANOG and REX-1. This study revealed that Type-A spermatogonia are pluripotent in nature. This work was supported by NAIP, New Delhi.

P119

Inhibition of polo kinase 1 (Plk1) affects resumption of meiosis in porcine oocytes

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Previous data of our laboratory revealed that activation of Plk1 preceded the increase of maturation promoting factor (MPF) activity in porcine oocytes matured *in vitro* (Anger, 2004, Mol Reprod Dev, 69, 11–16). The aim of this study was to investigate the implication of Plk1 in resumption of meiosis during *in vitro* maturation of porcine oocytes. When oocytes were treated with 1.5 μM of the Plk1 inhibitor BI2536, a substantial suppression of meiotic resumption occurred as only 11% of treated oocytes underwent germinal vesicle breakdown (GVBD) during 24 h of treatment. However, this inhibition was fully reversible as 92% of oocytes passed GVBD and reached mostly metaphase II (MII)-stage when treatment with BI2536 was followed by culture in control conditions. If the oocytes were pre-cultured for 8 h in control and subjected to Plk1-inhibitor the incidence of GVBD subsequently increased up to 62%. It suggests that the role of Plk1 in meiosis resumption decreased when chromatin condensation and other events leading to GVBD were already initiated.

P120

Periparturient profile of ceruloplasmin in plasma, colostrum and milk of cows

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In dairy cows, the periparturient period is characterized by marked hormonal, metabolical and immunological changes leading to the maturation of the fetus, termination of pregnancy, parturition, shedding of the placenta and initiation of lactation. During this time, immunological and energetic disturbances as well as imbalances of the antioxidative/oxidative status may occur leading to an increased risk of various diseases. In this study, the periparturient profile of ceruloplasmin activity as antioxidative and immunological marker was determined in blood plasma, colostrum and milk. Blood samples were collected from 22 healthy cows from 4 weeks antepartum (ap) until 5 weeks postpartum (pp) (nine samples). Colostrum was collected at parturition (first milking) and milk at 1, 3 and 5 weeks pp. Activity of ceruloplasmin was determined spectrophotometrically at 540 nm. In blood plasma, the activity of ceruloplasmin increased from 4 weeks ap (0.113 ± 0.010 U/g protein) to maximum activities of 0.216 ± 0.016 U/g protein at 1 week ap and then gradually decreased to 0.155 ± 0.013 U/g protein at 5 weeks pp ($p < 0.05$). The activity in colostrum (0.82 ± 0.03) increased to 1.0 ± 0.05 in milk at 1 week pp and then decreased to 0.80 ± 0.04 and 0.46 ± 0.03 at 3 and 5 weeks pp, respectively ($p < 0.05$). The results reveal dynamic changes in ceruloplasmin activities which might be a response to oxidative stress around parturition.

P121

Comparing conception rate of the repeat breeder vs. normal dairy cows following a combined treatment protocol during the warm season

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In this study, we aimed to compare conception rate after a combined protocol using progesterone based timed artificial insemination (TAI) and GnRH post AI in repeat breeders (RB; cows had more than three services with no clinically abnormalities of the reproductive tract and estrous cycle) vs. in normal cows (NC; cows had less than three services with no clinically abnormalities of the reproductive tract and estrous cycle) during warm season. Cows ($n = 123$) received the same protocol in RB ($n = 57$) and NC ($n = 66$) groups. All cows, received an ear implant containing progesterone and GnRH administration at day 0. At day seven an ear implant removed and $PGF_{2\alpha}$ injected. Second GnRH applied 56 h after $PGF_{2\alpha}$ and artificially inseminated 16–18 h after 2nd. GnRH. All inseminated cows received GnRH 7 days after TAI. All statistical procedures were performed using the computational software of SAS. Although response to first GnRH was higher ($p < 0.02$) in RB (39/57; 68.4%) than NC (32/66; 48.4%), synchronization rate was similar in RB (54/57; 94.7%) and NC (65/66; 98.4%) cows. Conception rate was not significantly different between groups; 38.6% (22/57) in RB and 46.9% (31/66) in NC. Response to post-AI GnRH tended to be lower ($p < 0.09$) in RB (42/55; 34.7%) than NC (58/66; 47.9%). Thus, the combined protocol was found effective in repeat breeder cows with similar conception rates when comparing normal cows in warm season.

P122

Equipure™ improves fertility of aged sub-fertile stallions after artificial insemination (ai) with cooled semen

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The aim of this experiment was to evaluate the effect of sperm selection by colloidal density gradient (silane-coated silica particles; EquiPure™, Nidacon) centrifugation on quality traits and fertility of stored semen after AI of single ovulating horse mares in cloprostenol-synchronized estrus. Gel-free ejaculates ($n = 32$) were obtained by a CSU- model AV from four Arabian stallions (18–22 year old) with a previous seasonal pregnancy rate < 60%. Semen was diluted 1:1 with a ready-to-use extender (INRA 96, IMV Technologies) and split into two portions. The 1st portion was centrifuged for removal of seminal plasma and the 2nd portion was centrifuged through discontinuous gradients of EquiPure™. Spermatozoa were re-suspended in semen extender at 50×10^6 sperm/ml, cooled-stored (6 h at 15°C) under aerobic conditions, evaluated for their quality traits and used for AI. Mares that developed follicles > 35 mm in diameter were treated with an IV dose of hCG (2500 IU, Chorulon®, Intervet) and inseminated with 10 ml of semen within 24–48 h before ovulation. Data were examined by ANOVA and chi-squared test. Stallion and semen treatment were the main sources of variation for all experimental endpoints. EquiPure™ increased the percentages of progressive motile sperm with intact plasma membrane and normal morphology ($p < 0.01$). Per-cycle 60-day pregnancy rates of untreated and EquiPure™ -treated semen were 35.9% (14/39) and 58.1% (25/43), respectively ($x^2 = 4$, $p < 0.05$). In conclusion, EquiPure™ improves stallion fertility in AI programs.

P123

Development of an ovine sperm selection (Ovipure™ -nidacon) kit

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Ten experiments were conducted over a one-year period and aimed at developing a centrifugation kit for selecting structurally and functionally competent spermatozoa. The kit was composed of three citrate-based media: a diluter, a colloid (silane-coated silica particles) and a washer. Asthenospermic ejaculates ($n = 270$) were collected from nine rams (6–9 year old). We examined the effects of media composition (serum albumin and polyvinyl alcohol), media osmolality (270–350 mOsm/Kg) and pH (6.8–8.5), media sterilization methods (filtration and γ-irradiation), colloid concentrations (70–100%) and processing steps, including semen dilution rates (1:1–1:19), semen

volume (0.25–1.5 ml) onto the colloid layer, volume (3.5–4.75 ml) of the aspiration fluid after sperm cell selection and volume (0.25–8.25 ml) of sperm washing medium. Semen evaluation end-points were sperm yield quantity and quality (progressive motility, plasma membrane integrity and morphology). Data were normalized and analyzed by ANOVA and Duncan's multiple range tests. Sperm traits were improved ($p < 0.01$) after adjustment of media osmolality and pH at 300 mOsm/Kg and 6.8, respectively. Colloid irradiation did not affect sperm yield. The highest sperm yield was obtained after centrifugation of 1 ml diluted semen containing $0.5–1 \times 10^9$ spermatozoa over a 4 ml-colloidal layer (80%), aspiration of the upper 4.25 ml and re-centrifugation of the selected sperm in 5.25 ml of washing medium supplemented with 1 mg/ml polyvinyl alcohol. In conclusion, OviPure™ kit is a simple protocol to improve semen quality.

P124

Leukotrienes: production, localization and concentration in bovine endometrium during the estrous cycle

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We showed *in vitro* that leukotrienes (LT)s are produced in bovine ovaries and its action depends on LT (LTC_4 vs. LTB_4) and cell type. Our *in vivo* study indicated that LTB_4 is luteotropic and supports corpus luteum during the estrous cycle and the early pregnancy inducing PGE_2 and P4 secretion, whereas LTC_4 is luteolytic stimulating $PGF_{2\alpha}$ secretion. However, it is not known whether LTs are produced and may act directly on the bovine uterus. We studied: mRNA expression (real time RT-PCR), histochemical localization for LT receptors (LTR-I for LTB_4 and LTR-II for LTC_4) and 5-lipoxygenase (5-LO), and concentration of LTB_4 and LTC_4 (EIA) in the endometrium during the estrous cycle (days: 0, 2–4, 8–10 and 16–18 of the cycle). LTR-I mRNA expression was the highest on days: 0 and 2–4 of the cycle, LTR-II mRNA expression was the highest on days 16–18 of the cycle ($p < 0.05$), whereas 5-LO mRNA expression was the highest on days 2–4 ($p < 0.01$) and unchanged in next stages of the cycle ($p > 0.05$). LTR-I immunohistostaining revealed the strongest reaction in estrus and days 2–4, LTR-II was the highest on days 16–18 and 5-LO was the highest in uterine vessels on days 2–4 and the lowest on days 8–10 of the cycle ($p < 0.05$). LTB_4 concentration was the highest in estrus whereas LTC_4 level was evaluated on days 16–18 of the cycle ($p < 0.05$). The results indicate that LTs are produced locally in the bovine endometrium dependently on the stage of the cycle and may act directly on the endometrium activating specific LT receptors during the estrous cycle.

P125

Corpus luteum – other source of lysophosphatidic acid in the bovine reproductive tract

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We have recently shown that lysophosphatidic acid (LPA) which is produced by the bovine endometrium, stimulates prostaglandin $(PG)E_2$ and progesterone (P4) secretion *in vivo* during the luteal phase of estrous cycle and early pregnancy. However, there is no data whether LPA can be produced in the bovine corpus luteum (CL). Therefore, the objective of the present study was to examine LPA concentration, protein localization and expression (immunohistochemical staining, Western blotting; respectively), and mRNA transcript quantification (Real-time PCR) for LPA synthesizing enzymes (*Phospholipase A2 – PLA2, Autotaxin – AX)* in the CL tissue during estrous cycle and early pregnancy. We confirmed the increasing concentration of LPA in the CL tissue towards the end of the estrous cycle and early pregnancy. Proteins for PLA2 and AX were found in the luteal cells and in some blood vessels during all examined phases of the cycle and early pregnancy. mRNA quantification and protein levels for PLA2 and AX in the CL did not differ during the estrous cycle ($p > 0.05$). However, on days 18–19 of pregnancy transcript abundance and protein levels for PLA2 and AX were the highest from all the examined phases of the estrous cycle and pregnancy ($p < 0.05$). In conclusion we demonstrated that the bovine CL, additionally to the uterus, is a source of LPA production in the bovine reproductive tract. Supported by MNiSW: DPN/DWM/MZ/5751/08/09.

P126

mRNA expression and protein immunolocalization for progesterone receptor membrane component 1 (PGRMC1) and SERBP1 protein in bovine endometrium during estrous cycle and pregnancy

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We have found that progesterone (P4) can affect bovine endometrial cell function *via* non-genomic pathway, presumably involving the membrane progesterone receptor PGRMC1, which forms active complex with serpine 1 mRNA binding protein (SERBP1). The aim of the study was to determine: (1) PGRMC1, SERBP1 and nuclear P4 receptor (PR) mRNA expression by real time PCR and (2) immunohistochemical localization of PGRMC1, SERBP1, PR proteins in bovine endometrium during estrous cycle and early pregnancy. Uteri were collected from cows on days 1–5, 6–10, 11–16 and 17–21 of the estrous cycle, and on weeks 3–5, 6–8, 9–12 of pregnancy ($n = 3–7$/each period). Obtained data showed, for the first time, the expression of PGRMC1 and SERBP1 mRNA in bovine endometrium during estrous cycle and pregnancy. The expression of PGRMC1 did not change during the estrous cycle, while SERBP1 and PR expression increased ($p < 0.05$) on days 11–16 and 17–21, respectively. Expression of the studied genes was the highest ($p < 0.05$) on weeks 3–5 of gestation. Immunostaining for PGRMC1, SERBP1, PR was detectable in bovine endometrium in all stages of the estrous cycle and pregnancy. The highest PGRMC1 and SERBP1 immunoreactivity was found in glandular epithelium and endothelium of blood vessel. Obtained data suggest that PGRMC1/SERBP1 complex is involved in non-genomic effect of P4 on endometrial cells and it may be a part of the mechanism participated in the protection of early pregnancy. Supported by grant NN311 348237.

P127

Effects of the *Tarantula cubensis* extract and trimethoprim + sulphadiazine in retained placenta in Holstein cows

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The study aimed to investigate the effect of administration of *Tarantula cubensis* extract (TCE) with trimetoprim + sulfadiazin bolus (STB) or STB alone on retained placenta (RP) in cows. Fourty cows with RP were divided into three groups. In group 1 (G1; $n = 20$) TCE + STB were administered 24 and 72 h after calving to cows with RP. In group 2 (G2; $n = 20$) only STB was applied 24 and 72 h after calving to cows with RP. In group 3 (G3; $n = 20$), no treatment was applied to cows without RP and served as control. Intervals for parturition to first estrus (FO), parturition to first insemination (FI), parturition to conception (PC), calving interval (CI), the number of services per conception (NS) and conception rate in first insemination (CR) were recorded in each group. FO, FI, PC, CI, NS and CR were, 45.80, 63.55, 74.15, 349.50, 1.36 and 70%; 46.55, 67.20, 85.10, 356.80, 1.70 and 40%; and 37.10, 50.95, 58.80, 332.65, 1.35 and 65% in G1, G2 and G3, respectively. FO, FI and PC are significantly ($p < 0.05$) shorter in G3 than those in G1. Likewise CI is significantly ($p < 0.01$) shorter in G3 than that in G1. However, no differences were detected between G1 and G2 for these parameters. The probability of nonpregnancy was 3.5 (0.95–12.96) times higher in G2 than that in

G1 to first service. In conclusion, both treatments resulted in optimal fertility parameters (G1; PC, CL and NS and G2; PC and CL) or fertility parameters in economic tolerance (G1; FO and CR and G2; FO and NS). TCE + STB treatment has a beneficial effect on cows with RP based on first service pregnancy rate.

P128

Effects of periparturient supplementation with ß-carotene on oestrous cyclicity and fertility of mares during the first 6 weeks after foaling

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In the horse, positive effects of β-carotene on ovarian and endometrial function have been suggested. However, contradictory findings have been reported as well and absorption of ß-carotene from the gastrointestinal tract has been questioned. In the present study, fertile broodmares of the Brandenburg State Stud were either fed β-carotene (1 g/day; Group CAR, n = 15) or left untreated (Group C, n = 15) from 2 weeks before the expected date of foaling to 42 days postpartum (d -14: basal value before experiment; d 0: day of foaling). Oral ß-carotene supplementation increased the concentration of ß-carotene in plasma (d -14: 0.02 ± 0.00 µg/ml in group CAR and C; d 0: 0.21 ± 0.04 µg/ml in group CAR and 0.06 ± 0.02 µg/ml in group C, $p < 0.05$). Concentrations of retinol and α-tocopherol at no time differed between groups. Foal heat began on average on day 9.2 ± 0.5 (±SEM) postpartum in CAR and d 10.7 ± 2.1 in C mares (n.s.), mean duration of oestrus was 4.7 ± 0.4 (CAR) and 4.6 ± 0.5 (C) days (n.s.). After breeding in foal heat 5/12 CAR and 7/7 C ($p < 0.05$) mares conceived. By the end of the breeding season 11/14 CAR and 14/14 C mares were pregnant (n.s.). Embryonic death did not occur in any of the mares during the examined period. Altogether, our data clearly demonstrate that β-carotene is absorbed from the gastrointestinal tract in horses but supplementation is without positive effects on fertility in adequately fed mares.

P129

Andrological characteristics of the kilis goat in Turkey

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The Kilis goat is one of the best-known dairy goat breeds in Turkey. These goats in the Southeast Anatolia region are well adapted to the extreme climatic conditions of Turkey. There is currently no published information on the reproductive characteristcs of the Kilis goat. In the present study, testes dimensions and spermatologic parameters of eight Kilis goats localized in Ankara were determined. From each buck, seven ejaculates were collected once a week using an artificial vagina during the breeding season. Ejaculates were evaluated for volume, motility, concentration, abnormal sperm, live sperm and pH. Moreover, morphometric sizes of testes of the goats were also recorded once per month. The spermatological parameters of samples were as following: ejaculate volume 1.09 ± 0.07 ml, percentage of sperm motility 87.33 ± 1.8%, sperm concentration 4371.87 ± 237 × 10^9/ml, total sperm abnormalities 4.57 ± 0.47%, sperm viability 91.5 ± 1.2% and pH 7.08 ± 0.03. The average scrotal circumference, volume, thickness, testis length and diameter were 29.21 ± 0.8 cm, 583.30 ± 33.26 ml, 0.39 ± 0.01 cm, 11.92 ± 0.30 cm, 5.83 ± 0.12 cm respectively. This is the first study which proved some reproductive characteristics of Kilis goats. In addition, all features of the semen samples were within normal limits but there were some differences in the characteristics compared to other breeds of bucks. Supported by grants from TUBITAK, Turkey (KAMAG-106G005).

