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Poster Abstracts

P1

Effects of L-NAME (a nitricoxide synthase inhibitor) on *in vitro* maturation of sheep oocytes

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Nitricoxide (NO) is a biological signaling molecule that is generated by NO synthase (NOS) from L-arginine. It has been demonstrated that NO has a crucial role in maturation of mammalian oocytes. In this study, the importance of NO/NOS system in in-vitro maturation of ovine oocytes was investigated. Different concentrations (0.1, 1, 10 mM) of L-NAME, a NOS inhibitor, were used to evaluate the effect of the inhibition of NOS on cumulus expansion and meiotic resumption of sheep oocytes. The results were evaluated by chi-square test and $p < 0.05$ was considered significant. L-NAME in the highest concentration (10 mM) inhibited total cumulus expansion as compared to control ($p < 0.05$). It also suppressed the meiotic maturation and the extrusion of first polar body in a dose-dependent manner. The percentage of oocytes at MII stage was 26.47%, 40.72%, 63.78% and 75.16% for 10, 1, 0.1 mM and control group, respectively. To evaluate if the effect is reversible, 0.1 mM sodiumnitroprusside (SNP, a NO donor) was added in the treatment containing 10 mM L-NAME. The concomitant addition of L-NAME with SNP reversed the inhibitory effect of L-NMAE on cumulus expansion and meiotic maturation. These results indicate that NO/NOS system is involved in maturation of sheep oocytes.

P2

Total lymphocyte counts are affected by *Neospora canium* during the peripartum period in dairy cows

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Neosporosis is a protozoan-parasitic disease that affects most warm-blooded animals. Bovine neosporosis is characterised by high rates of mid-gestational abortion. Aiming to study the effect of neosporosis on the peripartum immunity, 612 blood samples of 85 Neospora-seronegative and 17 Neospora-seropositive high-producing dairy cows were collected every 2 weeks during the last 2 months of gestation and the 1st week postpartum (six samples/animal). Total and differential leukocyte counts were automatically analysed using HEMA-VET®. Blood counts were analysed by repeated measures GLM ANOVA in regard to Neospora-seropositivity. Lymphocytes in Neospora-seropositive animals were significantly lower than in Neospora-seronegative animals on the first sample at 8th pre-partum week but increased and showed a peak (reaching seronegative animal's level) on the 4th pre-partum week ($p = 0.049$; within-subject effect). Meanwhile, Lymphocytes in Neospora-seronegative cows showed a slight decline during the pre-partum period. No postpartum differences were found among the two groups. During late gestation, the maternal immune system is recovering from the immune-depression of the second gestation-term. Lymphocytes peak observed at the 4th pre-partum week in Neospora seropositive cows would suggest that the immune response was highly activated during a punctual period of time during the third term of gestation compared to seronegative cows.

P3

Production of cloned caprine embryos through cumuls cell-whole cell Intracytoplasmic injection and ear fibroblast cell-fusion approaches

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At present, research on the production of cloned caprine embryos and offspring at global level is still not as advanced compared to other domestic animals such as bovine and ovine. Thus there are many factors that can be refined to improve the success rate of cloned caprine embryo production. The objective of the study was to evaluate some of the factors affecting the production of cloned caprine embryos *in vitro*. The factors were combination of cloning technique, type of donor cell and as well as the *in vitro* culture (IVC) medium. Combinations investigated were caprine cumulus-cell-whole cell intracytoplasmic cell injection-mSOF medium (CC-WCICI-mSOF), caprine ear fibroblast cell-fusion-mSOF (EF-F-mSOF) and caprine ear fibroblast cell-fusion-KSOM (EF-F-KSOM). The *in vitro* developments of the reconstructed oocytes were evaluated from day 2 post-activation to day 9. The cleavage rate of reconstructed oocyte in experiment EF-F-KSOM and EF-F-mSOF were higher compared to CC-WCICI-mSOF (82.23%, 69.63% and 13.51% respectively). Of the reconstructed oocytes in experiment EF-F-KSOM 5.92% managed to develop up to blastocyst, while no blastocyst was obtained using EF-F-mSOF or CC-WCICI- mSOF. The combination of EF-F-KSOM factors enables the production of caprine blastocyst. In conclusion, it is possible to produce cloned caprine embryos using both approaches; however in this experiment, KSOM medium is favourable to produce caprine cloned blastocyst.

P4

Sperm motility patterns in andalusian donkey semen

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The aim of this study was to detect the presence of discrete sperm subpopulations in Andalusian donkey ejaculates using a computer-assisted sperm analysis (CASA) system and to establish individual differences in the sperm subpopulation structure. Eight motility descriptors were assessed by CASA in fifteen fresh ejaculates from five donkeys. The data matrix consisted of 17186 observations. The FASTCLUS clustering procedure was used to separate the spermatozoa into their different motility subpopulations. As expected, four motile sperm subpopulations were identified. Subpopulation one consisting of slow and non-linear spermatozoa (5568 spermatozoa, 32.4%), Subpopulation two consisting of slow but linear spermatozoa (3111 spermatozoa, 18.1%), Subpopulation three consisting of rapid but non-linear spermatozoa (1136 spermatozoa, 6.6%) and Subpopulation four consisting of rapid and linear spermatozoa (7371 spermatozoa, 42.9%). Significant differences ($p < 0.001$) in the distribution of these subpopulations were seen in fresh ejaculates of five sampled donkeys. In conclusion, four well-defined motile sperm subpopulations were identified in the ejaculate of the Andalusian donkey. The relationship between the distribution of the sperm subpopulations and individual donkey shows that the spermatozoa of each have different motility patterns. Therefore, the study of discrete subpopulations of motile spermatozoa could lead to a substantial increase in information acquired during donkey semen analysis.

P5

Expression of sperm adhesion molecule-1 (SPAM1) in cow and sow oviduct

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The sperm adhesion molecule 1 (SPAM1) is a sperm surface glycoprotein with multiple roles in mammalian fertilisation. It has a hyaluronidase activity and also has a zona pellucida binding activity. The gene for the SPAM-1 has been known to be male reproductive tract-specific, however, studies in mice have reported that it is also synthesised in the female genital tract. The aim of this study was to analyse the presence of SPAM1 gene expression in the bovine and porcine oviduct. Oviducts were obtained from cows and sows from a slaughterhouse. Estrous cycle was detected according to the ovarian morphology and only late follicular phase oviducts were included in this study. Total RNA was obtained by scraping ampulla and isthmus-ampullar junction mucosa. The cDNA was synthesised and it was used as a template for polymerase chain amplifications using primers based on SPAM1 sequence from bovine (Fw: cctattacataccaaatgacag, Rev: cttgcatgaaactctttcctg) and porcine species (Fw: ctaacagacttggctactatc, Rev: gctgaaccaactcaatagac). Fragments of 210 pb and 243 pb were obtained in cattle and pig, respectively. The amplified products revealed a 80% and 72% sequence identity to human in cattle and pig, respectively. The results of this study reveal that the SPAM1 mRNA is expressed in cattle and pig oviducts. Further studies are needed to investigate the role of this protein in the oviduct during the fertilization. *This study was supported by MICINN-FEDER (AGL2009-12512-C02-01-02).*

P6

Effects of different superovulation and synchronization protocols on the ovarian response and embryo yield in angora goats

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This study aimed to compare effects of different superovulation and synchronisation protocols on the ovarian response and embryo yield during and out of breeding season in Angora goats. Sixty-nine Angora goats were used in this study. They were allocated to two groups. In the first group the oestrous cycles were synchronised using intravaginal CIDR (containing 0.3 g progesterone) for a period of 14 days. On day 9 following CIDR insertion, all nanny goats in this group were injected with PGF2α and FSH for superovulation (GI, $n = 49$ traditional protocol, in breeding season). FSH treatment was divided into six decreasing doses given twice daily (2.5, 2.5, 1.5, 1.5, 1.0, 1.0 ml, total dose of 700 IU). In the second group the cycles were synchronised with CIDR for 5 days. At the CIDR insertion, nannies were treated with PGF2α. eCG was given at CIDR withdrawal to synchronise ovulation and 36 h after CIDR withdrawal one dose of a GnRH analogue was injected to ensure ovulation (GII, $n = 15$, day 0 protocol, out of breeding season). Ovarian responses were determined by laparotomy 6.5 days after insemination and the number of CL was recorded. The difference in the mean number of CL's between GI (6.73 ± 0.76) and GII (4.66 ± 0.90) were not statistically different (chi-square test). The mean number of embryos recovered was significantly lower in GII ($p < 0.05$) than GI (chi square test). To conclude, day 0 protocol resulted in poor embryo yield compared to the traditional superovulatory protocol.

P7

Suitability of scrotal ultrasonography (US) for assessment of current and future semen quality in the ram

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Breeding soundness evaluation (BSE) is the primary assessment of reproductive potential in male animals. However, BSE is no more than a "snap shot' and it does not predict future semen quality in rams. Computerized analysis (CA) of US images provides information on histophysiological changes in male reproductive organs. We hypothesised that (i) present semen parameters correlate with US characteristics of the cauda

epididymis and (ii) testicular US images predict future semen quality. Six adult rams underwent BSE and scrotal US 60 d apart, during the breeding and non-breeding seasons. Image analysis utilized commercially available analytical software (Image ProPlus®; Media Cybernetics Inc., San Diego, CA, USA). An inverse correlation was found between numerical pixel values of the epididymes and percentage (%) of sperm with normal morphology ($r = -0.46$, $p < 0.05$). Pixel heterogeneity (standard deviation of pixel values, PH) correlated negatively with % of sperm with normal morphology ($r = -0.42$, $p < 0.05$) and directly with % of sperm with abnormal tails ($r = 0.43$, $p < 0.05$). PH of testicular parenchyma obtained approximately 60 day prior to semen evaluation inversely correlated with % of sperm with normal morphology ($r = -0.73$, $p < 0.01$) and sperm progressive motility ($r = -0.76$, $p < 0.01$), and directly with % of sperm with abnormal tails ($r = 0.72$, $p < 0.01$) and loose heads ($r = 0.79$, $p < 0.01$). We concluded that CA of epididymal and testicular echotexture in the ram was a valuable method for determining certain current and future semen parameters, respectively.

P8

Assessment of insulin-like growth factor- I as an indicator to predict endometritis and cystic ovarian disease in early postpartum dairy cows

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This study was carried out to assess IGF-I, non-esterified fatty acids (NEFA), beta hydroxybutyrate (BHB) and glucose concentrations during the pre- and post-partum period of dairy cows in normal condition, cows with endometritis puerperalis and cystic ovarian disease. The study was conducted on 87 lactating Holstein cows in Shiraz, Iran, fed the same diet as total mixed ration. Blood samples were collected every 2 weeks from 2 weeks before until 6 weeks after calving for IGF-I, non-esterified fatty acids (NEFA), beta hydroxybutyrate (BHB) and glucose analysis. Serum IGF-I was measured using ELISA kits (UK immunodiagnostic systems Ltd, IDS). Two, 4 and 6 weeks after calving, palpation of the reproductive tract was performed. At the same time, cows were first inspected for the presence of fresh abnormal discharge on the vulva, perineum, or tail and if discharge was not visible externally, a vaginal examination took place. Following palpation, transrectal ultra-sonography using a rectal linear probe (real time B-mode linear array scanner with a 5 MHz transducer, 500 V, Ami, Canada) was also performed to confirm palpation per rectum findings, and cyst diagnosis. Ovarian structures (follicle, CL and cyst) were scanned and measured with callipers. The proc mixed procedure of SAS (2005) sofware was used for analysis. The different groups were compared using duncan's multiple range test. Pre-partum IGF-I concentration was significantly lower (65.82 ± 21.19 arbitary units [AU]) in cows that developed cystic ovaries early postpartum than non cystic cows (82.81 ± 19.32 AU). Cows with clinical endometritis, had significantly lower concentration (70.31 ± 22.07 AU vs. 86.44 ± 16.75 AU) of IGF-I than normal cows. Parturition-first artificial insemination (AI) interval and ovarian cycle resumption after calving, was significantly shorter for cows without clinical endometritis than cows with clinical endometritis. In conclusion, among all metabolites that were measured in this study, IGF-I concentration was the main factor associated with occurrence of some of reproductive diseases after calving (endometritis and cystic ovarian disease) and it was a notable feature of the current study.

P9

Levels of free thyroid hormones in dairy cattle from estrus to pregnancy diagnosis

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Thyroid hormones play an important role in reproduction. However, few studies have been published concerning serum variations of free thyroid hormones during different phases of cow reproductive activity. The aim of this study was to evaluate in dairy cattle the plasma level of free T3 (fT3) and free T4 (fT4) during oestrus cycle and early pregnancy. The study was carried out in southern Italy on 11 Friesian cows (5 ± 1 y.o.). After gynaecological examination to assess the state of ovaries and uterus, a blood sample from each subject was taken the day before oestrus (T0) and at the appearing of the early clinical signs of oestrus (restlessness, female copulatory behavior and mucous discharge from the vulva) (T1). The subsequent blood samples were carried out at the time of A.I. (T2) and at 2 (T3), 7 (T4), 18 (T5) days later; the last sample was recovered at the first diagnosis of pregnancy (45 days – T6). Blood, collected in 10 ml vacutainer tubes, was centrifuged ($1150 \times g$ for 10') at 4°C; the obtained serum frozen in two aliquots. fT3 and fT4 was evaluated by enzyme immunoassay technique (EIA WELL®; RADIM, Italy) in each sample. In our results, changes in the concentration of fT4 and fT3 were almost overlapping. This indicates a complementarity between the production of fT4 and its enzymatic activity. The peak of fT4 was significantly different when comparing T6 with T0 and T1 ($p < 0.01$; paried student *t*-test). The T6 value, at 45 day of gestation, could be explained by the metabolic necessity of the developing conceptus which could act as a signal for the increased production of fT4 and its subsequent conversion to fT3 by the deiodinases.

P10

The effect of oxytocin and cloprostenol administration via umbilical artery on fetal membrane removal in cows with dystocia

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The aim of this clinical study was to investigate the effects of oxytocin and cloprostenol application via umbilical artery, which has been reported previously to be an appropriate way to administer uterotonics, on the time and ratio of fetal membrane removal in cows with dystocia immediately after parturition. Additionally, some serum biochemical parameters were determined in the cows. This study was performed on 60 cows with dystocia. All the cows were divided into three equal subgroups randomly. The first group was injected with 100 IU oxytocin, while the second group was treated with 0.15 mg cloprostenol and the third group with 10 ml 0.9% NaCl via intraumbilical artery (vessel with thick wall) immediately after dystocia. Blood samples (10 ml) were obtained at random by vein puncture from the jugular vein in 10 cows from each group immediately after parturition and before the

intraumbilical artery injections. No significant differences were determined between the groups with regard to the values of AST, ALP, CK, total protein and bilirubin, albumin, glucose, Ca, Na and K ($p > 0.05$). LDH and GGT level in the oxytocin group was significantly lower ($p < 0.05$) and significantly higher ($p < 0.05$) than the other groups, respectively. The metabolic profiles of cows indicates that all groups are similar to be biochemical parameters. Time and ratio of foetal membrane removal were not different between the groups ($p > 0.05$). In conclusion, the present study suggests that the administration of the agents used other uterotonic and enzyme combinations via umbilical cord should be investigated further regarding their effects on the fertility, time and ratio of foetal membrane removal in cows with normal parturition and dystocia at herd level.

P11

Soya-lecithin in extender improves the freezability and fertility of buffalo (*Bubalus bubalis*) bull spermatozoa

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Egg yolk is routinely used as a cryoprotectant in semen extenders. However, it may contain cryoprotective antagonists and there are hygienic risks associated with its use. Proteins of plant origin, like soya lecithin, lack these hazards. The aim of this study was to use soya lecithin as a cryoprotectant in extender and investigate its effects on *in vitro* quality (experiment 1) and *in vivo* fertility (expt. 2) of buffalo semen. In expt. 1, semen from five buffalo bulls was frozen in tris-citric extender containing either 2.5, 5.0, 10, or 15% soya-lecithin or 20% egg yolk; assessed for motility, plasma membrane integrity, viability and analysed by ANOVA. After dilution and equilibration, the values for motility, plasma membrane integrity and viability did not differ between egg yolk and soya lecithin, whereas after thawing all semen quality parameters were higher ($p \leq 0.05$) in the extender containing 10% soya lecithin compared to all the other semen extenders. In expt. 2, semen from two buffalo bulls was frozen in tris-citric extender containing either 10% soya-lecithin or 20% egg yolk. Data were analysed by chi-square. Based on 400 inseminations (200/group), fertility was higher ($p \leq 0.05$) in buffaloes inseminated with semen containing 10% soya-lecithin (56%) compared to 20% egg yolk (41.5%). The results suggest that 10% soya lecithin in extender improves the freezability and fertility of buffalo bull spermatozoa and can be used as an alternative to egg yolk in cryopreservation of buffalo semen.

P12

A case of mammary gland carcinosarcoma observed with segmental aplasia of the left uterine horn in a pekingese bitch

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This is a case report on a 9 year old Pekingese bitch with complaint of mammary gland mass in the left fifth gland that was presented to Obstetrics and Gynecology Clinic of Ankara University Faculty of Veterinary Medicine. Radical bilateral mastectomy was performed followed by an ovariohysterectomy. During the surgical procedure abnormality of one uterine horn was detected. The oval-shaped mass at the caudal of left inguinal mammary gland (fifth lobe) was $1.5 \times 0.5 \times 0.5$ cm in diameter, elastic in consistency and dark red homogeneous at cross-sectional view. Neoplastic epithelial cells, embryonic connective tissue and bone tissue with large areas of haemorrhage and necrosis were found in histopathological examination. Location areas of inflammation were observed with some neutrophils. Besides the carcinosarcoma defined mass, the ovaries and uterine tissue were removed via ovariohysterectomy. The removed tissue, weighing 25 g, was examined macro and microscopically. The right uterine horn was found to have bleeding mucosa while the left uterine horn had the formof a thin rope, with a lumen that was not very clear and severe hypoplasia. The right uterine horn contained tunica muscularis, lamina propria and provided broad haemmorhagic areas. The left horn consisted of tunica muscularis and serosa. Formation of medium-sized arteries and capillaries were also evident. This case was defined as segmental aplasia. Segmental aplasia of uterine horn, which is defined as mullerian duct defect, is oftenly observed in cows as white heifer disease, but also seen in ewes and sows. However in the bitch this abnormality is established incidentally during routine ovariohysterectomy. This is the first case of segmental aplasia of the left uterine horn observed with a mammary gland carcinoma in a single mammary gland.

P13

Investigation of kappa-casein, growth hormone and prolactin genes polymorphism in Turkish native cattle breeds by PCR-RFLP

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Kappa-casein (CSN3), growth hormone (GH1) and prolactin (PRL) genes are related to milk production and are also responsible for the synthesis of the major components of milk. These genes are important candidate genetic markers for growth and milk traits in livestock. There are no data on Turkish cattle breeds on these gene polymorphisms. In this study the main goal was to study DNA-polymorphism of the genes in Anatolian Black (AB) ($n = 44$), Turkish Gray (TG) ($n = 43$), East Anatolian Red (EAR) ($n = 44$), South Anatolian Red (SAR) ($n = 40$), Holstein ($n = 44$) and Brown Swiss (BS) ($n = 44$). A total of 259 cattle were genotyped for the CSN3-*Hind*III, GH1-*Alu*I and PRL-*Rsa*I polymorphism by the PCR-RFLP. The within breed variation, the deviation from Hardy-Weinberg equilibrium value (F_{IS}), allele frequencies, degree of genetic differentiation between populations (F_{ST}), and the heterozygosities (as gene variation indicates) were calculated using the Genetix software program version 4.05. The F_{ST} (Fixation index (F_{ST}) is a measure of population differentiation) value was calculated 0.053 and significant ($p < 0.001$). According to Nei's genetic distance values, SAR and EAR were found in most different breeds and this difference was significant. This study is the first report on CSN3, GH1 and PRL genotyping of Turkish cattle breeds. The results presented here demonstrate that these genes may be considered as a marker for dairy traits in cattle.

P14

Turkish shepherd dog akbash and some reproductive characteristics

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The purpose of this study is to introduce Turkish Shepherd Dog Akbash and its reproductive characteristics. Akbash was bred intensively in the past in Turkey. But now these white, graceful dogs with a well developed guardian instinct are bred only by a few people because of decreased sheep breeding. The first Akbash Dog arrived from Turkey to the U.S. in 1978. Observation and then the breeding of 'test' litters convinced the owner that this white dog was a regional breed that had developed unique, consistently inherited behavior, disposition, and appearance. About 5000 Akbash are told to exist in North America, though the number is estimated only as hundreds in its homeland where some breeding centers aimed to protect genetic characteristics of this dog are very new.Some knowledge related to Akbash were reviewed and about 1.5 years' retrospective reproductive records of 29 bitches of Akbash Dogs' Protection and Breeding Center were evaluated. As to the records, average (n = 29) puberty age was 14.41 ± 3.62 months. Estrus appeared mostly in May and June and then in December but it could be seen also every month (n = 42). Duration of estrus (n = 28) and interval from first mating in estrus to delivery (n = 17) were 7.60 ± 1.89 and 65.11 ± 1.26 days. Average litter sizes in first (n = 23), second (n = 11) and third (n = 7) delivery were 6.56 ± 1.70 (a), 7.3 ± 1.41 (b) and 5.58 ± 0.69 (c) respectively [a,b: $p < 0.05$; a,c: $p > 0.05$; b,c: $p < 0.05$]. In conclusion, Akbash, as one of the many white shepherd dog breeds, is needed to be protected because of its own genetic characteristics.

P15

Mechanism of antioxidant protection provided by GSH and vitamin e on cryopreserved semen in dogs

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The use of artificial insemination in dogs is potentially impaired by semen quality after cryopreservation, which could be due to the action of reactive oxygen species (ROS). An alternative to avoid the attack of ROS, is antioxidant treatment. The aim of this study was to evaluate the effect of reduced glutathione (GSH) and Vitamin E on the quality of cryopreserved dog sperm. Semen samples of twelve dogs were cryopreserved using Tris-egg yolk-citrate-glicerol extenders suplemented with 0, 1, 5 and 10 mM of GSH and 1, 5, 10 mM of Vitamin E. After thawing samples were evaluated for motility, vigor, integrity of membrane and acrosome, mitochondrial activity and as an index of lipid peroxidation and the TBARS assay. Statistical analyses were performed using SAS system for Windows (SAS Institute Inc). Results showed that GSH played a protective role on membrane integrity, when compared to the control (21 ± 2.84 and 6.21 ± 1.16). Vitamin E showed better results on mitochondrial activity if compared with control group. Samples treated with GSH showed a negative correlation between TBARS and membrane integrity ($r = -0.42$); samples treated with Vitamin E showed a positive correlation between TBARS and impaired mitochondrial activity ($r = 0.39$). Results indicate that GSH action was limited to the extracellular environment and that Vitamin E promoted an intracellular protection.

P16

Changes in the expression of toll-like receptors in the chicken testis during growth and in response to salmonella infection

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Infertility in male broilers is a major concern in the poultry industry. Bacteria species including Salmonella spp., have been implicated as causative agents of orchitis, epididymitis, and epididymo-orchitis in roosters, resulting in impaired fertility. Thus, protection of chicken male reproductive organs from pathogens is essential for maintaining their normal reproductive function. During the last years, Toll-like receptors (TLRs) have been identified as one of the key components of the innate immunity in vertebrate species and have been reported to be expressed in the reproductive organs in various female species. However, mechanisms of antimicrobial protection of male reproductive organs mediated by TLRs are poorly understood. The objectives of this study were to determine the expression of the entire family of the ten chicken TLR genes in the chicken testis, to investigate whether sexual maturation affects their testicular mRNA abundance and to determine whether Salmonella enteritidis (SE) infection alters their expression levels. RNA was extracted from the testis of healthy prepubertal, sexually mature and aged birds, and from sexually mature SE infected birds. RT-PCR analysis revealed that all TLRs, apart from TLR1-1, were expressed in the chicken testis. Quantitative real-time PCR analysis revealed that the testicular mRNA abundance of TLRs was developmentally regulated with respect to sexual maturation, while SE infection resulted in a significant induction of TLR2-1, 4, 5, 15 and 21 in the testis of sexually mature birds compared to healthy birds of the same age. These findings provide strong evidence to suggest a key role of TLRs in the protection of the chicken testis against Salmonella colonization.

P17

An atypical *Bacillus anthracis* infection in a bull causing a symmetrically swollen scrotal SAC – a potential health hazard for veterinary surgeons

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Bacillus anthracis infecting cattle is usually identified based on the typical symptoms: sudden death etc.*B. anthracis* causing atypical symptoms may remain undiagnosed and represent a potential health hazard to veterinary surgeons. This study describes an unusual case of anthrax diagnosed in a Bos taurus bull suffering from fever, in appetite and a symmetrically

swollen scrotal sac. The bull was treated with penicillin which initially cured the fever but not the swollen scrotal sac. Before the intended therapeutically castration, a punctuate consisting of 10 ml fluid collected into a vial from the scrotal sac was cultivated on blood agar at 37°C. After 24 h an almost pure culture of a completely non-hemolytic *Bacillus cereus* like bacteria was obtained. The strain was identified as *Bacillus anthracis* using Ba specific primers by the Finnish Food safety authority (EVIRA). After the diagnosis the bull was euthanized, the personnel treated with prophylactic antibiotics and the farm and clinic were disinfected. Later it was found out that 5 years earlier a cow on the same farm died suddenly and the reason was confirmed as anthrax. This is the first reported anthrax case expressed as a scrotal swelling associated with high fever responding to penicillin treatment but the fever relapsed after the end of the 5 day penicillin treatment.

P18

A novel mutation in the porcine TEX14 gene causes non-obstructive azoospermia and disruption of the intercellular bridges between the germ line cells, which are necessary for fertility in males

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Nine sterile azoospermic breeding boars with spermatogenic arrest at the spermatocyte stage were subjected for genetic studies. The animal material included nine affected Finnish Yorkshire boars and 21 unaffected control boars. Genotyping was conducted using the PorcineSNP60 BeadChip (Illumina Ltd, San Diego, CA, USA). The software package Plink was used for the association test. The Manhattan and the linkage disequilibrium plots and the most plausible haplotypes were produced with Haploview software. The most probable location for the defect causing mutation was on chromosome 12 within 32.6 Mb and 34.1 Mb. A good candidate gene was found at the position 32.8–32.9 Mb. Following gene sequencing an insertion in exon 27 was identified in the TEX14 gene. To evaluate the frequency of carriers among the AI boars, all Yorkshire AI boars used in AI during a week in October 2010 were studied. Fifteen percent of the boars were heterozygous carriers. In mice knockout studies it has been shown that the TEX14 gene is necessary for male fertility, but not for female fertility. In this study we describe for the first time a natural mutation in the TEX14 gene causing azoospermia. A gene test is ready for use to eliminate carrier AI-boars.

P19

Manual amnion rupture: an open window to reduce the twinning rate in dairy cattle

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Embryo reduction emerges as a chance to prevent the negative effects of twining in dairy herds. The risk of pregnancy loss after embryo reduction might depend on the time at which the reduction is performed. The aim of this study was to determine the optimum time (i.e. day post-conception with minimal risk of pregnancy loss) to conduct embryo reduction in cows with unilateral and bilateral twin pregnancies. On day 28–41 of gestation embryo reduction was conducted in 73 lactating cows bearing unilateral (n = 37) and bilateral (n = 36) twins by pressuring transrectally the amniotic vesicle of an embryo between the thumb and the transducer to cause its rupture. Pregnancy loss before day 60 was recorded in 23 unilateral and eight bilateral twin pregnancies (62.2% vs. 22.2%, χ^2 $p < 0.01$). Time for embryo reduction (mean ± SD) did not differ between cows that lost or maintained pregnancy neither in unilateral (33.4 ± 3.1 vs. 33.4 ± 3.7 days, Mann–Whitney $p = 0.31$) nor bilateral twin pregnancies (33.8 ± 3.7 vs. 33.9 ± 4.5 days, Mann–Whitney $p = 0.98$). Logistic regression indicated that gestation laterality significantly affected pregnancy loss. However, no significant effects of the time when embryo reduction is conducted or the interaction time by gestation laterality were found. The results show that embryo reduction may be conducted in both unilateral and bilateral twin pregnancies at any time between days 28 and 41 without differences on subsequent pregnancy maintenance.

P20

Expression of nitric oxide synthases in porcine uterus during pregnancy

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The exact mechanism of foetal losses during pregnancy in pigs is unknown. Nitric oxide is a key factor involved in myometrial smooth muscle relaxation and also regulation of adaptation-related changes in uterine and placental blood vessels. This study investigated the localisation and expression of endothelial (eNOS) and inducible (iNOS) nitric oxide synthase isoforms in porcine uterus from day 20 to 90 of pregnancy. Endometrial, myometrial and foetal membrane samples were collected on days 20, 30, 40, 60, 75 and 90 of pregnancy. The statistical analysis was carried out by one-way ANOVA followed by Dunett's *post-hoc* test (GraphPad PRISM). Western blot was used to determine protein expression and immunohistochemistry for cellular localization. Immuno staining of eNOS was found in trophoblast and endothelium of placental and maternal blood vessels, in luminal epithelium and endometrial glands. Stronger iNOS staining compared to eNOS was found in placental and endometrial blood vessels and in trophoblast. Expression of iNOS protein in endometrium and myometrium increased ($p < 0.05$) from day 40 to 90 of pregnancy both in placental and interplacental zones. On the contrary, a significant decrease ($p < 0.001$) in eNOS and iNOS protein level was found in foetal membranes on days 40 and 60 of pregnancy (respectively) when compared to remaining studied days. The gradual increase in eNOS expression during mid gestation may indicate a synergistic, supportive effect on uterine vascular and myometrial contractility in pigs affecting the maintenance of uterine quiescence. The dynamic increase in iNOS expression after day 60 of gestation suggests its contribution to uterine NO generation during the second half of pregnancy.

P21

Testing treatments for enhancing the refrigerated storage of red deer semen

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Long-term conservation of cooled semen allows to delay insemination, and it is of interest for assisted reproductive techniques such as sperm sexing or for storing semen from bad-freezer males. In this study, we aimed at testing different refrigeration conditions with semen obtained from red deer. Semen was obtained from four adult males during the breeding season (November). Stags were anaesthetized (Xylazine + ketamine) and electroejaculated. Semen was diluted with TES-Tris-Fructose, 20% egg yolk, and split in six treatments, packing the samples in straws: control, straw without air chamber, Trolox 1 mM, GSH 1 mM, 1% gelatin and 3.8% Oxyrase®. Semen quality was evaluated after 0, 24, 48 and 72 h at 5°C. Total motility (TM) and average path velocity (VAP) were assessed by CASA. Viability and acrosome status were assessed by flow citometry using propidium iodide and PNA-FITC. The effects of time and treatments were analyzed using linear mixed-effect models. Time decreased viability and intact acrosomes (0 h: 73 ± 2% and 88 ± 2%; 72 h: 67 ± 3% and 83 ± 2%; $p < 0.01$), but not motility (TM: 52 ± 1%; VAP: 61 ± 2 μm/s). Treatments yielded no significant effects, although Oxyrase tended to improve viability and acrosomal status at 72 h ($p = 0.1$). Our results suggest a negligible effect of the treatments on the quality of deer semen, although Oxyrase should be further investigated. *Supported by Junta de Castilla y León (LE019A10-2) and Ramón y Cajal program (RYC-2008-02560, MICINN, Spain).*

P22

Evaluating the simulated site of semen deposition by ultrasonography in training bovine artificial insemination

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The semen deposition site is critical for conception rates following AI in cattle. Ultrasonography has been used to identify deposition of different fluids loaded in straws when training AI-technique. A disadvantage is that fluid will disperse after deposition making evaluation uncertain. Moreover, it is questionable if deposition of media in the uterus of food-producing animals is allowed. Insemination of recoverable metal devices would be an alternative to overcome these problems. Beal et al. (1989, JDS, 72:2198-2202) reported a trial inseminating a brass bead tied to a nylon line, identified by ultrasonography. We modified this approach by using a metal rod tied to a nylon line fitted into an ordinary insemination gun. The rod was inseminated by a skilled inseminator randomly in the uterine horn, corpus or in the cervix, blindly for the ultrasound operator, into ten uteri *in vitro* and into ten uteri of live cows. The position of the rod was identified by the operator using a portable ultrasound machine. Location of the rod was noted by the inseminator and the operator independently and compared. Placing in a uterine horn near the bifurcation or in corpus was regarded as correct site of insemination. Sensitivity and specificity for the identification of the position of the rod was 100% in the vitro trial and 50 and 80% respectively *in vivo*. The method allows repeated deposition in live cows but demands some adjustments and subsequent validation in a larger number of animals before the use as training tool for inseminators.