P130

Expressions of Insulin-Like Growth Factor (IGF)-I, -II and their receptor genes are regulated in mare endometrium during estrous cycle and early pregnancy

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IGFs are known to associate with reproduction and are involved in the regulation of endometrial functions. The aim was to evaluate expression profiles of IGF-I, IGF-II and their receptors in equine endometrium during the estrous cycle and early pregnancy. Biopsies were obtained from mares on day of ovulation (d0, n = 4), late diestrus (LD, n = 4, high progesterone [P4]), and after luteolysis in the estrus phase (AL, n = 4, < 1 ng/ml P4) of the cycle. Biopsies were also taken on days 14 (P14, n = 4), 18 (P18, n = 4), 22 (P22; n = 4), and 60 (P60, n = 2) of pregnancy. Relative mRNA expression levels of genes were quantified using real-time RT-PCR. The normalized data was analyzed via one-way ANOVA and least significant difference test was employed to determine significantly different groups. IGF-I, IGF-II and the receptors were expressed in mare endometrium. Expression of IGF-I and IGF-IR were induced at LD while IGF-II and IGF-IIR expression were unaffected during the cycle. IGF-I, IGF-IR, and IGR-II expressions during early pregnancy did not change compared to d0 as IGF-IIR mRNA levels were decreased on P14 and P18 compared to d0, P22 and P60. Expressions of IGF-I, IGF-IR and IGF-IIR were significantly higher and IGF-II expression was lower at LD compared to P14. There were no differences in the expression levels of the studied genes between AL and p18. The results suggest that IGF system appears to be regulated in the equine endometrium by both cyclic changes and early pregnancy. (Funded by TUBITAK)

P131

Characterization of equine amnion-derived Mesenchymal Stem Cells (MSCs) after cryopreservation

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We already isolated and characterized equine amniotic epithelial (AESCs) and mesenchymal (AMSCs) stem cells showing that equine amnion represents a source of stem cells which may have clinical applications for their plasticity and reduced immunogenicity. The aim of the present study was to evaluate whether equine amnion stem cells can be frozen, stored and recovered without loosing their functional integrity in terms of morphology, renewal capacity and presence of specific stemness markers. Cells were frozen in DMEM supplemented with 50% FCS and 10% DMSO for 6 months and expanded after thawing to study cell population doubling (PD) and specific MSC-markers by RT-PCR and immunocytochemistry. Recovery rate after thawing was 60% for AESCs and 80% for AMSCs. After cryopreservation, AESCs and AMSCs conserved their shape: cuboidal epithelial and fibroblast-like respectively. After 31 days of culture 12.61 and 16.52 PD were observed for AESCs and AMSCs while fresh cells showed 13.08 and 26.5 PD respectively. Fresh and frozen cells expressed the same MSC-mRNA markers: CD29, CD44 and MHC-I mRNA but not CD34 and MHC-II. Frozen samples showed a decreased CD105 expression in respect to fresh ones. Only AESCs were positive for CD166. AESCs and AMSCs after thawing showed positive immunostaining for OCT-4, C-Myc and SSEA-4. In conclusion, freezing of these cells for banking purpose is faisable but the differences between freshly isolated and frozen amniotic cells require further studies.

P132

Three different fertility traits valued by Norwegian dairy farmers in relation to characteristics of their herds

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Fertility is a complex trait composed of different characteristics, like the ability (1) to show clear signs of heat, (2) to resume normal cyclic ovarian activity after calving and (3) to get pregnant after insemination. To get some information which one of these three characteristics the farmers give the highest value and which one they experience as most problematic a questionnaire was sent to Norwegian dairy farmers. Response was received from 3686 ones (40%). The answers were coupled with data from the National Dairy Herd Recording System and analyzed. More than 98% of the farmers considered the ability to show clear signs of heat and the ability to get pregnant after the first insemination as the most important traits. They where less concerned about the ability to show heat early after calving (70%). Holstein breeders did not consider this trait as important as the Norwegian Red Cattle breeders. Sixty percent of the farmers experience too many returns to heat after insemination, 54% would like more clear signs of heat and 45% think that the cows do not show heat early enough after calving. Farmers being satisfied with the three fertility traits had a higher herd fertility status. Fifty-three percent of these farmers had freestall barns. However, among farmers experiencing problems with the three traits 13% only had freestalls. In high yielding herds the farmers were generally more satisfied with fertility and specially the cows' ability to show heat early after calving. This is interesting since there is a negative genetic correlation between yield and fertility.

P133

Luteinizing hormone and follicle stimulating hormone induce COX -2 in the ovine cervix

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Cervical relaxation at oestrus is mediated by PGE_2 whose synthesis is regulated by COX-2. The high concentrations of FSH and LH during oestrus suggest that they stimulate COX-2 regulated synthesis of PGE_2 to mediate cervical relaxation. Our objective was to determine the effect of intra-cervical LH or FSH on COX-2 in the cervix of ewes, during oestrus. Eighteen ewes were assigned to four groups (FSH 2 mg; LH 2 mg; vehicle and no treatment). Ewes were synchronised using progestagen pessaries and 500 IU PMSG at pessary removal and the treatments were given 24 h after pessary removal. Cervices were collected 54 h after pessary removal and divided into six transverse sections; alternate sections were formalin fixed, wax embedded and sectioned at 7 μm. The expression of COX-2 was determined by immunohistochemistry in five cervical layers (epithelium, stroma, and circular, longitudinal and transverse muscle) and in three regions (vaginal, mid and uterine regions). Data were analysed by a hierarchical univariate ANOVA. Both LH and FSH significantly increased the expression index of COX-2 in the cervix (both $p < 0.05$). The expression index was highest in luminal epithelium and lowest in subepithelium stroma. The expression of COX-2 in vaginal region was significantly increased by FSH and LH ($p < 0.05$). The results indicate that both FSH and LH stimulated COX-2 in the sheep cervix at oestrus. The increased COX-2 induced by FSH and LH is probably associated with increased synthesis of PGE_2 and cervical relaxation during oestrus.

P134

The effect of intra-cervical LH and FSH on LHR mRNA in cervical tissue from oestrous ewes

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Higher concentrations of LH and FSH at oestrus stimulate cervical COX-2 by an unknown mechanism but one that may involve increased expression of mRNAs for the gonadotrophin receptors, particularly LH-R. Our objective was to determine the effect of intra-cervical LH or FSH on LH-R mRNA in the cervix of ewes, during oestrus. Eighteen ewes were assigned to four groups (FSH 2 mg; LH 2 mg; vehicle and no treatment). Ewes were synchronised using progestagen pessaries and 500 IU PMSG at pessary removal and the treatments were given 24 h after pessary removal. Cervices were collected 54 h after pessary removal and divided into six transverse sections; alternate sections were formalin fixed, wax embedded and sectioned at 7 μm. The expression of LH-R mRNA was determined by *in situ* hybridisation using a labelled LH-R riboprobe, in five layers (epithelium, stroma, and circular, longitudinal and transverse muscle) and three regions (vaginal, mid and uterine regions). Data were analysed by hierarchical univariate ANOVA. Both LH and FSH significantly increased LH-R mRNA in cervical tissue compared to the vehicle ($p < 0.05$) or the control group ($p < 0.05$). The expression of LHR mRNA was greatest in vaginal region compared to the middle ($p < 0.05$) and uterine regions ($p < 0.05$). The results show that local application of LH and FSH can increase the cervical expression of LH-R mRNA and suggest that in the ewe, regulation of LH-R in the cervix by LH and FSH is involved in the physiological mechanisms that control relaxation of the cervix during oestrus.

P135

Sperm viability, chromatin stability and sperm surface-ubiquitination in relation to field fertility of ai bulls from two age groups

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The aim of this study was to characterize semen from AI bulls at two ages, as unproven young bulls and as elite AI sires, thereby identifying parameters that (1) are able to predict elite sire fertility at the young bull stage and/or (2) can be used to calculate individual dilution of the semen. Ejaculate density, volume and motility before and after freezing were measured. Analyses performed on frozen-thawed (FT) semen were membrane integrity (viability), DNA fragmentation index (chromatin stability (DFI)) and sperm surface-ubiquitination (ubiquitin bound to sperm surface proteins (UBI)). Fresh and FT semen from 166 Norwegian Red AI bulls (15–16 months and 4–4.5 years) was analysed using light microscopy (motility) and flow cytometry (viability, DFI and UBI). Fertility of each ejaculate was estimated based on the non-return rate after 56 days (NRR56), calculated from ≥40 first inseminations per ejaculate. No differences in FT sperm characteristics were detected between the two groups. However, there was significant seasonal variation; DFI and viability were highest in autumn/winter, whereas UBI was lowest in autumn/winter. Viability for FT semen was similar in the two groups, while freezability of the semen improved with bull age. Ejaculate NRR56 ranged from 65.7% to 84.8%. Viability in FT semen from elite bulls showed a significant positive correlation with fertility, whereas UBI in young bulls showed a tendency of a negative relationship. We conclude that UBI shows promise as a fertility predictor for young bulls whereas viability after FT can be used for individual dilution of elite bull semen.

P136

Survival capacity of low quality bovine embryos after removing necrotic embryonic cells

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The incision of zona pellucida by a metal blade enables an atraumatic removal of disintegrated blastomeres from perivitelline space in embryos. The aim of this study was to evaluate the effects of this procedure on the developmental competence of bovine preimplantation embryos in *in vitro* culture (72 h) and on pregnancy rates after a transfer to recipients (synchronized Holstein-Friesian heifers, 340–370 kg) in farm conditions. Embryos were collected from superovulated donors on day 7. The embryos were selected by the morphological quality as well as by the developmental stages. Compacted morulae and early blastocysts of low morphological quality underwent the micromanipulation procedures. The treated embryos (experimental group) were compared with the intact embryos (control group). *In vitro* culture results revealed better survival rates of experimental embryos in comparison with the control embryos. *In vitro* development into blastocysts (Experiment: Control) was 78.9% (60/76): 53.8% (56/104), into expanded/hatching blastocysts 52.6% (40/76): 24% (25/104) and into hatched blastocyst 39.5% (30/76): 11.5% (12/104), ($p < 0.01$, χ^2 test). The pregnancy rate after the transfer was 51.4% (36/70) in the experimental and 35.2% (37/105) in the control group ($p > 0.05$, χ^2 test). The results show that the microsurgical evacuation of degenerated blastomeres significantly improves the viability of D7 bovine embryos *in vivo* and *in vitro*. It can be concluded that this micromanipulation represents an effective method for the treatment of low-quality bovine embryos before fresh transfer. Supported by a grant MSM 6215712403.

P137

Sperm chromatin structure of cooled-transported ovine semen

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The aim of this experiment was to study changeability of sperm chromatin structure during semen storage. Ejaculates ($n = 22$) with 70–85% sperm progressive motility and $2.5–4.5 \times 10^9$ sperm/ml were collected from 11 rams, split, diluted (0.1×10^9 sperm/ml) with milk-egg yolk and Ovixcell® (IMV Technologies) extenders, packaged in 0.5-ml straws and examined after 6, 24 and 48 h of storage at 5 ± 1°C. Semen evaluation end-points were computer-assisted sperm motion analysis, fluorescence-based analysis of sperm chromatin condensation and nuclear DNA integrity using chromomycin A_3 and acridine orange staining assays and fertility of preserved spermatozoa in terms of 50-days pregnancy rates after cervical insemination of progestagen-treated ewes ($n = 77$). Data were processed by a statistical Software (SPSS®) including multi-factorial ANOVA (extender × storage time × ram), Duncan's multiple range test, χ^2 analysis and Pearson correlation coefficient. Neither semen extender nor storage time had any effect on percentage of spermatozoa with de-condensed chromatin. Ovixcell® extender did not negatively influence sperm motility and DNA integrity. In milk-egg yolk extender, DNA abnormality increased ($p < 0.01$) and motility decreased ($p < 0.02$) after 48 h of storage. Compared with those in Ovixcell® extender, values of abnormal sperm DNA in milk-egg yolk extender were higher ($p < 0.01$) after 24 and 48 h of semen storage. A dramatic decline in fertility was observed when the percentages of abnormal DNA and de-condensed chromatin reached ≥4% and > 10%, respectively. In conclusion, semen extenders contribute to DNA integrity of stored ram spermatozoa.

P138

Modulation of acrosome reaction development in boar spermatozoa by caffeine can improve fertilization of porcine oocytes

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The purpose was to study the relationship between acrosome reaction (AR) progress in boar spermatozoa and their ability to fertilize matured oocytes. The spermatozoa were separated from cryopreserved semen of three boars on a Percoll gradient, untreated or treated with 1 mM or 2 mM caffeine and capacitated or co-incubated with oocytes. The motility, viability and acrosome integrity of spermatozoa and the efficiency of oocyte fertilization were assessed. After caffeine capacitation the percentages of motile and viable spermatozoa were significantly higher and the percentages of acrosome-intact spermatozoa were significantly lower ($p < 0.05$) compared with the untreated spermatozoa of all tested boars. The progress of acrosome reaction had a different character in each sire but, in all, the AR development was faster for spermatozoa treated by 1 mM than by 2 mM caffeine or left untreated. Similarly, in all boars, the total efficiency of fertilization was significantly higher ($p < 0.01$) for spermatozoa treated by 1 mM in comparison with those treated by 2 mM caffeine. It can be concluded that there is a relationship between the AR progress in cryopreserved boar spermatozoa and the efficiency of oocyte fertilization. The faster AR induced by 1 mM caffeine was more effective for *in vitro* fertilization. It is important to test sires before their semen is used. Supported by grant QI 101A166 of the Ministry of Agriculture and grant 523/08/H064 of the Grant Agency of the Czech Republic.

P139

Effect of three semen extender additives on fertilizing capacity and membrane status of ram sperm

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We tested the effect of several additives to semen extenders on fertilizing capacity of ram semen. Three fresh ejaculates collected from three rams of Lacoune breed were diluted in a Biladyl diluent, pooled together and divided into four groups according to tested additives: insulin-like growth factor I (IGF-I, 10 ng/ml), caffeine (1 mg/ml), glutathione (1 mg/ml) or none (control). Fertilizing capacity of fresh semen was tested by pregnancy rates (CR) of ewes 45 days after insemination. Penetration rate (sPR), using sperm penetration test on bovine oocytes and morphology of sperm head membranes, using transmission electron microscopy (TEM), were determined following storage of semen samples at 0–4°C for 5 days. Statistical analysis was done using Chi-square test. After a 5-day cooled storage, sperm of the controls were able to penetrate 76.5% of oocytes and reach 76.3% CR. An additional increase in sPR was caused by IGF-I (92.4%, $p < 0.05$), caffeine (92.3%, $p < 0.05$) and glutathione (83.0%, non-significant). CR were slightly ($p < 0.05$) improved by the addition of IGF-I (85.2%) or glutathione (81.25%), whilst caffeine (63.18%) showed a trend to decline CR below control (76.3%; $p > 0.05$). TEM showed a higher number of spermatozoa with intact plasma membranes and acrosomes in the IGF-I and glutathione groups. Caffeine, although it increased sPR and motility, caused membrane damages and loss of the acrosomal content. These results suggest that IGF-I and glutathione may exert a positive influence on fertilizing potential of cooled stored ram semen. The study was supported from the SRDA grant – APVV-0514-07and LA329 travel grant.

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Stallion spermatozoa prepared by single layer centrifugation with androcoll^{TM}-e are capable of fertilization *in vivo*

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1. Single Layer Centrifugation (SLC) with Androcoll^{TM}-E improves stallion sperm quality (Morrell et al, 2009, Theriogenology 72, 879–884). *Objective*: to test the fertility of SLC-selected stallion spermatozoa by artificial insemination (AI). *Methods:* Eight ejaculates from 1 stallion diluted in skimmed milk extender (Heitland et al, 1996, EVJ, 28, 47–53) were split into NT (not treated), T1 (centrifuged at 500 g for 13 min with 3 ml cushion fluid) and T2 (SLC through 15 ml Androcoll^{TM}-E at 300 g for 20 min). Sperm pellets were evaluated and used for AI after 24 h cooled storage. Data were compared using Student′s t test. One ejaculate from another stallion was used for T2 and AI. *Results*: Total motility (TM) and progressive motility (PM) were higher in T1 and T2 than in NT after 24 h and 48 h storage ($p < 0.05$), and were retained better in T2 than in T1 ($p < 0.05$) (24 h: TM(%) for NT, T1, T2 = 19.75 ± 4.27, 30.25 ± 6.65, 27.88 ± 8.22; PM(%): 6.25 ± 2.20, 12.13 ± 4.20, 13.9 ± 4.64. 48 h TM(%) for NT, T1, T2 = 10.33 ± 0.6, 18.67 ± 1.15, 20.00 ± 0.00; PM(%) 5.00 ± 1.00, 10.67 ± 2.51, 13.3 ± 0.56 respectively). Pregnancy rates were similar for T1 and T2 (5/11 and 3/8 respectively) and better than NT (20%). For the second stallion, sperm quality was improved after SLC (NT vs. SLC, motility 70% vs. 90%, normal morphology 71% vs. 85.5%, viability 66% vs. 81%); the mare became pregnant. *Conclusion*: spermatozoa selected by SLC are fertile after AI. Funded by the Swedish Foundation for Equine Research, Stockholm.