P23

The molecular machinery of the autophagy pathway is present in mammalian spermatozoa and could be playing an important role in these cells

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Cellular mechanisms involved in the response to stress in mammalian sperm are far from being completely understood. Autophagy is the bulk degradation of proteins and organelles. It is essential for the maintenance of homeostasis, viability, differentiation and development in somatic cells. The aim was to identify the molecular constituents of autophagy and to investigate the activation of this pathway in mammalian sperm. Men, dog, stallion and boar sperm were lysed and analyzed by Western blotting, indirect immunofluorescence (IF) and confocal microscopy using specific commercial antibodies (Ab). Using three different Ab, the isoforms A and B of LC3 (a main constituent of the autophagosome) were found in all the sperm lysates. Similar results were obtained with the class III of PI3K, which is also involved in autophagy in somatic cells. IF of these proteins in stallion sperm showed diffuse fluorescence, being more intense in the acrosome and the mid-piece. By using LC3-II as a marker of autophagy, we also detected an increase in the turnover of this pathway in response to 8Br-cAMP (1 mM, 3 h) and an inhibition of the pathway in cells incubated with H2O2 (100 μM, 1 h). These results show that LC3A, LC3B and Class III PI3K are expressed in mammalian spermatozoa mainly associated to the mid-piece and the acrosome. Interestingly, the autophagy pathway is regulated by physiological stimuli as well as by oxidative stress, indicating that it is not a reminiscence of spermatogenesis and could be involved in the regulation of important sperm functions. Supported by grants AGL2010-20758 and RZ2008-00018-00-00 by MICINN and by Junta Extremadura-FEDER (grant GR10010)

P24

In vitro maturation of canine oocytes in hormonal medium and spermatozoa

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The aim of this work was to study the influence of the addition of spermatozoa to the culture medium on maturation rates of canine oocytes incubated for up 72 h. A total of 171 COC (Cumulus-oocyte complexes) grade 1 were obtained from anestrous bitches and randomly allocated in two groups: G1

(n = 90) -oocytes cultured in TCM-199 medium with hormones (10 UI/ml hCG + 1 μg/ml progesterone + 1 μg/ml estradiol) for 72 h at 38°C, 5% CO2 in air; G2 (n = 81) -oocytes cultured in TCM-199 with hormones for 48 h and with hormones + spermatozoa for additional 24 h. Spermatozoa were added at a concentration of 5 × 106 sperm/ml. At the end of maturation, oocytes were denuded within 0.2% hyaluronidase solution by repeated pipetting and then, were stained with Hoechst 33 342 for evaluation of meiotic configuration. Statistical analysis was performed using the Chi-square test. The results demonstrated that even though oocytes co-cultured with spermatozoa showed a greater number of MI (8.6%, 7/81) and MII (7.4%; 6/81) stages compared to those cultured only with hormones (MI = 13.3%, 12/90; MII = 3.3%, 3/90), there was no difference between the groups. However, the percentage of degenerated oocytes has significantly increased in G2 (31%, 25/81) compared to G1 (18.8%, 17/90). In conclusion, the addition of sperm to the media had no benefit on retaken of meiosis or MII stage rates although further studies with a greater number of oocytes are needed.

P25

Investigation of semen parameters, freezability and testosteron levels in tushin rams during non-breeding season

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The aim of this study was to investigate changes of semen parameteres and testosteron levels and also freezability of Tushin Ram semen during non-breeding season. For this aim, semen was collected from tree Tushin rams by artifial vagina twice a week during non-breeding season (February, March, April and May = FMAM, Spring). Jugular blood samples were collected and blood plasma was obtained from each rams in order to determine testosterone levels twice a month during the non-breeding season. Data were analyzed by least squares analysis of variance using the General Linear Model procedure. Mean semen volume during FMAM was 1.3, 1.4, 1.3 and 1.2 ml, respectively ($p > 0.05$). Numbers of spermatozoa per ml during FMAM were 4.1×10^9, 4.1×10^9, 3.7×10^9 and 3.0×10^9 spermatozoa/ml, respectively ($p < 0.05$). Total spermatozoa per ejaculate during FMAM were 5.5×10^9, 5.8×10^9, 4.0×10^9 and 3.6×10^9, respectively ($p < 0.01$; except between Feb and March $p > 0.05$). Percentage of progressive motility during FMAM were 77.8%, 78.4%, 77.5% and 82.3%, respectively ($p > 0.05$). Testosteron levels in blood during FMAM were 1.9, 5.9, 10.5 and 3.0 ng/ml, respectively ($p < 0.05$). After thawing progressive motility (%) during FMAM were 23.75%, 34.54%, 27.70% and 35.00%, respectively ($p < 0.05$, except between March and May $p > 0.05$). In conclusion, native semen parameters of Tushin rams were detrimentally affected through non-breeding season (from Feb to May), while testosterone levels showed fluctuation during non-breeding season. Moreover, post-thaw progressive motility was considered, worst freezability was recorded in February although it was determined that Tushin ram semen could be cryopreserved during non-breeding season.

P26

Long-term flushing improves embryo quality-gene expression patterns in rabbit model

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Our aim was to study whether flushing effects embryo development and quality in rabbits. Does were fed a commercial diet (NANTA, Spain) (A; n = 12) or supplemented with 2% propylene glycol (PG) in water during lactation (29 days, B; n = 12) or during late pregnancy plus lactation (36 days, C; n = 12). They were inseminated and ovulation was induced with 1 μg Buserelin at Day 25 *postpartum*. Embryos were recovered 84 h later by laparotomy. Ovulation rate (OR) and viable embryos (VE) were recorded. In blastocysts mRNA transcripts was quantified by qRT-PCR to contrast relative levels of histone *H2AFZ* and genes related with glucose metabolism: solute carrier family 2 member 4 *(SLC2A4)*; insulin receptor *(IR)*; insulin-like growth factor receptors 1 *(IGFR1)* and 2 (imprinted gene: *IGFR2)*; oxidative stress: prostaglandin G/H synthase 2 *(PTGS2)*, nitric oxide synthase 3 *(eNOS)*, superoxide dismutase 1 *(SOD1);* apoptosis: tumor protein 53 *(Tp53)* and pregnancy outcome: placenta-specific 8 (*PLAC8*). ANOVA/χ^2 tests were performed. OR was significantly higher in A group than B and C groups (7.1 ± 0.3 vs. 3.9 ± 1.0, 4.3 ± 1.2, $p < 0.05$). VE rate was similar (84.7 ± 4.2, 93.0 ± 3.9, 90.0 ± 4.8%) but expression of *SLC2A4, IR, IGFR1, IGFR2* and *PLAC8* was significantly up-regulated ($p < 0.05$) in blastocysts from C group respect A and B groups. The expression pattern of *SOD1* was higher in A and C groups than B group ($p < 0.05$). *Tp53, eNOS* and *PTGS2* genes did not differ among them. Long-term supplement with PG improves quality of viable embryos enhancing their glucose uptake, imprinting and capacity for implantation.

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P27

Augmented familial incidence of vaginal septa in dogs

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In 2008 a 3 years old female Labrador Retriever with an 8 cm long and 1 cm thick vaginal septum was introduced to the Clinic for Animal Reproduction (also see: Arlt et al., 2009. Reprod Dom Anim, 44:S3, p. 76). Surgical treatment was conducted under general anaesthesia using a bipolar resectoscope (Karl Storz, Germany). By utilising a high-frequency electrosurgical unit (VIO 300 D, Erbe, Germany) the septum was resected. Three months later the bitch was naturally mated and subsequently delivered seven puppies (five males and two females) vaginally without complications. Both female pubs were examined with an endoscope (Dr. Fritz, Germany) at the age of 12 and 15 months, respectively. Both bitches showed a small vaginal septum with a diameter of 0.3 and 0.5 cm, respectively. Since the owners did not have an intent to breed both septa were not resected. Trying to obtain additional data of related dogs we retrieved information of one female littermate of the resected dog. It also had a small vaginal septum which was resected by a veterinarian. This bitch, nevertheless, was mated twice without success. In conclusion, the augmented familial occurrence of the congenital

abnormality indicates that hereditary factors seem to play a role in the pathogenesis of vaginal septa. Based on these four cases we suggest carefully weighing the risks of inheritance of vaginal septa in advance of a surgical resection.

P28

Quality of literature on bovine, equine and canine reproduction

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The objective of this study was to evaluate quality differences and deficits of published literature on reproduction in cattle, horses and dogs. A literature search in the databases Medline and Veterinary Science was conducted in 2009. About five times more articles on clinical bovine reproduction (n = 25 910) were found compared to canine (n = 5 015) and equine (n = 5 090) reproduction. A subset of 600 articles was randomly selected and exclusion criteria were applied. In total, 268 trials (86 for cattle, 99 for horses and 83 for dogs) were evaluated with the help of a systematic checklist and used for further analysis. For the field of canine and equine reproduction, there were fewer clinical trials with a control group compared to bovine reproduction (cattle 66.3%, horses 41.4% and dogs 41.0%). For all three species investigated, few publications were identified (3.7%) with the highest level of evidence, i.e. controlled, randomized and blinded trials or meta-analyses. In cattle 32.6% of the publications were graded adequate to draw sound conclusions. Only 7.2% and 11.1% was graded adequate in dogs and horses, respectively. Therefore, many publications in veterinary medicine comprise a suboptimal study design or reporting which does not provide necessary information for readers to interpret and apply study results. The veterinarian should always first assess the quality of articles before implementing information into practice to provide best available care for the animals. In conclusion, improvement of the quality of well-designed, conducted and reported clinical trails in veterinary medicine is required.

P29

Influence of superoxide dismutase and glutathione peroxidase antioxidants on frozen-thawed bull semen and effect of butylated hydroxytoluene on bull spermatozoa frozen in two different extenders

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This study evaluated the effect of antioxidants on bull sperm frozen in different extenders. Ejaculates from five Holstein bulls were pooled and diluted to 100 ml with a 2.9% sodium citrate extender containing 20% egg yolk plus antibiotics (CEY) for both experiments. Semen was divided into five aliquots, four experimental groups + one control. For exp. 1, each aliquot was further diluted with an equal volume of CEY without (control) or containing 100 U or 200 U SOD/ml, 50 U or 100 U GPx/ml. For exp. 2, each aliquot was further diluted with CEY or a Tris- (hydroxymethyl) aminomethane based extender alone or with added 0.5, 1.0, 2.0 or 4.0 mM BHT. Routine semen evaluation and statistical analyses were carried out using the General Linear Model procedures (GLM) of SAS followed by a *post-hoc* Tukey test to determine the level of significance among mean values. The lowest malondialdehyde (MDA) was obtained by addition of 100 U SOD/ml, 0.5 and 1 mM BHT to CEY extender compared with the other groups ($p < 0.05$). Sperm viability and motility were significantly higher ($p < 0.05$) when 0.5, 1 mM BHT and 100 U SOD/ml were added to CEY extender. Highest sperm viability was achieved by addition of 50 U GPx/ml to CEY extender. There was no significant difference in sperm motility between the treatment groups but sperm motility was significantly higher ($p < 0.05$) in samples extended in Tris–egg yolk (TEY) with 0.5 mM BHT compared to control group ($p < 0.05$). Our results suggest that the addition of BHT to TEY freezing extender only improves sperm motility whereas SOD and BHT can improve CEY extender.

P30

The effects of various levels of catalase antioxidant in two extenders on lipid peroxidation, viability and motility of frozen-thawed bull semen

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This study compared the effects of different catalase concentrations in bull semen extenders on post–thaw parameters considering semen quality and lipid peroxidation. Ejaculates from three bulls of proven fertility were pooled and split into two groups. Each group was diluted to a concentration of 30×10^6 spermatozoa either using citrate-egg yolk (CEY) or tris-egg yolk (TEY) extender. Both groups were divided into three aliquots, including a control and two test groups. Each aliquot was further diluted with an equal volume of extender either without (control) or with one of the following antioxidant concentrations: catalase either at 100 IU/ml (group 1) or 200 IU/ml (group 2). Statistical analyses were carried out using the General Linear Model procedures (GLM) of SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) followed by a *post-hoc* Tukey test which was used to define significances among the mean values. There was no significant difference in sperm viability and motility in the group diluted with CEY following addition of catalase at 100 IU/ml and 200 IU/ml. Highest sperm viability was achieved by addition of 100 IU/ml and 200 IU/ml catalase to TEY compared to the control group ($p < 0.05$). Malondialdehyde (MDA) levels did not change with addition of catalase compared to the control group. We postulate that the addition of catalase to TEY can be used to increase viability of frozen bull sperm. The results provide a new approach to the cryopreservation of bull semen and could positively influence intensive cattle production.

P31

Effects of first equilibration medium and co-culture with oviduct epithelial cells on the vitrification of sheep embryos derived *in vitro*

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Effects of two different vitrification protocols on the survival of sheep embryos were examined. Blastocyst stage embryos

produced *in vitro* with (C) or without co-culture (CC) with sheep oviduct epithelial cells were used in this study. Oocytes were collected from slaughtered ewes, matured in medium 199 supplemented with sodium pyruvate, FSH, LH and 10% FCS for 24 h, fertilized with fresh ram semen in bicarbonate buffered synthetic oviduct fluid with 2% sheep oestrous serum for 20 h and cultured in SOF medium. Glucose was added to culture medium on the 4th day of culture. Blastocysts were assigned two equilibration groups randomly; 20% ethylen glycol (EG) or 10% glycerol (G) for the first equilibration. After 5 min, all were kept in 20% ethylen glycol plus 10% glycerol for 5 min as the second equilibration. After 30 s in vitrification solution (25% ethylen glycol plus 25% glycerol), they were immersed into liquid nitrogen. Thawing was carried out in a water bath at 200°C for 15–20 s and blastocysts were transferred into 0.25 M sucrose for 5 min, washed in hepes buffered synthetic oviduct fluid, and cultured in synthetic oviduct fluid for 24 h. Survival rates of vitrified-thawed and cultured blastocysts were 62.10% in C-EG, 38.40% in CC-EG, 30.20% in C-G and 39.30% in CC-G groups. This study shows that vitrification of sheep embryos using ethylene glycol instead of glycerol as a first equilibration cryoprotectant could give reasonable survival rates and that co-culture of embryos with sheep oviduct epithelial cells could not improve survival rates.

P32

Ultrasound observation of ovarian dynamic after treatment of postpartum anoestrus dairy cows by GnRH and eCG

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The aim of this trial was to observe the ovaries after single application of GnRH or eCG analogues, in postpartum anestrous dairy cows. 21 cows from one dairy farm with acyclic ovaries (no CL, follicles <10 mm, P4 < 0.5 ng/ml) were identified during routine ultrasound examination using a 7.5 MHz probe. The cows were divided into three groups: Controls ($n = 5$); Group 1: treated with 250 μg GnRH ($n = 8$) and Group 2: treated with 750 IU eCG ($n = 8$). Follicles were measured daily and CL measurement took place on day 9 after the treatment. Follicular growth rate, reaction time on treatment and number of ovulations per cow were determined. Statistical analysis was performed by SPSS 13.0. The average growth rate was 1.3 ± 0.1 mm/day for both trial groups. Ovulation was confirmed by ultrasonographic detection of CL and rising P4 values >1.0 ng/ml. Resumption of cyclic activity occurred in 81% (13/16) of the treated cows (87% in Group 1; 75% in Group 2) and 20% (1/5) in controls. Cows treated with eCG or GnRH responded faster (4.6 ± 0.3 and 5.6 ± 0.6 days respectively) in comparison to the controls (23 days). Incidence of multiple ovulations was higher in Group 2 (in average 2.2 ± 0.5 ovulations) than Group 1 (1.5 ± 0.3). In conclusion, treatment with single dose of GnRH or eCG, in many cows with true postpartum anoestrus, caused resumption of follicular growth and ovulation. However, eCG treatment resulted in a quicker response, but higher ovulation rate compared to GnRH treatment.

P33

Induction and localization of five immediate early genes (IEGs) in the bovine corpus luteum (CL) at 30 min after prostaglandin F2 alpha (PGF) treatment

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IEGs are induced rapidly but temporarily in response to a wide variety of stimuli. The present study evaluated the effect of PGF on steady-state concentrations of mRNA in the bovine CL for 5 IEGs: jun proto-oncogene (c-JUN), FBJ murine osteosarcoma viral oncogene homolog (c-FOS), nuclear receptor subfamily 4, group A, member 1 (NUR77), member 2 (NR4A2) and early growth response 1 (EGR1). In addition, *in situ* hybridization (ISH) was utilized to investigate the cellular site of mRNA expression of c-FOS and NUR77 after PGF treatment. Lactating Holstein cows with a mature CL on day 7 after ovulation were allocated to receive intrauterine saline (control group, $n = 4$) or PGF (PGF group, 1 mg, $n = 5$). A biopsy of the CL was obtained using an ultrasound-guided biopsy tool prior to treatments and at 30 min after treatments. The mRNA concentrations for IEGs were analyzed by qPCR in duplicate using GAPDH as a housekeeping gene. Relative Expression Software Tool (REST2009) was used for statistical analyses. Compared to the 30 min control group, PGF increased mRNA for c-JUN (1.85-fold; $p < 0.009$), c-FOS (3.9-fold; $p < 0.016$), NUR77 (5.9-fold; $p < 0.016$), NR4A2 (4.8-fold; $p < 0.03$), and ERG1 (3.1-fold; $p < 0.004$). The increase in c-FOS and NUR77 mRNA were visualized and localized to specific cell types at 30 min after PGF treatment. Thus, all of these IEGs were induced at 30 min after PGF treatment and provide markers for cellular localization of PGF action in the CL.

P34

Factors affecting heatime activity clusters during the peri-ovulatory period in high yielding dairy cows

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The aims were to determine (i) Heatime's efficacy to detect 1st and subsequent ovulations, (ii) if health status influences efficacy and (iii) if conception rate to AI was influenced by Heatime activity score. Ninety-eight spring calving cows were monitored. Milk samples were collected twice weekly for progesterone assay to characterise resumption of reproductive activity. Reproductive tract health was assessed weekly by ultrasonography and vaginal mucus scoring. Body condition (BCS) and milk yield were assessed every 2 weeks. Heatime identified 72% of follicular phases from which 143 inseminations resulted in 68 conceptions. Thirty-two percent of clusters were false positives (high progesterone). Mean peak activity and cluster duration were highest for 2nd or subsequent ovulations followed, in descending order, by those during 1st ovulation, and high progesterone clusters ($p < 0.0001$). The odds of a cluster being in a follicular phase rather than a high progesterone phase improved by 29% for every 1 unit increase in peak activity and by 91% for every 2 h increase in duration. The probability of Heatime detecting a follicular phase was

improved if it was not a 1st ovulation, if BCS increased, if milk yield decreased and uterine infection was absent. Conception rate was influenced by AI on the same day (52.3%) or day after a cluster (32.3%) ($p = 0.045$). Identification of follicular phases improved as duration and peak activity level increased, and was influenced by uterine infection, BCS and milk yield. ***Funding***: Science Foundation Ireland (07/SRC/B1156) + Progressive Genetics

P35

Differences in tyrosine phosphorylation in epididymal and ejaculated boar spermatozoa

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The aim of this study was to determine the P-tyrosine phosphorylation pattern in epididymal (EP) and ejaculated (EJ) spermatozoa before and after a capacitation. Sperm samples were processed by Percoll gradient selection and incubation in TALP media up to 3 h. After immunofluorescence staining we evaluated the presence of tyrosine phosphorylation that in boar spermatozoa is characterized by a fluorescent signal present in the equatorial subsegment, triangular in appearance. A total of 200 spermatozoa per sample were evaluated in five replicates. Before capacitation, the EJ spermatozoa presented a lower percentage of phosphorilation than EP cells (EJ 15.9 ± 5.5% vs. EP 68.0 ± 7.4%, $p < 0.05$). However, at time 0, immediately after capacitation, the proportion of phosphorilated sperm increased in both groups (EJ 88.75 ± 6.58% vs. EP 86.5 ± 2.1, $p > 0.05$) and this high level of phosphorilation was maintained for 3 h of incubation (EJ 95.9 ± 1.7% vs. EP 82.4 ± 8.2, $p > 0.05$). The differences between EP and EJ before capacitation suggest that some components of the seminal plasma are implied in the sperm membrane stabilisation. However, the capacitation process is equally effective for both EP and EJ spermatozoa to induce phosphorilation signals. These results confirmed the previously obtained in Western-blot analysis of the same samples. *Supported by Fundación Séneca 08752/PI/08 and Spanish Ministry of Science and Innovation and FEDER Ref 12908.*

P36

Chemical communication in horses – volatile compounds in mare urine

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The aim of the study was to analyze chemical substances, especially p- and m-cresols, in urine from oestrous mares (OU) and urine including vaginal secretion from oestrous mares (UST) sampled when teased with a stallion. It has been suggested that p-cresol may act as a pheromone to stimulate libido in stallions. Eight mares, (9–19 years old) were sampled once per day from onset of oestrus until ovulation. Urine was also sampled once in three mares at dioestrus (DU). The urine was sampled at spontaneous urination. The samples were collected in small glass bottles and kept in −20°C until analyses. Solid-phase micro extraction (SPME) was used for collection and GC-MS (gas chromatography and mass spectrometry) for analysis of volatiles. The chromatograms showed numerous peaks, p- and m-cresols were identified at retention times around 33.10 and 33.25, respectively. In OU, p-cresol and m-cresol (area under curve) did not differ between days (D6–0 before ovulation), however in UST, m-cresol was significantly lower at D3 than at the other days. P-cresol but not m-cresol was found in DU. In UST compared with OU, p-cresol was higher at D 6 and 3, and m-cresol was higher on D 6, 3 and 1, respectively. Conclusion: The relative pattern of p- and m-cresols in OU and UST differed during oestrus. In general, UST contained higher amounts of p- and m-cresols than OU. Whether p- and m-cresols affect the sexual response in stallion remains to be further investigated, in a bioassay.

P37

Effect of hoechst 33342 on stallion spermatozoa incubated in KMT or modified INRA96-tyrode

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Currently the only known method to effectively produce separate populations of X and Y bearing spermatozoa is the Beltsville sexing technology. This technology implies that each individual sperm cell is interrogated for DNA content, measuring its fluorescence intensity after staining the spermatozoa with Hoechst 33342. Since there is no data regarding the toxicity of the staining on stallion sperm, 25 ejaculates were incubated up to 90 min in presence of 0, 8, 16, 45, 80, 96, 120, 136 and 160 μM Hoechst, in two media, KMT or INRA-Tyrodes. After 90 min of incubation, motility (CASA) and membrane integrity (flow cytometry after YOPRO-1/Eth staining) were evaluated. In KMT extender sperm motility significantly decreased when sperm was incubated in presence of concentrations of Hoechst of 80 μM or higher (79.2 ± 9.18 in controls vs. 62.6 ± 19.50 at 80 μM $p < 0.05$). However, membrane integrity only decreased significantly when incubated with 160 μM. Stallion spermatozoa stained in modified INRA96, tolerated higher concentrations of Hoechst and sperm motility only decreased significantly when incubated in presence of 120 μM or higher (70.9 ± 21.5 in controls vs. 63.0 ± 11.51 $p < 0.05$) and membrane integrity was not affected. Apparently the toxicity of Hoechst on stallion spermatozoa varies depending on the media, and INRA modified extender may be an alternative to KMT. *Supported by AGL 2010-20758 (GAN) and Junta de Extremadura-FEDER (GR 10010).*

P38

Reproduction parameters and uterine secretion of leucotrienes in cows with and without cytological endometritis

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Cytological endometritis (CE) is recognized as a cause of infertility in cattle but the problem is still poorly understood. The aim of this study was to compare the incidence, fertility parameters and uterine secretion of leucotrienes (LTB_4, LTC_4)

in cows with and without CE. We intended to evaluate the influence of CE on fertility parameters in 2 different herds, and leucotrienes concentrations served as a parameter of inflammation. In 215 clinically healthy cows from 2 herds on 4th and 6th week postpartum the cytobrush method was used to evaluate the presence of CE (18% and 10% of PNM's, respectively). The secretion of leucotrienes by endometrial cells was measured using EIA after incubation of cells from the smears. The prevalence of CE differed between the herds (55.3% vs. 40.8%) ($p < 0.05$) and generally decreased during postpartum (32.4% vs. 19%) ($p < 0.05$). In both herds fertility in the CE groups was diminished but in herds with higher rates of CE the reproduction parameters were lower (intercalving interval 413.2 vs. 380 days, conception rate after 1st insemination 41% vs. 55% and number of inseminations per conception 2.3 vs. 1.8). Surprisingly, we couldn't confirm elevated secretion of both leucotrienes in cows with CE because their levels in this group compared with cows without CE were comparable (LTB_4 2489.6 pg/ml vs. 2097.7 pg/ml; LTC_4 142.1 pg/ml vs. 132.7 pg/ml). This suggests non-inflammatory nature of CE, however information about leucotrienes secretion in such cows is very limited. It can be also concluded that CE reduces fertility in milk cows; however in both herds this problem has been differently pronounced.

P39

Testing bovine endometrial explants for survivability using cell proliferation reagent WST-1

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The aim of this study was to determine the suitability of Cell Proliferation Reagent WST-1 (Roche Diagnostics, Mannheim) to examine the survivability of bovine endometrial explants *in vitro*. The test is based on the measurement of cleavage from tetrazolium salt by mitochondrial dehydrogenase. Endometrial explants were collected from uteri directly after slaughter. Four uteri were cut open aseptically and small pieces of endometrial tissue were prepared carefully. Tissue cubes with one mm^2 in cross section were prepared from the central endometrial sample using a tissue chopper (McIllwain™) and transferred to a 96-well microplate into 150 µl of Dulbecco's Modified Eagle's Medium (DMEM). In each case eight explants from one uterus were cultured per well over a period of 216 h (9 days) at 37°C and 5% CO_2. In treatment group 1 no change of medium was performed. In treatment group 2, 50 µl of medium were replaced after 24 h of incubation. Viability of explants was tested after 1 h and every 24 h, respectively, until a total incubation time of 216 h. Therefore 10 µl of WST-test solution was added per well followed by incubation for 4 h at 37°C and 5% CO_2. Hundred microliter of supernatant was used for quantitative analysis of mitochondrial dehydrogenase activity. Absorbance from the formazan product was measured using a 96-well microplate reader (450 nm; reference wavelength 620 nm). Mitochondrial dehydrogenase activity as measure of cell viability was significantly higher ($p \leq 0.05$) in samples of treatment group 2 compared to samples of group 1. In group 1 (without exchange of medium) highest absorbance was measured after 24 h incubation, followed by constant decrease until 216 h incubation. In contrast formazan production in group 2 increased till 168 h. However, in treatment group 2 the measured viability after incubation for 216 h was comparable to the basic value of freshly incubated explants (1 h).

P40

Effect of yeast supplementation and mucus score on production and reproduction parameters on a commercial dairy farm

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The objectives were to i) investigate the effect of yeast culture supplementation (S. cereviciae1026) on the following production and reproduction parameters: vaginal mucus score at 3 weeks post partum, calving-1st service, calving-conception, number of serves, peak milk yield, milk fat and protein at 1st milk recording, milk yield during duration of the experiment (eight recordings, 1 every 2 weeks), body condition at calving and body condition at 1st service and ii) investigate the effect of vaginal mucus score post partum on the same parameters. one hundred and sixty-three spring calving cows on a commercial dairy farm were blocked on previous milk yield and enrolled in the experiment and assigned to either supplemented with yeast culture ($n = 72$) or control ($n = 91$). Body condition score was recorded on a scale from 1 to 5 at calving and at 1st service and vaginal mucus score was recorded between 3 and 4 weeks post partum on a scale from 1 to 3. All other parameters were collected at the end of the breeding season and all data were analysed using Chi square analysis. There was no effect of yeast supplementation on the parameters measured. Cows with a high vaginal mucus score (indicating uterine infection) at calving had a significant lower ($p > 0.05$) average milk yield in the experimental period. Cows with a BCS > 2.5 at calving tended ($p = 0.07$) to have a longer calving-1st service interval as did cows with low milk fat ($< 4.8\%$) in the 1st milk recording ($p = 0.08$).

P41

Relationships among crystallization, acetone and urea content in dairy cows cervical mucus

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The quality of cervical mucus can be defined by the urea and acetone content. The subject of this study was to determine the relationships among selected indicators of the quality of a cow's cervical mucus and its crystallization. At insemination, samples of cervical mucus were collected from 165 Holstein cows. An arborisation test of the cervical mucus was performed. The microscopy method for evaluation of crystallization of cervical mucus dried on a glass slide was used. The acetone content was determined on a gas chromatograph by the headspace method. The urea content was assessed photometrically. The statistical program SAS 9.1., GLM and MEANS procedures were used for analyzing the data. The highest average levels of urea (1 196.7 µg/g and 1 023.6 µg/g) were found in atypical crystallization and in samples without crystallized structures ($p < 0.05$). On the other hand, the highest average level of acetone (1.86 µl/g) was detected in fern-like patterns of crystallization ($p < 0.05$). Significant differences between the types of crystallization, acetone and urea content were not detected by the GLM method. The above-mentioned data are preliminary. We hypothesize that the acetone and urea content in the cervical mucus of cows has an influence on their fertility. However this requires further verification. *Funded by MSMT 6046070901, NAZV QI91A061 and FRVS 2395/2011/G4.*

P42

Expression of protein tyrosine phosphorylation in response to oviductal fluid varies with individual boars

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Differences in the response to the oviduct environment might decide the fertility of spermatozoa from a particular male. We hypothesized that spermatozoa from different boars might display different patterns of protein tyrosine phosphorylation (PTP), which is associated with acquisition of fertilizing potential, in response to oviductal fluid (ODF). Washed spermatozoa from four boars were incubated with porcine pre-ovulatory ODF at 37°C for 6 h in an atmosphere of 5% CO2 along with a noncapacitating control. Sperm PTP was assessed at hourly intervals by flow cytometry and confocal microscopy using an antiphosphotyrosine antibody. The percentage of spermatozoa with PTP was higher ($p < 0.05$) in the ODF treated group compared to controls. A clear difference was observed between boars in the expression of PTP in response to ODF. Spermatozoa from two boars responded ($p < 0.05$) to the ODF after 3 h incubation (17.2 & 22.6% spermatozoa showed PTP), while one boar responded after 1 h incubation (21.2%) and the fourth boar did not respond. The percentage of spermatozoa with PTP for this boar ranged from 2.3 to 10.4% and the average live born litter size for this boar was 11.5 while for the other boars it was 12.5. Our results clearly demonstrate expression of PTP in response to oviductal fluid varies with individual boars. Since PTP is related with sperm capacitation, hyperactivation and acquisition of fertilizing potential, its assessment *in vitro* could predict fertility in boars.

P43

Effect of season on the *in vitro* embryo production from prepubertal ovine oocytes

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Photoperiod positively affects reproduction in adult sheep through melatonin secretion by pineal gland during darkness, but little is known about photoperiod effects on oocyte quality in prepubertal ewes. Our aim was to determine the season effect on *in vitro* embryo production of prepubertal ovine oocytes. Slaughtered prepubertal Sarda sheep (4–6 weeks old) ovaries were classified according to the number of follicles larger than 2 mm on their surface: A) ≥30 follicles; B) 16–30 follicles; C) <15 follicles. COCs recovered from the three groups in autumn and spring were *in vitro* matured, fertilized and cultured up to the blastocyst stage. Blastocysts were vitrified/warmed and cultured *in vitro* for 24 h to evaluate re-expansion rates and cell number. Timing of blastocyst development at the 7th day of *in vitro* culture was lower ($p < 0.05$) during the autumn compared to spring in A (4.8 vs. 10.7%), B (1.9 vs. 9.1%) and in C groups (0 vs. 9.5). Expanded blastocyst output was lower ($p < 0.05$) in autumn compared to spring in A (14.1 vs. 20.8%, respectively), and B groups (12.7 vs. 20.5%, respectively), but it not in group C (10.2 vs. 16.2%). No differences were found in re-expansion of blastocoelic cavity after vitrification/warming nor in blastocyst cell number among the three groups. In conclusion, prepubertal ewes oocytes collected during the spring showed higher developmental competence in terms of kinetic of embryo development and blastocyst output.

P44

Effects of serum starvation and ionomycine treatments on somatic cell nuclear transfer in sheep

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In this study it was aimed to investigate the effects of serum starvation and ionomycine treatments on somatic cell nuclear transfer in sheep. Sheep oocytes collected from slaughtered ewes and matured in medium 199 supplemented with sodium pyruvate, FSH, LH and 10% FCS for 20 h were used. Cumulus cells cultured in medium 199 supplemented with 10% FCS were used as karyoplasts after serum starvation (0.5% FCS; SS) for 4 days or directly without serum starvation (10% FCS; S). After somatic cell nuclear transfer and electrofusion procedures, oocytes divided again into two activation groups. Oocytes were activated by ionomycine combined with 6-dimethylaminopurine (I+) or only with 6-dimethylaminopurine (I-). Cleavage rates were 37.25 (19/51), 44.12 (15/34), 34.62 (18/52) and 44.68% (21/47) in SS/I+, S/I+, SS/I- and S/I- groups respectively ($p > 0.05$). Some cleaved embryos (44/73) at the second day of culture were transferred into recipient ewes (4.59 ± 0.14 embryos/per recipient). Pregnancy rates according to progesterone analysis were 33.33 (1/3), 50.00 (1/2), 50.00 (1/2) and 100.00% (3/3) ($p > 0.05$). Only one pregnancy in the S/I- group continued after 40 days and due to a maternal problem (torsio uteri), the cloned lamb died 10 days before term. The results of this study reveal that somatic cell synchronization by serum starvation and ionomycine treatment for the activation of oocytes are not required for obtaining cloned animals.

P45

Energy source during *in vitro* culture (IVC) and sex ratio of bovine embryos

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Most systems for producing mammalian embryos *in vitro* use glucose as an energy source despite putative toxic effects. It is known that female embryos are more sensitive to negative effects of glucose during IVC. The aim of this work was to evaluate whether replacing glucose with myo-inositol and citrate during IVC affects sex ratio. Abattoir-derived oocytes were matured and fertilized *in vitro* using standard procedures. After 20–22 h of gametes co-incubation, zygotes were denuded and cultured in SOF containing either 1.5 mM glucose or 2.77 mM myo-inositol and 0.34 mM citrate, for 7 days. The percentages of blastocysts were recorded and the embryos (on average 122 per group) were sexed by PCR as previously

described (Alomar, 2008, Anim. Reprod. Sci. 107 48–61.). Differences in blastocyst rates and in the percentages of female embryos between groups were analyzed by Chi-Square test. The results of this study showed that myo-inositol-citrate increased both blastocyst yield (37.4 vs. 29.5%, respectively; $p < 0.01$) and the percentage of female embryos compared to glucose (61.5 vs. 45.6% respectively; $p < 0.05$). In conclusion, these results suggest to use myo-inositol and citrate in culture media to switch embryo sex ratio towards females.

P46

Tumor necrosis factor, ovarian steroids and oxytocin in the equine oviduct

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Many factors like hormones, angiogenic factors and cytokines, such as tumour necrosis factor a (TNF), are potential mediators of many cellular functions. On previous work on the mare oviduct we have shown a higher TNF immunostaining in the isthmus than in the ampulla and infundibulum, in the mid luteal phase. Thus, the aim of this study was to evaluate: (i) gene expression of TNF and its receptors (TNFRI; TNFRII) on mare's oviduct in the estrous cycle (follicular, early, mid and late luteal phases); (ii) *in vitro* nitric oxide (NO) production and angiogenic activity by mid luteal phase equine oviduct epithelial cells (OEC), under the influence of different hormones. Blood and mare oviducts were collected post mortem. Conventional PCR for gene expression was performed on oviducts (n = 20; 4/each phase). OEC isolated from mares (n = 6) oviducts were incubated with TNF (10 ng/ml), estrogen (10–9 M), progesterone (P4) or oxytocin (10–7 M each). TNF and its receptors mRNA were expressed in all oviduct portions, and NO production by OEC was higher when stimulated with P4 ($p < 0.05$). Angiogenic activity, indirectly determined by assessment of *in vitro* bovine aortic endothelial cell proliferation, conditioned by treated OEC, showed no difference. This study confirms that TNF and its receptors are expressed in equine oviduct. The major production of NO by OEC, under P4 influence, may be related with their different functions, such as cilia movement for clearance, as it was demonstrated in other tissues.

P47

Capacitation-like changes in sex-sorted boar spermatozoa

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Sex-sorting process induces several changes in sperm cell functionality which are defined as capacitation-like modifications. The aim of the present study was to evaluate protein tyrosine phosphorylation (TP) status and Chlortetracycline (CTC) staining pattern in boar spermatozoa after sex-sorting procedure and to compare these parameters with those of freshly ejaculated, capacitated and acrosome reacted cells. TP status was evaluated by immunofluorescence and three different patterns were recognized; each of them was considered to be typical of fresh (F), capacitated (C) and acrosome reacted (AR) cells. In sorted spermatozoa the most expressed pattern was F (80.2 ± 6.6% Mean ± SD), while C and AR patterns were 8.5 ± 5.9% and 11.3 ± 5.6%, respectively. These pattern distribution is similar to that observed in freshly ejaculated sperm cells (F 87.5 ± 7.3%; C 9.9 ± 6.1%; AR 2.6 ± 2%). CTC positivity was assayed in that it is considered a sensitive capacitation index. Sex sorted cells presented the following pattern distribution: F 68.4 ± 5.2%; C 26.3 ± 4.3%; AR 5.3 ± 0.9%, which are very similar to the those observed in capacitated spermatozoa (F 67.8 ± 6.1%; C 25.2 ± 2.5%; AR 7 ± 4.3%). These results suggest that sex sorting procedure induces a capacitation-like switch in sperm subpopulations of boar ejaculates, as registered with CTC technique. As for protein TP immunoreactivity, it evidences a fresh-like subpopulation trend, with an increase of AR pattern, probably due to mechanical damage. Further studies would be necessary to better define the pathways involved in sex sorting-induced modifications.

P48

Estrus synchronization using short- and long-term proestagen treatments and ram effect and ram effect + flushing in ewes outside the breeding season

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The objective of the present study was to determine the efficacy of the synchronization of estrus using short- and long-term progestagen treatments and ram effect (R group, n = 25) and ram effect + flushing (RF group, n = 25) in ewes outside the breeding season. A total of 147 Anatolian merino ewes, aged 3–5, and 15 rams, aged 2–3 years-old, were used in the trial. Intravaginal sponges containing 40 mg fluorogestone acetate (FGA) were inserted in the ewes for 6 (FGA1 group, n = 23), 8 (FGA2 group, n = 24), 10 (FGA3 group, n = 25) or 12 (FGA4 group, n = 25) days. In sponge groups, 125 μg D-cloprostenol and 400 IU pregnant mare serum gonadotrophin (PMSG) were injected i.m. the day before sponge removal. All ewes were hand-mated after detection of estrus. Hours to estrus and estrus response rates in sponge groups were higher than in R and RF groups ($p < 0.05$). There was no significant difference among the groups regarding pregnancy, lambing, multiple birth rates and litter size. Fecundity in FGA3 and FGA4 groups was higher than that in R and RF groups. The results of the present study show that intravaginal sponge containing 40 mg FGA for 10 or 12 days, but not 6 or 8 days, is superior to ram effect and ram effect + flushing for estrus synchronization in Anatolian merino ewes outside the breeding season.

P49

The effect of postpartum period on superovulation response in dairy cows

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The aim of this study was to compare the superovulatory response in cows that superovulated in various portpartum

periods. A total of 246 lactating Simmental cows were initiated to superstimulation between 30 and 150 days postpartum (between 30 and 45, 46 and 60, 61 and 75, 76 and 90, 91 and 105, 106 and 120, 121 and 135 and 136–150 days and n = 14, 35, 45, 53, 36, 24, 26 and 13 in groups 1, 2, 3, 4, 5, 6, 7 and 8, respectively) for this aim. Superstimulation was started between 8 and 12 days after the spontenoeus reference oestrus in all cows. All cows received follicle stimulating hormone (FSH) in 8 decreasing dosages over 4 days, at 12 h intervals. Luteolysis was induced by twice i.m. injection of 500 μg of cloprostenol with the 7th and 8th FSH injections. Following the last FSH injection, artificial insemination was performed 12, 24 and 36 h after the onset of oestrus. Ova/embryos were collected non-surgically at day 7 after oestrus by uterine flushings and evaluated and classified. Corpus luteum (CL) number in group 4 was higher than that in group 3, and unfertilized oocyte number in group 4 was higher than that in group 1 ($p < 0.05$). There was no significant difference between groups in terms of total ova/embryo, transferable embryo and degenerated embryo numbers. In conclusion, CL numbers of superstimulated lactating cows was affected by postpartum period in the present study. However, there was no significant difference according to postpartum period by means of total ova/embryo, transferable and degenerated embryo numbers.

P50

Body temperature early postpartum is higher in primiparous than in multiparous dairy cows

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Body temperature (BT) is the most common parameter for early identification of cows at risk for infectious diseases after calving. Body temperature values ranging from 39.2 to 39.7°C are commonly used as thresholds defining fever. There is anecdotal evidence that primiparous cows exhibit higher BT than multiparous cows early postpartum. The objective of this experiment was to study BT during the early postpartum period for both, healthy primiparous and multiparous cows. Vaginal temperature (VT) of 27 matched pairs consisting of a primiparous and a multiparous cow (parity 2.8 ± 0.9) that had calved with a maximal difference of 2 day was recorded every 10 min with a temperature logger (Minilog8, Vemco, Canada) inserted into the vagina from 2 to 10 day after calving. Using matched pairs ensured that temperature humidity index was identical during the course of the trial for primiparous and multiparous cows (70.6 ± 6.7 and 70.4 ± 6.7;$p > 0.01$). All cows gave birth to a single life calf without assistance and did not suffer from infectious or metabolic diseases during the observational period. From 2 to 5 days in milk (DIM) VT was higher in primiparous than in multiparous cows (DIM2: 39.6 ± 0.7°C vs. 39.3 ± 0.6°C, DIM3: 39.7 ± 0.7°C vs. 39.3 ± 0.6°C, DIM4: 39.6 ± 0.6°C vs. 39.3 ± 0.5°C, DIM5: 39.6 ± 0.5°C vs. 39.4 ± 0.5°C; $p < 0.01$). From 6 to 10 DIM VT did not differ ($p > 0.01$) between both groups. This study provides evidence of higher BT of first lactating cows in the first 5 DIM. Therefore, further research is warranted to determine optimal thresholds regarded as fever and depending on DIM and parity.

P51

Effects of different enterotoxigenic and verotoxigenic *E. coli* concentrations on boar sperm quality

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Enterotoxigenic (ETEC) and verotoxigenic (VTEC) *E. coli* have a high prevalence in farms, so high possibilities of accidentally semen contamination exist. If doses are contaminated, boar sperm quality will be altered, causing economic losses to artificial insemination (AI) centers. The aim of this study was to determine the effects of different concentrations of ETEC and VTEC on boar sperm quality. We inoculated boar extended doses with infective concentrations between 108 to 102 cfu/ml; non-inoculated doses were the negative controls. Inoculated tubes were kept at 37°C. Sperm motility, sperm viability and sperm morphology were assessed prior and after 24, 48, 72 and 96 h of semen's inoculation. Bacteria's presence after 24 h of semen inoculation was verified by PCR. Progressive motile spermatozoa significantly ($p < 0.05$) and progressively decreased over all the incubation period. The highest infective concentration was the most deleterious. A significant decrease in the percentage of viable spermatozoa was detected after 24 h of incubation in the 108 cfu/ml tube, and after 48 h in the tubes infected ETEC and VTEC from 108 to 103 cfu/ml No changes were observed in the percentage of mature spermatozoa. In conclusion, since ETEC and VTEC reduce sperm viability and motility at higher infective concentrations than 103 cfu/ml, their presence/absence in seminal doses should be assessed by PCR analysis before AI.

P52

The effect of the tissue culture medium (tcm) 199 supplemented with epidermal growth factor (egf) or insulin-like growth factor-1 (igf-1) on *in vitro* maturation of canine oocytes

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Current *in vitro* maturation (IVM) techniques of canine oocytes present limited success rates. Some particular features of the reproductive biology of the bitch, such as preovulatory luteinization and ovulation of the oocytes in germinal vesicle-stage, have been reported as the main reasons for the low successIVM rates. The aim of the present study was to assess the effect of the addition of EGF or IGF-1 to the culture medium TCM 199 on nuclear maturation of canine oocytes. Seven hundred and eighty-seven comulus-oocyte complexes (COCs) were sampled during different phases of the oestral cycle from 34 bitches submitted to elective ovariohysterectomy. The COCs were distributed into three groups: A (TCM 199, n = 199); B (TCM 199 + 100 ng/ml of EGF, n = 208) and; C (TCM 199 + 100 ng/ml of IGF-1, n = 204). Following 96 h of incubation at 38°C, in sterile atmosphere with 5% CO2, the nuclear maturation stage was evaluated following bisbenzimide staining (Hoechst 33342). The data were analysed with the Chi-square test, using the software SAS. The rate

of resumption of meiosis (i.e., the oocytes that surpassed the phase of germinal vesicle) was 28% in group A, 27% in group B and 20% in group C. Therefore, there was no difference between the control group (A) and the treatment groups (B and C). Oocytes presenting metaphase II were not found. In conclusion, the addition of EGF or IGF-1 to the TCM 199 did not favour the IVM of canine oocytes.

P53

Semen quality, dna integrity and protamination of young nelore bulls

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The aim of this study was to evaluate semen quality, DNA integrity and protamination of young (22–24 months age) Nelore bulls compared to adult animals (47–117 months age) from the same insemination centre. Frozen-thawed samples from three ejaculates of nine young Nelore bulls (Y) and five adult Nelore bulls (A) were evaluated for: motility, morphology, simultaneous evaluation of acrosome, membrane and mitochondrial potential (FITC-PNA, PI, JC-1), chromatin integrity (acridine orange – AO) and abnormal protamination (chromomycin A3 – CMA3). Motility (Y: 42.1 ± 12.4%; A: 45.7 ± 4.5%), sperm concentration (Y: 28.6 ± 3.7%, A: 29.2 ± 3.3%), damaged acrosome (Y: 68.1 ± 17.0%; A: 77.0 ± 6.3%), damaged membrane (Y: 60.9 ± 20.0; A: 83.8 ± 7.6), DNA integrity (Y: 0.4 ± 0.6%; A: 0.3 ± 0.1%) and impaired protamination (Y: 0.4 ± 0.4%; A: 0.4 ± 0.5%) showed no statistical differences ($p > 0.05$). Nevertheless, statistical differences ($p < 0.05$) were observed when total sperm defects (Y: 18.8 ± 6.4%; A: 10.1 ± 3.4%) and percentage of sperm with low mitochondrial potential (Y: 60.9 ± 20.0%; A: 83.8 ± 7.6) were evaluated. These previous results showed no major changes in semen features of young Nelore bulls. DNA integrity and protamination rates indicate that chromatin were efficiently packed and protected at the age of 22–24 months.

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P54

Effect of different concentrations of trans-10 cis-12 conjugated linoleic acid (10t, 12c CLA) in maturation of *in vitro* bovine oocytes

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Excessive lipid content in embryo cells is a consequence of embryo culture in the presence of serum. In previous studies the positive effect of CLA in co-culture with embryos was tested. In this work we aimed to examine CLA effects on oocyte maturation, using different concentrations of CLA. Abattoir-derived oocytes were matured in TCM199 + 10% serum + 10 μg/ml FSH + 100 μM GSH supplemented with 0 μM (N = 186), 50 μM (N = 212), 100 μM (N = 187) and 200 μM (N = 168) of CLA during 22–24 h, in a total of nine replicates. Matured oocytes (MO) were developed until the day 10. Cleavage was assessed 48 h after insemination. On day 7 and 8, embryos were evaluated for development and morphological status. Embryo development rates at D7 and D8 were calculated as number of morulae and blastocysts at those days per number of 2–4 cell embryos at cleavage. Data were analyzed using one-way ANOVA. The 200 μM of CLA showed the lowest rate ($p < 0.05$) of cleavage (68.1 ± 3.06, 75.2 ± 2.18, 72.7 ± 2.88 and 57.4 ± 3.41% for 0, 50, 100 and 200 μM CLA groups, respectively), and lowest rate ($p < 0.05$) of embryo development (80.9 ± 2.03, 81.4 ± 2.59, 81.1 ± 5.09 and 36.4 ± 3.87% for 0, 50, 100 and 200 μM CLA groups, respectively). The percentage of hatched embryos on day 10, was significantly ($p < 0.05$) higher with 100 μM CLA compared to 0 μM CLA (21.7 ± 4.01, 31.4 ± 3.96, 38.8 ± 4.34 and 0.0 ± 0.0% FOR 0, 50, 100 AND 200 μM CLA groups, respectively). In conclusion, the presence of CLA during maturation improved bovine oocyte competence to develop into higher quality embryos.

P55

Immunolocalization of estrogen receptor beta in ejaculated ram spermatozoa

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The presence of estrogen receptor beta has been recently reported in human spermatozoa. In addition, 17-beta estradiol has been shown to induce non-genomic activation of spermatozoa, which, together with progesterone, seem to modulate capacitation and acrosome reaction. The aim of this study was to verify the presence of estrogen receptor beta in fresh ejaculated ram spermatozoa. Sperm was obtained from nine different Rasa Aragonesa rams of proven fertility, and the studies were performed by immunoanalysis through western-blot, indirect immunofluorescence and immunocytochemistry. Western-blot analyses showed two bands of 50 and 60 kDa, compatible with the estrogen receptor beta molecular weight. Both indirect immunofluorescence and immunocytochemistry revealed three different sperm subpopulations according to the signal distribution: one subpopulation showed no antibody labeling; another subpopulation exhibited intense labeling on the apical region of the acrosome, and the third subpopulation presented an intense apical acrosomal signal with a weaker labeling on the post-acrosomal area. These three subpopulations, might be in relationship with the capacitation status and reflect the intrinsecal sperm heterogeneity in ram semen. *This work was supported by grants AGL2010-18975, AGL2008-01476 and DGA/A26-2010.*

P56

Gonadotropin-releasing hormone (GnRH) receptor expression and *in vitro* gnrh effects in early, mid and late-corpora lutea of mediterranean buffalo (*Bubalus bubalis*)

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The gonadotropin-releasing hormone (GnRH) receptor (GnRHR) expression and the *in vitro* GnRH effects on corpora lutea (CL) function was studied in buffalo at early (day 4), mid (day 10), and late (day 18) stages of diestrus. Immunohistochemistry evidenced the presence of GnRHR and

GnRH in the cytoplasm of luteal cells at all three stages; these immunosignals were more expressed ($p < 0.01$) during late phase. In *in vitro* cultured CL, GnRH analogue (buserelin) reduced ($p < 0.01$) progesterone and increased ($p < 0.01$) prostaglandin F2α (PGF2α) secretions at day 10 and 18, whereas PGE2 was increased ($p < 0.01$) only at day 18. Furthermore, buserelin engendered ($p < 0.01$) cyclooxygenase2 (COX2) and PGE2-9-ketoreductase (PGE2-9-K) enzymatic activities in day 10 and 18 CL, whereas COX1 was increased ($p < 0.01$) only at day 18. These results suggest that GnRHR and GnRH are constitutively expressed in buffalo CL independently of luteal stage. In addition, the present data evidenced that GnRH modulates directly CL hormone productions; in particular, GnRH down-regulates progesterone and up-regulates PGF2α synthesis at mid- and late-luteal stages, utilizing its cognate receptor with a post-receptorial mechanism that involves the increase of COX2 and PGE2-9-K enzymatic activities.

P57

Development of an *in vitro* bovine oocyte maturation system: effects of serum, hormones and epidermal growth factor on oocyte maturation

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The aim of the study was to evaluate the effects of follicle stimulating hormone (FSH), luteinizing hormone (LH), epidermal growth factor (EGF) and estradiol 17-β (E2) in maturation medium on nuclear maturation and cumulus expansion of bovine cumulus-oocyte complexes (COCs). COCs were classified as good and poor quality grades based on cumulus investment. COCs were subjected to *in vitro* maturation in TCM-199 in a humidified atmosphere of 5% CO2 in air at 38.5°C for 24 h. The combination of the hormones added to the medium was as follows: T1 = 10% (v/v) heat inactivated Fetal Bovine Serum (FBS); T2 = 5 μg/ml bovine LH, 0.5 μg/ml bovine FSH and 10 ng/ml EGF; T3 = FBS, bovine LH, bovine FSH and EGF; T4 = 1 μg/ml E2; T5 = FBS and E2. Supplementation of maturation medium with the FSH, LH, EGF irrespective of FBS supplementation stimulated expansion of cumulus around the oocytes compared to oocytes in other treatment groups ($p < 0.05$). The degree of cumulus expansion (good vs. poor) was significantly better for good COCs in all treatment groups ($p < 0.05$). However, there were no significant differences between treatment groups for nuclear development to the metaphase II stage. In conclusion the results confirm previous data effecting oocyte maturation. *This study was supported by a grant from Ondokuz Mayıs University (PYO.VET.1901.09.002).*

P58

The effect of fsh and pge analogue on the cervical penetration during the periovulatory period in mixed bred thai native goats

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The trans-cervical insemination to deposit semen within the uterus has a potential to improve the fertility rate in goat. However the tubular convoluted shape of the goat cervix limits the use of this technique. The aim of this study was to investigate the local intra-cervical administration of FSH and PGE analogue on the cervical penetrability during the oestrous period. Twenty mixed bred Thai native goats were assigned to 4 groups of 5. Goats were used in 3 replicates allowing 15 observations per treatment. Oestrous was synchronised using progestagen pessaries and 250 IU PMSG at pessary removal. The treatment was applied at 24 or 48 h after the pessary removal: Group1; controls, Group2; FSH 2 mg at 48 h, Group3; FSH 2 mg at 24 h and PGE 1 mg at 48 h, Group4; PGE 1 mg at 48 h. The cervical penetration was determined using the modified insemination pipette at 0, 24, 48, 54, 60 and 66 h after the pessary removal. The depth of penetration was analysed by ANOVA. The results show that depth of penetration increased gradually between 24 and 66 h and was significantly higher from 48 h to 66 h after pessary removal ($p < 0.05$). At 54 h and 60 h after pessary removal depth of penetration in group 2, 3 and 4 was greater than that in the control group ($p < 0.05$). The results suggest that local application of FSH or PGE analogue can relax the cervix and increase the depth of cervical penetration that may facilitate transcervical artificial insemination in goat.

P59

Basic fibroblast growth factor (bFGF) has a favourable effect on proliferation of pig granulosa cells cultured *in vitro* over prolonged period of time

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It was previously demonstrated that luteinizing human granulosa cells (GC) can be maintained in culture over prolonged periods of time in the presence of leukemia inhibitory factor (LIF). In the present study we investigated effect of LIF and basic fibroblast growth factor (bFGF) on proliferation of pig granulosa cells cultured *in vitro* over prolonged period of time. Granulosa cells were isolated from healthy small (SF-GC) and large (LF-GC) follicles and cultured in Knockout DMEM medium with supplements, in the presence of 10% of fetal calf serum. LIF and bFGF were used in the concentration of 1000 IU/ml and 10 ng/ml, respectively. To determine GC proliferation potential the newly synthesized DNA in cell cultures was measured in 72 h intervals by incorporation of 3H-thymidine using the technique of TCA precipitation and liquid scintillation counting. ANOVA was used to determine the significance of differences. bFGF stimulated ($p < 0.05$) proliferation of SF-GC at each of investigated time intervals, up to 21 days of culture. LIF had a stimulatory ($p < 0.05$) effect on SF-GC proliferation only after 72, 144 and 216 h of culture. In LF-GC, LIF stimulated ($p < 0.05$) proliferation after 72 h of culture while bFGF increased ($p < 0.05$) level of 3H-thymidine incorporation at each time interval up to 18 days of culture. The results of the study indicate a favourable effect of bFGF on survivability and proliferation of long term cultured pig granulosa cells.

P60

Media supplementation with seminal plasma proteins improves quality of bold sorted cryopreserved bovine sperm

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This study was conducted to investigate whether the addition of seminal plasma proteins (SPP) after flow cytometrical sorting has positive effects on cryopreserved bovine sperm. Ejaculates from eight bulls were bold sorted according to the Beltsville Sperm Sexing Technology. Sorted sperm from each ejaculate were divided into six aliquots: without seminal plasma and egg-yolk (0SP) and with 1% seminal plasma/20% egg-yolk (SP/EY) as well as supplementation with differing concentrations of SPP (0.5; 1; 2 and 3 mg/ml SPP/EY). Cryopreservation of sperm was performed identically for all groups. Sperm motility pattern (MS) were assessed by Computer Assisted Sperm Analysis (CASA). Percentages of plasma membrane integrity (PMI), acrosomal damages (AD), viable sperm with a high mitochondrial membrane potential (HMMP), sperm with a high degree of DNA fragmentation (%DFI) were tested flow cytometrically immediately (0 h) and 3 h (3 h) after thawing. Sperm motility at 3 h was higher ($p < 0.05$) in 2 mg/ml SPP-sperm (28.5 ± 11.3) compared to 0SP- (25.9 ± 10.1) and SP/EY-sperm (17.8 ± 9.0). PMI-0h was higher ($p < 0.05$) in 0.5 mg/ml SPP-sperm (45.4 ± 8.9) than in 0hSP- (35.9 ± 9.3) and SP/EY-sperm (36.3 ± 7.2). Percentages of DFI-3h were lower ($p < 0.05$) in 2 mg/ml SPP-sperm (0.6 ± 0.4) than in 0SP- (1.0 ± 0.6) and SP/EY-sperm (1.1 ± 0.4). No further effects ($p > 0.05$) were seen in any other comparisons. In conclusion, the results indicate that addition of seminal plasma proteins after bold sorting has positive effects on quality of cryopreserved sperm.

P61

Evaluation of different chemotherapy agents on well-being of bitches with transmissible venereal tumor (TVT)

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Aim was to evaluate efficacy of two different chemotherapy agents on TVT treatment in bitches and their effects on well-being of these animals. Bitches with TVT were either treated with Doxorubicin (DOX, 30 mg/m^2, n = 7) or Cisplatin (CIS, 70 mg/m^2, n = 7) in four applications with 21 days interval. Blood samples were collected before the first application and 24 h after the each application to monitor blood parameters such as hemocell counts, blood gases and different metabolic enzymes. In DOX group, six bitches were fully treated as tumor tissues and hemorrhage were completely disappeared with no complications while in CIS group, five bitches died during the course of treatment and remaining two had no visible improvement. Furthermore, various side effects such as anorexia, vomiting, tachycardia, tremor, diarrhea, and depression were observed in CIS group. Hemocell counts showed that bitches in both group developed leukocytosis and micrcytic-hypochromic-regeneratif anemia with remarkable decrease in leukocyte numbers. Metabolic acidosis was observed in both groups at the end of the treatment period. In CIS group, blood urea nitrogen (BUN), aspartat aminotransphersase (AST) and trigliserid (TRI) concentrations increased. Although creatine phosphokinase (CPK) increased numerically, an increase in phosphor and creatine indicated a decrease in glomerular filtration rate. In conclusion, DOX appeared to be safer and effective chemotherapy agent in bitches to treat TVT while CIS resulted in higher mortality rate and complications such as acute nephropathy

P62

Inhibition of cathepsin b does not affect the developmental competence of prepubertal calf oocytes and embryo quality *in vitro*

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Cathepsin B belongs to a family of lysosomal cysteine proteinases, which participe in a variety of proteolytic processes. Recently, the quantity of cathepsin transcripts in cumulus cells, especially cathepsin B, was found to be associated with low-developmental competence of adult bovine oocytes. In the present study, we investigated the effect of a cathepsin B inhibitor (E-64) during *in vitro* maturation of prepubertal calf oocytes on embryo development and quality. Cumulus-oocyte complexes (COCs) were matured in medium TCM 199 containing FCS and EGF (control), and supplemented with 1 (E1), 10 (E10) and 100 (E100) μM of E-64. After *in vitro* maturation, the oocytes were fertilized and cultured *in vitro*. Cleavage and blastocyst rates were determined on day 2 and 8 after fertilization. This experiment was repeated six times with 205–299 COCs per treatment group. No differences were observed in the cleavage rates among control group (77.7%), E1 (74.3%) and E10 (72.9%) groups while the E100 group reduced the cleavage rate significantly (67.3%; $p < 0.05$). At day 8 pi, blastocyst rate was significantly lower ($p < 0.05$) for E100 when compared to the other groups (12.5%, 11.5% and 8.7% for control, E1 and E10, respectively). When embryo quality was assessed by total cell number, no significant differences were observed among experimental groups (control: 135 ± 6.3; E1: 131 ± 8.1; E10: 137 ± 5.3 and E100: 124 ± 6.4). In conclusion, these data suggest that the inhibition of Cathepsin B during *in vitro* maturation does not improve the developmental competence of prepubertal calf oocytes.

P63

Effects of ysteine and ergothioneine on thawed merino ram sperm and biochemical parameters

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The study evaluated effects of antioxidants on thawed sperm parameters, lipid peroxidation and antioxidant activities of Merino ram sperm. Semen from rams was collected, diluted with an extender containing antioxidant and no antioxidant, then frozen. Straws were thawed at 37°C. Ergothioneine at doses of 2 and 4 mM increased rates of subjective motility (81.3 ± 2.3% and 80.6 ± 2.9%) compared to control (69.0 ± 2.3%). Ergothioneine at three different doses led to

higher rates of progressive motility (25.9 ± 2.2%, 31.0 ± 2.7% and 32.4 ± 2.2%) when compared to control (19.6 ± 1.1%, $p < 0.05$). Antioxidants did not show significant differences on rates of post-thaw sperm CASA motilities, in comparison to control. For sperm membrane integrity, cysteine 1 mM (72.8 ± 6.9%) showed a greater protective effect, compared to control (60.4 ± 1.8%, $p < 0.001$). Rates of sperm with high mitochondrial activity were increased with cysteine at doses of 1 and 2 mM (66.6 ± 10.6% and 67.4 ± 6.1%), compared to control (35.3 ± 7.0%, $p < 0.05$). CAT activity was only increased significantly in cysteine 1 mM (322.8 ± 16.5 mU/ml) compared to control (172.6 ± 37.1 mU/ml, $p < 0.001$). Cysteine at doses of 2 and 4 mM (226.0 ± 43.4 mU/ml and 253.7 ± 19.4 mU/ml) showed a tendency of increased activities of CAT when compared to control (172.6 ± 37.1 mU/ml). Ergothioneine supplementation in semen extenders was of greater benefit to motility and motion of frozen-thawed ram sperm.

P64

Lipid-encapsulated conjugated linoleic acid (CLA) supplementation: effects on reproduction in dairy cow

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High producing, multiparous Holstein Friesian cows were allotted into three groups. CLA1 group (n = 19) was supplemented with 70 g of Lutrell Pure® (BASF SE) containing 7–7 g of cis-9 trans-11 and trans-10 cis-12 CLA isomers from 21 day before calving until 10 day after artificial insemination (AI). CLA2 (n = 19) was supplemented from calving until 10 day after AI. Control dams (n = 20) received isocaloric, isonitrogenous and isolipidic diet. Between d49–63 postpartum (pp) animals were exposed to Pre-Synch protocol, followed by AI. Milk progesterone (P4) was monitored from calving until pregnancy check. On d33–35 after AI transrectal ultrasonography was performed and pregnancy-specific protein B was determined. Cows returning to estrus following AI were re-inseminated, and pregnancy was checked according to the farm routine. Day of first pp ovulation did not differ between groups. Dams from both supplemented groups re-conceived earlier compared to control ($p = 0.04$). P4 rise in supplemented animals tended to be more intensive between d3 and 6 following ovulation. Conclusion: CLA supplementation did not enhance resumption of pp ovarian cyclicity. However in the periparturient period CLA had beneficial effect on the intensity of luteinisation which may increase the survival rate of the conceptus and thus shorten transition period.

P65

Effect of NGF on *in vitro* maturation of bovine oocyte and subsequent developmental competence of embryo

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Aim of the study: Nerve growth factor (NGF) is known to be a member of neurotropin family. Since NGF is produced by granulosa cells, it may play an important role in oocyte maturation and embryo development. The present study was designed to investigate the effect of NGF on *in vitro* maturation and development of embryos to the blastocyst stage. Methods: Ovaries and oviducts were collected from a local slaughter house. Immature oocytes with more than three layers of cumulus cells were cultured in tissue culture media (TCM 199) containing 100 iu/ml penicillin and 100 μg/ml streptomycin with 10% fetal bovine serum (FBS), E2, and various concentrations (0, 1, 10 and 100 ng/ml) of NGF. Oocytes were incubated at 38°C in CO2 incubator containing 5% CO2 and 90% humidity for 24 h. Frozen/thawed Jersey semen was processed by density gradient method. Matured oocytes were fertilized in fertilization media for 18–20 h in CO2 incubator. An oviduct was disinfected and washed with sterile buffer followed by mechanical cell recovery into TCM 199. Embryos and oviduct cells were co-cultured. Results: Result indicated that the rate of nuclear maturation did not show any significant difference in comparison to the control group, whereas cleavage rates and blastocyst formation rates were significantly ($p < 0.05$) increased if more than 10 ng/ml NGF were added to the IVF system. Conclusion: Addition of NGF in maturation media may not influence the maturation rate but it might enhance the developmental competence of bovine embryos to the blastocyst stage, suggesting that the enhancing effects might promote oocyte cytoplasmic competence and thereby embryo development.