P141

In vivo characterization of pseudopregnancy in the Rabbit Doe

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Rabbits are induced ovulators and functional corpora lutea (CL) should not be present in the ovary of unmated females. Pseudopregnancy negatively affects the reproductive performance, since mostly primiparous does are not receptive and do not became pregnant, due to the high blood progesterone (P_4) levels. Pseudopregnancy can signify, also because of its frequency, an actual limitation on modern rabbit meat production. Ovulation and pseudopregnancy were induced in 24 hybrid rabbit does by the injection of 100 IU hCG. Sequential monitoring of dynamic changes in the ovaries was performed by ultrasonography on day 0, 2, 6, 15 and 17 of pseudopregnancy. Daily blood samples were taken for 18 day for RIA of P_4 and estradiol 17-β (E_2). The ovarian follicle distribution on day 0, before the ovulatory dose of hCG, was marked by approximately equal numbers of small and large follicles (LF). However, at day 2 the LF population was markedly depleted and the ovulation rate averaged 10.8 CL. By day 6 a repopulation of the ovary with LF (71.3%) occurred. The LF lost at ovulation had apparently been replaced by the time of luteal estrogen dependence at day 6 of pseudopregnancy. P_4 rose following hCG to reach highest values on day 10–12, then declined steadily, when CL were regressing, to < 1 ng/ml on day 16–18. During day 1–6, E_2 rose gradually: the appearance of new LF reported in this study may be reflected in the rise in E_2 by day 4–6 after ovulation. On day 15–17, when P_4 decreased, there was an increase of E_2, presumably due to higher LF activity. It seems interesting that CL regression occurred in the presence of the luteotropic hormone E_2. Further studies are needed to demonstrate a gradual decline in E_2 receptor in the CL and a gradual loss of E_2 responsiveness at the end of pseudopregnancy.

P142

Ultrasound-assessed ovarian dynamics and pregnancy rates in lactating rabbit does with different protein supplementation

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In an effort to sustain optimum milk production and simultaneous pregnancy, rabbit meat producers often increase nutrient density of females. This feeding strategy may lead to protein intakes in excess of requirements resulting in increased environmental pollution, as nitrogen excretion is strictly dependant on crude protein (CP) level. Plasma urea nitrogen concentrations (PUN) and reproductive efficiency were assessed in 90 multiparous hybrid rabbit does. At day 27 of gestation, the animals were equally divided into two groups fed a mixture at different protein levels: 18.5% (CP18.5) and 22% (CP22) on dry matter. Blood samples were taken on the day of AI (12 day postpartum) and assayed for PUN. Ovarian ultrasound scanning focused on the presence of large follicles after day 6 of pregnancy, since this follicular category, being steroidogenically active, should be crucial to the survival of the developing CL. CP22 rabbits exhibited significantly higher PUN compared to CP18.5 (34.1 vs. 24.6; $p < 0.01$), associated with reduced fertility: mean large follicles number and pregnancy rate increased when does were fed a diet not exceeding in protein content (14.4 vs. 8.1; 78.1% vs. 69.3%; $p < 0.01$). The results indicate that a high dietary CP may exert an adverse effect on reproductive efficiency by elevating PUN levels in the lactating rabbit doe. Further research is necessary to elucidate how ammonia, urea and some other toxic products of protein metabolism may interact at one or more steps to impair follicular growth, ovulation, fertilization, embryo transport and development or maternal recognition and implantation.

P143

New strategic approaches for short-term oestrus synchronization in dairy ewes based on progestagen, Gn-RH, $PGF_{2\alpha}$ and eCG treatments

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Two experiments were conducted in ewes to develop an oestrus-ovulation short-time synchronization protocol based on combined FGA (fluorogestone acectate), $PGF_{2\alpha}$, GnRH and eCG treatments. Experiment 1 was carried out on five treatment groups (N = 15): 1) Fe – control, FGA vaginal sponges (14 days) + eCG (Day14); 2) FPe, FGA (5 Days) + $PGF_{2\alpha}$ (Day 5) + eCG (Day 5); 3) PFe, $PGF_{2\alpha}$ (Day 0) + FGA (5 Days) + eCG (Day 5); 4) PFG, $PGF_{2\alpha}$ (Day 0) + FGA (5 Days) + Gn-RH (30 h after sponge removal, s.r.); 5) GPe, Gn-RH (Day 0) + $PGF_{2\alpha}$ (Day 5) + eCG (Day5). The ewes were checked for oestrus and hand-mated. The percentage of ewes in oestrus and the interval to oestrus, fertility (number of lambing ewes/number of treated ewes × 100) and prolificacy rates were: 93.3%, 30.3 h, 60.0%, 155.5% in Fe; 86.7%, 43.6 h, 86.7%, 146.1% in FPe; 92.3%, 36.0 h, 92.3%, 166.6% in PFe; 66.7%, 37.3 h, 33.3%, 175.0%, in PFG; 91.7%, 34.9 h, 66.7%, 165.5%, in GPe. The interval to oestrus was shorted in Fe and longer in FPe ($p < 0.01$). Compared to PFG (33.3%) fertility rates were significantly higher in PFe (92.3%, $p < 0.05$) and FPe (86.7%, $p < 0.01$). Experiment 2 aimed to evaluate ovulation time in relation to three treatment groups (N = 10): (1) FP, FGA (5 Days) + $PGF_{2\alpha}$ (Day 5); (2) FPG, FGA (5 Days) + $PGF_{2\alpha}$ (Day 5) + GnRH (30 h s.r.); (3) FPeG, FGA (5 Days) + $PGF_{2\alpha}$ (Day 5) + eCG (Day 5) + Gn-RH (30 h s.r.). GnRH advanced the ovulation time ($p < 0.05$) in FPG and FPeG (53.0 h vs. 61.6 h) as compared to FP with no GnRH. In conclusion, PFe or FPe short-term treatments could be utilized as a valid alternative to long-term methods for synchronization of oestrus before natural service in ewes during the breeding season.

P144

Effect of melatonin on seminal quality in cooled boar semen doses during 7-days storage

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Melatonin (MLT) is an efficient antioxidant that protects cells and tissues and also has receptor-mediated effects. Our aim was to evaluate the effect on seminal quality of 1 μM melatonin addition (0.003% ethanol) to commercially produced semen of pigs, preserved at 17°C for 1, 4 and 7 days in order to enhance the mean life of refrigerated boar semen. From the same boar, doses without melatonin (no addition, NA) and with ethanol served as controls (n = 62 doses). We evaluated motility parameters and percentage of static and progressive spermatozoa by CASA system, viability (SYBR-14/PI), acrosomal status (FITC-PNA/PI), membrane fluidity (MD540/YO-PRO-1) and mitochondrial status (JC-1) by flow cytometry. Statistical analysis was done by ANOVA using a general linear model (alpha = 0.05) and Pearson's chi-square tests. Motility characteristics were affected by melatonin addition. MLT treatment significantly enhanced the percentage of static spermatozoa through 7 days of storage (MLT: 11.9%-22.9%-38.5%/NA:10.1%-14.9%-19.6%; 1, 4 and 7 days respectively) and significantly reduced the percentage of progressive spermatozoa on day 7 (MLT: 48.9%; NA: 52.7%). Also, there was a significantly higher proportion of live spermatozoa on day 7 without melatonin (MLT: 86.09 ± 6.28; NA: 92.04 ± 1.55; mean ± SD). In our conditions the melatonin treatment did not enhance quality of refrigerated boar semen. Supported by PRI09A077 and GRU09019 Junta de Extremadura, Spain.

P145

Optimization of the digestion step for isolating genomic DNA from ram spermatozoa

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DNA isolation from spermatozoa is a difficult task because of their chromatin compaction, requiring higher concentration of proteinase K (PK) than other cell types, and a reducing agent (dithiotreitol [DTT] or β-mercaptoethanol [BM]). We aimed to find a quick and safe digestion method for isolating ram sperm DNA using small volumes. Cryopreserved sperm were thawed, diluted in somatic cell lysis buffer and centrifuged. Pellets were extended in four different digestion buffers, based on TNE-buffer (containing Tris, NaCl and EDTA) and 1% SDS (sodium dodecyl sulfate), plus: 15 U/ml PK, 1% BM (K15-BM); 3 U/ml PK, 1% BM (K3-BM); 15 U/ml PK, 1% DTT (K15-DT); or 3 U/ml PK, 1% DTT (K3-DT). Each solution was split among four tubes (100 μl/tube; 10^7 cells/tube), being incubated at 55°C for 4, 8, 12 or 24 h. At each time, DNA was extracted using phenol and phenol: chlorophorm, precipitated by adding NaCl (200 mM) and −20°C absolute ethanol. DNA was washed in 70% ethanol, dried and resuspended in TE-buffer. Samples were run in an agarose gel and bands were visualized. This experiment was replicated four times. Extraction was optimally achieved after 12 h, obtaining defined bands of high molecular weight with maximum yield for K3-DT. At 24 h, the yield was slightly higher, but signs of degradation were evident. We conclude that using 3 U/ml of PK, and DTT instead of BM and incubating for 12 h could be an appropriate method for obtaining DNA from ram spermatozoa. Supported in part by the Ramón y Cajal program.

P146

Detection of viral DNA in bovine embryos after *in vitro* maturation of oocytes in *Herpesvirus* type-5 -containing medium

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Certain infectious agents could affect the *in vitro* embryonic development, leading to a low production of embryos. This study was designed to verify whether the bovine *Herpesvirus* type-5 (BoHV-5) could be carried to the embryos after oocyte exposure to the virus, as well as its effect on the number of yielded embryos. Selected oocytes were divided into two groups: unexposed (UN; n = 179) and exposed (EX; n = 185). In the EX group, oocytes were transferred to drops of maturation medium (MM; TCM 199, GIBCO, Grand Island, USA) with virus (10^{-2} $TCID_{50}$/ml) for 1 h. Then, oocytes were washed and matured in drops of MM free of virus for 23 h. Following IVF, the presumptive zygotes were cultured in SOF medium up to day 7 postinsemination (pi). The fertilization rates and percentages of morula (M)/blastocyst (B)/ expanded blastocyst (EB) and B/EB/hatching blastocyst (HB) were recorded at 72, 144 and 168 h pi, respectively. BoHV-5 was determined by *in situ* hybridization (ISH) assay using a DNA probe for the glycoprotein- C gene. Data were analyzed using *t*-test for independent samples with $p > 0.05$ taken as significant. No differences ($p < 0.05$) were found between UN and EX for fertilization rates (89.8% vs. 80.4%), M/B/EB (31.1% vs. 31.2%) and B/EB/HB (28.8% vs. 29.3%), respectively. Thus, infected oocytes carried the virus to the embryos as evidenced by the positive signal for viral DNA, but its presence did not compromise the embryo development and production. However, these embryos might be a source of BoHV-5 transmission.

P147

Selection of boar sperm subpopulations by gradients for increasing the *in vitro* penetration performance

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Semen is composed of a heterogeneous population of sperm with varying degrees of structural and functional differentiation and normality. To optimize IVF, sperm selection methods have been used to isolate sperm subpopulations with high fertilizing capacity. The aim of this study was to evaluate the IVF performance using spermatozoa treated by 2-step, discontinuous gradient centrifugation, consisting of three different combinations of isotonic Percoll (P45/60, 60/75 and 45/90%) from fertile and normozoospermic boars. Oocyte penetration rate (n = 390) was higher when spermatozoa were selected by Percoll 45/90 (100%) than other gradients of Percoll combinations (P45/60: 79.0 ± 0.4%, P60/75: 80.0 ± 0.4%) and all of them higher than control group (no selected by gradient C: 30.3 ± 0.5%, $p < 0.001$). The mean number of spermatozoa per penetrated oocyte presented the same pattern with the highest number for the P45/90 group (C: 1.2 ± 0.4, P45/60: 2.7 ± 1.9, P60/75: 3.1 ± 1.9, P45/90: 4.3 ± 2.3, $p < 0.001$). Assessment of sperm quality after centrifugation showed that morphology is the main factor improved by selection by Percoll gradient 45/90 (morphologic normal sperm, C: 79.5%, P45/60: 78.4%, P60/75: 86.5%; P45/90: 95.2%, $p < 0.001$). However, acrosome status evaluated as normal apical ridge was not affected by Percoll treatment (ranged 84.1–88.8%, $p = 0.729$). Use of a 2-step, discontinuous gradient centrifugation with isotonic Percoll (45% and 90%) lead the optimization of the IVF system with high levels of oocyte penetration rates. Supported by Fundación Séneca 08752/PI/08.

P148

Influence of β-carotene and human chorionic gonadotropin on milk progesterone level and pregnancy rate in Holstein cows

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The effects of human chorionic gonadotropin (hCG) administered on day 7 after artificial insemination (AI) and β-carotene, given on the day of insemination, on the progesterone level and conception rate in Holstein cows were investigated. Following spontaneous estrus (day 0) and AI, cows were randomly divided as follows: group A (n = 17) received 1.500 IU of hCG intramuscularly (i.m., Schering-plough, the Netherlands); group B (n = 19) 200 mg of β-carotene i.m. (20 ml of Carofertin® Alvetra u. Werfft Gmbh, Austria) and group C (n = 18) served as a control without any treatment. Milk samples for ELISA progesterone analysis were collected on the day of AI and on days 14 and 20 after AI. Pregnancy was detected by rectal palpation between days 45–60 after AI. Milk progesterone level on day 14 was higher ($p < 0.01$) in group A (18.1 ± 12.3 ng/ml) compared to group C (7.5 ± 6.1 ng/ml), but not to group B (11.0 ± 10.8 ng/ml). Furthermore, milk progesterone concentration on day 14 tended to be higher in hCG-treated pregnant cows compared to hCG-treated non-pregnant cows (21.9 ± 70 vs. 14.7 ± 15.3 ng/ml, $p = 0.059$). Pregnancy rates were 47.1% (8/17), 36.8% (7/19) and 38.9% (7/18), in group A, B and C respectively, but obtained differences were only numeric ($p > 0.05$). This study indicates that only hCG treatment increased mid-cycle progesterone levels, but pregnancy rates were unaffected either by hCG or β-carotene application.

P149

Luteolysis in donkey evaluated by color Doppler ultrasonography

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Color Doppler ultrasonography is an efficient and non-invasive method to evaluate vascular perfusion. Corpus luteum (CL) vascularization has not been studied by Doppler ultrasonography in donkeys. In the first study, diestrus of 11 jennies was evaluated. $PGF_{2\alpha}$ (Dynolitic®) was administrated intramuscularly 8–10 days after ovulation. The jennies were monitored every 24 h by rectal palpation, color doppler ultrasonography (MyLab30, ESAOTE™) and progesterone determination. CL area was evaluated using MyLab30, CL color percentage (vascularization) by the analySIS® 2.1 program (GmbH™) and progesterone by RIA. Following $PGF_{2\alpha}$ administration, CL vascularization and progesterone reached basal values after 24 h, while the area decreased progressively over ther course of 3 days. CL vascularization showed high correlation with progesterone levels. The second study evaluated the evolution of these parameters during 24 h after the $PGF_{2\alpha}$ administration. Five jennies in diestrus (8–10 days after ovulation) were treated with 1 ml of Dynolitic®. The jennies were monitored each hour during 24 h after the inoculation. Uterine tone, CL vascularization and progesterone decreased significantly by 4 h after treatment in two jennies and by 5 h in three jennies. These results seem to indicate a quick functional luteolysis (4–5 h) after prostaglandin administration and a progressive structural CL regression. Color Doppler ultrasonography seems to be a good method to evaluate CL vascularization and activity.