P66

Susceptibility of dog sperm to different reactive oxygen species

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An alternative to overcome sperm damages caused by oxidative stress occurring during dog sperm cryopreservation is antioxidant treatment which requires the identification of which ROS are the most deleterious. Regarding this aim, semen samples from six adult dogs were collected, pooled and centrifuged. Samples were divided into two aliquots and centrifuged; seminal plasma (SP) was removed from one aliquot and the other was resuspended with SP. Samples were then incubated (1 h, 37°C) with 4 ROS inducer mechanisms: xanthine/xanthine oxidase (superoxide dismutase), hydrogen peroxide, malondialdehyde, ascorbate and ferrous sulfate (hydroxyl radical). Samples were analyzed for motility (CASA); mitochondrial activity (3,3′diaminobenzidine); membrane integrity (eosin/nigrosin); acrosome integrity (fast green/bengal rose); DNA fragmentation (SCSA); and malondialdehyde (TBARS), an index of lipid peroxidation.

Results showed that dog sperm is differentially modulated depending on the presence of SP and to the different ROS. Samples incubated with SP showed no differences on TBARS. On the other hand, samples incubated without SP showed higher lipid peroxidation when treated with hydroxyl radical when compared to the other ROS. Furthermore, while hydroxyl radical mostly altered mitochondrial activity in samples incubated with SP, hydrogen peroxide was the most deleterious without SP. The present results suggest that seminal plasma may play an important role in dog sperm susceptibility to oxidative stress.

P67

Malign melanoma in norduz goat: case report

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The goat had an ulcerated, black pigmented, hairless, apparently not painful and eliptic (10 × 4 cm) mass at the vulva. The mass was completely blocking to the vestibulum vagina. There was no known history of trauma to the perineal region. No other abnormalities were found on physical examination. Hair around the mass was clipped and the mass was cleaned povidone-iodine solution. Sedation was induced by i.v. treatment with 7.5 mg of diazepam. Lidocaine is injected at several sites around the base of the mass for regional anaesthesia. The mass was surgically excised. A section of the mass was submitted for histopathological evaluation. It had firmness consistency and 4 × 3.2 × 1.8 cm in diameter and weighed of 10 g. Histopathologically, polyhedral or spindle shaped anaplastic cells were extended from dermis to subcutis, which organised in nests or single cells. In cytoplasm of these cells were overloaded with brownish-black colour melanin pigment and their nuclei were inconspicuous. Histopathological findings obtained from both routine hematoxylin-eosin and Fontana masson staining were confirmed to diagnosis of malign melanoma. No abnormalities were observed about the blood parameters that were taken before the operation.

P68

Fine structures of the oocyte in relation to follicular fluid steroid hormones and IGF-I in the ovulatory follicles of camelus dromedarius

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Ovaries with no corpus luteum were recovered from adult she-camels within 30 min after slaughter and transported to the laboratory in a thermos containing normal saline at 32–35°C. Clear appearance ovulatory-sized follicles were then categorized based on their diameter into three classes: follicles with 9–13.9 mm diameter (n = 7), 14–17.9 mm diameter (n = 4), >18 mm diameter (n = 6). The follicles were aspirated, cumulus oocyte complexes were isolated for transmission electron microscopy and the follicular fluid was assayed for estradiol-17β (E2), progesterone (P4) and IGF-I. The mean (±SD) follicular fluid E2 concentrations was significantly (*t*-test; $p < 0.05$) higher in follicles with 14–17.9 mm diameter compared to that of the follicles with 9–13.9 mm diameter. The mean (±SD) follicular fluid concentrations of P4 and IGF-1 was also significantly ($p < 0.05$) higher in follicles >18 mm diameter compared to that of the smaller-sized group follicles. The oocytes collected from follicles ≥14 mm diameter showed more advanced signs of maturation including the increase in the number of microvilli in erect position, the more even distribution of the mitochondria throughout the ooplasm, the disappearance of the nuclear envelop and the increase number and size of vesicles in the ooplasm. In conclusion, final stages of *in vivo* oocyte maturation in dromedary camel is associated with increasing P4 and IGF-I concentrations and constant high E2 concentration in follicular fluid.

P69

Immunolocalization pattern of α-L-fucosidase in porcine sperm

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Previous biochemical studies have described the association of α-L-fucosidase with sperm in different species, including swine. The aim of this study was to determine the distribution of α-L-fucosidase in ejaculated porcine spermatozoa subjected or not to a treatment to undergo capacitation and acrosome reaction (AR). Sperm were divided in two groups: (i) Ejaculated sperm (ES) selected through a Percoll® gradient in TALP and treated with calcium ionophore to induce AR and (ii) Untreated ejaculated sperm (UES). The samples were incubated with a primary antibody (anti-human α-L-fucosidase, produced in rabbit, 1:100, 1 h) and then incubated with a secundary antibody (anti-rabbit IgG FITC, produced in chicken, 1:400, 1 h). AR for both groups was analysed by FITC-PNA using 200 sperm/sample. Percentage of sperm undergone AR was 28.5 ± 1.8^{a} (UES) and 73.2 ± 3.1^{b} (ES), respectively. Immunolocalization pattern was defined in 200 sperm with four replicates by imaging analysis (Leica Qwin V3.4.0). We have found significant differences between the percentage of α-L-fucosidase-positive sperm in UES (92.5 ± 1.04^{a}) and ES group sperm (34.9 ± 5.73^{b}). The fluorescent signal for both groups was located in the acrosome region and evenly distributed. Results indicate that α-L-fucosidase is located in the plasma membrane of the acrosomal region of porcine sperm and that it is mostly released after AR. Specific distribution of α-L-fucosidase in sperm suggests that this enzyme has a potential role in recognition and initial interaction of gametes during fertilization. *Granted by MEC AGL2009-12512-C02-01 and CARM 0452/GERM/06, 08752/PI/08*

P70

Immunophenotypic characterization of equine mesenchymal stromal cells using flow cytometry

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The therapeutic potential of mesenchymal stromal cells (MSC) has generated an increasing interest in equine veterinary medicine. Although many articles on the isolation and *in vitro*

differentiation of equine MSC are reported, only a few groups have undertaken an attempt to immunophenotype these cells. The lack of a single specific marker for MSC and the limited availability of monoclonal antibodies (mAbs) for equine MSC, are major complicating factors. In this study, we describe the expression of a panel of both cell surface antigens and intracytoplasmatic proteins to characterize undifferentiated equine MSC from umbilical cord blood using multi-color flow cytometry. These undifferentiated cells were first characterized by differentiation towards osteocytes, chondrocytes and adipocytes. At least 10 000 cells were analyzed using a two laser FACScanto flow cytometer (Becton Dickinson Immunocytometry systems), and FACSDiva software. All data were compensated and corrected for autofluorescence as well as for unspecific bindings. Isolated MSC from equine umbilical cord blood expressed CD29, CD90, CD44 and lacked expression of CD79α, MHC class II antigens and a monocyte marker. Unfortunately, none of the different clones of mAbs used to detect CD34, CD45 and CD73 respectively, were able to recognize equine epitopes on equine MSC as well as on equine positive control cells, indicating that these clones do not cross-react with the horse.

P71

Detection of bovine herpesvirus-1 (BoHV-1) in semen

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In countries where BoHV-1 is endemic, to prevent virus spread by contaminated semen it must be screened. In 2010, 2.878 extended semen belonging to 530 bulls, of European and Zebu breeds, of seven Brazilian AI centers were analyzed for BoHV-1 by nested PCR. The primers were B gene which amplifies, respectively, 653 and 274bp. Positive samples in nested PCR were also submitted to viral isolation in monolayer MDBK cell lines, in individual culture tubes. From the total, six were positive for BoHV-1 in nested PCR and in viral isolation. Although PCR is more sensitive, the viral isolation corroborate the PCR, showing that the virus was infective. All the positive semen belonged to the same AI center: three batches was of one red angus bull that eliminated BoHV-1 in two subsequent weeks; another red angus bull eliminated BoHV-1 in only one ejaculated. Other two positive batches were from one limousin eliminated BoHV-1 in two subsequent ejaculated with three days of intervals. It is believed that the origin of the outbreak was due to adverse climatic conditions like high temperature and humidity. As european breed is less adapted to tropical climate, it probably reactivated the latent BoHV-1 and spread to the other bulls. In Brazil, the IBR/IPV is endemic and one of the measures to prevent and control this disease is to analyze all batches of semen produced in AI centers.2011Abstract

P72

Alternative treatment of ovarian cysts with *Tribulus terrestris* extract: a rat model

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Tribulus terrestris has long been used in traditional medicine to treat impotency and improve sexual functions in man. Its main constituents are saponins, diosgenins, alkaloids and amides. There is little information about the treatment of female reproductive disorders with *T. terrestris*, The aim of the present study was to evaluate the Tribulus terrestris extract efficiency in the treatment of polycystic ovaries in Wistar rat model. The 15 mature rats received 2 mg/case estradiol benzoate i.m. at day 0 of the study to induce polycystic ovaries. The rats were randomly divided into three groups (control, low-dose, high-dose group) which received 0, 5 and 10 mg of *T. terrestris* extract, intraperitonealy. The treatments were given on days 50 and 61 after estradiol injection; at the same time a vaginal smear was prepared. The ovaries were removed on day 62 and histological sections were prepared accordingly. The number of corpora lutea and their diameters, the thickness of the theca interna layer and the number of all follicles were evaluated in both ovaries. In comparison to control group, the number of corpora lutea and primary and secondary follicles significantly increased following *T. terrestris* treatment in high-treatment group (0 ± 0 vs. 3.6 ± 2.7; 0 ± 0 vs. 6.8 ± 2.2; 2.6 ± 1.4 vs. 20.6 ± 2.7, respectively), but the number of the ovarian cysts decreased (5.4 ± 1.1 vs. 1 ± 1). It can be concluded that *Tribulus terrestris* have a luteinizing effect on ovarian cysts, which may relate to its gonadotropin-like activity; also high doses of the extract can efficiently remove ovarian cysts and resume ovarian activity in the rat.

P73

The effect of butylated hydroxytoluene on the functionality of boar spermatozoa undergoing sex sorting and cryopreservation

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Sex sorted pig spermatozoa by flow cytometry are known for their sensitivity to cold shock, which is related mainly with the amount of unsaturated fatty acids present in the membranes. This study evaluates the protective effect of a lipid-soluble antioxidant (butylated hydroxytoluene; BHT) on the quality of sex sorted and frozen-thawed boar spermatozoa. Sperm were sex-sorted using a MoFlo SX, collected in Tes-Tris-glucose + 2% egg-yolk media supplemented with 0 (as control), 0.04, 0.4 and 4 mM of BHT; and cryopreserved using a 0.25 ml straw procedure. Sperm membrane integrity (flow cytometry) and sperm motility (computer-assisted analysis) were evaluated after sorting and through the cryopreservation process (after centrifugation for sperm concentration prior to freezing, at the end of cooling period at 5°C, and either 30, 90 and 150 min after thawing). Data were processed by SPSS 15.0, including a multi-factorial ANOVA. As expected, the time after thawing had a significant negative effect on sperm motility and membrane integrity ($p < 0.05$). However, when the BHT effect was evaluated, plasma membrane integrity and motility were not different among groups, but a positive tendency (approximately 10%) for the percentage of membrane integrity was observed when 0.4 mM of BHT was used, suggesting a positive effect on the functionality of the cells. *Supported by MICINN (AGL2008-04127/GAN), Séneca Foundation (GERM04543/07) and Sexing Technologies (TX, USA).*

P74

Free radicals detection by the fluorescein probe (CM-H2DCFDA) and flow cytometry is a good predictor of fertility in ram sperm samples

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Semen analysis aims to determine the potential fertility of each sample in a simple and low cost manner. Evaluating individual male fertility by artificial insemination is expensive and laborious. Therefore, there is a need to develop techniques to assess *in vitro* sperm characteristics capable to predict *in vivo* fertility. We studied flow cytometry as a tool to predict *in vivo* fertility of frozen-thawed ram sperm. Semen samples were collected by artificial vagina from six rams of Manchega breed and afterwards frozen. In order to evaluate the *in vivo* fertility, intrauterine inseminations were carried out in 551 ewes with a mean fertilization rate of the 42%, ranging from 22 to 62%. Frozen semen samples were thawed and incubated for 2 h in freezing extender and in SOF medium (37°C, 5% CO2), simulating physiological conditions in the female reproductive tract. Sperm viability and apoptosis were assessed by the fluorochromes, propidium iodide (PI) and YoPro-1, respectively. Furthermore, we evaluated the peroxidation process by the BODIPY 581 fluorochrome and lipid and radical production by fluorescein (CM-H2DCFDA). Our results were analysed by multiple regression analysis and showed that after incubating these samples for 2 h in SOF, there was a negative relationship between fertility and the amount of free radicals as measured by the fluorescent probe CM-H2DCFDA ($p < 0.05$; $r = -0.895$). For the other parameters evaluated no relationships with fertility *in vivo* were found.

P75

Influence of the sperm fertilization doses on chromosomal abnormality rates of 4-day-old bovine embryos

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This cytogenetic study was undertaken to quantify, by chromosomal analysis, the incidence and type of chromosomal abnormalities on *in vitro*-produced (IVP) bovine embryos. Four groups of 150 matured oocytes were *in vitro* fertilized for 18 h with a final sperm concentration of 1×10^4, 1×10^5, 1×10^6 and 1×10^7 cells/ml. Presumptive zygotes were cultured for 96 h, collected and fixed individually for chromosomal analysis. A total of 225 embryos were suitable for chromosomal analysis following a standard method. Rates ofhaploidy, diploidy, aneuploidy and polyploidy were determined individually. There were no differences ($p > 0.05$) in chromosomal alteration rates at concentrations of 1×10^4, 1×10^5 and 1×10^6 cells/ml. The incidence of polyploidy strongly increased ($p < 0.01$) at concentration of 1×10^7 cells/ml. The fertilization rate obtained with the lowest concentration (1×10^4 cells/ml) was significantly lower ($p < 0.05$) than those obtained with the other concentrations. Acceptable fertilization rates with low chromosomal abnormalities can be achieved with a final concentration of 1×10^5 or higher. In conclusion, the use of low sperm concentration doses in the IVF process in cattle drastically reduces the number of chromosomal abnormalities, without affecting the embryo yielding.

P76

Ultrasonic characteristics of follicular dynamics during the estrous cycle in native jennies and mares in upper Egypt

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The aim of the current study was to compare follicular dynamics and other related parameters in native Egyptian jennies and mares throughout the estrus cycle using ultrasonography. Eight estrus cycles in eight clinically healthy mature native jennies and 18 estrus cycles in nine clinically healthy mature native mares – using an ultrasonic device with a 6/8 MHz probe – were studied from February to June. The results revealed that the length of the interovulatory interval, estrus and diestrus in mares were 21.9 ± 0.42, 7.3 ± 0.81 and 14.7 ± 0.95 days, respectively. The corresponding figures for the jennies were 24.25 ± 1.26, 8.6 ± 0.61 and 17.25 ± 0.72 days. One and two follicular waves per cycle were recorded for jennies and mares throughout the recorded cycles, respectively. The largest follicle of the first follicular wave was first detected at D −1.75 ± 0.47 and −0.80 ± 0.84 (Day 0 = ovulation day, where days in minus mean days before ovulation) in mares and jennies, respectively, the difference was significant. The difference in the growth rate of the dominant follicle was not different between the mares (2.78 ± 0.14 mm/day) and jennies (2.32 ± 0.18 mm/day). In mares, the largest follicle of the second follicular wave reached a maximum diameter of 42.70 ± 2.63 mm at D 19.25 ± 0.43. The CL regressed earlier in mares than in jennies. Results of the present study indicated that compared to mares jennies are mono-ovulators with one follicular wave per cycle. The dominant follicle in jennies was detected earlier than in mares and had a slower growth rate. CL developed earlier and regressed gradually in jennies as compared with mares.

P77

Efficiency of artificial insemination in mediterranean italian buffalo during two seasons

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The aim of this work was to compare the efficiency of AI in buffalo in two different periods of the year. The trial was carried out during autumn, i.e. the favorable reproductive season, and midwinter, characterized by a daylight transition towards increasing photoperiod at Italian latitude. In particular, we evaluated if the increase of daily light hours affects early embryonic mortality (EM) rate. Buffalo cows were synchronized by OVSYNCH protocol and artificially inseminated in autumn (n = 131) and midwinter (n = 125). Twentyfive and 45 days after AI, the buffaloes underwent ultrasonography. Buffaloes pregnant on day 25, but not on day 45, were considered to have undergone EM. Differences between the two periods were analyzed by X-square test. In

autumn, pregnancy rate on day 25 after AI was 62.6% and declined to 58.0% by day 45, with an EM rate of 7.3%. In midwinter pregnancy rate on Day 25 after AI was 59.2% and declined to 45.6% by day 45, with an EM rate of 23.0%. The pregnancy rate on day 45 was higher during autumn compared to midwinter ($p < 0.05$). The EM rate was significantly different ($p < 0.05$) being higher in midwinter. This study showed that pregnancy rate on day 45 after AI is significantly improved during the months with decreasing daylight hours. As no difference was found in pregnancy at day 25 between the two periods, the increased pregnancy rate recorded in autumn at day 45 was mainly due to the reduced incidence of EM.

P78

Induction of estrus in awassi ewes in non-breeding season

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Objective of the present study was to investigate induction of estrus and the fecundity rate in Awassi ewes induced by progesterone and eCG during the period approaching the breeding season. Ewes (n = 622) were recruited between July 21 and August 15 in central Turkey and were inserted vaginal sponge containing 20 mg flugestone acetate for 12 days. At sponge removal, ewes received 400 IU eCG i.m. Estrus detection was performed using teaser rams starting 24 h after sponge removal. Ewes at estrus were allowed to mate with a ratio of 1 ram for 5 ewes. Pregnancy rate, lambing rate, litter size and fecundity were determined according to the lambing record. Estrus induction rate was 91% while pregnancy rate was 55%. Lambing rate was detected to be 79%. Multiple birth rate was 46% (38% twin, 7% triple, and about 1% quadruplets). Abortions (3.5%), stillbirths (8.4%) and lamb mortality (3.3%) were mostly observed in multiple pregnancies. Fecundity was 1.42 and increased by 34%, compared to the previous breeding season results (1.08) in the same herd. In conclusion, synchronization method used in this study appeared to be efficient to increase estrus rate, multiple pregnancy, and fecundity in non-breeding season in Awassi ewes. *Funded by TUBITAK-TEYDEB-309O404.*

P79

Comparison of two methods to assess the concentration of dog semen

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The aims of this study were to: (i) compare two used techniques to determine dog semen concentration; (ii) assess the influence of dilution rate on photometric readouts. Twenty one ejaculates were collected from four dogs. Each ejaculate was analyzed for sperm concentration by the Thoma chamber (golden standard) and the photometer SpermaCue. Thereafter, the original semen samples were diluted to obtain three dilutions of known concentration ($100–150 \times 10^6$, $151–200 \times 10^6$ and $201–300 \times 10^6$ sperm/ml) and analysed for sperm concentration with a SpermaCue. Each semen sample was assessed five times with each method, and the repeatability, expressed as intra-class correlation coefficient (ICC), was determined. Data were compared by ANOVA and Pearson's correlations. Significant ($p < 0.001$) and high correlations ($r = 0.759$) were found between the results obtained by the two methods used in this study. The SpermaCue also showed a very good repeatability for assessing sperm concentration of raw semen based on ICC (0.90). For extended semen, the repeatability of the SpermaCue was very good for the three dilutions (ICC > 0.94). Based on our findings, the SpermaCue was sufficiently accurate to be used for the determination of sperm concentration of raw and extended dog semen, and it makes it possible to examine a large number of samples very easily and in a short period of time.

P80

Serum fatty acid composition of dairy cows in different reproductive states

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Dietary fat is beneficial to the reproductive system in dairy cows with a particular importance for PUFAs (polyunsaturated fatty acids). Fatty acids of the n-3 and n-6 families (omega 3 and omega 6) are'essential' and must be supplied by the diet. They provide the precursors for prostaglandin synthesis and can modulate steroid metabolism. The aim of this study was to evaluate fatty acid composition in blood serum of cows with different physiological and pathological reproductive conditions. In total, 42 Holstein-Friesian cows from one dairy farm were examined and were divided in five groups according to ultrasound findings: 1. Regular cyclicity n = 9; 2. Pregnant prepartum n = 5; 3. Puerperium n = 13; 4. Ovarian cysts n = 4; 5. Static ovaries n = 11. Fatty acids were analyzed by Hydrolytic Extraction Gas Chromatography. Significantly higher concentrations ($p < 0.05$) of total serum lipids were detected in Groups 1 and 2 (0.42 ± 0.12 and 0.42 ± 0.09 g/100 g resp.) than in Groups 3, 4 and 5 (0.27 ± 0.04; 0.26 ± 0.06 and 0.27 ± 0.04 g/100 g resp.). There was a significant dominance of PUFA's vs. SFA (saturated fatty acids) in Groups 1, 4 and 5 (61.4%; 64.4% and 59:4%). Higher % of CLA (Conjugated linoleic acid) was found in cows with regular cyclicity (6.92%) than in other cows (0.0; 0.6; 2.8 and 2.9% for Groups 2 to 5, resp.). In conclusion, dietary fat and adequate PUFA concentrations in serum are associated with better reproductive performance in dairy cows.

P81

Ovarian response to estrous synchronization protocol based on use of reduced doses of cloprostenol in cyclic goats

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The aim of this study was assess the effectiveness of the conventional and two reduced doses of a prostaglandin analogue (cloprostenol) to induce luteolysis and estrus. The first trial was carried out using twenty does, which were grouped to the following treatments according to the cloprostenol dose: high (H) 87.5 μg, 1 ml, n = 7; medium (M), 43.75 μg, 0.5 ml, n = 6; and low (L), 26.25 μg, 0.3 ml, n = 7). Ultrasonic examination was done on Days 0 (day of

injection), 3, and 7 to determine the presence or absence of corpus luteum, number of corpora lutea and the area of luteal tissue. In the second trial, 24 does were assigned to the same treatments: H, M and L groups (n = 8, each), which were treated with two i.m. injections of cloprostenol 10 days a part. Ultrasound examination was carried out to determine the presence or absence, size and number of viable corpora lutea on Days −10 (day of first injection), 0 (day of second injection), and 11. In addition, ultrasound scanning was performed daily on Days 0, 1, 2, and 3 to assess the development of ovulatory follicles. Estrus detection was done each 12 h during 72 h after the 2nd injection. In Experiment 1, all does showed luteolysis. In Experiment 2, the percentage of goats in estrus and treatment-estrus onset interval were 100% and 49.5 ± 3.0 h; 100% and 51.0 ± 3.0 h; 75% and 56.0 ± 3.5 h for H, M and L groups, respectively. Development of preovulatory follicles and the number and sizes of corpora lutea after the treatment were similar among the groups. In conclusion, a 43.75 μg dose of cloprostenol is capable to induce luteolysis and synchronization of estrus in cyclic goats. *Founded by CDCH-UCV*

P82

Effect of different synchronization protocols on estrus response and fertility during the transition period in merino ewes

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Aim was to investigate efficacy of different synchronization protocols in the transition period on estrus response and pregnancy rates in merino ewes that had not become pregnant after the breeding season. Three different protocols were used as follow: I) Ewes (n = 30) were inserted intravaginal progesterone sponge (florogestan asetat; 30 mg) for 6 days and injected PGF2α (125 μg) at the time of sponge removal, II) Ewes (40) were treated same as in group 1 and also were injected PMSG (250 IU) at time of sponge removal, III) Ewes (n = 38) were only injected PGF2α at the same time ewes in the other two groups received PGF2α. Estrus was detected by addition of rams 24 h after PGF2α and ewes were mated. Pregnancy was diagnosed 60 days after mating by ultrasonography. Estrus detection rates were 100% for Group I, 93% for Group II, and 82% for Group III. Pregnancy rates were 73% for Group I, 89% for Group II, and 100% for Group III. Both the estrus detection rate and pregnancy rate was significantly different between Group I and III ($p < 0.05$). In conclusion, during the transition period, synchronization protocol I yielded higher estrus detection while protocol III gave greater pregnancy rates.

P83

The usefulness of the synthetic bitch sex pheromone for stimulation of reproductive reflexes in dogs (*Canis familiaris*)

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The aim of the experiment was to estimate the usefulness of the bitches synthetic sex pheromones (BSSP) for the stud dogs stimulation. Eau De Estrus® (Symbiotics USA) containing the bitches sex pheromone is designed for the male stimulation during semen collection with the lack ofbitches in estrus. We evaluated the usefulness of this atractant in two experiments. In the first experiment the 10 stud dogs have been used. The dry swabs, containing BSSP and natural pheromones obtained from the vagina of the bitches being in estrus, were presented to the dogs. In the second experiment bitches being in anoestrus (n = 5) were presented to the stud dogs (n = 3). Next, he same females were presented to the dogs after aplication of a few drops of the BSSPto the area of vulva of those bitches. In the next step, the bitches in estrus (n = 3) were presented to the dogs. The evaluation of the results was based on the obserwation of the males behavior. The intesive snifing and general stimulation were observed when the swabs with natural pheromones and bitches in estrus were presented to the stud dogs. Unfortunately, in both experiments we did not notice the positive reaction (described as a intensively snifing and sexually aroused) to the dry swabs, anoestral bitches and swabs or bitches treated with synthetic pheromone. We concluded that in this model of experiment the BSSP is not useful for dogs stimulation.

P84

Maternal temperament and body condition score and their impact on ovine and caprine fetal development: assessment by b-mode and non-invasive color Doppler ultrasonography

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A prospective study was performed in order to investigate whether B-mode and non-invasive color Doppler sonography (NCDS) can be used as diagnostic tool to describe the influence of maternal temperament and body condition score (BCS) on foetal and pregnancy related parameters in small ruminants. On the basis of Arena tests, animals were divided into more fearful (MF: seven sheep, seven goats) and less fearful (LF: six sheep, six goats) groups [BCS3 (seven sheep, eight goats) and BCS4 (six sheep, five goats)]. Ultrasonic parameters were assessed every 2 weeks from conception until week 18 of pregnancy. Amniotic vesicle (AV; $p < 0.001$), foetal chest and orbita diameters, biorbital breadth, occipito-snout and liver length ($p < 0.001$–0.01), as well as metacarpus length [$p < 0.03$: effect of fearfulness (FE)] was greater in LF or BCS4 goats than in MF or BCS3 goats. The pulsatility index (PI) and resistance index (RI) ($p < 0.03$, 0.001, resp.) of uterine (UTA) and umbilical (UMA) arteries was lower in LF or BCS4 than in MF or BCS3 goats. Identical results were observed in sheep, except AV, placentome diameter ($p < 0.01$–0.05, FE) and UTA-RI ($p < 0.01$, FE). UMA parameters were affected mainly by FE, especially in goats (RI: $p < 0.01$). In conclusion, maternal temperament and BCS severely affected foetal growth, as well as maternal and placental ultrasonic parameters. Therefore, they should be considered as important factors in the interpretation of results established by B-mode and NCDS.

P85

Progesterone concentrations and pregnancy rates of repeat breeder cows following post insemination prid or GnRH treatments

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The objective of this study is to determine the effects of application of progesterone (PRID) and GnRH injection after artificial insemination on serum progesterone concentrations and pregnancy rates in repeat breeder cows. In this experiment, 45 repeat breeder Holstein cows were divided into three treatment groups. In group I, repeat breeder cows received an injection of GnRH on day 12 after artificial insemination. In group II, repeat breeder cows received a PRID from day 4 to day 11 and in group III, materials received a PRID from day 11 to day 18. Before, 7 and 21 days after the treatments, blood samples were obtained from all treated cows to assay serum progesterone concentrations. In control group 15 repeat breeder cows did not receive any treatment. On 4, 7, 10, 13, 16, 19 and 21 days blood samples were obtained from all control repeat breeder cows to assay serum progesterone concentrations. Pregnancy rates were 20% in group I, 26.6% in group II, 40% in group III and 20% in group IV. There were no significant differences between four groups ($p > 0.05$). After 7 days from application day, serum progesterone concentrations were greater in group I and group III ($p < 0.05$) compared with the control group. In group II, progesterone concentrations were numerically greater than control groups but it was not statistically significant. In conclusion, application of PRID and GnRH injection after artificial insemination statistically did not improve pregnancy rates in repeat breeder cows, despite the fact that serum progesterone concentrations were higher in treatment groups.

P86

Adding hormones sequentially could be an effective approach for IVM of dog oocytes

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There are no successful and repeatable methods for *in vitro* embryo production in the dog. The aim of the present study was to examine the effects of adding hormones sequentially, to mimick the dog's *in vivo* endocrine milieu, on maturation of immature canine oocytes *in vitro*. In the treatment group, hormones were added to the medium each day for 4 days (0–24 h: 2 μg/ml FSH + 20 μg/ml β-estradiol; 24–48 h: 2 μg/ml FSH + 20 μg/ml β-estradiol + 1 μg/ml LH; 48–72 h: 10 μg/ml FSH + 5 μg/ml β-estradiol + 10 μg/ml LH + 4 μg/ml progesterone; 72–96 h: 2 μg/ml FSH + 2 μg/ml progesterone). In the traditional group, 10 μg/ml FSH, 20 μg/ml β-estradiol and 10 μg/ml LH were added daily during the entire culture period and no hormone added to the control group. The sequential hormone addition was more beneficial for IVM rates than the traditional hormone addition and control groups (48.1%, 38.9% and 23.0% respectively; $p < 0.0001$). As a result, hormone addition sequentially may be an effective approach for the IVM of the immature canine oocytes. We suggest that attempts to define the adequate conditions for IVM in the dog should extend towards this new perspective.

P87

Phosphotyrosine glycogen synthase kinase 3 expression in boar epididymal and ejaculated spermatozoa

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Glycogen synthase kinase-3 (GSK3) is a multifunctional protein directed Ser/Thr kinase. Two isoforms exist of GSK3, α (51 kDa) and β (47 kDa), both strictly regulated via phosphorylation. The aim of this study was to investigate the changes in tyrosine phosphorylation of GSK3 in spermatozoa during sperm epididymal maturation and after ejaculation. Undiluted epididymal samples from three boars were obtained by cannulation of four epididymal regions (proximal and distal caput, corpus and cauda), whereas, ejaculated samples were obtained using the gloved hand method and manual masturbation. Epididymal and ejaculated sperm samples were washed twice with PBS at $600 \times g$ for 10 min at 4°C to obtain the sperm fraction. Washed sperm samples were incubated during 30 min at 4°C with lysis buffer to obtain sperm protein extracts. Epididymal and ejaculated sperm samples were analyzed by SDS-PAGE and Western Blot with anti-phospho-GSK3 (Tyr279/Tyr216) antibody (05-413; Millipore, Temecula, CA, USA). A 51 kDa band corresponding to α-GSK3 isoform was present in all epididymal sperm samples, but it was absent in ejaculated samples. Nevertheless, β-GSK3 47 kDa band only appeared in spermatozoa from the cauda epididymis, being its expression increased significantly in ejaculated spermatozoa. In conclusion, these data suggest that tyrosine phosphorylation of β-GSK3 and dephosphorylation of α-GSK3 isoform is a key process implicated in activation of spermatozoa after ejaculation.

P88

Cryopreservation of bovine ovarian tissue and subsequent *in vitro* embryonic development, using an effective approach for oocyte collection

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This study aimed to assess dissection/puncture combined technique for collecting bovine oocytes and to determine the effect of ovarian tissue cryopreservation on subsequent *in vitro* embryonic development. Ovaries ($n = 31$) were cut into small fragments using a scalpel blade and then randomly assigned to fresh tissue or cryopreservation by slow freezing or vitrification groups. Oocytes were collected from fresh and frozen-thawed ovarian tissue by the puncture method. Data were analyzed by one-way ANOVA followed by LSD. The advantage of this technique was demonstrated by the recovery rate of morphologically good quality cumulus-oocytes complexes (COCs) from fresh tissue (31.7 ± 2.0 oocytes/ovary). However, the cryopreservation affected the post thawing recovery rates of total and good quality COCs from slowly frozen (26.6 ± 2.0 and 23.5 ± 2.3 oocytes/ovary, respectively) and vitrification groups (21.7 ± 1.1 and 17.6 ± 1.8 oocyte/ovary,

respectively). The maturation rate was higher for fresh tissue (94.1 ± 1.1%) than the two cryopreservation groups, while the slowly frozen group had better maturation rates than the vitrification group (80.1 ± 1.3 and 73.0 ± 1.9%, respectively). No statistical differences were observed in the cleavage and embryonic developmental rates between fresh tissue and cryopreservation groups. In conclusion, dissection method followed by puncture of bovine ovaries greatly maximizes the number of good quality oocytes recovered, as well as the embryo yield. Ovarian tissue can be successfully cryopreserved by slow freezing and vitrification.

P89

Determination of sex ratio in bovine semen by quantitative sybr green real time PCR

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At present, the only proven method for producing sexed semen in mammals is the cell sorting by flow cytometry, based on DNA difference. Cell sorting by flow cytometer provides a powerful tool for artificial insemination and production of predefined sexed embryos. Due to high costs and decrease in fertility rate, the extensive use of sexed semen in livestock depends extremely on sorting purity of sperm cells. Validating the accuracy of sperm sexing requires reliable procedures, therefore real time PCR assay can determinate sex ratio as reliable assay. In this study a SYBR Green real time PCR assay was used to determinate sex ratio in bovine sperm. Two primers were designed on specific X- and Y- chromosome genes. The Y-specific primers pair were designed on a conserved region of the bovine Y- chromosome-linked SRY gene that is responsible for male sex determination. the Y-product amplification length was 120 bp (GenBank accession no. HQ908797). Oligonucleotide X-specific primers were designed to amplify a 149 bp DNA fragment on the bovine proteolipid protein gene (PLP) (GenBank accession no.HQ875721). Two certified standard curves were obtained using of two plasmids containing the X- and Y- amplicons. The method was validated by a series of accuracy, repeatability, and reproducibility and by testing two sets of sorted and unsorted samples for X- and Y-chromosomes. The evolution of X-chromosome bearing sperm content in unsorted samples showed an average of 50.3 ± 0.97% for ejaculates and 51.60 ± 0.18% for the commercial semen. Accuracy (95.2%), repeatability (CV = 3.19%) and reproducibility (CV = 2.24%) was shown. This new method for quantification of the sexual chromosome content in spermatozoa might be reliable approach and providing a valid support to the sperm sexing technologies with low costs.

P90

Heterologous *in vitro* fertilization using bovine oocytes and stallion fresh and frozen semen

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The present experiment was designed to evaluate the interaction between fresh/frozen-thawed stallion spermatozoa and zona pellucida free bovine oocytes, to analyze the capacity of fertilization of fresh/frozen-thawed semen of stallion by heterologous fertilization and flow citometry. After semen collection from two stallions using an artificial vagina, sperm evaluation were immediately performed. Part of the semen sample was cryopreserved. Sperm viability and motility were reassessed immediately after thawing. The acrossome and plasma membrane integrity of the spermatozoa were evaluated on flow citometry, using propidium iodide (PI) and fluorescein isothiocyanate conjugated with Penut Agglutinin (FITC-PNA). *In vitro*-matured cow oocytes were inseminated with different percent live stallion sperm (hight (> 50%) or low (< 40%) viability stallion sperm). After 18 h of co-incubation, the oocytes were fixed, stained with 4′,6-diamidino-2-phenylindole (DAPI) and examined for the two polar bodies. The results of fertilization with fresh and frozen-thawed hight viability spermatozoa penetrated 51.9 ± 2.25% and 34.4 ± 2.7%. Lower rates of penetration were observed for fresh and frozen-thawed low viability spermatozoa 48.1 ± 6.95% and 13.7 ± 1.60% showing significant differences ($p < 0.05$). In flow citometry, we observed that the fresh and frozen-thawed hight viability have better results for the acrossome integrity 91.04 ± 0.7% and 76.4 ± 7.79%, than the semen with lower viability 27.44 ± 2.59% and 25.92 ± 2.97% and for the plasma membrane integrity the fresh and frozen-thawed hight viability semen have better results 83.6 ± 1.55% and 57.69 ± 9.01% than the lower viability 16.57 ± 3.17% and 6.87 ± 1.05%. These findings suggest that bovine oocytes provide a useful model for assessing the penetration potential of frozen-thawed stallion sperm.

P91

Analysis of IGF-I and IGF-II gene expression in goat oviduct cells using real-time PCR

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Oviducts are dynamic organs that support gamete transport, maturation, fertilization and early embryonic growth and development. Oviducts are targets of estradiol and progesterone produced in response to Leutinizing hormone (LH) and Follicular Stimulation hormone (FSH) by stimulation of ovaries. In our previous study the expression of growth factors such as IGF-I & II ligand by oviduct cell under influence of hormones using RT-PCR method did not show any difference in stimulatory effect when compared with control group (without hormonal treatment). How ever in our new study we accurately analyzed the comparison of expression using real time-PCR.

In this study similar cell culture protocol were followed, we isolated the oviduct epithelial cells from goat oviduct slaughtered at local abattoir. Cells were cultured in TCM 199 medium supplemented with 10% FBS and various concentrations of estradiol, progesterone, LH & FSH. Total RNA were extracted from cell at 0, 24, 48 h culture and also from confluent monolayer stage, Quantitative real-time PCR was applied using two sets of IGF-I & IGF-II primers.

Quantitative real-time PCR showed significant differences in transcript levels between estradiol, progesterone, LH & FSH treatment for IGF-I and IGF-II genes, where as in previous result we visualized the expression of IGF-I (293 bp) and IGF-II (77 bp) ligand in all treated and non treated control goat oviduct epithelial cell.

Using method of Quantitative real-time PCR showed that E2 and P4 regulate gene expression in oviduct and these hormones have a direct effect on expression of IGF-I and IGF-II genes.

P92

Ultrasonographic evaluation of mammary neoplasia in bitches using B-mode and doppler mode

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The aim of this study was to evaluate the sensitivity of ultrasound for the diagnosis of mammary tumors in bitches. Sixty mammary tumors were evaluated, which were divided into two experimental groups (1-benign; 2-malignant). The ultrasound evaluation allowed assessment of echogenicity, surface regularity and presence of other findings. Doppler evaluation allowed assessment of vascular index. Histological diagnosis and immunohistochemistry for evaluation of vascular endothelial growth factor were performed. Twenty-four benign and 36 malignant tumors were diagnosed. No correlations regarding surface regularity, echogenicity and ultrasound findings were found between the experimental groups by conventional ultrasound examination ($p > 0.05$). Likewise, no significant correlation was found between presence of vascularization and its characteristics between groups using color-flow Doppler ultrasound ($p > 0.05$). Power Doppler ultrasound yielded average maximum velocities of 28.71 cm/s for malignant tumors and 19.91 cm/s for benign tumors, which were significantly different ($p = 0.0117$). No significant differences were found for minimum velocity, RI and PI ($p > 0.05$). When analyzing vascular endothelial growth factor (VEGF), an average of 2.22 was found for group 2 and 1.66 for group 1; this difference was statistically significant ($p = 0.0315$). Positive correlation was found between presence of VEGF, presence of vascularization and maximum velocity ($p = 0.0469$). Therefore, Doppler evaluation can be used to assist in diagnosis malignancy of mammary tumors in bitches.
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P93

Sexual behaviours in synchronized estrus goats

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During estrus goats exhibit sexual behavior to attract the male. The objective of this study was to documentand measure anogenital sniffing, tail wagging and contact with other female goats. Two groups ($n = 10$, each) of multiparous 3–4 year old goats were used. The goats in estrous and controls weighed 39.8 ± 0.9 and 41.1 ± 1.2 kg and had body condition scores of 1.6 ± 0.1 and 1.6 ± 0.1, respectively. Estrus was synchronized with intravaginal sponges impregnated with 20 mg fluorgestone acetate for 10 days. Forty-eight hours before sponge removal, 250 IU eCG and 250 mg sodium cloprostenol were administrated i.m. The control group was treated with intravaginal sponges for 10 days; 48 h before removal 2 ml of sterile distilled water was injected i.m. Following sponge removal, the proceptive behavioural incidents were counted by video for 1 h every 12 h during 72 h in both groups. Repeated measures analyses were conducted using Kendall's W test and Wilcoxon signed rank test. The proceptive behaviours were expressed as median and minimum-maximum. The goats in the treated group showed more anogenital sniffing at 36 h: 5 (1–29), 48 h: 2 (1–7) and 60 h: 3 (1–13) than the controls ($p < 0.05$). They also showed more tail wagging at 24 h: 5 (1–258), 36 h: 120 (14–352), 48 h: 22 (2–142) and 60 h: 4 (3–15) than the controls ($p < 0.05$) and similarly more touching with another goat at 24 h: 2 (1–13) and 36 h: 7 (3–14) ($p < 0.05$). The control group did not express proceptive behavior ($p > 0.05$). In conclusion the estrous goats expressed more intense anogenital sniffing and tail wagging, followed by touching with another goat.

P94

Correlation of lactate blood levels with neurological and cardiorespiratory status of canines neonates born by cesarean section

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The aim of this study was to evaluate lactate levels of bitches and to compare these with their offsprings born by cesarean section and to correlate with Apgar score and neurologic examination (pain, sucking, anogenital, magnum and flexion reflexes). Seven healthy bitches with at least one live fetus were submitted to cesarean section under general anesthesia using morphine, propofol and isoflurane protocol. The dams were examined clinically, tested for blood gases and lactate. Fetal cardiac frequency was recorded by ultrasound. Apgar score and neurologic responses of 23 newborn dogs were recorded immediately after birth (M0) and repeated after 10 min (M10). Blood samples were collected from the jugular vein of the bitch and the offspring for lactate determination. The results were evaluated with ANOVA. No significant differences were observed between lactate levels of the bitch and Apgar score and lactate levels of the offspring ($p > 0.05$). Nevertheless, offspring lactate ($x = 5.57$ mM) showed correlation ($p = 0.02$) with fetal cardiac frequency (196 bpm). Lactate levels of the bitches (2.48 mM) were significantly lower than those of the offspring (5.57 m). Concerning the M0 and M10, Apgar score was higher for M10 (M0 = 3.64, M10 = 5.77). Maternal lactate cannot be utilized as a single method to evaluate post cesarean fetal depression. However, when associated with other clinical evaluations and the fetal heart rate, it can provide an auxiliary method to evaluate neonatal viability, indicating tissue hypo-perfusion due to the anesthetic effects on the offspring.

P95

Training of dogs for detection of oestrus specific scent in saliva of cows

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Efficient oestrus detection is a permanent challenge for successful reproductive performance in dairy cattle. Heat detection is time consuming and expensive. Dogs have been trained to identify oestrus specific odour in vaginal fluid, milk,

urine and blood samples under laboratory conditions with accuracy of more than 80%. Also dogs could be trained to work as heat detection dogs direct on farm. Therefore it would be beneficial in terms of hygiene and safety, to have the dog working on the feed alley with cows fixed in head lockers. The objective of this study was to test if dogs can be trained to detect oestrus specific scent in saliva of cows. Saliva samples were collected from cows in oestrus (n = 46) and dioestrus (n = 68). Thirteen dogs were trained in this experiment. In the test and training situation dogs had to detect one positive out of four samples. A false indication was ignored and documented in the test situation. For determination the accuracy dog handlers were blinded regarding the position of the positive sample. The overall percentage of correct positive indications was 58.8% (n = 316) with a range from 40 (one dog) to 75% (three dogs). To our knowledge this is the first report that dogs are able to detect oestrus specific scent in saliva of cows. Although the accuracy of detection was lower as for vaginal fluid, a dog should be able to identify cows in oestrus sniffing at the mouth and nose.

P96

Sex ratio of the offspring in relation to the uterine horn of gestation in dairy cattle

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Asymmetric distribution of the sexes within the uterus of pregnant animals has been described in numerous species. In cattle, however, limited information is available regarding sex ratio of the offspring in relation to the uterine horn of gestation. In two recently published reports concerning beef cattle, by Hylan et al. (2009) and Giraldo et al. (2010), a significantly greater proportion of males were gestated in the right uterine horn and a greater proportion of females in the left uterine horn. It was concluded a need of further investigation to prove this phenomenon. Therefore the specific objective of this study was to determine the sex ratio of calves gestated in the right and left uterine horn in dairy cows. In a total number of 303 pregnant Norwegian Red cows, diagnosed by rectal palpation, the side of corpus luteum graviditatis and the pregnant horn were recorded. No transuterine migration was found. The pregnancies were in 63.0% (no191) of the cows in the right uterine horn and in 37.0% (no 112) in the left uterine horn. When the cows delivered, the calving data, including calf sex, were collected. The overall sex ratio in this study was 49.5% (150/303) males and 50.5% (153/303) females. In the right uterine horn the proportion of males was 50.3% (96/191) and the proportion of females was 49.7% (95/191). The sex ratio in the left uterine horn was 48.2% (54/112) males and 51.8% (58/112) females. The difference in the sex ratio of the calves between the two uterine horns was not significant.

P97

Use of anti-oestrogens and aromatase inhibitors to prevent heat induction in anoestrous bitches treated with deslorelin implants

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GnRH agonist implants are available for chemical sterilization in male dogs, but in anoestrous female, these compounds lead to estrus induction in 93% of the cases. Strategies using progestagens and GnRH antagonists have been proposed to avoid this phenomenon, but from now, none had given relevant results. Our aim was then to test the use of aromatase inhibitors and anti-oestrogens to prevent this effect. Eleven anoestrous adult beagle bitches were included in this trial. All bitches were administered a deslorelin implant (Suprelorin® 4.7 mg, Virbac, France) in the post-umbilical region and concomitantly received either the aromatase inhibitor anastrozole 0.1 mg/kg (Group 1, n = 3, Arimidex®; Astrazeneca, France) or the anti-oestrogen clomifen acetate 5 mg/kg (Group 2, n = 8, Clomid®, Sanofi-Aventis, France) during 15 days per os. Bitches were kept under observation during treatment and the month after: vaginal swabs, quantitative progesterone assays and ovarian ultrasounds were performed to detect signs of oestrus and ovulation. In Group 1, 1/3 bitch did not exhibit any oestrous sign whereas the two others presented bloody discharge and keratinization of the vaginal epithelium after 5 and 6 days post-implantation. Ovulation was confirmed in all of these bitches. In Group 2, no bloody discharge was observed in 6/8 bitches whereas in the two others it was seen 7 and 8 days post-implantation. Keratinized cells were observed in vaginal smears of all bitches, but characteristic endoscopic appearance of pro-oestrus/oestrus was only observed in the two bitches presenting vaginal discharge. Ovulation occurred in 5/8 bitches between 16 and 18 days post-implantation. Therefore, these compounds cannot be considered as valuable alternatives to prevent the induced estrus occurring in anoestrous bitches.

P98

Effect of GnRH on enhancement of pregnancy rates of artificial insemination in cross bred cattle

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The aim of this study was to investigate the effect of different doses of GnRH along with artificial insemination and 12 days post artificial insemination (AI) in crossbreed dairy cattle. A total of 110 apparently healthy cross breed dairy cattle with the history of two and more services were selected. The cows were randomly divided into five equal groups: G1 cattle (n = 22) were treated intramuscularly with 20 μg Buserelinacetate (Receptal) at the time of AI; G2 (n = 22) had the same procedure as G1 but with the dose of 10 μg Buserelinacetate. G3 cattle (n = 22) were given the 10 μg GnRH analogue 12 days post AI. The G4 cows (n = 22) had a double insemination with a 6–12 h interval. The last group (G5) was given a single service which was considered as the control group. Pregnancy diagnosis was conducted by transrectal examination after 3 months. The pregnancy rates were found to be 72.73, 63.64, 54.55, 45.46 and 36.37% in the G1, G2, G3,

G4 and G5 groups, respectively. The first two groups showed a significant statistical variation ($p < 0.05$) as compared to control group (G5). The other findings that were observed, were that treated cows became more responsive to the therapy than the cows with a significant variation ($p < 0.05$). The GnRH is cost effective to use as well as to avoid the economic losses from the milk in every cycle as compared to other hormonal protocols.

P99

Peri-parturient metabolic status and reproduction in hungarian dairy herds

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In high producing dairy cows metabolic status determines the most important reproduction parameters (calving interval, number of AI/pregnancy, post partum (PP) uterine diseases and late embryonic loss, LEL). In 2010, blood samples were collected in three high producing Hungarian dairy herds (mean milk production is between 9200 and 10 500 kg/cow/year) from different nutritional groups (dry cow, DC/n = 13/, transition cows before calving, TCb/n = 23/and PP, TCpp/n = 24/, milking cows 10–30 days PP, MC/n = 24/). Metabolic parameters (β-carotene, ferric reducing ability of plasma/FRAP/, NEFA and BHB) were measured from sera/plasma and the most important fertility parameters were also recorded: uterine treatments, pregnancy rate and rate of LEL. The range of mean β-carotene serum concentration (cc) varied in different groups between 2.55 and 4.55 μM, and significant difference was found between DC and MC groups ($p < 0.001$). The highest difference in BHB plasma cc was recorded in TCb and TCpp groups (0.17 vs. 0.35 mM, $p > 0.1$), and similar tendency of mean plasma NEFA cc was measured in TCb and MC groups (0.26 vs. 0.58 mM, $p < 0.01$). No significant differences were recorded in FRAP plasma cc (range 312–378 μM). Data analysis showed significant correlation between the plasma NEFA cc and LEL ($p = 0.065$) in the TCpp and MC groups. Higher plasma NEFA cc in TCpp and MC cows also associated with lower pregnancy rate ($p < 0.01$). Based on these findings it can be concluded that increased plasma NEFA cc until 30th days PP should be a risk factor of LEL followed the first successful AI. *Supported by grant OTKA 73805, Hungary*

P100

Expression of genes influencing the calcium oscillation in pig oocytes

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The aim of this study was to observe the changes in the expression of four genes SERCA2 (sarco/endoplasmic reticulum Ca transporting ATPase), Orai2, STIM2 (stromal interaction molecule) and STX5 (syntaxin) in different development stages of the pig oocytes. We collected the oocytes with the follicular fluid. The *in vitro* maturation took 44 h in TCM completed with LH, FSH and EGF. The GV (germinal vesicle) stage oocytes were used right after collection, the MI (metaphase) stage after 22 h and MII phase after 44 h of maturation. The experiments were carried out three times with all groups and repeated the RT-PCR twice. Ninety-three GV, 92 MI and 92 MII oocytes in the first, 70 of each group in the second and 80 in the third experiment were used. After mRNA extraction the transcribed cDNA was used for the RT-PCR reaction containing the primers for the four genes, a control gene and a negative control. With the help of Delta Delta Ct method we compared our genes to a control gene: YWHAG (C094522). The statistical analyzes was carried out with the help of SAS program (Tukey's Test). The expression of two observed genes (Orai2 and STX5) reduced significantly ($p < 0.05$). SERCA2 and STIM2 did not change significantly. The reduction of the expression of STX5 is necessary as its protein bounds to polycistin-2 and blocks the Ca^{2+} flow through the ER membrane. The protein of SERCA2 is an important molecule of other membrane transport processes so the expression of its gene should be constant. The proper ratio of STIM2 and Orai2 is essential of the Ca^{2+} oscillation in the oocytes so if the expression of Orai2 reduces STIM2 should stay at the same level. *This project was supported by TAMOP 4.2.1/B*

P101

Angiogenesis regulation by cytokines in the equine corpus luteum

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As pleiotrophic factors, cytokines are able to coordinate different determinant events for luteal function, during the estrous cycle. In the present study, the influence of tumor necrosis factor alpha (TNF), interferon gamma (IFNG) and FasL on angiogenesis coordination has been addressed. After cell isolation from early (ELP, n = 5), mid (MLP, n = 5) and late luteal phase (LLP, n = 5) corpus luteum (CL), cell cultures were stimulated with: (i) no exogenous cytokines (negative control); (ii) TNF (10 ng/ml); (iii) INFG (10 ng/ml); (iv) FASL (10 ng/ml); or (v) FASL + TNF + INFG (10 ng/ml). The mRNA expression of pro-angiogenic vascular endothelial growth factor (VEGF) and its receptor (VEGFR1), anti-angiogenic trombospondin 1 (TSP1) and its receptor CD36 was quantified by real time PCR. During ELP, mRNA expression of the complex VEGF/VEGFR1 was increased by TNF ($p < 0.05$), while all factors alone, or in association, reduced CD36 mRNA expression ($p < 0.001$). In the MLP, TNF maintained the same inhibitory effect over CD36 ($p < 0.05$). During LLP the same cytokine, as well as the association FASL + TNF + INFG reduced VEGFR1 mRNA expression ($p < 0.05$) but increased mRNA expression of TSP1 and CD36 ($p < 0.05$). Thus, TNF may support angiogenesis during CL establishment, while synergic action of cytokines FASL + TNF + INFG during LLP may determine vascular regression mediated by the increase of anti-angiogenic factors.

P102

Analysis of spermatozoa from venus transgenic boars produced by *sleeping beauty* transposition

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Recently, we described a method for producing transgenic pigs using a non-autonomous Sleeping Beauty (SB) transposon. *In vivo* developed porcine zygotes were co-injected with Venus transposon and a hyperactive SB100 transposase. A total of 141 *in vivo* developed zygotes were injected and transferred to synchronized foster sows. We analyzed reproductive parameters of two transgenic boars, which showed a ubiquitous expression of the Venus reporter in somatic cells. The spermatozoa from transgenic boars were found to be Venus positive by fluorescence microscopy and flow cytometry [FACScan; BD Bioscience, Heidelberg, Germany; argon laser (488 nm; 15 mW), Filter for green fluorescence (530/30 nm)]. Molecular analysis of ejaculated sperm cells suggested three independent integrations of the transgene. Interestingly, all mature spermatozoa tested were phenotypically Venus-positive and gave a distinct fluorescence peak in flow-cytometric measurements. The motility parameters of the spermatozoa were measured with a computer-assisted sperm analyser (CASA; Hamilton Thorne Bioscience-IVOS, Beverly, USA). The motility parameters measured were as follows: MOT% (percentage of total motile sperm), PMOT% (percentage of progressive motile sperm), VSL (progressive velocity, μm/s), VCL (curvilinear velocity, μm/s) and LIN (linearity of track %, VSL/VCL). Motility of the transgenic sperm indicated no drop in percentage of motile sperm. Sorting of sperm cells into X- and Y-chromosome bearing populations, did not reveal any differences in Venus-fluorescence with respect to the sex chromosome load. To test the fertility of transgenic sperm, 16 wild-type sows were artificially inseminated. A clear segregation of the transgenic trait could be seen in the offspring, 10% of the offspring was negative.

P103

Histological study of the ovarian tissue: deleterious effect of the fixative agent on the follicular morphology in domestic species

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Histological examination is commonly used for the evaluation of the morphological integrity of ovarian tissue. The objective of this study was to compare the effect of 5 fixatives [neutral buffered formalin (NBF), Bouin's solution, paraformaldehyde 4% (PAF), Davidson's fixative and Karnovsky's fixative] on the morphological preservation of small follicles in domestic species, in order to define the histological protocol inducing the least artifacts. For each fixative agent and for each species, ovaries were fixed (24 h, standardised conditions), then processed for histology and stained with H&E; 100 follicles from five ovaries were observed. In the doe rabbit, Karnovsky's fixative and PAF allowed the observation of 98.4 ± 0.9% and 84.8 ± 5.1% of normal follicles respectively; in contrast, <4% of normal follicles were observed after fixation with Bouin's solution ($p < 0.05$). In the queen and the bitch, Davidson's fixative allowed the observation of 86.8 ± 2.5% and 91.6 ± 4.5% of normal follicles respectively; after fixation with NBF, <4% of normal follicles were observed in both species ($p < 0.05$). In the ewe, Davidson's fixative and Bouin's solution preserved 86.8 ± 3.8% and 76.8 ± 4.2% of the follicles; in contrast, <4% of normal follicles were observed when fixed with NBF ($p < 0.05$). In the cow, PAF preserved 79.0 ± 6.9% of normal follicles whereas no intact follicle was observed after fixation with NBF ($p < 0.05$). This study confirms artifacts induced by the fixation depend on both the fixative and the species. It also illustrates how the choice of the fixative may be critical for the accurate assessment of the integrity of the ovary in cryopreservation studies.

P104

Prediction of the timing for parturition and of the kittens birth weight at term

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The aim of this study was to predict both the accurate onset of parturition, using ultrasonographic measurements of the femur, and the kitten's birth weight. For this purpose, a prospective study was performed in 24 purebred queens with normal pregnancy. Cats were scanned from 35 days before parturition to the day of term, using a micro-convex probe (8–12 MHz). Lateral and ventrodorsal radiographs were used to determine the litter size. For each foetus, the maximal femur length and the transversal biparietal diameter were measured. The parturition time and the kitten's birth weight were estimated using linear mixed-effects models on R software because of random effects (several foetuses for one queen and a few paired measurements) and fixed effects (litter size, weight, wither height and age). The best linear regression of the parturition time was $y = 37.864–0.193 \times 1 + 1.227 \times 2–0.615 \times 3–0.832 \times 4$. The variables were the femur length (×1), the weight of the queen before pregnancy (×x2), the litter size (×3) and the age of the queen (×4). The 70% prediction level was $y \pm 1.6$ days. The kitten's birth weight was correlated to the calculated femur length at birth (x6) and the wither height (×5). The estimated weight (w) was determined using the following formula: $\log(w) = 0.692 + 0.011 \times 5 + 0.005 \times 6$. The best predicted level was obtained using femur length as compared to biparietal diameter. The duration of the gestation was increased with the weight of the queen before mating ($p < 0.01$). The onset of the parturition was sooner when the femur was longer, and when the queen was older ($p < 0.01$). By predicting the kitten's weight at birth, the model is also informative for their prognosis for life.

P105

eCG is superior to FSH in out-of breeding season pregnancy rate in Mazandarani river buffalos

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Much experience has been acquired in the induction of ovulation in cows during the last 30 years and only recently

such experiments have been performed with water buffaloes. The present investigation was undertaken to examine the pregnancy rate in river buffaloes of myankale (an area in north of Iran, in Mazandaran province) in out-of breeding season following the administration of equine Chorionic Gonadotrophin (eCG) and follicle stimulating hormones (FSH). Ten primiparous noncycling river buffalo from the myankale native herds were used in this study. All the buffaloes received GnRH plus CIDR on day 1 of the experiment. Then they were divided into two groups randomly. Group A: FSH (400 mg, 2 im injections every day of decreasing doses for 5 days) was injected from day 9, then prostaglandin F2α (PG) was injected 48 h later, and CIDR were removed 12 h after PG injection. Group B: One dose of eCG (3000 iu) was injected intramuscularly on day 11, with PG injected and CIDR removed as in group A. By using a teaser bull, all bufalloes from 36 to 72 h after the prostaglandin injection were carefully observed every 4 h for estrus symptoms, particularly for the moment of standing heat, and AI was done twice (at a 12 h interval). Around 35 days after insemination, pregnancy diagnosis was done by ultrasonography. The results showed that one buffalo in group A and four buffaloes in group B were pregnant. All the five pregnant buffaloes had single pregnancy and delivered one fetus in term. This study showed that ovulation in out of breeding season in myankale buffaloes can result in pregnancy and eCG seems to give better results as FSH.

P106

Effect of a GnRH-agonist implant on reproductive funtion in queens – preliminary results

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Slow release GnRH-agonist implants have been proven to be efficient for reproduction control in several species. The aim of this study was to examine the effect of a 4.7 mg deslorelin implant (Suprelorin®) on queens in season. Cats were treated either during oestrus (OE) or after the end of oestrous signs (IOE). Blood samples were taken for determination of estradiol-17ß (E2) and progesterone (P4); cats were observed for oestrous signs, side effects and further changes (body weight etc.). Comparing results for E2 and P4 from treatment to week 12 afterwards, hormone concentrations varied significantly in both groups ($p < 0.0001$). In oestrous queens, E2 decreased from 34.9 (2.1) (day 0) to 12.8 (1.8) (day 7) to 3.5 (1.6) pg/ml (week 12) while P4 increased from 0.3 (2.4) (day 0) to 30.7 (1.9) (day 14) and decreased to 0.6 (2.4) ng/ml (week 12). In IOE queens, E2 slightly increased from 8.3 (1.3) (day 0) to 15.6 (1.3) (day 2) and decreased to 5.3 (1.5) pg/ml (week 12); P4 was 7.7 (4.8) (day 0) initially, increased to 10.9 (5.3) (day 7) and decreased to 0.5 (2.9) ng/ml (week 12). Results indicate that in OE group ovulation and/or luteinisation of follicles was induced, while a slight stimulation of E2 was observed in IOE group. Preliminary results show that treatment with 4.7 mg deslorelin implants is suitable for suppression of reproduction.

P107

Gene expression pattern of adiponectin and adiponectin receptors in bovine dominant and non-dominant follicles

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Recent studies have revealed that adiponectin, a novel adipocytokine, and its two receptors (AdipoR1 and AdipoR2) are involved in regulation of ovarian function in various farm animal species. The aim of this study was to investigate the gene expression pattern of Adiponectin and its receptors in ovarian cells of dominant (DFs) and non- dominant follicles (NDFs) in the bovine. Based on the estradiol/progesterone ratio, two largest follicles from ovaries collected at a slaughterhouse were classified as NDFs and DFs. In addition, the stage of the estrous cycle (follicular or luteal phases) of the slaughtered cows was determined by macroscopic observation of the ovaries and the uterus. The relative expression of adiponectin, AdipoR1 and AdipoR2 mRNA in theca and cumulus cells were determined by qRT-PCR. Adiponectin and its receptors genes were clearly expressed higher ($p < 0.05$) in theca and cumulus cells of DFs than those of NDFs during the follicular and luteal phases of bovine estrous cycle. Positive correlation ($r = 0.7$; $p < 0.01$) was observed between adiponectin mRNA level in ovarian cells of DFs and follicular fluid estradiol concentration in follicular phase. Adiponectin mRNA abundance in ovarian cells of NDFs showed a significant negative correlation with follicular fluid progesterone concentration in follicular phase ($r = -0.7$; $p < 0.01$) and luteal phase ($r = -0.80$, $p = 0.01$). In conclusion, this work has revealed the novel association of adiponectin and its receptors genes with follicular dominance.

P108

Seasonal variation in testicular volume and serum testosterone concentrations in the brown bear (Ursus Arctos)

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The aim of this study was to improve the knowledge on the reproduction of brown bear. To extrapolate the results to the wild population, the changes of testicular volume and serum testosterone were studied in six adult brown bears housed in half-freedom regime in Cabarceno Park (Cantabria, Spain). At this latitude, premating season spans from February to mid April, mating season from late April to late June and postmating from July to August, with slight interannual variations. Previous teleanesthesia (tiletamine + zolazepan 7 mg/kg, ketamine 2 mg/kg), the length and width of each testis were measured using digital calipers. Testicular volume was estimated based on the formula for a cylinder with spherical ends $[(\pi\text{width}2 \times (\text{length} \times \text{width})/4) + (\pi\text{width}3/6)]$. The volumes for right and left testes were combined to obtain total testicular volume for each male. Blood samples were obtained via cephalic venipuncture and serum testosterone concentrations were determined by enzymatic immunoassay. There were seasonal differences in testicular volume and circulating serum testosterone concentrations, with the greatest volume testicular ($206.6 \pm 11.1\ cm^3$) and testosterone concentration (970.5 ± 191.0 ng/dl) in premating period.