P150

Motility patterns of spermatozoa from subfertile stallions filtered by silica gel double layer (Equipure™)

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Seven stallions with fertility problems were used. Aliquots of filtered semen were divided into four treatment groups: (1) Control, diluted 1:5 in Kenney extender, analyzed at 0 h, refrigerated and reanalyzed at 24–48 h; (2) centrifuged semen, pellet resuspended in Kenney extender, analyzed at 0 h, refrigerated and reanalyzed at 24–48 h; (3) immediate filtration with EquiPure™ (EP), analyzed at 0 h, refrigerated and reanalyzed at 24–48 h; (4) 1:5diluted semen, stored at 4–7°C, filtered with EP at 24–48 h and analyzed. Sperm viability and abnormalities were analyzed by eosin-nigrosin stain, motility by CASA and DNA fragmentation by Holosperm™. Neither treatment nor time affected sperm abnormalities or DNA fragmentation. Semen centrifugation or EP filtration improved sperm viability and motility. However, EP filtration resulted infaster, straighter and more active spermatozoa movements than the other treatments. Filtered semen cooled and stored for 24–48 h maintained acceptable motility characteristics while centrifuged semen showed a significant decrease at 24–48 h. Four motile sperm subpopulations (SP) were observed in fresh semen and maintained in each treatment and time. EP filtration increased the percentage of spermatozoa included in a SP with very high velocity, linearity, very high lateral head amplitude and beat frequency. EP filtration after semen cooling and storage showed higher global sperm motility parameters, however, SP with most rapid, linear and energetic spermatozoa increased with EP filtration before refrigeration and decreased if semen was refrigerated and filtered.

P151

Pellet-freezing improves post-thaw motility and viability of dromedary camel sperm

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Efficient methods for the preservation of dromedary camel semen have yet to be developed. Cryopreservation of dromedary camel semen in straws or as pellets was compared in an attempt to improve the efficiency of dromedary camel semen cryopreservation. Ejaculates (n = 21, n = 5 males) were collected, assessed (Morton, 2008, The continued development of artificial insemination in alpacas, RIRDC publication), diluted 1:1 in Green Buffer, then further diluted 1:1 with Clear Buffer and frozen in 0.5-ml straws or as 200-μL pellets on dry ice (Morton, 2007, Reprod Fertil Dev, 19, 792–96). Data were analysed by ANOVA after arc-sin transformation using GenStat. Semen was highly viscous (38.5 ± 2.1 mm; range: 18–50 mm), the volume averaged 1.9 ± 0.2 ml (range: 0.5–6.0 ml) and contained 194.7 ± 36.7 × 10^6 sperm/ml. After collection, 19.5 ± 3.4% of sperm displayed oscillatory motility, which increased to 57.9 ± 4.2% and became progressive after liquefaction and dilution. Pellet-frozen sperm showed higher 0-h and 3-h post-thaw motility (pellets 0 h: 46.0 ± 2.5%, 3 h: 32.8 ± 3.8%; straws: 0 h 30.8 ± 3.0%, 3 h 18.8 ± 3.2%; $p < 0.05$), sperm membrane integrity (pellets: 0 h 46.4 ± 3.0%, 3 h: 38.3 ± 2.8%; straws 0 h: 26.4 ± 2.8%, 3 h: 31.4 ± 2.9%; $p < 0.05$) and sperm viability (pellets 0 h: 62.0 ± 3.0%, 3 h: 54.6 ± 3.3%; straws: 0 h 50.5 ± 3.4%, 3 h: 46.7 ± 4.3%; $p < 0.05$) compared with freezing in straws. However, there was no difference in sperm acrosome integrity (0 h: 85.6 ± 0.6% vs. 86.1 ± 1.1%; 3 h: 82.8 ± 1.5%, 81.0 ± 3.0%; $p > 0.05$). It is concluded that pellet-freezing improves the post-thaw sperm parameters of Dromedary camel semen.

P152

How to decrease calving interval in lactating dromedaries (*Camelus dromedarius*)

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During milking, camels are considered to be in ''lactation anoestrus'' and are mated first after weaning. This practice results in a long calving interval, and therefore, it would be vital to decrease the number of days open. The aims of this study were to follow up ovarian activity in milking camels and monitor daily milk yield in pregnant animals. Eleven, multiparous camels in mid lactation were selected for the study. The camels were milked by a milking machine twice a day and milk quantity was recorded. Ovarian activity was monitored with ultrasonography. The camels were mated when the size of the dominant follicle reached 1.2–1.5 cm. Pregnancy was diagnosed by ultrasonography and progesterone determination. Average milk production of 2 week periods was compared from 2 months before until 2 months after conception. The effect of pregnancy on milk production was tested with analysis of variance. All camels had follicular development and were mated (20 cycles). Seven of 11 animals conceived 284 ± 21.5 days post-partum. There was a significant effect of time ($p < 0.001$), pregnancy ($p < 0.05$) and interaction ($p < 0.001$) on average milk yield. In non-pregnant dromedaries, milk decreased slowly over time. In pregnant camels, a slow decrease until Day 30 was followed by a sudden drop from 8.8 ± 0.24 to 6.3 ± 0.16 kg/day by Day 60 of gestation. We conclude that the calving interval of dromedaries can be shortened by mating during lactation as follicular development was regular and lactation anoestrus did not occur in our study. However, shorter calving interval would also mean major economic loss in a dairy operation.

P153

Chromosomal abnormalities as causes of reproductive failure in cattle

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Since reproductive performance is a very important characteristic of domestic animals, cytogenetics has been used as a diagnostic tool to identify the chromosomal abnormalities which particularly influence the reproductive performance of the carriers. The role of chromosomal abnormalities as causes of reproductive failure is very important and involve both the chromosome number and the chromosome structure which are very often associated with developmental abnormalities, embryonic death and various levels of infertility. In cattle populations where artificial insemination is used, inherited abnormalities can quickly become widely distributed and cause great economical losses. Considering that the chromosomal abnormalities may cause reproductive disorders the karyotype analysis of 100 Romanian Black Spotted bulls was performed. In this study we identified five bulls with abnormal configuration of the chromosomal complement: one bull presented 1/29 translocation and four bulls had chromosomal instability, expressed by mono- and bi-chromatidic breaks on autosomes and heterosomes, gaps and loss of chromosomal fragments. Although the carriers had a normal phenotype, the analysis of their reproductive behavior and the parameters of the semen demonstrated once again the role of these structural defects in the etiology of different levels of infertility. The carriers were eliminated from the reproduction during the first year of the test cycle and consequently the dissemination of the abnormalities in the offspring population was stopped and the expenses for the complete test cycle were avoided.

P154

Preliminary serological study of *Coxiella burnetii* antibodies in dairy herds in Northeast Spain

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Coxiella burnetii is an obligate intracellular Gram-negative bacterium that causes Q fever, a zoonotic disease endemic worldwide. The reservoirs are almost all animal species. In infected animals *C. burnetii* infection is harmless; however the disease has been related to reproductive problems in dairy cows. The objective of this study was to provide data on the prevalence of *C. burnetii* infection in subfertile herds in high producing dairy farms of north-eastern Spain. Eight hundred and seventeen serum samples were collected from cows of three different farms. A commercial indirect ELISA (CoxLS Ruminants kit) was used to determine antibody-carriers of *C. burnetii*. The antigen of the kit was isolated from domestic ruminants by INRA (France). A cocktail of antigens (phases I and II) was used to detect total IgG anti-*C. burnetii*. In addition, bulk-tank milk (BTM) samples from each farm were analysed with a polymerase chain reaction (PCR) to detect the presence of *C. burnetii*. The preliminary results suggest high seroprevalence in *C. burnetii* antibodies in the tested dairy farms, for 403/817 cows were serologically positive (49.3%) with a prevalence of 53%, 45.5% and 30% in each farm. The PCR of BTM demonstrated the presence of *C. burnetii* DNA in the three farms. The serological results suggest that Spain may be suffering from a Q fever outbreak and that further investigation may be necessary to identify the herds and animals that are spreading the bacteria. PCR from BTM of dairy farms can be a very useful tool to identify the presence of the disease in dairy herds.

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Differences in blood concentrations of leptin, igf-1, insulin and nefa in dairy and beef cows during transition period

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The aim of the study was to compare blood metabolic indices in cows with different genetic and physiological background with regard to the regulation of milk production and energy status. Slovak-Holstein spotted dairy breed (DC, $n = 16$) and Charolais beef breed (BC, $n = 8$) cows were used in this study. Body condition score antepartum in dairy cows was 3.5–4 in 5 scale and in beef cows 6.5–7 in 9 scale (1-poor and 9-obesity). Blood samples for analysis of leptin, IGF-1 (insulin-like growth factor 1), insulin and NEFA (nonesterified fatty acid) were collected twice weekly from 3 weeks antepartum (predicted) to 21 days postpartum (p.p.). Leptin was assayed by a multi-species leptin RIA kit (Linco) validated for bovine leptin, IGF-1 by RIA using human kit S-2143 (Linco) validated for bovine analysis and insulin by ELISA (2340, Roner). NEFA was determined in blood serum by spectrophotometry. Significantly higher concentrations of leptin were recorded in BC vs. DC in the third and second week antepartum (5.59 ± 1.14 vs. 3.91 ± 0.74 ng/l) ($p < 0.05$) but not postpartum (4.2 ± 0.80 vs. 3.2 ± 0.58 ng/l). Significantly higher concentrations of IGF-1 in BC vs. DC (141.7 ± 12.07 vs. 98.4 ± 17.4 ng/ml) were found in the first week p.p. ($p < 0.01$). Significantly higher of concentrations of insulin in BC vs. DC (3.98 ± 0.55 vs. 2.84 ± 0.32 ng/l) were found in the first and second week p.p. ($p < 0.05$). Significantly lower concentrations of NEFA in BC vs. DC (0.19 ± 0.05 vs. 0.39 ± 0.15 mmol/l) ($p < 0.01$) were recorded in the first and second week p.p. (Funded by Ministry of Education of Slovak Republic, VEGA 1/0263/09).

P156

The use of progesterone-supplemented co-synch and ovsynch for estrus synchronization and fixed-time insemination in Saanen goat

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The effect of choronogest supplemented Ovsynch and Co-synch protocols on the estrus synchronization, follicular development and fertility after fixed-time insemination was evaluated in goats during the breeding season. Co-synch (n = 24) and Ovsynch (n = 25) were applied to nulliparous does with a body weight >30 kg. The onset and duration of estrus were determined with teaser bucks. Follicular development and ovulation were monitored by ultrasound (4–8 MHz probe). The does were laparoscopically inseminated with 11.5×10^6 motile spermatozoa/straw at the 2nd GnRH injection for Co-sync and 8 h after the 2nd GnRH injection for Ovsynch. Estrus was detected in 92% and 84% of the does for Co-synch and Ovsynch, respectively. The onset and duration of estrus were 31.1 h and 30.9 h, and 34.4 h and 29.4 h for Co-synch and Ovsynch, respectively ($p > 0.05$). The follicle diameters at the 2nd GnRH injection and the number of ovulations were 0.72 cm and 0.68 cm and 2.6 and 2.8 for Co-synch and Ovsynch, respectively. There were no significant differences with respect to NRR_{30} (62% and 40%) and pregnancy rates (38% and 24%) determined on the 30th day for Co-synch and Ovsynch, respectively. Kidding and prolificacy rates were 38% and 24% and 1.4 and 1.2 for the Co-synch and Ovsynch treated goats, respectively ($p > 0.05$). In conclusion, during the breeding season, the Co-synch protocol yielded better results than the Ovsynch protocol. Further studies should be done for determining the best time for fixed time insemination and the acceptable insemination dose to improve fertility results.

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Efficiency of the replacement of cird-device on the synchronization treatment used in ewes during breeding season

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The aim was to assess the efficiency of two long synchronization protocols based on ovulation rate, corpus luteum (CL) diameter and blood progesterone (P4) concentration. Twenty-three adult ewes were divided into two groups (GI, n = 11 and GII, n = 12). Estrus was synchronized with the progesterone (P4) device CIDR for 14 day. Whereas in GI the CIDR-device remained the 14 day, in GII it was replaced by a new one on day 7. Doses of 2.5 mg of dinoprost, were administered on day 0 and 14. All ewes received 300 IU of eCG on day 14. Ultrasonographic observations (US) were accomplished every 8 h after the end of treatments for 4 day to determine the ovulation. Measurement of corpus luteum (CL) diameter by US and serum P4 determination were performed at days 5, 10 and 15 post-ovulation. Data were analyzed by ANOVA, Chi Square and Tukey tests using the software package SAS. The variables analyzed did not show differences between the two groups ($p > 0.05$). All the ewes ovulated after the end the protocols. The ovulation per animal in GI was 1.54 ± 0.82 and GII was 1.33 ± 0.77. The diameters of CL at day 5, 10 and 15 post-ovulation were 10.25 ± 1.36, 11.68 ± 1.81 and 9.74 ± 1.50 mm in GI and 9.83 ± 1.16, 11.16 ± 1.72 and 10.00 ± 1.48 mm in GII, respectively. The maximum size of CL was on day 10 for both groups ($p < 0.01$). The mean P4 levels at these times respectively were 4.10 ± 2.27, 5.94 ± 3.79 and 3.27 ± 2.33 ng/ml in GI and 3.56 ± 1.65, 5.03 ± 2.17 and 2.78 ± 1.75 ng/ml in GII. There was significant difference only between day 10 and 15, for both groups ($p < 0.05$). We concluded that the both protocols, with or without replacement of CIDR-device, were effective in promoting ovulation and normal luteal function in Santa Inês sheep. Financial support: FAPESP.

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Effects of placentome removal on reproductive performance in heifers and maternal behaviour on calves

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The effects of removal of placentomes on reproductive performance was evaluated on 14 Holstein heifers. Additionally it was assessed whether the duration of the process had an impact on maternal behavior. Immediately after spontaneous delivery of healthy calves, three placentomes were removed per vaginam. The heifers received an intrauterine treatment (i.u) with 2000 mg chlortetracyline hydrochloride (Devamisin®/Vetaş, Turkey). During this process the calves were nursed in a warm and clean room and regular controls and treatments were carried out. After 1.5 h they were returned to their mothers. The mean interval between calving and first service was 84.5 ± 21.9 days (range 64–145 days). Calving to conception interval was 119.78 ± 37.7 days (range 64–200 days). Retained fetal membranes (RFM), endometritis prulenta and RFM-endometritis complex were observed in 4 (28%) and two heifers (14%) and one heifer (7%) respectively. Number of services per conception was 2.0 ± 0.8. The control results were compared with usual data obtained from the farm. The mean interval between calving and first service, calving to conception interval and number of services per conception were 79.2 ± 3.0, 101.0 ± 2.3, 1.6 ± 0.6 respectively. All heifers were restless after separation of calves but afterwards they spent the first few hours licking them. All calves were able to suckle and nursed within 2 h. Three of calves (21.4%) could not stand up until 26 h after parturition. They were fed with an artifical feeding bottle and they were required extra treatment. Such pathology is very unusal in newborn calves. This study seems to indicated that placental removal in heifers had negative impact on reproductive performance and maternal nursing on calves. *Supported by (TUBITAK-TOVAG/107O259).

P159

Expression of inflammatory cytokines and concentration of matrix metalloproteinases and their inhibitors in fetal membranes and fluids collected from horse mares at delivery

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In humans, delivery has been described as a controlled inflammatory process with degrading of placental tissues. To investigate a possible role of comparable mechanisms in the regulation of delivery in horses, allantoic fluid (ALF), amniotic fluid (AMF) as well as tissue from amnion (AMN) and allantochorion (AC) were collected from mares (n = 13) after undisturbed gestation at spontaneous delivery. In fetal fluids, matrix metalloproteinases (MMP) two and nine were detected by gelatin zymography and tissue inhibitors of metalloproteinases (TIMP) 1, 2 and 3 by reverse zymography. Real time PCR was used for detection of mRNA of IL-6 and IL-8 calculated against β-actin as endogenous control and endometrium as calculator. Between AMN and AC, differences in mRNA expression of IL-6 (AMN 15.2 ± 4.4; AC 0.6 ± 0.2; $p = 0.053$) and IL-8 (AMN 23.8 ± 5.6; AC 0.6 ± 0.2; $p < 0.05$) were detected. Concentrations of MMP-2 and MMP-9 were higher ($p < 0.05$) in AMF (MMP-2: 33.6 ± 2.3, MMP-9 43.4 ± 3.9) than in ALF (MMP-2 15.9 ± 1.5, MMP-9 4.5 ± 0.6; $p < 0.05$), and the concentration of their tissue inhibitors TIMP-1, 2 and 3 was higher in ALF compared to AMN ($p < 0.05$). A high concentration of MMP-9 in equine fetal fluids at term is similar to humans where MMP-9 during labor is the major MMP in fetal membranes. The higher concentrations of MMP-9 and cytokines in AMN compared to ALF may suggest a primary role of AMN for the induction of labor.