During this season, serum testosterone concentrations were positively correlated ($p \leq 0.001$, $r = 0.739$) with testicular volume, otherwise it were not correlated during mating and postmating period. *Supported by MICINN (CGL 2010-19213/BOS) and CANTUR S.A.*

P109

Forskolin improves vitrification ability of *in vivo* derived porcine zygotes

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The aim of this study was to determine the effect of chemical delipidation with forskolin on the viability of porcine *in vivo* derived zygotes vitrified by Superfine Open Pulled Straw (SOPS) or Solid Surface (SS) methods. Zygotes (n = 237) were obtained from weaned sows (n = 14) on day 2 of the cycle (D0: onset of estrus). Zygotes were randomly allocated to two groups and cultured for 24 h in NCSU-23 with 10 μM forskolin (F) or without forskolin (WF). After culture, embryos from both experimental groups were vitrified by SOPS or by SS method. Vitrification and warming were performed as described by Sanchez-Osorio et al. (Theriogenology 2010, 73:300-8). Vitrified-warmed embryos progressing to the blastocyst stage after 96 h of culture were considered viable. Viable embryos were fixed to assess the total cell number (CN). There were no differences in embryo viability between SOPS (59.9 ± 7.3%) and SS (67.2 ± 7.2%) methods. Zygotes cultured with forskolin showed higher ($p < 0.02$) viability (71 ± 7.2%) after vitrification and warming than those zygotes cultured without forskolin (56.1 ± 7.3%). No differences were found among the four experimental groups for the CN (range: 27.4 ± 4.7 to 29.8 ± 4.7). In conclusion, the chemical delipidation with forskolin enhances the vitrification ability of *in vivo* derived zygotes, which can be successfully vitrified using the SOPS or SS methods. *Supported by SENECA (GERM 04543/07) and MICINN (AGL2009-12091).*

P110

Individual *in vitro* bovine embryo production does not alter mrna expression profiles of several quality linked genes

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Without the interfering effects of other oocytes or embryos, an individual IVP system is the tool of choice for studies on oocyte quality and oocyte/embryo metabolism. Before routinely using this system, the current study aimed to check the quality of completely singly produced embryos by analyzing mRNA expression profiles of several quality linked genes. Therefore, grade I COCs (slaughterhouse ovaries) were randomly assigned to two treatments: an individual IVP protocol or routine group IVP (control). Individual maturation, fertilization, and culture were performed in 20 μl droplets under oil on a cumulus cell monolayer; group maturation and fertilization in 500 μl per 100 COCs, and group culture in 50 μl per 25 zygotes under oil. Ten blastocysts per treatment (five replicates) were collected 7 days p.i, and the mRNA abundance relative to H2AFZ of 10 quality linked genes (TP53, BAX, SHC1 SHC, IGFR2, COX2, AKR1B1, PLAC8, SLC2A1, MNSOD and GPX1) was determined. The expression levels did not differ between the treatments, except for GPX1, which was significantly downregulated (ANOVA, $p < 0.05$) in singly produced blastocysts. GPX1 is involved in detoxification and mtDNA protection to oxidative stress. Its overexpression may reflect overall mitochondrial dysfunction. In conclusion, gene expression profiles of individually and in group produced embryos are comparable, thus the single IVP system can be applied as a tool in oocyte and embryo quality studies.

P111

Low-dosage sperm-intra-fallopian-transfer (SIFT) in dairy cows

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The regular insemination dose in cattle is $15–20 \times 10^6$ spermatozoa for frozen-thawed semen or $2–3 \times 10^6$ spermatozoa for sexed, frozen-thawed semen. A new approach has been evaluated in a preliminary field trial to further minimize the required sperm dose in lactating cows to as little as 0.85×10^6 spermatozoa. In a field study 93 spontaneous ovulating cows were used on a large commercial dairy unit. In 47 cows, an average of 0.85×10^6 frozen-thawed bull spermatozoa diluted in 13 μl Andromed™ extender were transferred non-surgically with a specially designed catheter directly into the oviductal isthmus on the ipsilateral side to the dominant follicle-bearing ovary. In another 23 cows, 1.1×10^6 frozen-thawed bull sperm diluted in 17 μl Andromed™ extender were transferred and in a further group of 23 cows 0.85×10^6 frozen-thawed bull spermatozoa extended in 13 μl of Tris-EY extender were transferred in the same way. As control 165 were inseminated with a semen dose of 15×10^6 sperm into the uterine body parallel to the SIFT groups. In all three trial and in the control group two bulls were used. From the three SIFT groups 23.4%, 26.1% and 26.1% of the transferred cows were pregnant 35 days after SIFT, respectively. The pregnancy rates were statistically not different for either semen dose, extender or bull used (Chi-Square-Test, $p > 5\%$). The pregnancy rate for bull one was 30.0% in all groups and for bull two 15.2% after SIFT. In the control group 22.9% of the cows got pregnant after AI with bull one and 35.4% after AI with bull two. The SIFT method was successfully applied employing highly reduced sperm numbers with a non-surgical sperm transfer method directly into the oviductal isthmus in a large commercial diary unit. The results indicate a bull effect when using very low semen dosages.

P112

Effects of a prolonged equilibration time on quality of bovine sperm cryopreserved with egg yolk and soybean extenders

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The effects of a prolongation of equilibration time on quality of sperm cryopreserved with egg yolk and soybean extenders

were investigated. In each of 10 bulls four ejaculates were cryopreserved after equilibration times of 6 and 24 h at 4°C by using an egg-yolk- (EY) and a soybean (SB) extender. Sperm quality was evaluated flowcytometrically by determining the percentage of plasma membrane and acrosome intact sperm (PMAI) immediately after thawing using FITC/PNA staining and calculating the percentage of sperm with a high degree of DNA fragmentation (%DFI) 3 h after thawing performing the SCSA. After 6 h equilibration PMAI was higher ($p < 0.05$) in sperm extended with EY than in sperm extended with SB, but no difference ($p > 0.05$) between sperm extended in both extenders could be observed after an equilibration time of 24 h. For both extenders a positive effect ($p < 0.05$) of a prolongation of equilibration time on PMAI was noticed. %DFI did not differ ($p > 0.05$) between sperm extended in EY and SB after 6 h equlilibration, but after 24 h equilibration %DFI was lower in sperm extended in SB compared to sperm extended in EY. The prolongation of equilibration time led to a decrease ($p < 0.05$) in %DFI in sperm extended in SB, but not ($p > 0.05$) in sperm extended in EY. The results show that the prolongation of equilibration time has positive effects on quality of cryopreserved sperm extended with egg-yolk as well as in soybean extender, but the effects were more distinct using the latter mentioned extender.

P113

Stallion sperm are highly susceptible to hydroxyl radicals

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The use of chilled sperm is crucial for equine artificial insemination. However, studies indicate that quality of chilled semen is highly impaired due to oxidative damages due to Reactive Oxygen Species. The aim of this study was to identify the most deleterious ROS to equine sperm. Semen samples from four stallions were collected, diluted with chilling media and transported to the laboratory under 15°C. Samples were then incubated (1 h, 37°C) with 4 ROS: superoxide anion, hydrogen peroxide, hydroxyl radical and Malon-di-aldehyde (MDA). Samples were analyzed for motility, mitochondrial activity, membrane and acrosome integrity, DNA fragmentation and the measurement of thiobarbituric acid reactive substances (TBARS) an index of lipid peroxidation. Results showed that the hydroxyl radical was more harmful to the mitochondrial activity if compared with samples treated with hydrogen peroxide (24.6 ± 5.9 vs. $43.7 \pm 6.8\%$, respectively). Similarly, TBARS was higher in samples treated with hydroxyl radical when compared to those treated with both superoxide anion and hydrogen peroxyde (2037.7 ± 154.8, 681.2 ± 170.1 and 789.4 ± 124.5 ng/10^6sperm). A positive correlation was found between TBARS and mitochondrial activity indicating that the higher the susceptibility of sperm against oxidative stress is, the lower the mitochondrial activity. TBARS also correlated negatively with motility. The present results suggest that stallion sperm is highly susceptible to the hydroxyl radical, a damage mechanism apparently related to the mitochondrial activity.

P114

Case report – perineal hypospadia in a dog

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Hypospadia is a developmental defective closure of the urogenital folds involving the penis urethra remaining open (Ader et. al., 1978, J. An. Anim. Hosp. Assoc., v.14 p. 721). This reports a Random-bred dog, 9 months age, seen at UNESP Veterinary Hospital – Jaboticabal Campus, with a vestigial penis, without urethral orifice, prepuce ventral opening, and perianal urethral fistula presence, characterizing a perineal hypospadia. A surgery intervention was done, amputating the vestigial penis as well the prepuce ventral opening occlusion, maintaining the urethral perineal fistula. Probably this abnormality occurred due the low testosterone fetus production during the genitalia development, besides it is important point out that the dog is a cub from an incest mate (mother and son).

P115

Leukotriene pathway genes are regulated at mRNA levels in equine endometrium during estrous cycle and early pregnancy

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Leukotrienes (LTs) are mainly involved in inflammatory and immunological responses. There is evidence that they may also be involved in the luteolytic mechanism. Arachidonic acid is converted to LT in a series of enzymatic reactions that include arachidonate 5-lipoxygenase (5-LO), 5-LO activating protein (FLAP), LTA4 hydrolase (LTA4H), and LTC4 synthase (LTC4S). Actions of LTs are exerted through their receptors LTB4R, CysTL1, and CysTL2. The aim of the study was to elucidate the expression profiles of LT pathway genes in the equine endometrium during the estrous cycle and early pregnancy. Endometrial biopsies were obtained from mares on the day of ovulation (d0), at late diestrous (LD, $n = 4$) and after luteolysis during the estrus phase (AL, $n = 4$), as well as on days 14 (P14; $n = 4$), 18 (P18, $n = 4$) and 22 (P22, $n = 4$) of pregnancy. mRNA levels were quantified in duplicates by qPCR. A mixed model was fitted on the normalized data and LSD test was employed. Expression of 5-LO was greater on d0 and LD, declined at AL and was suppressed during pregnancy. LTA4H was increased at LD and at all days of pregnancy, but its expression was significantly greater at LD compared to P14. LTC4S expression was only induced at LD. CysTL1 and 2 expressions were decreased by both cyclic changes and pregnancy whereas FLAP and LTB4R expressions were not affected by pregnancy or cycle. In conclusion, the expression of LT pathway genes are demonstrated in the equine endometrium and their expression levels are regulated during luteolysis and pregnancy.

P116

Evidence-based medicine: quality and comparability of clinical trials investigating the efficacy of prostaglandin F2α for the treatment of bovine endometritis

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The decision making process of practicing veterinarians should be driven by objective and science-based information. Unfortunately, there is a dearth of high level evidence research results in veterinary medicine. In addition, a remarkable variation in the quality of studies in veterinary science was found. Postpartum uterine infections occur commonly in dairy cattle, with a prevalence of up to 57.7%, and are reported to have an immense negative impact on reproductive performance. With regard to the treatment of endometritis, there is a wide discordance between research results investigating the efficacy of PGF2α. Therefore, the objective of this study was to evaluate the quality and comparability of research publications and to summarize the effect of PGF2α for the treatment of endometritis. A comprehensive literature search was conducted utilizing online databases revealing a total of 2723 references. In addition, four articles were retrieved by reviewing citations. After applying specific exclusion criteria defined before the search, a total of 65 publications, comprising 68 trials, were eligible for further analysis. These articles were evaluated utilising specific evidence parameters listed in an evaluation form developed by Dicty [1] such as the involvement of control groups, blinding, randomization, and sample size. The analysis revealed that more than half of the trials (51.5%) were older than 20 years. Furthermore, we found that about one third (36.8%) of all trials was controlled and randomized, while three of those (4.4%) were also blinded. Among the 68 trials, there was a wide variety of diagnostic methods applied. In more than half of all publications (54.4%), the number of treatments administered to each cow was not precisely quantified. Of those trials (n = 22) which calculated a calving to first service interval, 36.4% of the authors observed a decrease. Thereof, about two thirds (22.7%) were statistically significant. Concerning the calving to conception interval, 50% of the authors claimed decrease, which was in 23.3% of the cases statistically significant. Overall, 70.6% (n = 48) of all trials referred to a conception rate. Of those, 35.4% of the authors described the conception rate to increase while less than half (12.5%) provided statistically significant results. To the best of our knowledge this is the first systematic analysis to demonstrate that an impressive percentage of studies addressing the efficacy of PGF2α for the treatment of endometritis are severely flawed in the study design, and comparability between publications is limited due to considerable differences (e.g. definition of endometritis, calculation of reproductive performance parameters).

P117

Ultrastructural alterations in vitrified immature bovine oocytes

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Our previous study showed that addition of 5% butanediol to the vitrification solution (VS) significantly improves the viability and subsequent development of immature bovine oocytes. Therefore, this study was directed towards examining the ultrastructural changes in immature bovine oocytes induced by different levels of 1,3-butanediol (BD) following vitrification/warming processes. Four groups of immature bovine oocytes were incubated in holding solution (HS; M199 + 20% FBS) + 7.5% ethylene glycol (EG) + 7.5% DMSO for 12 min followed by 1 min in three different vitrification solutions (A: Control, B: HS + 15% EG + 15% DMSO + 0.5 M sucrose, C: B + 5% BD, and D: B + 10% BD). Oocytes were then mounted on cryotop devices and plunged into liquid nitrogen. After 10 days, oocytes were warmed and processed for transmission electron microscopy. Results indicated that in control oocytes, a number of cumulus cell projections (CCPs) were traversing the zona pellucida (ZP). Adherent-type junctions were found between distended end of CCPs and microvilli (MV) at the perivitelline space which shows the effective communications between these two types of cells. Reduction in number of MV and swelling of the ZP were observed in all treated groups. However, they were least and most obvious in groups C and D oocytes, respectively. Except group D, other groups showed well preserved mitochondria, endoplasmic reticulum, and cortical granules. Incidence of cytoplasmic vacuoles was significantly higher in treated oocytes than control groups. In conclusion, these data suggest that vital contact between cumulus cells and immature oocyte can be better preserved by addition of 5% BD into the vitrification solution.

P118

Effects of using kerkol (Prangos platychlaena Boiss. ex Tchihat) plant as feed supplementation during mating period on live weight and reproductive traits in does

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The purpose of the study was to determine the effects of Kerkol (Prangos platychlaena Boiss. ex Tchihat), a locally utilized feed crop, when utilized as supplementary feed for flushing purpose during mating period on live weight and reproductive traits in does. Forty-five Hairgoat does were assigned into three groups and were subjected to a 6 week feeding program (3 weeks before and after buck introduction). Basic nutrient requirements of all groups were provided with clover hay and animals in two groups were supplemented with barley or Kerkol. Live weights of control, barley and Kerkol groups at buck introduction were 40.5 ± 2.0, 42.3 ± 2.0 and 45.2 ± 2.0 kg, respectively ($p > 0.05$), whereas they were 41.4 ± 1.7, 44.5 ± 2.2 and 47.4 ± 2.9 kg at the end of feeding program. Mean live weight of Kerkol group at this time were significantly heavier than other groups ($p < 0.05$).

Supplementary feeding with either barley or Kerkol did not affect ($p > 0.05$) pregnancy rate (control, 0.87; barley, 1.00; Kerkol, 0.93), litter size (control, 1.31; barley, 1.29; Kerkol, 1.21) and twinning rate (control, 0.31; barley, 0.28; Kerkol, 0.22). It could be said that in order to determine the effects of Kerkol feeding on live weight and reproductive traits more detailed studies with larger populations are needed.

P119

Large volumes of bull semen separated by slc with a species-specific colloid, Androcoll^{TM}-B

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The possibility of processing whole bull ejaculates in a simple one-step manner would allow separation of spermatozoa from seminal plasma and dead/moribund spermatozoa, all of which can lower the quality and lifespan of the remaining sperm population. Our hypothesis was that purified spermatozoa would potentially tolerate freezing better than non-purified samples. One to three ejaculates from each of seven Estonian Holstein bulls were tested for motility (CASA) and membrane integrity (HOS-test) immediately after ejaculation, after Single Layer Centrifugation through Androcoll™-B (SLC), and post-thaw, both with and without a preceeding colloid treatment. The SLC-selected spermatozoa had significantly higher overall and progressive motility and membrane integrity compared to the fresh sample ($p < 0.05$ – $p < 0.01$). The overall and progressive motility of semen processed through SLC before freezing had significantly higher post-thaw motility than samples frozen in the conventional way ($p < 0.05$). On average, the membrane integrity in fresh vs. SLC semen increased from 46% to 66% and the post-thaw motility in SLC processed semen increased from 55% to 70% compared to semen frozen routinely. Therefore, SLC of bull semen through Androcoll-B could be beneficial when an improvement of semen quality/freezability is needed for bulls with high genetic merit but varying semen quality.

P120

The effect of cell types on *in vitro* development of ovine embryos in co-culture system

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The present study was to investigate the impact of type of co-culture cells on developmental competence of ovine oocytes matured and fertilized *in vitro*. The oocytes were matured for 22–48 h, fertilized with fresh semen and the presumptive zygotes were then cultured for 9 days either in synthetic oviductal fluid (SOF) or co-cultured with mesenchymal stem cells (MSC), embryonic fibroblast cells (EFC), or oviductal epithelial cells (OEC). The cleavage rate in EFC group (66%) was lower than other groups (83%, 85% and 86% in SOF, OEC, and MSC, respectively). The rate of compacted morula at day 5 in SOF (19.4%) was lower ($p < 0.01$) than other groups (31.7%, 33.1% and 30% in OEC, EFC, and MSC, respectively). The proportions of embryos that developed to the blastocyst in SOF and OCE at day 8 (32.6% and 29%, respectively) were higher ($p < 0.01$) than EFC and MSC (9% and 10%, respectively). The highest (56%) and lowest (18%) hatchability rates were achieved in OEC and EFC, respectively. In conclusion, despite the higher speed of embryo development to the compacted morula stage in co-culture system, the proportion of embryos reached to the blastocyst stage in the absence of co-culture cells (SOF) was higher than co-culture system. Moreover, the developmental competence of ovine embryos was inversely affected by the presence of EFC and MSC during *in vitro* culture.

P121

Cold Shock protective effects on boar spermatozoa at 5°C by synergistic effects of additives to a semen extender

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The effects of 10% seminal plasma (SP), bovine serum albumin (BSA) or a cold shock protecting agent (CSP) added to a commercial extender on chilling sensitivity of boar sperm to 5°C were investigated. Sperm rich fractions of six boars were diluted 1:1 with a semen extender (Androhep w/o BSA), cooled to 17°C, centrifugated, and the residual pellet diluted in eight variants of the extender with different combinations of SP, BSA, and CSP. Split samples were stored in parallel at 17, 10, and 5°C. After 24 h motility was evaluated with a computer-assisted sperm analyzer and the rate of sperm with intact plasma and acrosomal membrane determined on a flow cytometer after staining with propidium iodide (PI) and fluorescein isothiocyanate conjugated peanut agglutinin (FITC-PNA). Analysis of variance revealed that BSA or CSP itself were major sources of variance for the amount of PI & FITC-PNA-negative sperm and total motility ($p < 0.01$). At 17°C all eight combinations were in a close range for membrane integrity and total motility. However, at 5°C the combination of all three components (SP, BSA, and CSP) resulted in a higher amount of membrane intact sperm (75.0 ± 7.3%) and total motility (79.1 ± 76.9%) compared to all other combinations ($p < 0.05$). In conclusion, synergistic effects of 10% seminal plasma, BSA and CSP seem to minimize chilling stress on boar spermatozoa.

P122

Apoptosis and proliferation in the canine endometrium during the oestrous cycle

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During the oestrous cycle, canine endometrium undergoes morphological changes including cellular proliferation, apoptosis and differentiation. This study evaluated pathways involved in apoptosis regulation during the oestrous cycle, through the localization and semi-quantification of pro- and anti-apoptotic proteins (FAS, FASL, BAX, BCL-2) by immunohistochemistry. Apoptosis and proliferation were detected by TUNEL and Ki67 antigen, respectively. The highest proliferative index was observed in all cell groups during the follicular phase and again, only in basal glands at early diestrus. This was associated with a low apoptotic index

and a strong co-expression of pro and anti-apoptotic proteins. The highest apoptotic index was detected in the basal glandular epithelium at mid diestrus, which was coincidental with a decrease of BCL2 expression in those cells. An increase in the apoptotic index of crypts, stromal and endothelial cells was observed at the end of diestrus. These results indicate that the regulatory pathways of proliferation are different under estrogen or progesterone dominance. The high BCL2 expression in both the follicular and early diestrus stages seems to oppose the action of pro-apoptotic proteins but, in the absence of embryo implantation (until day 20–22 of diestrus) basal glandular cells will become apoptotic. In conclusion, pro- and anti-apoptotic proteins regulate the balance between cell proliferation and death in the canine endometrium during the oestrous cycle.

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P123

Freezing of dog semen after cool storage: preliminary results

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The aim of this study was to assess the effect of cryopreservation on dog sperm motility after 24 h of cool storage using a low cost Styrofoam box. Semen samples were collected weekly by digital manipulation from four dogs of different breeds. After collection, three ejaculates from different dogs were pooled. The experience was replicated four times. Semen samples were divided into two aliquots. One of them was immediately frozen after collection (control) following a commercial procedure (CaniPro™ Freeze). The other one was cooled for up to 24 h in a Styrofoam box and then frozen following the same procedure. Total and progressive sperm motility was assessed by CASA and compared between fresh and frozen-thawed semen samples by ANOVA. Cryopreservation caused a significant ($p < 0.05$) decrease on post-thaw sperm motility both in control as well as in samples that first underwent cool storage for 24 h. However, no differences were found ($p < 0.05$) in post-thaw sperm motility between the control and the previously stored samples. In conclusion, dog semen can be chilled for up to 24 h in a Styrofoam box and then frozen obtaining similar post-thaw sperm motility as semen samples frozen immediately after collection.

P124

Incidence of diseases in dairy herds in northern Germany

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Management and animal health are important factors for reproductive performance in dairy cows. It was the aim of this study to record common diseases in dairy cows in Northern Germany and describe associations between the lactation number (LN: 1, 2, >2), first test-day milk yield (Mkg1: <30 kg, ≥30 kg) and herd milk yield (Hmkg: ≤7500 kg, >7500 kg), the latter parameter serving as a proxy for herd management (extensive vs. intensive). Data were collected on 98 dairy herds in Schleswig Holstein with a total of 6439 lactations over a period of 2 years. Data were processed on cow level using logistic regression models (GLMMIX) with LN, Mkg1 and Hmkg as fixed, and herd as random effects. Lactational incidences of the following diseases were: hypocalcaemia (HC: 5.0%), retained placental membranes (RFM: 7.2%), clinical metritis/endometritis (ME: 4.9%), clinical mastitis (CM: 15.3%), ketosis (1.6%), displaced abomasum (DA: 0.4%), lameness (L: 15.4%). An increase in somatic cell count above 200.000/ml as indicator of subclinical mastitis (SM) occurred at least once during lactation in 61.9% of the cows. Dystocia was recorded in 13.2% of the cows. Number of lactation (2, >2 vs. 1) was a risk factor for HC (OR 3.7, 23.0), RFM (OR 1.7, 2.5), CM (>2 vs. 1 OR 2.1) and L (OR 1.3, 2.1). LN 1 was a risk factor for dystocia (OR 2.7, 2.8). Mkg1 (≥30 kg) was not a risk factor of disease except for SM in first parity animals (OR 1.3). Hmkg (>7500 kg) was a risk factor for ME (OR 1.9), DA (OR 7.8), L (OR 1.6) and HC (only in cows with high Mkg1 (OR 2.3)). In conclusion, not individual milk yield but management practices resulting in different herd milk yields and age were major factors of various diseases.

P125

The anatomy of cervix and the cervical penetrability during oestrus in the Thai goat

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In goats, intra-uterine deposition of semen leads to higher fertility results than intravaginal or intracervical insemination. However the trans-cervical artificial insemination (TCAI) in goats is limited by the convoluted tubular shaped cervix. This study was to investigate the anatomy of the cervix and the cervical penetrability in the crossbred Thai goat. Sixty cervices were collected from an abattoir near Bangkok. They were classified as follicular or luteal cervices based on the presence of a corpus luteum on at least one of the ovaries and subdivided into nulliparous or multiparous based on the morphology of the uterus. The length of the cervix, number of cervical folds and cervical folds were evaluated. The depth of penetration was measured by passing an insemination pipette into the cervix. In follicular cervices, multiparous had longer cervices than in nulliparous (4.2 ± 0.2 cm vs. 3.5 ± 0.2 cm; $p < 0.05$) with no difference in the number of internal rings ($p > 0.05$). The depth of penetration in multiparous was greater than in nulliparous (3.8 ± 0.2 cm vs. 2.3 ± 0.2 cm; $p < 0.05$). The cervical folds in nulliparous were rosette, flab and slit type (22.73%, 27.27% and 50% respectively) and in multiparous were rosette, flab, slit and papilla type (58.06%, 16.13%, 22.58% and 3.23% respectively). In luteal cervices there were no differences in cervical length, number of cervical rings or depth of penetration. Multiparous goats have more potential for a successful TCAI due to the greater cervical penetrability and rosette folds allow an easier cervical passage during follicular phase.

P126

Resumption of reproduction cycle in postpartum primiparous Kalkouhi ewes

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The main target of the present study was to figure out the moment of postpartal resumption of ovarian cyclicity in Kalkouhi ewes divided into two different weight groups. In total 30 primiparous ewes divided into two different groups (over 45 kg (n = 15) and under 45 kg (n = 15)), participated in the study which took place from August to November. Blood samples were collected every other day by jugular vein puncture starting at day one after lambing until day 25. Blood samples were centrifuged at 3000 rpm for 10 min for serum separation. Levels of oestrogen and progesterone were measured by ELISA. The results showed that the first follicular wave occurred on average at day 7 postpartum in both weight groups. The second ovulation identified by a significant rise in the peripheral progesterone level, occurred on average on day 15 in over 45 kg ewes while on day 11 in the lighter animals. The difference between the two groups was however not statistically significant. These observations show that the first ovulation occurs at the same time in both weight groups, while the second ovulation occurs (not significantly) earlier in ewes with a body weight of <45 kg.

P127

Effects of egg yolk concentration on quality of frozen-thawed goat sperm collected by artificial vagina and electroejaculation

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The aim of this work was to study the effects of adding to a freezing extender (Tris-Citrate-Fructose) different egg yolk concentrations (0, 1.5, 10 and 20%) on goat sperm motility (SM), acrosome integrity (NAR) evaluated subjectively and viability assessed by flow cytometry. For that, semen from six males was obtained by both artificial vagina (AV) and electroejaculation (EE). After the addition of media, semen samples were frozen over nitrogen vapors and thawed for their evaluation. In general, samples collected by AV showed better quality after thawing in relation to EE. SM resulted significantly higher ($p < 0.05$) for AV collection for all extenders. NAR was significantly higher in samples obtained by AV when 0% and 10% egg yolk extenders were used in relation to EE (72% vs. 58.5%; 88.25% vs. 64.65%). Finally, viability resulted significantly higher ($p < 0.05$) in samples collected by AV when freezing extender with 1.5 and 10% egg yolk were used compared to EE. Regarding to the different concentrations of egg yolk, for both methods no differences were found for SM and NAR while viability was significantly lower ($p < 0.05$) for semen samples frozen with 0% egg yolk freezing extender. In conclusion, semen collection by AV shows better results than EE.

P128

Oestrus induction of sows with peforelin, new insights from the field

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Peforelin is a GnRH that specifically induces the release of Follicle Stimulating Hormone in pigs. It is registered for induction of oestrus (Maprelin®, 75 μg peforelin/ml). In a multi-center blinded placebo field trial, the effect of peforelin treatment on reproductive performance was evaluated. Eleven farms were included, totaling 4333 sows, randomly spread over parities and treatments. Treatments [peforelin (M) and placebo (P)] were performed according the registered indication (gilts: 2 ml 48 h after last altrenogest dose, primiparous sows: 0.5 ml 24 h after weaning, multiparous sows: 2 ml 24 h after weaning). Wean to oestrus interval (WOI), farrowing success rate (FSR = litters per 100 treatments) and litter size (total born) was recorded. For statistical analysis, comparisons controlling for farm were performed. WOI tended to be shorter for M (M 5.26 days, P 5.41 days). FSR was higher for M (81.9%) than for P (79.4%) ($p < 0.05$). Litter size was similar (M 13.67, P 13.67). Two response profiles could be distinguished: In farms with a strong positive effect on FSR in primiparous sows (M 88.2%, P 74.7%) ($p < 0.001$), no difference was recorded in gilts and multiparous sows. In farms with a strong positive effect on FSR in gilts and multiparous sows (M 81.9%, P 77.0%) ($p < 0.01$), no difference was recorded in primiparous sows. Optimum dosing in relation to the body condition at weaning is the most likely cause for the differences. Body condition loss is most likely in primiparous sows, but can be a problem at farm level. In new trials, the effect of dosing in relation to body condition will be tackled.

P129

Impact of oestrus induction of sows with peforelin on subsequent litter performance

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Increased litter size correlates with piglet mortality and a decrease in birth weight and litter homogeneity. Reduced follicle development affects the quality of the follicles and of the ovum within. The production of good quality piglets already starts at the onset of follicle development. Peforelin is a GnRH that specifically induces the release of Follicle Stimulating Hormone in pigs. It is registered for induction of oestrus (Maprelin®). In a multi-center blinded placebo field trial, the effect of peforelin treatment on subsequent litter performance was evaluated. Full farrowing batches from nine farms were included, totaling 361 litters. Treatments [peforelin (M) and placebo (P)] were performed according the registered indication. At birth all piglets were tagged and weighed individually. At weaning (24 days), all surviving piglets were weighed again. Litter homogeneity, body weight gain and piglet mortality was calculated. The statistical analysis was performed with SAS®.186 M litters were compared to 175 P litters. Litter size (M 12.59, P 12.46 life born piglets), birth weight (M 1443 g, P 1444 g) and litter homogeneity (M 0.19, P 0.20) were comparable. The combined effect of peforelin on piglet mortality (M 14.1%, P 16.1%) and body weight gain (M 5431 g, P 5332 g) resulted in an increased litter weight gain (M 56.61 kg, P 53.27 kg) ($p < 0.05$).Stimulation of follicle development with

perforelin positively impacts the performance of the subsequent litter. Although the mechanisms leading to this improvement are not yet understood, the effects are relevant in view of the challenges of increased prolificacy of modern genetics.

P130

Effects of immunization against androstenedione or bone morphogenetic protein 15 (BMP15) on reproductive performance in sheep

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Ewes (100 +/group) were immunized for 3 years against androstenedione (A), BMP15 (B) or no antigen (control). Vaccination with A altered (χ^2) the distribution of ewes with 1, 2, 3 or >4 corpora lutea in all years compared to controls and in 2 years for B. Distribution of ewes birthing 0, 1, 2 or >3 lambs was altered (χ^2) by B in all 3 years but only in 1 year for A. Weaning rate for A and B groups averaged 107–137% and 96–129% of controls. Changes in distribution (χ^2) of ewes weaning 0, 1, 2 or 3 lambs were observed in 1 year for A and 3 years for B groups. No differences were detected in proportion of ewes (χ^2) conceiving in the first cycle or partial failure of multiple ovulations. Modelling indicated that both sex and litter size affected birth weight, but no detectible effect of treatment was found. Growth rate was significantly affected by sex, birth weight and number of lambs raised but not treatment. In conclusion, immunization against androstenedione or BMP15 increased ovulation rate, leading to variable changes in number of lambs born and weaned. Androstendione vaccination increased lambs weaned, although not always significantly. BMP15 vaccination altered the pattern of the number of lambs weaned, but often no increase in lamb production was observed as more ewes produced 0 or 3 lambs. Vaccination did not significantly affect embryo/fetal survival or lamb performance independently of the effects on ovulation rate.

P131

Effect of the change in the administration time of PGF2α and gnrh on the growth of the ovulatory follicle during cosynch protocol in presynchronised cows

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The study was designed to assess effect of the change in timing for the administration of PGF2α and second GnRH in the Cosynch protocol on the growth of the ovulatory follicle. Following the determination of corpus luteums by ultrasonography in 14 cows who had completed 45 days postpartum, 2 ml of PGF2α (0.075 mg D-Kloprostenol) was injected intramuscularly to induce presynchronization. An electronic chip was placed over the sacrum of the cows to detect estrus. The Cosynch protocol commenced in both groups on the 6th day of the cycle. Group I (n = 7) received a 2 ml of GnRH (25 mg Lecirelin acetate, i.m.) on day 0. 7 Days later, the cows were given a second dose of PGF2α. Cows were inseminated 56 h after the PGF2α injection and a second dose of 2 ml GnRH was injected. Group II's (n = 7) treatment differed from Group I's in that the second GnRH injection was administered 8 days after the PGF2α injection. Follicular development was monitored daily by ultrasonography. Diameter of the dominant follicle was determined as 14.0 ± 4.9 mm in Group I and as 11.6 ± 2.2 mm in Group II with the PGF2α injection during the Cosynch protocol period ($p > 0.05$). CL growth during the same period was observed as 25.5 ± 4.1 mm in Group I and 23.5 ± 3.9 mm in Group II ($p > 0.05$). No significant difference was determined between the groups in terms of the ovulatory follicle diameter at the time of insemination ($p > 0.05$). In conclusion, a change in the time of administration of PGF2α in the Cosynch protocol does not have an effect on the growth of the ovulatory follicle.

P132

Dose depend effect of *Tribulus terrestris* L. extract on the ovarian activity in rabbits

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The pharmacological activity of the *Tribulus terrestris* L. herb is estimated by its content of steroidal furastanol saponine as a protodioscin. In the present study, we studied the dose depend effect of the Tribulus extract on the follicular activity in rabbits. The experiment was conducted with 35 New Zealand rabbits of 35–40 days old divided in five groups: one control and four experimental. The experimental animals were treated with different doses of the Bulgarian *Tribulus terrestris* L.: 2.5, 5, 7.5, 10 mg/kg of body weight during 42 days. A standard diet of food and water was supplied *ad libitum*. The body weight was measured every 7 days. A routine histological estimation of the ovaries was carried out including follicular dynamics, the number of follicles and their size as measured in serial 5 μm sections of the whole ovary. Data were analyzed using the Statistica software programme (Stat Soft Inc., Ver.6.0). The group treated with 2.5 mg/kg herb showed the highest ovarian activity. In this group we observed all categories of follicular structures with predomination of the tertiary preovulatory follicles. The 10mg/kg dose provoked follicular atresia: in the secondary follicles we observed degenerated cells, in the tertiary – widespread atretic bodies. The daily weight gain was correlated with ovarian activity. The maximum growth intensity (13%, $p < 0.05$ vs. control group) as well as the highest ovarian activity without pathological changes was observed in the group treated by 2.5 mg/kg of Tribulus terrestris L extract.

P133

Thyroid hormones profiles in high-producing dairy cows with different patterns of postpartum luteal activity

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Seventy five multiparous clinically healthy high-producing Holstein dairy cows were used to characterize the relationship between the thyroid hormone levels and the occurrence of

different patterns of postpartum (pp) luteal activity. Transrectal ultrasound scanning and blood sample collection were performed twice weekly. Serum progesterone (P4), beta-hydroxybutyrate (βHB), thyroxine (T4), 3,30,5-tri-iodothyronine (T3), free T4 (fT4) and free T3 (fT3) were measured from the 1st to the 8th week pp. The data were statistically analyzed using the mixed procedure in SAS 6.12. Based on the serum P4 profile of the cows, 33.4% had normal luteal activity (NLA), while 40%, 13.3%, 8%, and 5.3% had prolonged luteal phase (PLP), delayed first ovulation (DOV), anovulation (AOV), and short luteal phase, respectively. The serum T4 concentration in PLP cows was higher than in the NLA cows at the third week pp ($p < 0.05$) and did not change thereafter ($p > 0.05$), while there was a significant increase ($p < 0.01$) in the serum T4 concentrations during the subsequent weeks in the NLA cows. The mean serum fT4 concentrations in the DOV and AOV cows were significantly lower ($p < 0.05$) than in the NLA cows. In addition, AOV cows had higher mean serum βHB and T4 concentrations than the NLA cows ($p < 0.05$). In conclusion, the serum thyroid hormones profile differs in high-producing dairy cows showing PLP, AOV and DOV in comparison with the cows with normal postpartum luteal activity.

P134

MHC-I and -II expression in uterine tissues after induced and spontaneous canine abortions

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The aim of the study was to investigate the expression of major histocompatibility complex (MHC)-I and –II in uterine tissues after induced and spontaneous canine abortions (days 25–45). Abortion was induced with aglepristone (IA, $n = 10$), in comparison to a group of spontaneous abortions (SA, $n = 5$), and a further group of normal healthy pregnant animals that were ovariohysterectomized (controls, $n = 7$). The expression of MHC-I and –II was investigated by immunohistochemistry and RT-PCR at placental and interplacental sites of uterine horns and the corpus uteri. MHC-I and –II mRNA was detected in all tissues studied. At placental sites, the average number of MHC-II positive cells was significantly higher after SA and IA then in controls; in deep endometrial glands layer (SA = 450.80 ± 262.36, IA = 124.20 ± 28.56, control = 66.86 ± 66.22, $p < 0.01$) and in glandular chambers layer (SA = 479.40 ± 133.73, IA = 149.90 ± 59.85, control = 97.14 ± 50.32, $p < 0.01$). The same was observed in deep endometrial glands layer (SA = 260.60 ± 134.69, IA = 178.60 ± 132.36, control = 59.57 ± 58.24, $p < 0.01$) and in surface endometrial glands layer (SA = 260.60 ± 134.69, IA = 178.60 ± 132.36, control = 59.57 ± 58.24, $p < 0.01$) of interplacental sites as well as in the corpus uteri (SA = 111.60 ± 29.80, IA = 82.0 ± 17.68, control = 28.0 ± 9.46, $p < 0.01$ and SA = 121.40 ± 39.53, IA = 50.50 ± 21.96, control = 71.14 ± 24.20, respectively). We suppose that in dogs, induced but especially spontaneous abortions are associated with an increase in the number of endometrial MHC-II positive cells.

P135

Conception rate in lactating dairy cows after artificial insemination with sexed semen

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The aim of the study was to determine the conception rate after artificial insemination (AI) with sexed-sorted semen in lactating dairy cows. Only cyclic cows (n = 292) were randomly assigned into two groups at the time of AI. Cows with a follicle between 12 and 18 mm diameter at the time of AI and clear vaginal discharge were inseminated with either frozen-thawed conventional semen (CS; n = 146) or sexed-sorted semen (SS; n = 146) of one bull (Sylvester, 011H06440, Alta-Genetics, USA). All cows had a deep uterine insemination in the uterine horn ipsilateral to the dominant follicle. Statistical analyses were conducted by using SAS. Pregnancy per AI (P/AI) at 31 day tended to be greater ($p < 0.10$) in CS (37.7%) than SS groups (28.8%), and P/AI at 62 day was lower ($p < 0.05$) in SS (24.0%) than CS (34.9%) group. In addition, parity ($p < 0.05$) and season ($p < 0.01$) were significantly effect on P/AI at 31 day. Primiparous cows had a greater P/AI than multiparous at 31 day in SS ($p < 0.02$; 40.3% vs. 21.3%) or CS ($p < 0.002$; 50.7% vs. 26.5%). Similar results were observed at 62 day in SS ($p < 0.04$; 33.3% vs. 17.9%) or CS ($p < 0.007$; 46.2% vs. 25.3%). During the warm time of the year (June–July–August) P/AI at 31 day in SS group (20.6%) was detected lower ($p < 0.05$) when compared to the cold time of the year (34.9%, from September to May). Similarly, P/AI at 31 day in CS was lower ($p < 0.005$) during the warm (25.3%) than the cold (46.9%) time of the year. In conclusion, P/AI at both 31 and 62 day was greater in primiparous than multiparous cows with SS. P/AI was lower during the warm time of the year in both SS and CS groups. Sexed-sorted semen can be successfully used in primiparous cows, and during the cold time of the year in lactating dairy cows.

P136

Clinical and endocrinological effects of two different treatments for the induction of abortion in bitches

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The aim of the study was to compare the clinical and endocrinological effects of two different treatments for the induction of abortion in bitches. In group 1 (G1, n = 7), only aglepristone (10 mg/kg bw, two injections 24 h apart) was administered. In group 2 (G2, n = 7), aglepristone (as in G1) and cloprostenol (1 μg/kg bw) were combined. To measure progesterone (P_4), relaxin and estradiol (E_2) concentrations, blood samples were collected starting the beginning of treatment (h0) and every 6 h until abortion was completed. Abortion was started at day 2.4 ± 1.3 and 2.4 ± 0.5 in G1 and G2, respectively. Pregnancies were terminated 5.5 ± 1.8 and 5.0 ± 1.5 days after h0 in G1 and 2, respectively ($p > 0.05$). In G1, E_2 levels were always higher than in G2,

but significantly at 42, 48, 54 h and at the beginning of abortion ($p < 0.05$). The average plasma P_4 levels were always higher in G1 than in G2, but significantly from h6 to the end of abortion, when concentrations decreased significantly in G2 and maintained at lower levels until the end of abortion ($p < 0.05$; $p < 0.01$). In conclusion, duration of abortion was not significantly different between groups and the blood concentration of hormones here does not seem to be relevant for the course of abortion. The question remains, by which mechanism $PGF_{2\alpha}$ influences the relaxin + E_2 concentration.

P137

The effectiveness of progesterone support administrations of GnRH, hCG and PGF2α on fertility of Tuj sheep during the non-breeding season

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This study was conducted to evaluate the effectiveness of the administration of different hormones on the fertility parameters of Tuj sheep during the non-breeding season. Ewes assigned to Group I (G1; n = 15) received an injection of 2 ml GnRH (0.0042 mg Buserelin acetate, i.m.) 7 days prior to the implantation of intravaginal sponges (Flugeston acetat, 20 mg). The sponge was then left in the vagina for 14 days, with a second GnRH injection administered 7 days after the sponge implantation. An injection of 1.5 ml PGF2α (5 mg Dinoprost, i.m.) was administered on the same day the sponge was removed. Group II (G2; n = 15) received the same treatment, but the GnRH was replaced by hCG (1000 IU, IM). Animals in Group III (G3; n = 15) were only treated by intravaginal application sponges, while Group IV (G4; n = 15) was the control group. Immediately following the removal of the sponge, the ewes of all groups were introduced to the ram, and those showing estrus were serviced. Estrus rates were established in G1 as 66.6%, 60% in G2, 73.3% in G3, and 20% in G4. The pregnancy rates following the first estrus cycle in the groups were 10.0%, 77.8%, 0.0%, and 33.3%, respectively. The lambing rate in G1, G2, G3 and control group was determined as 66.6%, 100%, 46.6% and 0% respectively. The ewes in G2 displayed a progesterone value of 3.6 ± 2.1 ng/ml on the seventh day following the removal of the sponge while in G4, the progesterone value was established as 1.2 ± 0.4 ng/ml ($p < 0.007$). In conclusion, the promising results we observed in the rates of pregnancy and lambing for Tuj ewes treated by a combination of hCG and vaginal sponge during the non-breeding season may be of further interest.

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Selection of housekeeping gene for quantitative gene expression in cyclic and early pregnant equine endometrium

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The aim was to evaluate a set of Housekeeping Genes (HKGs) to be used in the normalization of gene expression in the equine endometrium. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), hypoxanthine ribosyl transferase 1 (HPRT1), ubiquitin B (UBB), tubulin alpha 1 (TUBA1), ribosomal protein L32 (RPL32), beta-2-microglobulin (B2M), 18S rRNA (18S), and 28S rRNA (28S) HKGs were evaluated using real-time PCR and were compared in different physiological stages of the endometrium. Endometrial biopsies were obtained from mares on day of ovulation (d0, n = 4), at late diestrus (LD, n = 4), after luteolysis (AL, n = 4) of the cycle and on days 14 (P14; n = 3), 18 (P18, n = 3) and 22 (P22; n = 3) of pregnancy. A model based on REML with support of descriptive statistics was proposed in accordance with the experimental design and was further confirmed using the principal component analysis (PCA). Results were compared with widely used softwares including geNorm, BestKeeper, and NormFinder. Results indicated that GAPDH was the most stable HKG and RPL32 was ranked as the second best. 18S and 28S were found to be the least stable. The proposed model, PCA, geNorm, and BestKeeper were in agreement in detecting the most stable and the least stable HKGs in the equine endometrium during the estrous cycle and early pregnancy.

P139

The effect of preovulatory follicle size at the time of insemination on pregnancy in lactating dairy cows

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The aims of this study were to determine the factors that affect follicle size at the time of AI and to detect the relationship between maximal follicle size at the time of AI and pregnancy per AI (P/AI) in dairy cows. Overall 1428 follicle size measurements were performed in Holstein-Friesian (HF) and Swedish Red (SR) cows which were inseminated following a fixed-timed AI protocol (TAI). Pregnancy diagnosis was performed by ultrasonography twice at 31 and 62 day after TAI. Pregnancy loss was recorded when the second check founded negative. All statistical procedures were performed using the multivariate statistical model of SAS. Follicle size was affected by breed ($p = 0.0001$), milk production (MP, $p < 0.02$), parity ($p = 0.05$), season ($p = 0.04$) and type of TAI protocol ($p = 0.001$). Follicle size was greater in HF (15.55 ± 0.19 mm) than SR cows (14.88 ± 0.23 mm) and in multiparous (15.35 ± 0.20 mm) vs. primiparous (15.07 ± 0.21 mm) animals. The effects of DIM, BCS and service number were not significant. Follicle size significantly affected P/AI. Cows that were inseminated with a follicle between 14.5 and 17.5 mm were at significantly higher risk to be pregnant ($p < 0.01$) at 31 and 62 day after insemination. Embryonic losses decreased ($p < 0.01$) in cows that were inseminated with follicles between 13 and 17 mm. Follicle size in dairy cows seems to be affected by breed, MP, parity and season, while P/AI and embryonic loss are significantly related to follicle size at the time of AI.

P140

Chromatin integrity and fertility of frozen ram spermatozoa

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This study aimed at evaluating the influence of thawing temperature on chromatin integrity and fertility of frozen ram spermatozoa. Ejaculates (n = 97) with $\geq 3 \times 109$ sperm/ml and 80% sperm motility were collected from 12 rams, diluted with a soybean lecithin-based medium, frozen in 0.25-ml straws and thawed at either 38 or 48°C for 30 s. Semen evaluation end-points were assessment of sperm chromatin structure using aniline blue, Feulgen, chromomycin A3 and acridine orange staining techniques as well as 50-day pregnancy rate after laparoscopic insemination of ewes (n = 103) in progestagen-synchronized oestrus. Data were analysed using multi-factorial ANOVA and chi squared tests (thawing temperature × ram × ram age × insemination time), Duncan's multiple range test and Pearson correlation coefficient. The results showed that the ram was the main source of variation for all experimental end-points. At 38°C-thawing temperature, pregnancy rate (75%) of mature rams (4–5.5 year old) was higher ($p < 0.05$) than that (45.7%) of young ones (1–2.5 year old). Thawing of frozen semen at 48°C decreased incidences of chromatin de-condensation and DNA denaturation and improved fertility of young rams ($p < 0.05$). Ewes inseminated after 66 h of sponge removal had a significantly higher pregnancy rate (70.6%) than that (50%) of ewes inseminated after 60 h. A significant relationship ($r = -0.55$; $p < 0.05$) was found between chromatin instability and semen fertility. In conclusion, ram age, thawing temperature and insemination time contribute to the success of artificial insemination with frozen semen in sheep.

P141

The effect of presynchronization on reproductive parameters in ewes at the breeding season

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To compare the conception rates obtained in the first and the second oestrus following progesterone and prostaglandin based oestrus synchronisation at the breeding season, 202 ewes were divided into four groups. Ewes in group I (n = 50) and II (n = 50) were synchronised with two cloprostenol ($PGF_{2\alpha}$) (125 μg) injections 11 days apart while ewes in group III (n = 51) and IV (n = 51) were synchronised with intravaginal sponges containing 40 mg flourogestone acetate (FGA) for 11 days. Oestrus was determined using teaser ram at 8 h intervals for 5 days after the last application in all groups and ewes that showed oestrus were recorded in groups I and III while they were hand-mated in groups II and IV. Moreover, ewes that showed oestrus in groups I and III were hand-mated during oestrous determination 16 days after the last application for 5 days. Data were analyzed with chi square analysis for percentage and with ANOVA for time intervals and fecundity. Onset of oestrus for insemination periods in groups I, II, III and IV was 440.2 ± 3.52, 52.5 ± 1.36, 446.5 ± 3.31 and 58.3 ± 1.36 h, respectively. Oestrous rate (%) per insemination period in group III was higher than that in the other groups ($p < 0.05$). In addition, pregnancy and lambing rate (%) in group III was higher than that in group II and IV and fecundity in group III was higher than that in group II ($p < 0.05$). The results of the present study show that presynchronisation can be an alternative to the standard synchronisation protocols in terms of pregnancy and lambing rates in ewes at the breeding season.

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Investigation of pregnancy rates obtained from vitrified holstein embryos

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In the present study, in vivo embryos, obtained following a superovulation protocol with decreasing doses of FSH (Folltropin-V) of donor Holstein cows, were frozen by vitrification and subsequently transferred into recipient cows from the same breed. Composition of vitrification solutions were VS1 [0.1 M Sucrose (S); 0.1 M Xylose (X); 1% Poly Ethylene Glycol (PEG); 10% Glycerol (G)], VS2 [0.2 M S; 0.2 M X; 2% PEG; 10% G; 10% Ethylene Glycol (EG)] and VS3 (0.3 M S; 0.3 M X; 3% PEG; 20% G; 20% EG). By flushing the uterus on day 7 after AI 10 expanded blastocysts of good quality were obtained and vitrified. For thawing, the straws were removed from liquid nitrogen, held in air for 5–6 s and then thawed completely in water at 20°C. For removal of cryoprotectants embryos were washed in 0.5 and 0.25 M sucrose solutions for 5 min each and transfered into PBS with 20% FSC. Embryos were checked for vitality under the stereo microscope at 25°C and then loaded into 0.25 ml straws (one embryo/straw). Within 30 min, embryo were transfered into synchronised recipient cows. Before embryo transfer (ET), rectal palpation of the recipients was carried out to confirm the presence of a corpus luteum. After upper epidural anesthesia ET was performed ipsilaterally and with a non-surgical method. Four of the 10 recipient cows were determined to be pregnant by rectal palpation after day 60 of ET. All pregnancies obtained resulted in births. *In vivo* embryos could easily be frozen by vitrification method, and pregnancy could be obtained by ET into recipients after thawing.

P143

Protamine deficiency in a nelore bull with high levels of dna strand breaks – case report

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The aim of this study was to evaluate whether a Nelore bull with high levels of DNA strand breaks (acridine orange test–AO) also had impaired protamination through chromomycin A3 (CMA3) staining. Frozen-thawed samples from three ejaculates were evaluated for: motility, morphology, simultaneous evaluation of acrosome, membrane and mitochondrial potential (FITC-PNA, PI, JC-1), chromatin integrity (AO) and protamination (CMA3) by fluorescent microscopy and flow

cytometry (Attune™ – 10.000 cells). To contrast these results we selected five sexually mature Nelore bulls with normal values for the same parameters (group B). No significant differences ($p < 0.05$) between Bull A and group B were observed for motility and cells with low mitochondrial potential. Nevertheless, Bull A had much greater percentages ($p < 0.05$) of total morphological sperm defects (A: 50.8 ± 6.5%; B: 4.7 ± 2.7%) chromatin damage (A: 13.8 ± 9%; B: 0.6 ± 0.5%), deficient protamination (A: 2.1 ± 0.4%; B: 0.6 ± 0.1%), and presented lower values for acrosome (A: 24.3 ± 3.3%; B: 76.9 ± 8.9%) and plasma membrane integrity (A: 24.5 ± 6.1%; B: 75.7 ± 9.3%). Although bovine spermatozoa seem to have low rates of protamination deficiency, the effects of nuclear condensation and protamination on fertility are largely unknown. In conclusion, in this study the integrity of DNA alterations appeared to be related to protamine deficiency and greater percentages of spermatozoa with morphological defects.

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P144

The accuracy of transvaginal ultrasonography during early pregnancy in Saanen goats

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Data on the use of transvaginal ultrasonography in pregnant goats are scarce, although transabdominal and transrectal routes have been repeatedly investigated. The aim of this study was to evaluate the accuracy of real-time transvaginal ultrasound during early pregnancy in goats. Fifty multiparous Saanen dairy goats were included. Following estrus synchronization and mating, a B-mode real-time scanner with a 5–7.5 MHz multi-frequency endocavitary probe was used to examine the animals in standing. The value of visualizing at least one gestational sac (with or without any fetal pole) in order to predict the birth of at least one live lamb was assessed at weeks three through eight post mating. The concordance among the sonographically observed embryos and actual number of live births was also determined. The accuracy (proportion of true positives and true negatives to the overall study population) for predicting a live birth increased gradually from 65% at week 3–93% at week 8. Sensitivity values followed a similar pattern (61% at week 3, increasing to 100% at week 8). Positive and negative predictive values at week 8 were 90% and 100%, respectively. However, the concordance rate between the observed number of sacs and the actual number of live births was only 17% at week 3, increasing to about 60% at week 8. In conclusion, transvaginal ultrasound during early pregnancy in Saanen goats (especially after 5 weeks postmating) can effectively predict live births but not the order of multiple pregnancies. Further investigations comparing the utility of transrectal and transvaginal routes in pregnant goats should be encouraged.

P145

Leukotrienes as cytokine mediators in ovarian follicles development, maturation and ovulation in cattle

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Cytokines have multiple functions in the reproductive tract. Leukotrienes (LT)s are commonly known for their proinflammatory action, presence, expression and effect on ovarian and uterine functions in physiological conditions have recently been shown. The aim of the present study was to examine LTs role as mediators of cytokines, during development, maturation and ovulation of ovarian follicles in cow. Granulosa cells were isolated enzymatically from 5 to 10 ovarian follicles (day 8–10 of estrous cycle) and stimulated for 24 h with: LTC4 and B4 (each 10^{-6} M), cytokines (TNFα in combination with INFγ, each 10 ng/ml), antagonists of LTC4 (Azelastine, 10^{-7} M), LTB4 (Dapsone, 10^{-7} M), combination of cytokines with LTs and cytokines with LT antagonists. Experiment was repeated four times ($n = 4$ for each experimental group). Although LTC4 with cytokines had no effect ($p > 0.05$), Azelastine with cytokines increased mRNA and protein expression for LH and FSH receptors ($p < 0.05$). LTB4 with cytokines stimulated 3βHSD mRNA and protein expression, whereas Dapsone with cytokines increased FSH receptors mRNA and protein expression ($p < 0.05$). Progesterone output was increased by cytokines with Azelastine and decreased by LTC4, Dapsone with cytokines ($p < 0.05$). 17β-Estradiol output was stimulated by LTB4 with cytokines and inhibited by Dapsone and LTC4 with cytokines ($p < 0.05$). Results suggest that LTC4 is not cytokine mediator, in contrast with LTB4 which seems to enhance cytokine action during follicular development and maturation.

P146

Evaluation of superovulation responses in some domestic ewe breeds of Turkey

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Aim was to compare multiple ovulation response of three domestic sheep breeds of Turkey. Daglic ($n = 19$), Herik ($n = 27$) and Norduz ($n = 30$) ewes were exposed to a superovulation program during the breeding season. Ewes were treated with an intravaginal progesterone sponge for 12 days and administered reducing doses of FSH. After sponge removal, ewes in estrus were mated with fertility-proven rams and given 750 IU hCG. The ovarian response 7 days after mating was assessed by laparoscopy and oviducts were flushed for embryo collection. Ewes were considered superstimulated if the combined number of corpora lutea (CL) and large unovulated follicles on ovaries on day 7 was at least 3. Superovulation was defined as at least 3 ovulated follicles (indicated by presence of CL). Eleven Daglic (58%), 21 Herik (78%) and 24 Norduz (80%) ewes were superstimulated. Superovulation response was 63% for Daglic, 66% for Herik, and 76% for Norduz. Although there were no significant differences, Daglic ewes seemed least responsive to the superstimulation program. Superovulatory response was similar among breeds. The average number of CL and embryos did not differ among breeds and were 7.71 ± 3.95 CL and 4.29 ± 5.06 embryo for Daglic, 9.30 ± 3.86 and 3.00 ± 4.55

for Herik, and 10.33 ± 6.07 and 3.33 ± 3.79 for Norduz, respectively. Minumum fertilization (embryo) and recovery rates (embryo + unfertilized oocytes) were 55% and 68% for Daglic, 32% and 63% for Herik, and 32% and 62% for Norduz, respectively. Ewes of the Daglic breed yielded the lowest number of unfertilized oocytes in the present study. In conclusion, in Turkey different local sheep breeds appear to respond to the mentioned superovulation program similarly.

P147

Development of AI in small ruminants in Turkey

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The introduction and application of artificial insemination (AI) in Turkey begun in 1925 as the Russian scientist Mihailov demonstrated its implementation in horses to Turkish veterinarians at the Karacabey Farm. Since this application gave successful results in horses, it was tested in sheep and cattle and highly successful results were obtained. For example, approximately 20 000 ewes were inseminated artificially in 10 AI centers, of which two were portable, in Bursa in 1936. The number of inseminated sheep went up to 271 000 in 1968 and was intensively used for cross breeding with the Merino breed in Anatolia. However, a continuity for the AI applications could not be provided and either completely ended or had been limited because of many reasons such as economic, social and technical. Moreover, the success rate had not been high. Today, unfortunately, according to the data of the Ministry of Agriculture, there are no inseminations in small ruminants registered, and AI in small ruminants has only been applied in some universities or research institutes. In this study, developments in the AI attempts applied in small ruminants during recent years in parallel with developments in animal breeding were examined at the Ege University. In this context, as a result of studies carried out in 2010 at the Department of Animal Science of the Agricultural Faculty of Ege University, in total 216 Saanen goats were intracervically inseminated in three operations in total. Mean conception rate following first insemination was 50.0% in goats inseminated with fresh semen, 27.9% in goats inseminated with frozen semen, and 37.5% overall.

P148

The influence of ovariectomy on serum concentrations of thyroid hormones and histological structure of thyroid gland in dogs – preliminary results

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Purpose of the present study was to estimate the influence of gonadectomy on the secretion and histological structure of the thyroid gland in bitches. Determination of the concentration of thyroid hormones: thyroxine (T4), free thyroxine (fT4) and triiodothyronine (T3) in serum was performed with Radioimmunoassay human tests RIA-gnos Cisbio Bioassays in 28 non castrated and 63 castrated bitches. In the second stage thyroid glands were collected at necropsy from seven non castrated and 10 castrated animals. The organs were fixed in buffered 10% formalin solution, and embedded in paraffin. Tissue samples were stained with hematoxylin-eosin and evaluated histologically by means of a microscope (Nikon Eclips 80i DS-Ri1). Two-way analysis of variance showed significant differences between castrated and non-castrated bitches in fT4 and T4 concentrations. The mean value of fT4 was 16.25 ± 5.60 pg/ml in non-castrated and 11.25 ± 4.58 pg/ml in castrated animals. The mean value of T4 amounted 22.16 ± 8.78 ng/ml in intact and 18.22 ± 7.61 ng/ml in ovariectomized bitches. There were no statistically significant differences in T3 levels. Histological examination revealed a higher incidence of thyroid vesicles lined with squamous epithelium than with cuboidal epithelium in castrated bitches in comparison to intact ones. Moreover the mean height of both the cuboidal and the squamous epithelium was lower in castrated animals. In conclusion, both the peripheral level of thyroid hormones and the histological image suggest a decrease in the activity of the thyroid gland in castrated bitches. These results show a tendency to hypothyroidism without clinically visible signs in castrated bitches.

P149

Bladder eversion caused by chronic cystitis in a Arabian race horse

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Bladder eversion is rare and can occur as a consequence of excessive straining during or immediately following parturition in mares. The present abstract describes a bladder eversion caused by chronic cystitis in a non-pregnant mare. An Arabian racehorse (mare, 3 years old) was admitted to The Racehorse Hospital of the Turkish Jockey Club with a history of lumbar pain, excessive straining and frequently taking the position of urination. During physical examination tenesmus and stranguria were observed. Due to the tenesmus a mucosal structure became visible from the ventral commissure of the vulva. Laboratory findings revealed leucocytosis, increased urine pH, proteinuria, pyuria, and hematuria. *Streptococcus equi* (zooepidemicus) and *E. coli* were isolated and identified in urine culture. Thickening of the bladder wall and turning inside out of the bladder through the urethral sphincter could be demonstrated during transrectal ultrasonographic examination. After reposition of the bladder, cytoscopy was performed during which a severe hyperaemia, erosion and ulcers in the bladder mucosa could be detected. Antibiotic treatment based on the urinary culture in combination with steroid and nonsteroid anti-inflammatory drugs were used in the treatment of chronic cystitis. Based on the clinical, laboratory, ultrasonographic, and cytoscopic findings, we came to the conclusion that the bladder eversion was caused by a chronic cystitis. It was concluded furthermore that severe inflammation, pain, and tenesmus due to a chronic bacterial cystitis may result in bladder eversion, while the bladder eversion could be improved by an effective medical treatment of the chronic bacterial cystitis.

P150

Investigation of BMP15 and BMPR-1B gene mutations in profilic Sakiz sheep

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The Sakiz (Chios) sheep breed is mainly present in the Mediterranean and Aegean coasts of Turkey and is characterized by a high litter size. Previous studies have suggested that fertility characteristics of Sakiz sheep may be controlled by an unidentified major F gene. Bone morphogenetic protein 15 (BMP15) and bone morphogenetic protein receptor-1B (BMPR-1B) genes were identified as major determinants for the increased ovulation rate and litter size in different prolific sheep breeds. Objective of this study was to investigate effects of BMP15 and BMPR-1B genes on female productivity of Sakiz sheep. Blood samples and phenotypic data were collected from a Bafra sheep (Sakiz × Karayaka cross) population in which the higher litter size phenotype has been inherited. Exon flanking PCR primers were designed for BMP15 and BMPR-1B genes. PCR-RFLP analyses were conducted for determination of Booroola, Hanna and Inverdale genotypes. Except exon-9, of BMPR-1B gene, all BMP15 and BMPR-1B exons were amplified by PCR and possible sequence variations were investigated by DNA sequencing. PCR-RFLP and DNA sequencing analyses demonstrated that Booroola and the six different previously reported BMP15 genotypes were absent in Sakiz sheep and also sequence polymorphism was not detected in all analyzed exons. Candidate gene analyses illustrated that BMP15 and BMPR-1B genes did not have an effect on the litter size of Sakiz sheep. There is a need for analyzing other candidate genes attributing to the higher litter size in this breed.

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P151

Pregnancy rates in holstein cows after insemination with sex-sorted sperm

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The aim was to assess insemination (AI) efficiency with 2.2×10^6 X-chromosome bearing sperm (XS) of cows in spontaneous estrus (SE) and using Ovsynch protocol. A total of 470 cows were assigned to five groups composed from non-inseminated cows or non-pregnant after previous 1–3 AI with unsorted semen (US). GR1 – Ovsynch treated cows, first AI after calving with XS (n = 49). Milk progesterone (P4) was measured in this group to determine the effect of ovarian function on response to synchronization and pregnancy rate. GR2 – Ovsynch treated non-pregnant cows, AI with XS (n = 186). GR3 (control) – Ovsynch treated non-pregnant cows, AI with 15×10^6 US (n = 174). GR4 – non-pregnant cows, AI at SE with XS (n = 28). GR5 (control) – non-pregnant cows, AI at SE with US (n = 33). Pregnancy was diagnosed in all groups at Day 90 after AI by palpation of the uterus per rectum. In GR1 the pregnancy rate was 30.6%. According to the P4 profiles, 33 cows (67.3%) responded to treatment. The pregnancy rate of responded cows was 45%. In other groups pregnancy rates were: GR2 – 36.6%, GR3 – 46%, GR4 – 50% and GR5 – 57.6%. The pregnancy rate after the first AI with XS in treated cows (GR1) was not different ($p > 0.05$) from those in Ovsynch treated non-pregnant cows. However, the pregnancy rates in both groups were significantly lower ($p < 0.05$) than in cows from AI with XS at spontaneous estrus. Insemination with sorted sperm was more efficient at spontaneous estrus than that of Ovsynch treated cows. Using the Ovsynch protocol, pregnancy rate depended on the response of cows to synchronization.

P152

Predicting ovulation time based on estrous signs, periestrual hormone level and periestrual cervical mucus characteristics in Zebu cattle (*Bos indicus*)

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This study was designed to explore the temporal pattern among various estrous signs, cervical mucus characteristics, peripheral progesterone and luteinizing hormone levels and ovulation in Zebu cattle, and to identify the reliable sign(s) of estrus to predict the ovulation time. The onset, intensity and expression of various signs of estrous were continuously recorded till ovulation in 60 Sahiwal cows. Cervical mucus was collected prior to the AI from mid cervix and immediately analysed. Ovulation Time was determined by ultrasonography at 2 h interval. To study the changes in peripheral progesterone and LH level, blood was collected from 20 cows (synchronized and natural estruses; 10 each) and analysed using bovine-specific ELISA kit. Mucus discharge, vulvar swelling and tumefaction of mucus membrane appeared early in relation to the ovulation time (31.27 ± 1.97, 31.05 ± 2.98 and 30.79 ± 2.53 h, respectively) in comparison to mounting (27.67 ± 2.33 h) and standing to be mounted signs (25.37 ± 2.11 h). The mounting and standing to be mounted signs also persisted significantly less hours ($p < 0.01$) than mucus discharge, vulvar swelling and tumefaction of mucus membrane. The duration from estrus to LH-peak and LH-peak to ovulation was 2.2 ± 1.62 and 29.45 ± 1.73 h in natural estrus and 3.3 ± 0.67 and 32.75 ± 3.62 h in synchronised. The peak LH value was higher in synchronized (15.26 ± 2.8 ng/ml) than natural (12.67 ± 1.96 ng/ml). The estrus to ovulation duration was significantly ($p < 0.05$) higher when the progesterone level was >1 ng/ml on the estrus day of (36.17 ± 0.73 h in natural and 37.57 ± 4.32 h in synchronised) than when it was below <1 ng/ml (31.28 ± 1.74 h in natural and 28.83 ± 3.61 h in synchronised). The cervical mucus discharge was copious in 70.17% of the estrus, thin in 63.16% of the estrus and with typical arborisation pattern in 57.89% of the estrus. The mean pH, conductivity and spinnbarkeit value of mucus were 7.59 ± 0.06, 15.21 ± 0.15 mS/cm and 11.06 ± 1.06 cm respectively. Mucus discharge, vulvar swelling and tumefaction of mucous membrane can be good predictor ovulation if combined with cervical mucus characteristics in this species.