P160

Efficacy of hCG and GnRH with respect to follicular size in cosynch protocol in cattle

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The aim of this study was to compare the efficacy of hCG and GnRH with respect to follicular size in Cosynch protocol based on pregnancy rates (PRs) in cattle. All animals (n = 165) received injections of GnRH (buserelin acetate, 0.02 mg) and $PGF_{2\alpha}$ (dinoprost trometamol, 25 mg) seven days apart. Concurrently, an ear implant containing norgestomet (3 mg) was inserted at GnRH injection and removed prior to $PGF_{2\alpha}$ injection in all animals. At GnRH and $PGF_{2\alpha}$ injections the presence of visible corpora lutea, and at $PGF_{2\alpha}$ injection of preovulatory follicle (small [SF] <10 mm or large [LF] ≥ 10 mm) were determined with transrectal ultrasonography (USG). Animals were assigned randomly to receive either GnRH (0.01 mg; group I: Cosynch, n = 83) or hCG (1500 IU; group II: modified Cosynch, n = 82) at timed artificial insemination (TAI) 56 h after injection of $PGF_{2\alpha}$. Pregnancies were diagnosed with transrectal USG 32–35 days after TAI. Overall pregnancy rates (PR) following TAI did not differ between group I (42.2%; 35/83) and group II (35.4%; 29/82). However, logistic regression analyses of animals without anoestrus and premature luteal regression indicated significant ($p < 0.05$) interaction effect of follicle size by treatment. PR was lower in animals with small follicles (SF) (35.7%; 10/28) than those with large follicles (LF) (58.3%; 21/36) in group I. In contrast, PR was similar in animals with SF (42.9%; 12/28) and LF (38.1%; 8/21) in group II. In conclusion, follicle size could be considered when using different hormones in TAI protocols, although overall PRs did not differ between groups.

P161

Expression of ACTH, dopamine and GnRH receptors and PPARγ in bovine corpus luteum during diestrus

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The corpus luteum (CL) is a transient organ, essential for successful pregnancy, which develops from ruptured follicles after ovulation. If pregnancy fails to occur, the CL undergoes luteolysis, a dynamic regression process that ends with its complete functional and structural demise. The aim of the present study was to evaluate by immunohistochemistry the presence of the receptors for ACTH, dopamine and GnRH and PPAR γ(peroxisome proliferators-activated receptor γ) in early-, mid-, late- and regress-CL during diestrus cycle of bovine. Polyclonal antibody for ACTH receptors (melanocortin-2 receptor) immunostained strongly the cytoplasm of luteal cells in early, mid and late stages and weakly in regress-CL. GnRH receptor polyclonal antiserum immunoreacted weakly in the luteal cell cytoplasm of early-, mid- and late-CL whereas this receptor was not found in regress CL. D2, D4 and D5 dopamine receptor types were evidenced by polyclonal antisera in the cytoplasm of luteal cells: D2 and D4 immunosignals were weakly detected in early-, mid- and late-CL; conversely, D5 was moderately evidenced in all four CL stages. Finally, PPARγ monoclonal antibody immunostained the cytoplasm and nuclei of luteal cells during early, mid and late, but not regress diestrus. Our results suggested that the life-span of bovine CL is regulated by a multifactorial system that includes various hormonal regulators that up to date are not yet well studied.

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Effect of intra-oviductal laparoscopic manipulation on future reproductive performance of sows

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Currently, intra-oviductal deposition of sperm cells or zygotes by laparoscopy is emerging as a useful tool for improving the efficiency of several reproductive biotechnologies in pigs. The aim of this study was to evaluate the effect of intra-oviductal laparoscopic manipulation on the future reproductive performance of sows. Twenty weaned sows were used in the experiment. Ten sows were subjected to only laparoscopic manipulation on the first day of estrus. During laparoscopy, 100 µl of PBS were injected into each oviduct close to the ampulla (LP group). The remaining 10 sows were used as control (C group). Sows from both groups were allowed to follow a natural cycle and were inseminated 19–21 days later with 3×10^9 sperm dose at 12 and 24 h after onset of estrus. Analysis by ANOVA showed no differences between LP and C groups for pregnancy rates, farrowing rates and litter sizes (100%, 90%, 11.8 ± 0.92 and 90%, 90%, 11.5 ± 0.71 for LP and C groups, respectively). In conclusion, the mechanics of intra-oviductal manipulation by laparoscopy do not affect future reproductive performance of sows. Supported by MICINN (AGL2008-04127/GAN; Madrid, Spain) and Seneca Foundation (GERM, 04543/07), Spain.

P163

Researches Related to Age Influence on Certain Reproduction Traits in Tigaie Sheep Bred in Romania

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The research were applied on a Tigaie breed flock, located in the North-Eastern area of Romania. The main goal was to achieve original data, in order to optimize the reproduction activity. Two hundred ewes were chosen and divided into eight groups (each containg 25 animals) based on their age (e.g. the 1st one included sheep aged less than a year, while the 8th one included sheep aged 8 years). The following reproduction traits were studied, in order to reach research goals: fecundity, prolificacy, birth rate and gestation length. Lambs weight after lambing was correlated with parturition type (single lamb or twins). The achieved results have been put into a data base and used to run a statistical analysis using the REML algorithm. The fact that over 80% of the ewes aged less than a year exteriorized sexual cycle and have been mated indicates that Tigaie breed has a very well precocity level. Related to fecundity, highest values occurred after the 2nd sexual cycle, which corresponded to the 10–27 October period. Fecundity varied between 88% (ewes aged less than 2 years) and 100% (ewes aged between 4 and 7 years). The prolificacy index analysis revealed its increase, from 104.76% (ewes aged less than a year) till the maximum of 120% (ewes aged 4 years). It decreased then at 104% (ewes group aged 8 years). Significant differences occurred for gestation length ($p < 0.05$) between most groups. Therefore, ewes age is an important factor influencing the average length of gestation. The studies we carried out showed the semi-tardive characteristic of Tigaie breed and the fact that ewes could be used in reproduction, from the very first year of life, if optimal husbandry conditions are provided.

P164

Subclinical endometritis of dairy cows in Hungary

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Subclinical endometritis (ScE) is a condition characterized by a substantial neutrophil granulocyte (NG) infiltration of the endometrium at the late involution period. Neither cervical discharge, nor other visible or per rectum palpable clinical sign can be observed. Nevertheless, it has a great economic impact due to a decreased conception rate. Diagnosis is made using endometrial cytology by estimating the proportion of NG. Prevalence and effect of ScE on reproduction performance was studied in Hungarian dairies. Uterine wash fluid collected between 40–72 days post partum was used as sample to determine NG infiltration. Both, cows with a healthy involution (n = 26) and cows previously suffering from puerperal metritis (PM) (n = 35) were included in the study. Samples, in principle, were prepared and evaluated as previously described (Gilbert, 2005, Theriogenology, 64, 1879–1888), however, we applied Naphtol-AS-D-chloroacetate-esterase, a NG specific immuno-hystochemical method instead of Giemsa staining. NG proportion of > 5% was considered to be ScE. Overall prevalence of ScE was found 37.7% with almost equal distribution over the previously PM (37.1%) and non PM (38.5%) cows. ScE in cows with no previous involution complication resulted in a 17.5% lower conception rate accompanied with many more days open, also requiring a higher number of inseminations. Even in a combined analysis of the data i.e. by grouping on ScE only, regardless of previous uterine disease, interval to first insemination as well as days open were significantly longer in cows with ScE (187 ± 92 vs. 233 ± 98 days).

P165

Relationship between apoptosis-related parameters and *in vivo* fertility with refrigerated ram semen

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The objective of this study was to determine the relationship between certain sperm quality parameters, especially those related to apoptosis, and the *in vivo* fertility rate after cervical AI. A total of 608 ewes from 34 different farms were inseminated with 71 refrigerated sperm samples obtained from 15 mature *Rasa Aragonesa* rams. We evaluated sperm motility, capacitation state and parameters related to apoptosis such as phosphatidylserine (PS) exposure, DNA damage and caspase activity of samples obtained both in breeding (B:August-February) and non-breeding season (NB:March-July). The proportion of intact sperm (PS-) was higher ($p < 0.01$) and with damaged-DNA was lower in B than in NB season (43.5 ± 2.1 vs. 27.4 ± 2.0 and 18.5 ± 1.4 vs. 21.9 ± 1.8, respectively). The percentage of live sperm with no caspase activity was very low in both seasons (1.0 ± 0.1 and 1.4 ± 0.3). However, the percentage of live non-capacitated sperm was higher ($p < 0.05$) in NB (21.3 ± 2.4) than in B (14.4 ± 1.5) season. Fecundity was correlated with the percentage of DNA-damaged ($r = -0.33$, $p < 0.01$) and live non-capacitated ($r = 0.31$, $p < 0.05$) sperm. When results were divided in groups depending on the distance between collection and insemination place, the correlation between fertility and DNA-damaged sperm (−0.585) was very significant ($p < 0.01$) in the most remote farms, suggesting the importance of this parameter. CICYT-FEDER AGL 2007-61229, 2008-01476, UZ2008-BIO-20.

P166

Use of the heatime® system for heat detection in beef heifers

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The efficacy of the HEATIME® heat detection system based on general activity measurement was investigated in 36 Charolais heifers (15–16 months old) housed in an experimental INRA station during the breeding period (February-May 2009). Heat detection was performed twice a day with a vasectomised bull. When the HEATIME® system and/or the bull spotted potential heat, a blood sample was systematically collected to measure progesterone concentration as a reference method to identify estrus. With all information, successive cycling periods were identified for each heifer. A total of 151 detection events occurred with the bull and 186 were spotted by HEATIME®. Sensibility, specificity, positive and negative predictive values were calculated in reference to the occurrence of an estrus period and averaged respectively 85%, 95%, 95% and 83% using bull detection, compared to 85%, 75%, 81%, 80% using HEATIME®. Then, as a decision rule, HEATIME® alert was considered as an estrus only when the date corresponded to 1 or 2 regular previous estruses. When applying this rule, sensibility, specificity, positive and negative predictive values reached 81%, 96%, 95% and 84% respectively. To conclude, when combining HEATIME® alerts with 1 or 2 regular cycles, the HEATIME® system detects estrus periods in beef heifers with the same efficiency as a bull. Under farm conditions, this decision rule implies to set up the HEATIME® system 6 weeks before the breeding period.

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Three step synchronization treatment and fixed time artificial insemination in primiparous vs. multiparous nelore zebu cows considering body condition score

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The purpose was to evaluate the results of a three step synchronization protocol performed in Mato Grosso (Brazil) on 116 primiparous and multiparous Zebu cows comparing pregnancy rate (PR) at first insemination and verifying if the success of fixed time artificial insemination (FTAI) was related to variations of nutritional status. The protocol was applied on fertile cycling females: Day 0 – CIDR® device application together with an injection of 0.2 mg of estradiol benzoate (2.0 ml Estrogin®); Day 7 – inoculation of 12.5 mg of dinoprost trometamine (2.5 ml Lutalyse®); Day 9 – withdrawal of P4 device and contemporary injection of 400 IU eCG (Novormon®) and 0.5 mg of estradiol cipionate (0.25 ml of ECP®). On Day 11 FTAI was performed by the same operator with a single lot of frozen/thawed Valdostana breed semen. The PR was 46.51% for the primiparous and 47.95% in multiparous cows ($p > 0.05$). Subsequently, PR in both groups was correlated with the body condition score (BCS; 2–2.5 poor nutrition and 3–3.5 good nutrition). For primiparous cows with a BCS of 2–2.5 and 3–3.5 the PR was 38.09% and 54.55% respectively; for multiparous cows with a BCS of 2–2.5 and 3–3.5 the PR was 46.66% vs. 50.00%. The three step treatment demonstrated to be efficient to synchronize fertile oestrous. The response of eCG treatment is satisfactory considering the good PR at first AI in both groups without statistical difference in PR. The limiting factor of poor BCS doesn't affect PR results even if a positive trend of results can be noticed in good nutritional status subjects.

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Endometrial characterisation of induced 'pseudopregnancy' in the mare

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Non-surgical transfer of embryos to mares induced into prolonged dioestrus by transrectal manually rupture of the conceptus after liberation of the 'maternal recognition of pregnancy' signal resulted in no pregnancies, suggesting an inadequate endometrial environment for conceptus development. The aim of the present study was to compare the ultrastructure of the endometrial glands in mares induced to pass into prolonged dioestrus with those in mares at an equivalent stage of gestation. Endometrial biopsies were collected on eight mares on Day 26 of pregnancy, 8 days after manual rupture of a day 18 conceptus and on Day 8 of dioestrus. Samples were processed in 2% glutaraldehyde and fixed in OsO4 1% (both in 0.1 M cacodylate buffer) before progressive dehydration in propylene oxide. Sections were contrasted with uranyle acetate/lead citrate. Descriptive statistics and an analysis of covariance with the number of vacuoles as a function of 'stage' and 'mare' were performed. The number of vacuoles per glandular cell present 8 days after rupturing the conceptus was significantly ($p < 0.001$) lower than those at the 'equivalent stage of gestation' (6.5 vs. 12.7) and within the same quantity range as in dioestrus. Thus, it was concluded that the presence of a conceptus in the uterus stimulates the secretion of histotroph secretions by the glandular epithelium. For this reason, the term 'prolonged dioestrus', rather than 'pseudopregnancy', would seem more appropriate to describe a non-pregnant mare that exhibits failure of cyclical luteolysis.

P169

Influence of cryopreservation on the chromatin integrity of dog spermatozoa

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Recently, increasing attention has been paid to sperm chromatin structure as one of the parameters determining male fertility. Sperm chromatin structure alterations (DNA breaks) can affect triggering and regulation of activity of paternal genes, thereby leading to either zygote mortality early after fertilization or early abortions. Cryopreservation is known to reduce sperm quality and thus also the fertilization outcome. There is some information about changes of chromatin integrity during storage of preserved semen in several domestic animals such as bulls, stallions and boars, but information on dogs is still lacking. The aim of the study was to ascertain what happens with the chromatin integrity during the cryopreservation process. Ejaculates of 20 dogs were collected by digital manipulation and analyzed. Ejaculates were cryopreserved in Tris-citric acid-fructose extender with egg yolk and 6% of glycerol. Sperm chromatin integrity, motility, morphology, acrosomal integrity and viability were evaluated after collection and after thawing. Sperm chromatin integrity was analyzed by Sperm chromatin structure assay using flow cytometry. Percentage of mature spermatozoa with increased chromatin damage was expressed as DNA fragmentation index (DFI %). Even though all parameters of conventional sperm analysis were significantly altered by cryopreservation, sperm chromatin integrity did not change during sperm freezing/thawing. The mean sperm DFI was 2.1 ± 2.2% and 2.3 ± 2.4% in fresh and frozen/thawed semen, respectively. (Funded by GACR 523/08/P561 and MZE 0002716202)

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Leptin as indicator of lipomobilisation during lactation in simmental cows

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The aim of the study is to determine potential use of leptin as indicator of lipomobilisation during early lactation in Simmental cows. High milk production and its direct impact on health and metabolism of the cow needs to be followed routinely in the dairy industry. It is worldwide accepted to use BCS change as indirect parameter of lipomobilisation of the cow. BCS is easy to apply in cows of fine constitution such as Holstein cows but not so easy in rough and robust Simmental cows, which still produce significant amount of milk in Croatia and some other countries. In such animals an alternative indicator of lipomobilisation is needed. Using metabolic parameters such as NEFA or BHB is not accurate, since it is strongly influenced by onset of cyclicity during and after puerperium. So we needed some other parameter, closely related to fat tissues mobilisation. In our study we followed lactation in 69 Simmental cows during first 10 weeks after parturition. All cows, aged 4–6 years, produced more than 25 l of milk per day and were healthy by all means. Blood was collected weekly, at 7 a.m. during 10 weeks. Sera samples were stored at −20°C and analysed using LINCO multispecies RIA kit for leptin. All cows were inseminated at the first visible estrus, and half conceived (32 vs. 37). We found strong linear correlations between onset and increase of milk production and decrease of leptin concentration. Furthermore, the average leptin values decreased three times (from 6 to 2 ng/ml) during the first 3 weeks after parturition. The onset of pregnancy did not influence leptin values probably due to the predominant influence of energy demands for lactation. Our research has shown that leptin concentrations vary markedly after calving in Simmental cows. After validation by comparison with other parameters (such as BCS, NEFA or BHB) a more rapid and practitioner-based method (rapid test) can be developed for use on-farm.

P171

Toluidine blue as stain for optimal morphometric evaluation of frozen-thawed bull spermatozoa

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This study was designed to compare the performance of Toluidine blue stain (TBS) and two protocols of Hemacolor (H1, H2) (Merck, Darmstad, Germany) for staining the bull sperm head in frozen-thawed semen samples collected from eleven Brahman bulls. Manufactors' instructions were followed for H1, increasing by two min the time proposed for each step in H2. Morphometric analysis was performed using Sperm-Class Analyzer (Microptic, Barcelona, Spain) to assess the sperm head size (length, width, area and perimeter). In order to determine the adequacy of the staining techniques for capture and digitalization of the images, at least 100 spermatozoa from each slide, 1 per staining and per animal, were captured and subsequently analysed, wich added up to 5783 spermatozoa over the entire semen samples. TBS was found to be the best procedure for staining the frozen-thawed bull sperm ($p < 0.0001$) based on the highest percentage of sperm correctly analyzed (88.29%) and the lowest coefficient of variation on the image processing and morphometric measurements (4.54) in comparison to H1 (63.69/CV) and H2 (50.77/CV) respectively. TBS provided good colour intensity and optimum contrast of the sperm head with the surrounding background that allows efficient boundary detection and reduces the number of stained foreign particles. The staining methods affected significantly the sperm head dimensions ($p < 0.0001$). The values of H1 were higher than H2 and these higher than TBS (H1 > H2 > TBS). Hemacolor® provides more intense grey-level values, resulting in enlarged cells, which influence the

size morphometric parameters. Based on these findings, we recommend the TBS for its accurate and reproducible morphometric results.

P172

Evidence for the presence of separate boar sperm subpopulations based on mitochondria activity

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This work analyzed the presence of separate sperm subpopulations in boar ejaculates based on their mitochondrial activity, assessed by JC-1 stain. The stain was evaluated by confocal laser microscopy and further image analysis of sperm midpieces. This established an orange: green staining ratio for each sperm. Results were then analyzed using a multivariate cluster analysis. Fresh boar ejaculates had a mean orange: green ratio of 8.2 ± 0.1 (arbitrary values). The cluster analysis rendered the presence of four separate sperm subpopulations. Subpopulation 1 (27.7% total sperm) had the highest activity (mean orange: red-green intensity: 14.7). Subpopulation 2 (37.3% total sperm) included cells with high activity but lower than Subpopulation 1 (mean orange: red-green intensity: 6.7). Subpopulation 3 (31.6% total sperm) had a mean orange: red-green proportion of about 3.0. Subpopulation four had the lowest activity (mean orange: red-green proportion of about 0.8); this represented 3.7% of overall sperm. Our results are the first to reveal the existence of sperm subpopulations, with regard to mitochondrial activity, and this may be useful to analyze sperm heterogeneity in an ejaculate.

P173

Caprine sperm survival after an osmotic test which simulates cryopreservation

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This work was carried out to assess sperm survival after an osmotic test that simulates cryopreservation protocols. Semen was collected from 10 Alpine bucks (30 ejaculates), seminal plasma was removed and spermatozoa were resuspended in an isoosmotic medium. Aliquots of diluted sperm were exposed to hyperosmotic conditions: 300 (control), 1200 and 2100 mOsm/Kg for 15 min. Isoosmolarity was reestablished by adding suitable hypoosmotic solutions. Firstly, sperm were exposed to hyperosmotic conditions at 23°C (15 ejaculates); secondly, at 5°C (hyperosmotic solution) and 37°C (hypoosmotic solution), respectively (15 ejaculates). Plasma membrane and acrosome membrane integrity were assessed during hyperosmotic conditions and after restoration of isoosmolarity. Proportion of both plasma membrane-intact and acrosome-intact spermatozoa decreased significantly ($p < 0.05$) as hyperosmolarity increased; a further decrease was manifested when isoosmolarity was reestablished ($p < 0.05$). These changes in plasma and acrosome membranes were more severe at 5 and 37°C than at room temperature: 26 vs. 18% for plasma membrane and 26 vs. 14% for acrosome membrane integrity. Caprine sperm exposition to hyperosmotic conditions and subsequent restoration of isoosmolarity by hypoosmotic solutions produced quantitative damage to plasma and acrosome membranes. This approach provides insights on the possible use of this sort of osmotic tests to predict sperm cryosurvival; however, inter-male sperm susceptibility to freeze-thawing protocols (''good'' and ''bad freezers'') as well as other freezing variables different from osmotic changes that affect sperm cryosurvival should be considered.

P174

Intravaginal application of the PGE1 analog misoprostol improves induction of abortion in bitches

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The goal was to compare four treatments for induction of abortion in bitches. Bitches (n = 28, days 25 to 35 of gestation) were grouped as follows: *group I* (n = 7): aglepristone (10 mg/kg/day sc 2 days; *Group II* (n = 7): aglepristone + cabergoline (5 μg/kg, po, daily until abortion was complete) + misoprostol (200 μg for ≤20 kg, 400 μg for >20 kg, intravaginally, daily until abortion was complete); *Group III* (n = 7): aglepristone + misoprostol; *Group IV* (n = 7): aglepristone + cloprostenol (1μg/kg, sc, 2 days, together with aglepristone). All bitches were examined clinically and sonographically on a daily basis. Statistical analyses was performed using an One-Way ANOVA and the Mann-Whitney-U test (SPSS software®, Version 14, SPSS Inc., Chicago, USA). In Group III, the abortions were completed 6 days after the beginning of treatment. At the same day in Group I, II and IV, pregnancies were terminated in 57.1, 85.7 and 42.8% of cases; between day 8 and 10, all pregnancies were terminated. In Group IV, on days 2 and 1 before the beginning of abortion and the day the abortion started serum concentration of progesterone 4 was lower than in the other groups ($p < 0.01$). Side effects (vomiting) only occured in Group IV. In conclusion, the combined use of aglepristone and misoprostol significantly shortened duration of abortion in bitches.

P175

Effect of a laser system of injecion and a high pyruvate medium on the development of equine embryos produced by ICSI

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We tested the effect of laser assisted ICSI and embryo culture in a high pyruvate medium on equine embryo development in a cross 2 × 2 design. ICSI was performed using two techniques: conventional (group C; n = 147) vs. laser assisted micromanipulation (group L; n = 166). Injected oocytes were equally cultured in two groups: control (0.5 mM pyruvate, group CPYR; n = 168) vs. high pyruvate (4.5 mM pyruvate, group HPYR; n = 145). We compared the rates of successful injection, cleavage and degeneration at day 2 between the ICSI techniques. No difference was observed between the rate of successful injection (83% group L vs. 88% group C, p = 0.218) neither cleavage at day 2 of culture (26% group L vs. 34% group C, p = 0.189). The rate of degeneration tended to be higher in the group L (40% group L vs. 29% group C, p = 0.078). In the second part of the study we compared the rates of cleavage, arrests at the pronucleus stage and degenerated embryos. The embryos cultured in the HPYR medium presented a reduced number of arrests at the PN stage (1% HPYR vs. 7% CTRL, p = 0.025) and a tendency for a lower percentage of degeneration at day 2 (27% HPYR vs. 42% CTRL, p = 0.07). No statistical difference was observed for the cleavage rate (34% HPYR vs. 26% CTRL, p = 0.189). In summary, the laser system do not prevent embryo development. High concentration of pyruvate in the medium tends to reduce the degeneration of embryos and arrests at the PN stage during the first two days of culture.

P176

The Efficacy of single dose prostaglandin F2α and anti-prostaglandin F2α antibodies in sheep

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The efficacy of single dose prostaglandin $F_2\alpha$ analogues such as dinoprost tromethamine, cloprostenol sodium and d-cloprostenol was studied in 60 nulliparous and multiparous sheep. We also aimed to detect endogenous anti-prostaglandin $F_2\alpha$ antibodies and the relationship between these antibodies and fertility parameters. Prostaglandin $F_2\alpha$ was administered to the animals one week after the first estrus and blood samples were collected three times (at 0, 24 and 48 h after the injection). The concentrations of progesterone and estradiol in the samples were measured using commercial ELISA kits (Fertigenix-Easia, Biosource Europe, SA). The presence of anti-prostaglandin $F_2\alpha$ antibodies in these samples was detected by scintillation counter. There was a significant ($p < 0.01$) decrease in the progesterone levels in both nulliparous and multiparous animals, to which cloprostenol sodium was administered on the day of administration and after 24 and 48 h. There was no significant difference between the groups in terms of pregnancy rates and estrus recurrence rates. Anti-prostaglandin $F_2\alpha$ antibodies were detected in all blood samples. However, it was considered that these antibodies did not have a sufficient level to affect parameters such as pregnancy rates and estrus recurrence rates in sheep. Supported by a grant from Firat University Scientific Research Projects Unit.

P177

Presence and distribution of fungi and bacteria in the reproductive tract of healthy stallions

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A saprophytic bacterial flora is present on the penis and the distal part of the urethra of stallions. Little is known about the fungal flora of their reproductive tract. The aim of the study was to describe the distribution of fungi and bacteria in stallion genital apparatus. From 11 healthy stallions, samples were collected in five different locations: urethral fossa, penis/prepuce, urethra pre- and post-ejaculation and semen. For fungal and for bacteriological examinations, samples were taken in three and one occasions ($n = 165$ and 55), respectively. A difference ($p < 0.05$) in the presence of fungi was seen between urethral fossa or penis/prepuce (45.4%) and urethra pre- or post-ejaculation or semen (15.1%, 6.0% and 0%, respectively). Yeasts were isolated in 9.1% of the samples, never in semen. The most represented fungi were *Penicillium spp*., *Aspergillus spp*., *Scopulariopsis spp*., *Trichosporon spp*. and Mucoracee. A difference ($p < 0.05$) in proportion of samples showing a high (>50,000 CFU/ml) total bacterial count was observed between urethral fossa and urethra pre- or post-ejaculation or semen. Differences among sampling locations were observed also for Staphylococci coagulase positive and negative, these being the most represented bacteria. *Taylorella equigenitalis*, *Salmonella abortus equi* and sulphite reducing clostridia were never isolated. *Escherichia coli* and coliforms were always either low or absent. In conclusion, the study shows, that despite the presence of bacteria and fungi on the external genital tract of the tested stallions, only a low bacterial count was found in the semen and fungi were always absent.

P178

High developmental capacity of cloned pig embryos following trichostatin a-mediated epigenomic modulation throughout *in vitro* maturation of oocytes pre-treated with roscovitine

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The aim was to determine the effect of *R*-roscovitine (RSCV) and trichostatin A (TSA) on the *in vitro* maturation (IVM) of porcine nuclear recipient oocytes and *ex vivo* development of nuclear-transferred (NT) embryos descended from sow dermal fibroblast cells. Cumulus-oocyte complexes (COCs) were pre-matured with 50 μM RSCV for 22 h. Afterwards, the COCs were incubated for 20 h in TC 199 medium supplemented with 0.1 IU/ml human menopausal gonadotropin, 10% porcine follicular fluid, 0.6 mM *L*-cysteine and 10 ng/ml recombinant human epidermal growth factor, followed by their continuous IVM for 22–24 h in the same medium enriched with 80 nM TSA. Reconstituted oocytes were simultaneously fused and electrically activated, and then cultured *in vitro*. The treatment with RSCV prior to IVM in the TSA-depleted, and subsequently TSA-enriched medium led to the reaching of meiotic maturity by almost all the oocytes (89/91; 97.8%) as compared to a control group (67/83; 80.7%). Moreover, the percentages of morulae (48/82; 58.5%) and blastocysts (27/82; 32.9%) developing from NT oocytes that had been previously exposed to RSCV and TSA were significantly higher than in the RSCV- and TSA-untreated group (22/59; 37.3% and 10/59; 16.9%, respectively). In conclusion, the abundance in a formation of morulae and blastocysts suggests the improved reprogrammability of fibroblast cell-inherited nuclear DNA in an epigenomically-matured cytoplasm of recipient pig oocytes undergoing sequential exposure to RSCV and TSA.

P179

Influence of hormonal stimulation on pregnancy rate in 5/8 girolando cows in postpartum treated with progestogen and estrogen

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It is known that increasing reproductive efficiency is five times more profitable than improving milk quality and three times more profitable than genetic progress. The objective of this research was to evaluate the influences of the ovarian follicular status on ovulation and pregnancy rates in 5/8 Girolando cows. For this purpose 20 Girolando cows 60 days postpartum were implanted subcutaneously with 3 mg of norgestomet and received 2 mg of estradiol benzoate i.m. Transrectal ultrasound examinations were performed to evaluate the population of ovarian follicles. The amount of follicles present in each ovary and the diameter of the largest follicles were noted. The norgestomet implants were removed after seven days and the animals received 2 μg Prostaglandin $F_2\alpha$ and 10 animals received 1mg of estradiol cipionate administered i.m. 12 h after estrus was detected. All the females were inseminated and examined every 12 h until the ovulation occured. From all animals implanted with norgestomet, only in one cow no big or medium follicles were detected. All the females presented estrus between 48 and 60 h after the removal of the implants. It was also verified that all the females ovulated between 12 and 24 h after the end of the estrus, receiving estrogen or not. From twenty cows that were treated with norgestomet, 13 were pregnant. Acknowledgments: CNPQ and FACEPE.

P180

Effectiveness of optidyl®, bioxcell® and egg yolk tris-based extenders to freeze Brown-Swiss and Holstein bull semen

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The aim of this study was to compare the effectiveness of egg yolk Tris-based extender and two commercial extenders (Bioxcell® and Optidyl®) to freeze bulls semen. Ejaculates from Holstein (n = 36) and Brown-Swiss (n = 36) were divided in three aliquots and diluted in Tris-based, Optidyl® and Bioxcell® extender, respectively. Thereafter they were frozen and thawed following a standard protocol. The effectiveness of freezing extenders was assessed according to post-thaw sperm motility evaluated by CASA, acrosomal and total abnormalities examined microscopically and plasma membrane integrity measured using the hypoosmotic swelling test. Regarding to Holstein bulls, the highest percentages of subjective (53.1 ± 1.8%, $p < 0.01$), CASA progressive (22.7 ± 1.5%, $p < 0.001$), and CASA toal motility (64.7 ± 0.8%, $p < 0.001$) were found in semen diluted in Optidyl®. Optidyl® extender also provided best protection to acrosome (4.1 ± 0.5%) and plasma membrane integrity (60.4 ± 1.7%) compared to other extenders ($p < 0.001$). Regarding to Brown-Swiss bull, the lowest percentages of post-thaw subjective (28.6 ± 1.6%, $p < 0.01$), CASA total motilities (36.2 ± 1.1%, $p < 0.001$) and membrane integrity (34.6 ± 1.2%, $p < 0.001$) were obtained in the semen samples diluted in Bioxcell®. The percentage of progressive motility was found to be higher in Optidyl® (17.7 ± 3.1%) than Bioxcell® (7.2 ± 1.1%) ($p < 0.01$). The highest percentages of acrosomal (11.2 ± 0.6%; 10.6 ± 1.3%) and total abnormalities (20.1 ± 1.4%; 16.8 ± 1.6%) were found when Bioxcell and Tris extender were used. In conclusion, Optidyl® extender could be used for successful cryopreservation of Holstein and Brown-Swiss bull's semen.

P181

Investigation of seasonal exocrine function of the testes in Hungarian black racka rams

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Hortobágyer Racka is a native Hungarian sheep breed, which is bred strictly seasonal. Aim of the study was to determine how seasons effect testicular exocrine function of Racka rams. Nine mature (18–20 months of age) Black Racka rams were included into the year long trial. Size of the testes was measured every 2 weeks, whilst semen was collected weekly. Ejaculate volume was noticed and motility was assessed under phase contrast microscope and classified between 1 and 5. Concentration of the semen was determined in Buerker-chamber. Morphology was evaluated after Cerovsky-staining. Circumference of the testes increased continuously from 22.58 ± 1.43 cm (winter) to 31.55 ± 1.16 cm (autumn). A significant correlation was found between testes circumference and day length ($r = 0.38$, $p < 0.001$), as well testes circumference and monthly average temperature ($r = 0.68$, $p < 0.001$). Maximum ejaculate volume was recorded in autumn (0.89 ± 0.34 ml), whilst minimum was measured in spring (0.52 ± 0.29 ml). The highest sperm concentration was detected in summer ($6.68 \pm 2.06 \times 10^9$/ml, the lowest one in winter ($5.07 \pm 1.64 \times 10^9$/ml, $p = 0.017$). Total sperm number was the highest in autumn (4.78×10^9). There was no significant difference in motility during the trial (4.81 ± 0.58%). The lowest morphological anomalies were detected in autumn compared to other seasons (8.73%, $p < 0.01$). In conclusion slight seasonal differences could be detected in testicular function of Racka rams. (Founded by OTKA-K 76371)

P182

Variations in iron-status in Spanish purebred mares during the estrous cycle

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In women during sexual cycle, there are fluctuations in fluid volume and significant blood loss. These changes are associated with variations in iron status (Kim, 1993, Am J Clin Nutr, 58, 705–709). The aim of the present research was to analyze the effect of estrous cycle on hemoglobin concentration (HB), packed cell volume (PCV), iron (FE) and ferritin (FERR) concentrations in Spanish mares. Venous blood samples were taken from 20 reproductive Spanish mares during follicular (FF; follicle diameter > 3.5 cm) and luteal phases (LF, from the fifth day diestrous). HB and PCV were analyzed by Sysmex F-820 and microhematocrit, respectively. FE was determined by spectrophotometry (METROLAB 2300 Plus V3®) and FERR were analyzed by a turbidimetric method using reagents from Spinreact® with a coefficient of variation intra-assay of 5.0%. There were no differences in the HB (FF: 12.4 ± 1.6; LF: 12.4 ± 1.3 g/dl), PCV (FF: 35.0 ± 4.1; LF: 35.2 ± 3.3%), FE (FF: 164.3 ± 32.3; LF: 183.7 ± 47.5 μg/dl) or FERR (FF: 161.4 ± 77.2; LF: 156.7 ± 77.9 μg/dl) concentrations between both phases of cycle. The absence of changes throughout the estrous cycle suggests that examination of these parameters have no diagnostic value for reproduction in the mare.

P183

Evaluation of isolation and analysis of RNA quality and integrity for bovine placenta; including separated caruncle and cotyledon evaluation

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Acid guanidium phenol preparations such as Trizol allow the reproducible isolation of high-quality total RNA from various sources. In order to establish an economical and reproducible method for the high-quality RNA extraction from bovine placenta, total RNA was isolated with 1 ml acidic solution containing guanidinium thiocyanate, sodium acetate, phenol and chloroform, followed by centrifugation. Total RNA was precipitated with isopropanol and no purification kit was used. The RNA quality was determined by spectrophotometry using the optical density (OD) absorption ratio (OD260 nm/OD280 nm should be > 1.7). The ratio was between 1.70 and 2.10. Integrity of the RNA was verified by agarose-gel electrophoresis. The results of electrophoresis showed three clear bands of 28s, 18s and 5s rRNA, respectively. First strand cDNAs were amplified with extracted RNA using a kit (GeneAmp Gold RNA PCR Core Kit), followed by basic PCR. The RT-PCR products were successfully derived from the extracted RNA. Total content and quality of isolated RNA tended to be lower in the cotyledon (3200.45 ± 1515.91 ng/μl) than in the caruncle (4055.65 ± 1692.80 ng/μl). This RNA extraction method represents an economical and reproducible method in obtaining high-quality RNA from fetal and maternal parts of bovine placenta with Trizol, without any purification method. Supported by TUBITAK-TOVAG/107O259.

P184

Effect of trichostatin a treatment on the preimplantation development of nuclear-transferred goat embryos

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The purpose of the study was to examine the *in vitro* developmental potential of caprine cloned embryos reconstructed with cell nuclei of adult dermal fibroblasts following their treatment with a histone deacetylase inhibitor, trichostatin A (TSA). Somatic cells were cultured *in vitro* up to a total confluence and then exposed to TSA (50 nM; 23 h) to enhance their reprogramming efficiency in nuclear-transferred (NT) embryos. Enucleated *in vitro*-matured oocytes were electrofused with the TSA-treated (Group I) and untreated fibroblast cells (Group II). After a 2-h delay, the reconstructed oocytes were chemically activated with calcium ionomycin, followed by treatment with 6-dimethylaminopurine for 2 h. The NT-derived embryos (Groups I and II) were incubated in B2 medium for 24–48 h. Afterwards, cloned embryos were cultured in B2 medium supplemented with 10% FBS for 140–168 h up to morula/blastocyst stages. In Group I, out of 39 cultured NT embryos, 32 (82.0%) were cleaved. The frequencies of cloned embryos that reached the morula and blastocyst stages yielded 27/39 (69.2%) and 17/39 (43.6%), respectively. In Group II, from among 61 cultured embryos, up to 47 (77.0%) underwent cleavage divisions, but only 24 (39.3%) and 11 (18.0%) developed to morula and blastocyst stage. In conclusion, it has been shown that treatment of caprine dermal fibroblasts with TSA before starting the cloning procedure resulted in the high blastocyst formation rate by enhancing the efficiency of donor cell nuclear reprogramming.

P185

Cytoskeletal characteristics in relation with meiotic competence and ageing in porcine and bovine oocytes matured *in vitro*

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We investigated the relationships between the cytoskeleton morphology, the meiotic competence and the effect of culture duration in *in vitro* matured (IVM) porcine and bovine oocytes. Nuclear stage, microfilaments and spindles in oocytes were evaluated by epifluorescent and confocal laser scanning microscopy after staining with Hoechst 33,342, rhodamine-phalloidin and anti-α-tubulin followed by Alexa Fluor 488-conjugated secondary antibody. Porcine oocytes cultured for 33 h, 44 h and 52 h and bovine oocytes cultured for 12 h, 22 h and 30 h of IVM were evaluated. The frequencies of cytoskeletal anomalies between metaphase-I (M-I), metaphase-II (M-II) or total oocytes in both species were compared by ANOVA. Higher rates of M-I oocytes at 44 h of IVM in pigs or at 22 h of IVM in cattle showed breaks in the continuity of the cortical F-actin compared with M-II oocytes (58.1% vs. 15.6% and 51.1% vs. 5.6%, respectively) ($p < 0.05$), whereas no difference was found in the frequencies of spindle abnormalities. Extension of IVM duration had no effect on spindle and cortical microfilament anomalies in both species. However, in pigs, the extension of IVM caused an increased rate of oocytes showing degradation of the supranuclear actin caps (11.8% and 28.5%, respectively). Our results indicate that during IVM in both species, altered microfilaments are associated with meiotic arrest at the M-I stage and that extended IVM affects the supranuclear F-actin.

P186

A survey of Turkish veterinarians' attitudes to control of reproduction in small animals

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Data were collected from 302 practices located in Istanbul, Ankara and Bursa and from 35 faculty members of veterinary schools of Istanbul, Ankara and Uludag (Bursa) by performing a face-to-face interview to investigate the academic and practicing veterinarians' beliefs and practices regarding prevention of breeding in female companion animals. Three hundred and thirty-eight interviews were completed (response rate 69%). Medroxyprogesterone acetate was the primary choice for medical contraception (68.8%) and 24% respondents considered using human contraceptives. However, most responders (60.4%) favored surgical contraception and ovariohysterectomy was the most common surgical technique (97%). Spaying was most commonly performed when the animals were approximately 7 months or older (dogs: 70.3%, cats: 69.2%). For the surgical procedure, ventral midline incision was more preferred in dogs (85%) than in cats (35%). The most popular induction agents were xylazine and medetomidine. For the maintenance of anaesthesia, most veterinarians (79%) preferred injectable agents. Ketamine (88.3%) and isoflurane (73.5%) were the most common injectable and inhaled agents for the maintenance. The most preferred ligature materials used for tying the reproductive organs were chromic catgut and polyglactin 910. The majority of respondents (59.5%) did not administer any kind of analgesics. For pain management, only nonsteroidal anti-inflammatory drugs were used and the most popular drug used was carprofen. The analgesics were most commonly (49%) used for only 24 h.

P187

Contamination of *in vitro*-derived bovine embryos after fertilization of oocytes with frozen semen previously exposed to BoHV-5

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Bovine herpesvirus type 5 (BoHV-5) has already been isolated from bull semen and aborted fetuses, but there are few reports about its presence in *in vitro* -produced bovine embryos. This study was carried out to determine whether oocytes *in vitro* fertilized with semen exposed to BOHV-5 could lead to production of infected embryos. One bull ejaculate was diluted in egg yolk-Tris-glycerol extender and infected with virus (10^{-2} $TCID_{50}$/ml). After a 15 min viral co-incubation interval at 27°C, the diluted semen was packed into 0.5 ml straws and cooled to 4°C for 5 h. Then, semen samples were frozen in a Styrofoam box in liquid N_2 vapor for 20 min before freezing by plunging into nitrogen. PCR assay was performed in both fresh and frozen semen to confirm the viral DNA absence and presence, respectively. The thawed semen was prepared by Percoll gradient and the matured oocytes were co-incubated. The presumptive embryos were transferred to drops of modified SOF and cultured up to Day 7 post-insemination. The *in situ* hybridization assay (ISH) confirmed the presence of the viral DNA in the embryos. A positive ISH signal showed that *in vitro* -produced embryos were susceptible to contamination by BoHV-5, indicating that sperm were capable of carrying the virus into the embryo. Thus, further investigations must examine the viability of these embryos in an ET program, as well as the implications of viral transmission to recipients.

P188

Non-enzymatic antioxidative defence mechanisms in plasma of sows during the periparturient period: vitamin C and glutathione

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The aim of the study was to determine plasma profile of non-enzymatic antioxidants (vitamin C and glutathione) in periparturient sows. The study involved 24 sows aged 1–3 years. All sows were from one closed-cycle production farm. The plasma values of vitamin C and glutathione (GSH) were determined spectophotometrically. The mean vitamin C value on days 13–14 prepartum was 0.49 (±0.19) mmol/g protein and decreased ($p < 0.05$) at 24–48 h postpartum to 0.33 (±0.19) mmol/g protein. On days 6–7 and 13–14 postpartum, vitamin C values further decreased to 0.17 (±0.006) and 0.15 (±0.007) mmol/g protein, respectively. The mean GSH value on days 13–14 before delivery was 0.071 (±0.009) mmol/g protein and decreased ($p < 0.05$) at 24–48 h before delivery to the value of 0.062 (±0.018) mmol/g protein. In this period, the mean GSH value was similar to that observed during the first 24–48 h postpartum. On day 6–7 after delivery, the value of GSH reached those observed on days 13–14 and 6–7 prepartum. On days 13–14 postpartum, the value of GSH was 0.115 (±0.029) mmol/g protein and was higher ($p < 0.001$) than that on days 13–14 prepartum. These study indicate that porcine values of vitamin C and glutathione decrease during the periparturient period, possibly resulting in impaired non-enzymatic mechanisms of antioxidative defence.

P189

Prediction of stillbirth by monitoring endocrine and metabolic parameters in dairy cattle

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A gradual increase in stillbirth rates has been observed during the last few decades. Only half the stillborn calves are directly related to calving difficulties therefore the objective of this study was to investigate the effect of hormonal and metabolic disturbances on the incidence of stillbirth. In two studies, 94 and 86 dairy cattle were sampled 3 or 2 times at drying-off, 3 weeks prior to expected calving, and within 1 h after calving, respectively. Cows were grouped according to the incidence of stillbirth. The following hormones and metabolic parameters were measured: P4, E2, cortisol, T3, T4, insulin, IGF-1, PAG, glucose, BHB, NEFA, urea, TP, albumin, AST, β-carotene, urine pH, and NABE. Results were compared between the two groups (control vs. stillbirth group) using Student-t test (at individual time points) and repeated measures ANOVA (considering time and group effect together). Differences in P4 (significantly higher: $p < 0.001$), E2 (significantly lower: $p < 0.01$), PAG (lower tendency) and NEFA (slightly higher tendency) concentrations within 1 h after calving might be associated with stillbirth in dairy cattle; however, more work is needed. The other hormone and metabolic parameters were not associated with the incidence of stillbirth. OMFB-173/2006 research fund.

P190

Correlations between concentrations of steroid hormones, ovarian cortex and endometrium state in sows with ovarian cysts

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The aim of this study was to evaluate the relationships between ovarian cysts and concentrations of 17β-estradiol (E2), progesterone (P4), testosterone (T), androstendione (A4) both in plasma and cyst fluid, and morphological changes in ovarian cortex and endometrium. The values of hormones were determined by RIA. The samples for histometrical evaluations were procured after slaughter of 18 sows divided into three groups of six sows: (1) with polycystic ovaries, (2) with single cysts (3) without cysts. The number of follicles, corpora lutea, albicans, atretic follicles and cysts were counted. Ciliated, secretory and parabasilaris cells of uterine epithelium were counted in 1.0 mm segments, and uterine glands to a 1.0 mm depth. The cysts were negatively correlated with all sizes of follicles but positively with process of atresia in all stages of follicles development. Sows with polycystic ovaries had lower concentrations of A4 but higher concentrations of T in blood plasma ($p \leq 0.01$). The presence of ovarian cysts was positively correlated with concentrations of E2, T and A4 in cysts fluid, number of all kinds of cysts, atresia of primary follicles (a correlation coefficient r from 0.72 to 0.97, $p \leq 0.05$), number of secretory and parabasilaris cells (0.82 vs. 0.63, respectively) and number of secretory uterine glands (0.80, $p \leq 0.01$). Ovarian cysts were negatively correlated with the number of ciliated cells (−0.784, $p \leq 0.01$). These changes may lead to persistent infertility in sows.

P191

Evaluating telomere length in bovine spermatozoa before and after flow cytometric sex sorting using quantitative fluorescence *in situ* hybridisation (qFISH)

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Previous studies observed a delayed onset of first cleavage in bovine embryos produced with sex sorted sperm (Bermejo-Alvarez, 2010, Reprod Fertil Dev, 22, 426–436). We hypothesised that one reason for this finding could be damaged telomeres, a short, highly repetitive DNA sequence playing an essential role during fertilisation and early cleavage (Liu, 2002, Dev Biol, 249, 74–84), whose exposed position at the end of each chromosome might have made them vulnerable to sperm nucleases possibly activated by the sorting process. To test this hypothesis a qFISH technique was applied using a telomere specific PNA-oligonucleotide probe to measure telomere length in single sperm. Ejaculates were collected from three fertility proven bulls. For qFISH, sperm cells were divided into three groups: sorted, unsorted and unsorted but stained with Hoechst 33342. The latter group was added in order to differentiate between possible effects due to the DNA stain Hoechst 33342, whose fluorescence is used to differentiate between X- and Y- chromosome carrying sperm, and effects actually due to the sorting process. Per bull and treatment group 100 spermatozoa were evaluated. Three technical replicates were performed. Fluorescene intensities were compared and data was analysed by ANOVA. No significant differences were found between groups. However, due to strong interactions between the factors group and bull, the testing of higher animal numbers would be required for a definitive statement.

P192

Reproductive disorders in alpacas and lamas at a referral center

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This study reports on the main conditions seen at our clinic over 12 years on 2435 female and 164 male alpacas and llamas. The major complaints in females were repeat breeding (74%), pregnancy loss (18.5%), reproductive emergencies (5.1%) and genital abnormalities (2.4%). Uterine disorders, primarily endometritis and endometriosis were the most common diagnosis (54%). In maidens, the main disorders were ovarian hypoplasia (30%), persistent hymen (17.9%) and vaginal aplasia (9.7%). Chromosomal abnormalities were seen in 34% of these cases. Luteal insufficiency, frequently associated with obesity, was the cause of recurrent pregnancy loss in 18% of the cases. In males, infertility was primarily due to testicular hypoplasia (45%) and testicular and epididymal cysts (17%). Testicular degeneration due to heat stress was the leading cause of infertility in proven males. Reproductive emergencies were dominated by uterine torsion (54.4%) and dystocia (25.6%). The most common causes of the dystocia were head and limb deviations and breech presentation. Neonatal death (n = 46) in the first week of life was due to maladjustment or sepsis associated with dystocia (63%), prematurity or long pregnancy length (>370 days) (17.4%) and congenital abnormalities (13%). This retrospective analysis may direct veterinarians to determine education and degree of expertise needed to set up a camelid theriogenology service. The diagnostic approach is similar to that used in horses. Experience in imaging techniques and knowledge of camelid reproductive peculiarities and medical complications are important in order to provide a high standard of care.

P193

Optimisation of the reproductive capacity in ross-308 breeders (Hens), using delayed photostimulation

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Aviculture practice proved that certain dysfunctions could occur in fowl reproductive status if photostimulation schedule is not correlated with somatic development. Photostimulation onset was postponed by a week to allow the fowl to reach the optimal live weight required to sustain an appropriate laying intensity and to produce high quality incubation eggs. The experiment comprised 11,992 Ross-308 breeders, randomly divided in two groups (Lc and Lexp) reared on permanent litter, using the same technology, in two halls identically equipped. The same lighting schedule was applied for both groups as the producer recommended. In the experimental group, when the hens were 21 weeks old photostimulation began and light intensity was increased (to 60 lux). Postponing photostimulation of the hens induced better live weight uniformity (v = 10.89%, compared to 11.90% in control group) and higher eggs yield (+4.69 eggs/hen), while the feed conversion ratio was calculated to be 271.26 g feed/egg (compared to control: 281.49 g/egg); casualties were 0.55% lower than for fowl reared in accordance with the management guide. The eggs produced by the hens under belated photostimulation proved to have better quality traits than those laid by the control group.

P194

Effect of progestagen pretreatment on fertility of ewes

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The duration of exposure to progestagens prior to artificial insemination (AI) or natural mating was examined in Karagouniko ewes during the transition to the breeding season. Progestagen intravaginal sponges were inserted in all ewes and remained *in situ* for either 14 days (Long exposure, groups LA and LM) or 6 days (Short exposure, groups SA and SM). All ewes received 400IU eCG and 53–55 h later intracervical AI was performed in groups LA (n = 79) and SA (n = 99) using fresh diluted semen (500×10^6 spermatozoa/ml). In groups LM (n = 70) and SM (n = 68) ten fertile rams were introduced for oestrus detection and natural mating. In the latter groups progesterone concentration was assessed in blood samples collected daily for 4 days starting at sponge removal (day 0). Pregnancy diagnosis was performed by ultrasonography 55 days later. On day 3 progesterone concentration was higher ($p < 0.05$) in group SM compared with LM. Onset of oestrus after sponge withdrawal did not differ between groups. Pregnancy rate was greater ($p < 0.05$) in group LA (40.5%) and SM (35.3%) compared with SA (25.3%) and LM (17.1%), respectively; litter size was 1.75, 1.58, 1.52 and 1.50, respectively. These results indicate that fertility rate after progestagen pretreatment depends on the method of insemination. Further experiments are underway to define a fixed-time AI protocol in Karagouniko ewes subjected to short progestagen pretreatment.

P195

Progestagen treatment associated with different doses of eCG to advance the breeding season in churra galega bragançana ewes

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To assess the effects of long-term progestagen treatment associated with different doses of eCG (equine Chorionic Gonadotropin) to advance the breeding season in Portuguese Churra Galega Bragançana ewes, we used a group of 41 females (two were later rejected) aged between 2 and 7 years. In mid May, all ewes were treated with an intravaginal sponge impregnated with 20 mg fluorogestone acetate (FGA) for 12 days. Half the ewes received an injection (i.m.) of 500 UI eCG and the other half an injection (i.m.) of 750 UI eCG at sponge removal. Blood samples were taken twice a week for two weeks before sponge insertion and daily for 5 days after eCG injection for progesterone determination. Four intact rams with harness marker were used to identify oestrus. Transrectal ultrasound scanning was performed for pregnancy diagnosis. In early May, 63.4% of all ewes had low progesterone values. Treatments resulted in 97.4% ewes in oestrus and in 89.7% ewes with corpora lutea (CL) ($\chi^2 = 60.6$; $p < 0.001$). The higher dose of eCG had a positive effect on oestrus (94.4% vs. 100.0%; $\chi^2 = 6.2$; $p < 0.05$) and pregnancy (61.1% vs. 81.0%; $\chi^2 = 9.7$; $p < 0.01$) and fertility ((number of lambing ewes/total number of ewes) × 100) (61.1% vs. 76.2%; $\chi^2 = 5.2$; $p < 0.05$) rates. The eCG dose had no effect in ewes with CL (88.9% vs. 90.5%; $\chi^2 = 0.053$; $p > 0.05$) nor on prolificacy (1.5 ± 0.8 vs. 1.7 ± 0.6; $p > 0.05$). In conclusion, long-term FGA treatment may be used to advance the breeding season in Churra Galega Bragançana ewes and the reproductive response depends on the eCG dose.

P196

The effect of elevated non esterified fatty acid concentrations during bovine oocyte-complex maturation on subsequent embryo viability

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Negative energy balance (NEB) in dairy cows leads to elevated non esterified fatty acid (NEFA) concentrations in follicular fluid which is associated with impaired oocyte development. This study focused on the effect of NEFA exposure during oocyte-complex (COC) maturation on COC amino acid turnover; a marker of early embryo viability. During serum-free maturation, 734 bovine COCs were exposed to (1)

physiological NEFA concentrations = CONTROL (150 μM of total NEFA, i.e. oleic, stearic & palmitic acid), (2) elevated stearic acid concentrations = HIGH SA (75 μM SA) or (3) elevated NEFA concentrations = HIGH COMBI (425 μM total NEFA). Zygotes were subsequently cultured in SOF + 5% FCS medium. Day 7 embryos were cultured singly for 24 h and the amino acid composition in spent medium measured non-invasively by HPLC. Maturation in HIGH COMBI and HIGH SA resulted in reduced blastocyst production (14.9 and 18.3%, respectively) compared to CONTROL (25.2%) ($p < 0.05$). HIGH COMBI embryos had higher amino acid consumption (factor 1.8) ($p < 0.01$), production (factor 1.7) ($p = 0.02$) and total turnover (factor 1.7) ($p < 0.01$) than CONTROL embryos. HIGH SA embryos had higher amino acid consumption (factor 1.9) ($p < 0.01$) and turnover (factor 1.6) ($p < 0.03$) compared to CONTROL EMBRYOS. This study demonstrates that embryos from COCs matured under NEB conditions are metabolically more active than CONTROL embryos. Up-regulated embryo metabolism has previously been associated with elevated DNA damage and low embryo viability.

P197

Relationship between Body Condition Score (BCS) and Reproductive Score (RS) on estrous cycle of standardbred maiden mares

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Standardbred maiden mares are generally in poor physical condition as a result of incorrect feeding management at the end of a racing career. The aim of this study was to investigate the influence of Body Condition Score (BCS: 1–5 scale) and Reproductive Score (RS: 1–5 scale) on ovarian activity of mares during the same breeding season. A total of 29 standardbred maiden mares (7 ± 2 year old) were divided in two groups. Group A (BCS ≥3.0; n = 12) and group B (BCS ≤2.5; n = 17). BCS was determined by visual and palpation inspection before breeding season starting 15 January; day 0. On day 17, RS was determined by gynaecological and ultrasound examination (Aloka SSD 500®, probe 5.0 MHz) of ovaries and uterine horns. On ovulation day (OD) the subjects exited from the study. Regression equations for the prediction of days to first seasonal ovulation (y) were created; the best equation was: $y = 1.74BCS^3 - 50.72BCS - 28.26RS + 242.16$ ($R^2 = 0.89$; SE = 11.6 day; $p < 0.01$). Highly significant relationships were shown in all mares between BCS and RS (0.81; $p < 0.01$) and between OD and BCS (−0.81; $p < 0.01$) and RS (−0.92; $p < 0.01$). In conclusion, combining BCS and RS allows a good prediction of OD in standardbred maiden mares.

P198

Influence of different follicle populations upon quality of equine oocytes

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In the last few years, horse breeders have become interested in embryo production *in vitro*, but only few foals have been born using this technology. While the rate of chromosomal maturation of *in vitro* cultured horse oocytes is high, fertilization rates *in vitro* are still not good. The aim of our study was to characterize cytoplasmic and structural changes of horse oocytes during the development of the follicle. Fourteen Mecklenburger Warmblood mares underwent repeated transvaginal ultrasound guided follicle aspiration. Aspiration sessions were performed 44 times during oestrus of the mares to obtain oocytes from preovulatory follicles and subordinate follicle populations and 79 times oocytes were recovered from growing, progressive follicle populations before a dominant follicle had developed. Every oocyte was analysed for cumulus morphology, chromatin configuration, glucose-6-phosphate dehydrogenase activity (G-6-PDH) as well as mitochondrial activity and aggregation. There was no impact of the follicle population upon the G-6-PDH-activity and no links between the G-6-PDH-activity and other parameters were seen. But progressive follicle populations contained more oocytes with a compact cumulus, fibrillar diplotene and higher mitochondrial activity, while subordinate follicle populations had more oocytes with an expanded cumulus, condensed diplotene and lower mitochondrial activity ($p < 0.05$). In conclusion, follicle population origin significantly influences oocyte quality.

P199

Evaluation of oocyte harvest by laparoscopy on biochemical and blood parameters of goats

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Twelve female goats had oocytes harvested four times at intervals of 15 days. Blood samples were collected in two periods: immediately before and 24 h (24 h) after laparoscopy for evaluation of urea and creatinine to assess renal function; aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyltransferase (GGT) to evaluate liver function; and *erythometric* and leukometric parameters. The average values of blood total cells were not different, regardless of period evaluated. There was an increase in total white blood cells 24 h after laparoscopy ($p < 0.01$). Hematocrit and hemoglobin values remained within the normal range for goats throughout. Mean values of AST increased ($p < 0.05$) 24 h after laparoscopy, possibly due to muscle tissue irritation caused by a single intramuscular injection. A reduction ($p < 0.01$) in ALP was observed at 24 h post-surgery but GGT did not show any change between periods. An increase in mean creatinine ($p < 0.01$) occurred 24 h after laparoscopy, possibly due to dehydration caused by with-hold of water (24 h) or due to surgical positioning. Values of urea decreased ($p < 0.01$) 24 h after laparoscopy possibly due to fasting time required to perform surgery safely (36 h). We concluded that laparoscopy did not unduly disturb renal and liver function nor red and white blood cells; and, thus, can be made four times at an interval of 15 days in goats.

P200

Equine spermatozoa recovered from epidydimes stored at 4°C for up to 48 h and cryopreserved can penetrate zona free bovine oocytes

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Equine spermatozoa recovered from epididymides stored at 4°C for up to 96 h after castration can be cryopreserved successfully with motility and viability higher than 60% (Vieira et al., 2010, In: Reproduction Technologies in Horses. EAAP Scientific Series). However, these sperm, despite being alive, might have lost some functional features that severely modify fertilizing capacity. In a first experiment, we confirmed that stallion epididymal frozen-thawed sperm had capacity to decondense heads in the bovine oocyte after ICSI. In a second experiment, we evaluated the sperm penetration capacity of frozen-thawed epididymal spermatozoa recovered from epididymes stored at 4°C for up to 48 h. Due to difficulties in developing a homologous equine IVF system, we used bovine oocytes free of zona pellucida as alternative model (Choi et al., 2003, Theriogenology). In a total of 185 oocytes, we detected effective penetration and male pronuclear formation in 7.6% without any influence of different days of storage (0, 24 and 48 h); and after 18 h of coculture, spermatozoa still had progressive motility. These preliminary data indicate that epididymal stallion spermatozoa could be used in the production of offspring using assisted reproductive technologies (AI, IVF or ICSI) and zona-free bovine oocytes could be a good method to evaluate stallion epididymal spermatozoa. Supported by Fundacion Seneca 08752/PI/08.

P201

Observer variability in cytological diagnosis of endometritis in mares

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During the breeding season, an efficient diagnosis of fertility problems in broodmares is essential. Although cytological examination of the endometrium has been effectively introduced into the routine broodmare practice, science-based evidence of this method is currently not available. The objective of this study was to determine inter-observer variability and the influence of different sampling techniques on the diagnosis of endometritis based on cytological observations. A total of 65 mares were included in this study. Uterine cytological samples were performed from each mare using a cytological brush and a swab. They were immediately smeared on a microscopic slide and stained with haematoxylin-eosin. In each sample, 300 cells were examined microscopically and proportion of polymorphonuclear cells (PMN) evaluated independently by two similar trained observers. Samples were classified as negative (<2% PMN) or positive (>2% PMN) for endometritis. For reliability of agreement between different observer Cohen's κ-statistics were used. Additionally Spearman's rank correlation was calculated to describe variability between investigators and between techniques. The inter-observer variability for two independent observer revealed poor agreement ($\kappa = 0.146$; $r_s = 0.207$; $p > 0.05$). A weak positive correlation could be determined for results of the two techniques ($r_s = 0.392$; $p < 0.05$). Due to the high inter-observer variability, care is required in interpretation of cytological results, but the study requires observations with more observers Additional techniques should also be considered to diagnose equine endometritis.

P202

Are bovine endometrial cells the source of lysophosphatidic acid?

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Lysophosphatidic acid (LPA) influences secretory functions and cell viability of bovine endometrium, strongly augmenting prostaglandin E2 secretion by stromal cells. The objective of the present study was to examine if cultured bovine endometrial epithelial and stromal cells were able to produce LPA. LPA concentration was measured in the medium and in cells. Protein expression (Western blotting) and mRNA transcript quantification (Real-time PCR) for LPA synthesizing enzymes (*Phospholipase A2 – PLA2, Autotaxin – AX)* were evaluated in cells. Moreover, different types of the receptors for LPA (*LPARs – LPAR1, LPAR2, LPAR3 and LPAR4)* were studied in cells. Both stromal and epithelial cells were able to produce LPA. LPA concentration in epithelial cells did not differ from LPA concentration in stromal cells ($p > 0.05$). However, higher mRNA and protein expressions for *AX* and *PLA2* occurred in stromal than epithelial cells ($p < 0.05$). The was also higher mRNA transcript abundance and protein expressions for all *LPARs* in stromal than epithelial cells with strong overexpression of *LPAR2* and *LPAR3* ($p < 0.05$). In conclusion, the present study demonstrated that LPA mainly produced by stromal cells may act as an autocrine factor in the bovine endometrium via active LPARs. Supported by MNiSW: DPN/DWM/MZ/5751/08/09.

P203

Involvement of Prostaglandins (PGs) and Oxytocin (OT) in the adverse effect of Polychlorinated Biphenyls (PCBs) on the contractions of bovine myometrium *in vitro*

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The aim of the study was to investigate the effect of PCBs, (recognized environmental pollutants) on uterine contractions. Uteri and oviducts were collected from cows at 1–5 day of the estrous cycle, while ovaries were obtained on 8–12 day after ovulation. Endometrial, myometrial, epithelia of oviduct, granulosa and luteal cells as well as myometrial strips were exposed (2–72 h) to PCBs (0.1–100 ng/ml): mixture (Ar1248), individual congeners (30, 77, 126 or 153) or hydroxy-metabolites (30 OH, 50 OH). Next, viability of cells, expression of genes involved in synthesis of PGF2α (COX-2, PGFS) in myometrium and OT (NP-I/OT, PGA) in ovarian cells, secretion of PGF2α and PGE2 from uterine and oviductal cells and OT from ovarian cells or force of myometrial strips contractions (basal and OT-stimulated), were measured. Some strips were also pre-incubated with indomethacin, which blocks PG synthesis. PCBs did not affect ($p > 0.05$) the viability of studied cells but did increase ($p < 0.05$) the expression of studied genes and the secretion of OT and PGF2α, and PGE2 from oviduct epithelium . PCBs also increased ($p < 0.05$) the basal and OT-stimulated force of myometrial strips contractions. Basal contractility was reduced ($p > 0.05$) when the synthesis of PGs was inhibited. We conclude that synthesis of PGF2α and OT, and further PG and OT secretion is a part of the mechanism by means of which PCBs may affect the force of myometrial contractions in cattle. Supported by grant N N311 006536.

P204

Effect of post-ai cephapirin on conception rate in repeat breeder cows

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We aimed to test the effect of Cephapirin administration after a combined protocol using progesterone-induced timed artificial insemination (TAI) and GnRH on conception rate in repeat breeder cows (RB). Holstein Freisian and Swedish Red cows (n = 331) had more than three services with no clinically abnormalities of the reproductive tract or estrous cycle were selected as RB in this study. All cows received an ear implant containing progesterone for 7 days between day 0 and 7 of Ovsynch protocol and all inseminated cows received GnRH administration 7 days after TAI (GnRH-Ear Implant-7d-PGF-56 h-GnRH-16–18 h-AI-7d-GnRH). Inseminated cows were randomly assigned into two groups; 160 cows received intrauterine Cephapirin administration 12 h after TAI and171 cows were untreated. Response to 1st GnRH of Ovsynch (67.5%; 108/160 vs. 69.6%; 119/171), synchronization rate (98.8%; 158/160 vs. 98.3%; 168/171), response to 3rd GnRH (88.1%; 141/160 vs. 83.6%; 143/171) and conception rate (43.8%; 70/160 vs. 46.2%; 79/171) were not different between cephapirin treated and control cows, respectively. In conclusion, post-insemination intrauterine cephapirin administration did not affect conception rates in repeat breeder cows.

P205

Influence of vitamin E and selenium on udder health – a meta-analysis

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Vitamin E and selenium are micro components in animal nutrition. They act as anti-oxidants supporting the oxidative stability of lipids in the cell membrane and thus protecting cells from perioxidative damage caused by free radical activity. By supplementing vitamin E and selenium, the incidence of placental retention, metritis and ovarian cysts in cattle can be reduced. The aim of the present study was to examine the relatively unknown effects of vitamin E and selenium supplementation (prior and post partum) on factors associated with udder health, using a meta-analysis. In total, 1843 publications were found related to this subject but only 19 were included in the meta-analysis. By supplementing vitamin E and selenium, the mean relative risk for mastitis was reduced by 34%. The effect was stronger (−40%) when supplementing with only selenium, in comparison vitamin E alone (−30%). On average, the somatic cell count was reduced by 24,000 cells per ml milk, when supplementing with vitamin E and selenium. Milk yield was increased (on average +1.0 kg milk per animal per day); the effect of vitamin E was considerably greater than that of selenium (on average +6.5 kg vs. +0.4 kg milk). A pre- and postpartal supplementation of vitamin E and selenium is, therefore, advisable.