P153

Procaine does not activate equine oocytes

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In horses, conventional *in vitro* fertilisation (IVF) is very difficult probably due to insufficient capacitation of stallion sperm *in vitro*. In a recent study (McPartlin *et al*. 2009)

however, equine spermatozoa were successfully hyperactivated with procaine with cleaving embryos as a result. Since procaine may induce cleavage, we investigated whether it can cause oocyte degeneration and/or parthenogenesis. Equine oocytes were matured *in vitro* during 24 h in DMEM-F12 based medium at 38.5°C in 5% CO_2. After denudation, oocytes were incubated for 24 h in modified-Whittens medium with (MWP) or without (MW) 5 mM procaine. Then oocytes were cultured *in vitro* in DMEM-F12 medium with 10% fetal calf serum. Oocyte viability was evaluated using Hoechst. Oocytes with one pronucleus were considered parthenogenetic, with metaphase I or II were non-parthenogenetic and without visible nuclear material and a shrunken cytoplasm were degenerated. At 60 h post incubation (hpi), the proportion of non-parthenogenetic (30% for MW and 30% for MWP), parthenogenetic (33% for MW and 30% for MWP) and degenerated (37% for MW and 40% for MWP) oocytes was not significantly different between MW and MWP oocytes ($p = 0.91$, Fisher's exact test; $N = 120$). At 120 hpi, procaine did not influence the proportion of non-parthenogenetic (15% for MW and 8% for MWP), parthenogenetic (20% for MW and 10% for MWP) or degenerated (65% for MW and 82% for MWP) oocytes ($p = 0.08$, Fisher's exact test; $N = 133$). No parthenogenetic cleavage was noted. We can conclude that procaine did not induce oocyte parthenogenesis or degeneration.
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P154

The Effect of FSH or PGE1 analogue on the mRNA expression for EP 2 and EP4 in the goat (*Capra hircus*) cervix

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There is a degree of natural relaxation of cervix at oestrus associated with the increases in plasma FSH and the accompanied changes in prostaglandin synthesis in cervical tissues. Moreover, intracervical application of both FSH and Misoprostol (PGE1 analogue) has been reported to increase penetrability of cervix. The aim of this study was to investigate if they do so by having their effect on mRNA expression of prostaglandin E receptors; EP2 and EP4, in the cervix. Oestrus was synchronised in 20 Thai goats using progestagen pessaries and PMSG. Intra-cervical hormone was applied at 24 or 48 h after the pessary removal: Group 1; controls, Group 2; FSH 2 mg at 48 h, Group 3; FSH 2 mg at 24 h and PGE 11 mg at 48 h, Group 4; PGE 11 mg at 48 h. Cervices were collected at 54 h after sponge removal and divided transversely into three regions (vaginal, mid and uterine) and stored at −20°C. The mRNA expression for EP2 and EP4 was determined in cervical tissues by RT-PCR using β-actin as reference. Data on the relative expression levels of EP2 and EP4 mRNA were analysed by ANOVA. PGE1 alone or with FSH increased ($p < 0.05$) EP2 mRNA expression, whereas FSH alone or with PGE1 increased ($p < 0.05$) EP4 mRNA expression compared to controls. We found that combination of FSH and PGE1 increased ($p < 0.05$) both EP2 and EP4 mRNA expression compared to the controls. The results suggest that the mRNA expression for EP2 and EP4 is differentially regulated and both the receptors play an important role in cervical relaxation of goat at oestrus.

P155

Efficacy of intratesticular vs. intraepididymal injection of chlorhexidine solution as chemical sterilant in dogs

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The efficacy of testicular injection site (intraparenchymal vs. intraepididymal) of 1 ml solution at 5% chlorhexidine digluconate, as a method for chemical castration, was compared in 42 healthy dogs divided in two equal groups: (i) into the dorsal cranial portion of each testicle; (ii) in the tail of both epididymes. At day 60, testicular echography revealed a local hyperechogen nodular lesion surrounded by normal parenchyma in group A; epididymis hyperechogenicity and normal parenchyma in group B. Libido was reduced in both groups. Computer Assisted Sperm Analysis (day 0 vs. 60) showed a significant decrease in ejaculate volume (ANOVA test: 2.2 ± 0.6 vs. 1.1 ± 0.4 ml; $p < 0.01$) and oligospermia in group A; in group B a significant reduction in ejaculate volume (ANOVA test: 5.8 ± 1.2 vs. 2 ± 1.1 ml; $p < 0.01$) and azoospermia. At day 60, histological exam showed a necrotic area in the injection point but still normal spermiogenic activity in other areas in group A; an area of necrosis and fibrosis besides the epididymis extending to the tubuli seminiferi recti, rete testis and ductuli efferentes, and degeneration of the seminiferous tubules associated with a significant alteration of the germinal epithelium cells in group B. These findings show that a single percutaneous administration of a 5% chorhexidine digluconate solution injected into the cauda epididymis of both testes is a reliable an effective non-surgical sterilization method in dogs. On the contrary, the injection into the testicular parenchyma is not effective. This study was approved by a local Ethics Committee.

P156

Effects of melatonin against oxidative stress on flow cytometry sorted buffalo sperm and subsequent *in vitro* embryo development

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Melatonin (MLT) can scavenge a variety of toxic oxygen and nitrogen-based reactants. The latter suggests its possible role as an antioxidant in protecting sperm from oxidative stress. In the present study, MLT (10^{-4}M) was instantly supplemented in each extender before the staining, sorting and freezing procedure during the sperm sorting process by flow-cytometry. To analyse the effect of MLT supplmentation, the quality of buffalo sperm was evaluated by Laser Tweezers Raman Spectroscopy (LTRS) after the staining, sorting and freezing-thawing procedure. The results of the spectroanalysis showed that buffalo sperm frozen in media supplemented with MLT displayed a lower intensity at all Raman spectra than those frozen without MLT (except for 1302 cm^{-1}). The blastocyst rate after IVM/IVF using sperm treated with MLT was 27.21%, which was significantly higher than without MLT (18.24%; $p < 0.05$). These results suggest an antioxidant role of MLT in helping the sexed buffalo sperm against oxidative stress and subsequently enhance the developmental potential of IVF embryos, and also imply the potential of LTRS

technique as a rapid, effective and noninvasive tool in assessing the quality of buffalo sperm cells. The knowledge from this study can attribute to optimize sperm sexing procedures and thus facilitate its commercial use in buffalo species.

P157

Stereological analysis of tissue composition in bovine teat explants

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Bacteria enter the mammary gland through the teat canal. The first tissues they encounter are the Fürstenberg's Rosette (FR) and the teat cistern (TC). On the basis of explant cultures we studied mRNA transcripts encoding for the immune-related genes CXCL8, CCL20, S100A8,-9,-12, IL-1β and TNFα after stimulation with 1 μg/ml lipopolysaccharide (LPS). Since different tissue- and cell-types might influence the results of quantitative gene expression studies, the tissue composition of 64 teat explants from the FR and the TC of two cows were analyzed by quantitative stereological investigations: the volume densities (VV) of connective tissue, musculature, epithelium and CD11a/18 positive immune cells in the explants were determined in equidistant serial sections. In all analyzed samples, the VV of connective tissue was significantly higher in the FR than in the TC, whereas the VV of muscle tissue was significantly higher in the TC than in the FR. The VV of epithelial cells was significantly higher in the FR than in the TC. The Vv of CD11a/18 positive immune cells exhibited a considerable variability in different explants. Thus, FR- and TC-explants differ significantly in their cellular composition, which has to be taken in account when analyzing and comparing gene transcript data from these regions. The data support that bovine teat explant cultures are a valid *in vitro* model system.
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P158

Pregnancies in mares inseminated with spermatozoa selected by single layer centrifugation and stored for 48 or 72 h

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Poor semen quality, poor handling of sperm and prolonged times from collection to artificial insemination (AI) are causes of failure of pregnancy in mares. Sperm quality (motility, chromatin integrity, membrane integrity and normal morphology) can be improved by Single Layer Centrifugation (SLC) through a species-specific colloid (Androcoll-E). This improved sperm quality is retained during storage. The objective of the present pilot study was to test the fertility of SLC-selected semen after cool (+4 to 8°C) storage for 48–72 h. The gel-free ejaculate from three stallions, collected using a phantom and an artificial vagina, was extended with warm INRA96 (IMV, l'Aigle, France) to a final concentration of 100×10^6/ml. Aliquots were layered on top of Androcoll-E and centrifuged at $300 \times g$ for 20 min. The sperm pellets were resuspended in INRA96, cooled and stored for either 48 or 72 h before AI. Seven standard bred mares (age 10–19 years) were each inseminated once with $300–600 \times 10^6$ spermatozoa. Five mares (71%) were confirmed pregnant at day 16–18 after ovulation with normally developed embryos. Pregnancy rate after AI with SLC-selected spermatozoa stored for 48 h was 75% (3/4 mares) and with SLC-selected spermatozoa stored for 72 h was 67% (2/3 mares). The maximum interval between SLC and ovulation followed by conception was 5 days. Conclusion: SLC-selected spermatozoa are fertile after an extended storage time, enabling increased usage of cool stored semen.

P159

No effect of supplemental feeding with glycerol or propylene glycol in early lactation on the fertility of swedish dairy cows– preliminary field study results

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The aim of this study was to evaluate the fertility in dairy cows with supplement of glycerol or propylene glycol under field conditions. Within 17 conventional tie-stall herds, 965 cows were randomized to one of three treatments; 450 g of glycerol, 300 g of propylene glycol or no supplement. Supplements were fed daily 0–21 days post partum as a top dressing on the concentrates. Data on monthly milk yield, fertility and veterinary treatments were collected. In a subset of 294 cows in seven herds, milk samples were collected twice weekly for progesterone measurements. Resumption of ovarian cyclicity was defined as the first progesterone rise >4 ng/ml. The effect of the supplements on number of days between calving and first or last insemination (CFI and CLI, respectively), calving interval (CI) and days to resumption of ovarian cyclicity (OC) were investigated using linear mixed regression models. The full model fixed effects included treatment, breed, parity, calving season and milk yield. Results are presented as overall LSMeans for treatment with glycerol or propyelene glycol and control, respectively. CFI was 86, 83 and 83 days respectively. CLI was 113, 117 and 117 days respectively. CI was 388, 399 and 395 days respectively. OC was 26, 26 and 27 days respectively. No differences were found between the treatments for CFI, CLI, CI and OC ($p = 0.35$; 0.77; 0.17 and 0.63 respectively). Our preliminary results indicate that supplement feeding with glycerol or propylene glycol to cows regardless of their physical state, does not improve their fertility.

P160

Pregnancy-specific protein bovine (PSPB) concentrations in whole and demi embryo derived early pregnancies

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The objective of this study was to evaluate plasma PSPB concentrations in early pregnancies (n = 99) following the

transfer of one whole (n = 66) or one demi (n = 33) embryo to virgin dairy heifers. The experiment was designed to evaluate the effects of embryo size at transfer (whole or demi) on Day 7 of the estrous cycle (Day 0 = estrus), and intravaginal progesterone (P4) supplementation on Days 7–19, on plasma PSPB concentrations measured by ELISA on Days 7, 21, 25, 35, 42, 49, 56 and 63 of pregnancy. Concentrations of PSPB were similar in non-supplemented whole and demi embryo pregnancies. In contrast, in P4 treated recipients, demi embryo pregnancies had higher ($p < 0.05$) PSPB concentrations on Days 25–42 than whole embryo pregnancies. Overall, treatment with P4 had a positive effect ($p < 0.01$) on PSPB concentrations on Days 35–63. In conclusion, whole and demi embryo derived conceptuses produced similar plasma PSPB concentrations. Secretion of PSPB was stimulated by exogenous P4 treatment. These observations corroborate the presence of a compensatory growth and placental function of demi embryos until implantation.

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P161

Coxiella burnetii sero-positivity is related to placenta retention in high producing dairy cows

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The possible relationship between Coxiella-seropositivity and the reproductive performance of cows during the year preceding the serological screening was examined in three high producing dairy herds, with particular emphasis on placental retention (fetal membranes retained longer than 12 h after parturition). The three herds were tested positive by the polymerase chain reaction test for *C. burnetii* in the bulk tank milk with an excretion higher than 10^4 Coxiella/ml. Antibodies against *C. burnetii* were detected in 50.2% of 781 parous cows analyzed, ranging from 46% to 53% for the individual herds. From 440 pregnancies recorded, 16.8% (74/440) suffered pregnancy loss: 15% during the early fetal period (from pregnancy diagnosis to 90 of pregancy) and 2.1% after day 90 of gestation. Logistic regression analysis indicated no significant effects of herd, lactation number and Neospora caninum seropositivity on retained placenta. Based on the odds ratio, the likelihood of a placenta to retain increased by factors of 1.75 or 8.1 in cows showing *C. burnetii* seropositivity (6.4% in seronegative vs. 12.5% in seropositive) or twin pregnancies, respectively. No significant interactions were found. Relationships between *C. burnetii* infection and reproductive disorders such as abortion, stillbirth, weak offspring, postpartum metritis and infertility have been suggested, but to our knowledge, *C. burnetii* seropositivity has not been described before as a predisposing factor for retention of the placenta.

P162

Single administration of PGF2α on days 15–21 postpartun failed to improve subsequent fertility of dairy cows

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Presence of intrauterine fluid (FIU) and its echogenicity detected by ultrasonography at days 15–21 postpartum (pp) has been related to an impairment of subsequent fertility in dairy cows. Administration of PGF2α as a treatment for inadequate uterine involution is still under discussion. The aim of this study was to evaluate the effect of a single PGF2α injection at 15–21 days pp in cows with FIU. Sixty-two high-producing Holstein–Friesian dairy cows with (n = 22) or without (n = 40) corpus luteum (CL) with either echogenic, i.e. purulent (FIU2) (n = 43) or anechogenic, i.e. mucous (FIU1) (n = 19) FIU on days 15–21 pp, were randomly assigned to a control (n = 25) or a PGF2α treatment group (n = 37). Cows were reexamined on the following week for FIU improvement (evolution from FIU2 to FIU1 or from FIU1 to disappearance) or permanence. Oestrus was detected by using pedometers and confirmed by examination of the genital tract at AI. After first AI, pregnancy status of each cow was recorded by ultrasound on days 28–32. FIU content improved in 22 treated and 16 control cows (59.5% vs. 64%, $p = 0.72$), while nine treated and 10 control cows were diagnosed pregnant (24.3% vs. 40%, $p = 0.19$). Logistic regression analysis indicated no significant effect of PGF2α administration, presence of CL and the interaction PGF2α by CL neither on FIU disappearance nor on pregnancy status at first AI. In conclusion, PGF2α administration in cows showing FIU on days 15–21 pp failed to reduce FIU and to improve subsequent fertility regardless the presence of a CL.

P163

Preliminary approach of viscous media use for ram sperm freezing

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Storage in solid or viscous media could improve AI or sperm criopreservation after long storage, by decreasing the cell metabolic rate and changes in extender composition. Our objective was to improve post-thawing results of ram sperm extended in viscous media, testing several media and the effect of removing the media before freezing. Three ejaculates were collected from two rams by artificial vagina. Each ejaculate was divided and extended with TES-Tris-Fructose, 4% glycerol, 20% egg yolk, alone (TTFYG) or supplemented with 1.5% gelatin (G), 1% guar gum (GG) or 2% methylcellulose (MC). After 3 h at 5°C, samples were frozen (−20°C/min). Part of the samples were washed prior to freezing (1/5 in TTFY, $600 \times g$ 6 min) and frozen without viscous media. Samples frozen in viscous media were washed after thawing to allow evaluation. Quality post-thawing was assessed according to motility (CASA) and viability (SYBR 14+/PI−). Pre-freezing washing decreased ($p < 0.05$) total motility (42 ± 7% vs.

58 ± 6%) and viability (43 ± 5% vs. 65 ± 2%). Motility and viability were higher ($p < 0.05$) for TTFYG alone (60 ± 4%; 66 ± 4%) than for GG (39 ± 7%; 48 ± 8%) or MC (24 ± 5%; 42 ± 8%), whereas G was similar (65 ± 9%; 61 ± 6%). Our findings suggest that pre-freezing removal of viscous media is detrimental and that gelatin might be further tested for application in ram semen storage.

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P164

Low plasma progesterone at diestrus is accompanied by reduced luteal blood flow and increased size of the dominant follicle, and relies on the quantity of luteal tissue in cows

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The aim of the study was to investigate the influence of low plasma progesterone (pP_4) on luteal and follicular development as well as its dependency on luteal size, blood flow and gene expression. In 15 dairy cows, pP_4, area of luteal tissue (LTA) and follicular fluid (FFA), luteal (LBF) and follicular (FBF) blood flow were determined daily until D(ay) 4 of the estrous cycle (D1 = ovulation) and then every 2 days until D9 of the subsequent cycle. Volume of luteal tissue (LTV), relative LBF (rLBF), relative (rLP_4) and absolute (LP_4) luteal P_4, as well as luteal mRNA expression of important steroidogenic factors were determined at diestrus [D9 and 11(±1), respectively]. Cows were allocated according to pP_4 (means of D7–15), either < 2 ng/ml (P_4L; n = 7) or > 2 ng/ml (P_4H; n = 8). In the treatment cycle, LTA was smaller in P_4L than P_4H on D13 ($p = 0.01$) and 15 ($p = 0.03$), LBF was lower on D15 ($p = 0.02$), and FFA (dominant follicle, 1st wave) was larger on D13 ($p = 0.03$), 15 ($p = 0.03$) and 17 ($p = 0.01$). In the subsequent cycle, FBF was lower ($p = 0.01$) in P_4L on D7. Plasma P_4 was related with LP_4 ($r = 0.43$, $p = 0.04$), LTA ($r = 0.65$, $p = 0.0001$) and LTV ($r = 0.43$, $p = 0.02$), but not with rLBF ($p > 0.05$); rLP_4 and gene expression were not related ($p > 0.05$). Results indicate that low pP_4 at diestrus is accompanied by smaller LTA, reduced LBF and larger FFA, and relies on the quantity of luteal tissue.

P165

Case report – female pseudohermaphroditism in 1-year-old mixed dog

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Pseudohermaphrodite animals have only one gonadal sex that is in accordance with the cromossomic sex, but its phenotype is the opposite (Meyers-Wallen, 2001, *Recent Adv. S. Anim. Reprod.*). The female pseudohermaphrodite has the ovaries as gonads and some masculinization grade (Mickelsen and Memon, 1997, *Trat. Med. Inter. Vet.*, 4. ed., v.2, p.2326–2331). An 1-year-old mixed dog was seen at Veterinary Hospital – UNESP – Jaboticabal Campus with a penis exposition failure, testicles absence in the scrotal bag and an urinary incontinence. During the clinical exam it was observed a penis hypoplasy and the testicles absence into the scrotal bag was detected. The inguinal subcutaneous palpation cast aside the ectopic testicles presence. The surgery was performed, observing the two ovaries presence, uterine corn and the uterine body. Neither ectopic testicles nor prostate were found. The ovaries histopathology evaluation was done, and it was asserted being functionals and without morphologic alteration, characterizing a female Pseudohermaphroditism. This abnormality cause is not evident, however is known that it can occur due the androgens administration in the dog during pregnancy, changing the female fetus development.

P166

Pure Natural Orifice Translumenal Endoscopic Surgery (NOTES) for ovariohysterectomy in bitches: a preliminary feasibility study

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Ovariohysterectomy (OHE) often leads to complications and requires rescue analgesia. Few studies focused on the development of new minimally invasive spay in dogs have been performed. The aim of the present study was to assess the feasibility of OHE totally performed through a transvaginal laparoscopic aproach (NOTES) in bitches. Four shelter-owned healthy bitches weightin 12.2 kg (SD ± 1.3 kg) were studied. The animals were premedicated with a mixture of acepromazine (0.02 mg/kg IM), midazolam (0.4 mg/kg IM) and morfine (0.5 mg/kg). Anesthic induction was performed using propofol (5 mg/kg IV to effect) and maintenance with isoflurane in 100% oxygen following tracheal intubation. The surgical procedure started with caudal traction of the vaginal fornix and sharp incision of the vaginal mucosa. The submucosa layer was bluntly dissected and a 12 mm trocar was inserted through the vaginal wound. Following CO_2 pneumoperitoneum, a 10 mm rigid telescope with working channel was introduced through the trocar. The right ovary was grasped using laparoscopic Babcock forceps and raised for transabdominal suspension suture. The ovarian pedicle was resected using bipolar coagulation/cut forceps. The same step was carried out on the opposite side and the left ovary was grasped and pulled into the trocar. The trocar was withdrawn and the uterus was completely exteriorized through the vagina. The uterine body and vessels were coagulated and the stump was repositioned within the abdomen. Routine post-op care was carried out. Surgical time ranged from 42 to 68 min (mean 54 min). Mild vaginal bleeding occurred in all patients, which ceased within the first 24 h. No rescue analgesia was required at any time point. Pure NOTES-OHE was proven to be technically feasible in bitches. Since no abdominal incision was required, we hypothesize that pure transvaginal (NOTES) OHE may cause less pain due to absence of somatic stimuli. Further trials are needed to assess such hypothesis.

P167

The quality of ram cooling-stored semen under influence of insulin-like growth factor I (IGF-I)

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The study was aimed at examining the effects of IGF-I on ram sperm traits after hypothermic (4°C) storage. Sperm ejaculates from three Lacaune rams were diluted in Triladyl, pooled and divided into the groups of IGF-I doses added (0, 10, 100 or 200 ng/ml). Following 72 h of storage the sperm samples were stained for a plasma membrane integrity (peanut agglutinin, PNA-FITC), membrane stability (annexin V-FITC) and apoptosis (Yo-Pro-1) and analyzed under a fluorescent microscope. Sperm motility was determined by CASA (at 0, 24 and 48 h) and fertilizing ability (at 48 h) – by an *in vitro* fertilization (IVF) test on bovine oocytes. IGF-I, (100 and 200 ng/ml), decreased the portion of spermatozoa with disrupted plasma membranes (15.9 and 16.5% resp. vs. 28.3% in control, $p < 0.05$) and, given at all tested doses, reduced the rate of apoptotic sperm (6.2%, 4.9% and 4.4% respectively vs. 9.7% in control, $p < 0.05$). The proportion of spermatozoa with annexin V-labeled membrane changes was reduced by IGF-I from 9.6% (control) to 4.9% (100 ng/ml) and 6.1% (200 ng/ml). IGF-I (10 ng/ml), elevated sperm motility measured after 24 h storage (83.3% vs. 69.2% in control); the higher doses had no effect. IGF-I, given at 100 ng/ml, increased sperm fertilization rate (67.8% vs. 47.0%, $p < 0.05$). In summary, IGF-I improved ram sperm functions during cooling storage and their effects were reflected in fertilizing ability *in vitro*.

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P168

Effect of Time of Ovulation on the Efficiency of Oestrus Detection in Dairy Cows by ALPRO

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The efficiency of the activity sensor ALPRO® (DeLaval International AB, Tumba, Sweden) was evaluated for oestrus detection (OD) in relation to resumption of ovarian cyclicity. Milk from 115 multiparous cows was taken twice weekly for progesterone (P4) measurement by EIA. Each ovulation/oestrus event was determined from the P4 profile and compared with the respective activity alarms of ALPRO®. A receiver operating characteristic curve was used to find out the optimal cut-point for the relationship between OD efficiency and days in milk (DIM). Differences in OD efficiencies before and after optimal cut-point were tested using Fisher's exact test. DIM to first, second, third and fourth oestruses were 38 ± 2.4 (mean ± SEM), 53 ± 2.2, 76 ± 2.45 and 100 ± 3.3 days, respectively. OD efficiency at the first oestrus was 44% (51/115), second oestrus 78% (88/113), third oestrus 82% (78/97) and fourth oestrus 66% (49/74). OD efficiencies at the first and fourth oestruses were lower in cows which ovulated before 22 DIM and before 89 DIM, respectively, compared to the cows which ovulated after 22 DIM (OR = 4.1; $p < 0.01$) and after 89 DIM (OR = 3.7; $p < 0.05$). OD efficiencies at the second and third oestruses were higher in cows which ovulated before 50 and 63 DIM, respectively, compared to the cows which ovulated after 50 DIM (OR = 0.2; $p < 0.001$) and after 63 DIM (OR = 0.1; $p < 0.05$). OD efficiency was dependent on the time of ovulation.

P169

Role of peroxisome proliferator-activated receptor γ in corpora lutea of pseudopregnant rabbits

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The peroxisome proliferator-activated receptors (PPARs), a family of three (a, d, g) nuclear receptor/transcription factors, are involved in critical processes of ovarian function, such as steroidogenesis, angiogenesis, and apoptosis. PPAR agonists present contradictory actions on granulosa and luteal steroid secretion. To this end, experiments were devised to examine by immunohistochemistry the occurrence of PPARgin rabbit CL (days 4 and 9 of pseudopregnancy) as well as its effects on the endocrine activity using PPARg agonist (15d-PGJ2) and antagonist (T0070907) *in vitro*. To evaluate the role of PPARg on the acquisition of luteal capacity to PGF2a, the dynamic changes in gene expression for PPARg were analyzed by RT-PCR in day 4 and 9 CL after PGF2a analogue (alfaprostol) injection. Immunohistochemical results revealed that PPARg was localized in the cytoplasm and nuclei of luteal cells. In both luteal stages, PPARg agonist increased ($p < 0.01$) progesterone and decreased ($p < 0.01$) PGF2a *in vitro* secretion, while the antagonist showed an opposite effect. In day 9 CL, PGF2a determined a PPARg mRNA down-regulation ($p < 0.01$). This study indicates that PPARg acts as a luteotrophic factor by affecting the endocrine activity of rabbit CL and that PGF2a regulates luteolysis in day 9 CL also via PPARg gene expression modulation.

P170

Expression of toll-like receptors in porcine granulosa cells

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Toll-like receptors (TLRs) are a group of pattern recognition molecules that recognize various microbial components and play a crucial role in the activation of the innate immune system in vertebrate species. Sequencing of the porcine genome revealed that it consists of ten TLRs, namely TLR1-10. Although TLRs have been studied in various pig organs and cell lines, little is known about their expression and function in the porcine ovarian follicles. The aim of this study was to investigate the expression of the complete repertoire of TLRs family in the porcine ovarian follicles and to determine possible differences in their expression between small and large follicles. Granulosa cells isolated from small (< 3 mm) and large(> 3 mm) follicles were used for RNA extraction, followed by RT-PCR analysis. Results showed that all the

members of porcine TLRs were expressed in the granulosa cells, with higher expression levels detected for TLR1, 3, 4 and 8. No differences in the expression levels of these genes were observed between small and large follicles. The expression of all types of TLRs in porcine granulosa cells, covering the recognition of Gram-positive and Gram-negative bacteria, double-stranded RNA, bacterial flagellin and single strand RNA, strongly suggests that these molecules are involved in the defense mechanism of the porcine ovarian follicles against a broad spectrum of microorganisms. Further experiments are underway in order to determine the changes in the expression levels of these genes as a response to various infections in the porcine ovarian follicles.

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P171

Effect of pentoxifylline on motility pattern of fresh boar spermatozoa

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Contradictory effects of Pentoxifylline (PTX) on sperm motility and motion characteristics of motile spermatozoa have been described for both fresh and cryopreserved semen in some mammalian species. The aim of this study was to determine the influence of PTX on the motility pattern of fresh boar spermatozoa. Fifteen sperm-rich ejaculate fractions collected from five mature fertile boars were diluted with BTS-extender alone (as control) or supplemented with 4, 8 and 16 mM of PTX and incubated during 30 min at 37°C. Total sperm motility (TSM) and the individual kinematic pattern of motile spermatozoa, assessed by the computer-assisted motility analyzer (ISAS), were recorded at 0, 15 and 30 min of incubation time. The TSM was lower ($p < 0.05$) in PTX samples than control, showing the lowest TSM values in the samples with the highest PTX concentrations ($p < 0.05$). Four sperm populations (P), defined by eight kinematic parameters, were identified after cluster analysis of the 108 327 individual motile spermatozoa: (P1) hyperactive-like cells, (P2) poorly progressive cells, (P3) progressive cells swam forward rapidly, and (P4) progressive cells swam forward slowly. The PTX has a detrimental effect ($p < 0.05$) on motility pattern. The P1 and P3 decreased and P2 increased as PTX concentration increased. The results suggest that PTX decreased both the number of motile spermatozoa and quality of sperm movement in fresh diluted boar sperm samples.

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P172

Evaluation fluorescent stains and flow cytometry for viability assessment of gilthead sea bream (*Sparus aurata*) spermatozoa

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Viability evaluation using flow cytometry is a very important tool for sperm quality assessment. Fluorochromes and flow cytometry have been used in several fish studies, but staining protocols have not been thoroughly adapted to teleost species. The objective of this study was to evaluate viability fluorochromes in gilthead sea bream sperm, analyzing the stain dynamics and its repeatability. Cryopreserved spermatozoa were thawed, diluted in PBS (10^6/ml) and stained with 1.5 μM propidium iodide (PI), alone or combined with either 5 μM Hoechst 33342 (H342) or 0.1 μM SYBR-14. Tubes were incubated at room temperature and analyzed by flow cytometry at 1, 5, 10, 15, 30, and 60 min. The experiment was performed in triplicate. Fluorescence intensity and proportion of spermatozoa stained with PI (%PI+, dead) were fitted to asymptotic curves (vs. time), CV% of triplicates were obtained for each time, and % of dead spermatozoa were compared among methods (Bland-Altman agreement coefficients). For PI and PI/H342, fluorescence and %PI+ increased quickly, reaching a plateau by 15 min (˜45% viability, CV% ˜10%). CV% were higher for 1 and 5 min, implying less repeatability at short incubation times. PI/SYBR-14 was more dissimilar and showed higher CV% (22% for 15 min). This study contributes with basic knowledge for the use of fluorochromes in teleost species.

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P173

Exposure of porcine *in vitro* matured oocytes to sybr 14 and fluorescence limits their developmental competence

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This study evaluated the effect of exposure of porcine *in vitro* matured oocytes to SYBR 14 and fluorescence on intro fertilization (IVF) parameters and embryo development. Matured oocytes were divided into five groups: (i) oocytes stained with 5 μg/ml SYBR 14 for 10 min (S), (ii) oocytes stained with 5 μg/ml SYBR 14 for 10 min and exposed to fluorescence for 5 s (SF5), (iii) oocytesstained with 5 μg/ml SYBR 14 for 10 min and exposed to fluorescence for 30 s (SF30), (iv) oocytesstained with 5 μg/ml SYBR 14 for 10 min and exposed to intermittent pulses of fluorescence for 5 s (SFI), and (v) untreated oocytes (C). After treatments, the oocytes were incubated for 5 h with 1000 frozen-thawed spermatozoa per oocyte and cultured for 18 h ($n = 615$) to assess IVF parameters or 7 days ($n = 1502$) to evaluate embryo development. No differences were observed in sperm penetration and monospermy among groups. Oocytes from SF5, SF30 and SFI showed higher ($p < 0.001$) percentages of degenerated presumptive zygotes (range: 20.7 ± 5.7–28.0 ± 5.7%) than in S and C groups (0%) and a lower ($p < 0.001$) cleavage rate (range: 14.9 ± 5.2–31.6 ± 5.2%) and blastocyst formation (range: 2.1 ± 2.9–7.4 ± 2.9%) than the S and C groups (62.6 ± 5.2% and 66.3 ± 5.2%; 28.5 ± 2.9 and 27.9 ± 2.9%; respectively). These findings indicate that the exposure of matured oocytes to SYBR 14 and fluorescence for periods as short as 5 s significantly decreased their developmental ability.

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P174

Comparison of the effect of different antioxidants on frozen-thawed and incubated ram semen samples

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The addition of antioxidants to semen could improve its quality by means of limiting reactive oxygen species (ROS) production and subsequent damage from the oxidative stress. Our objective was to test four antioxidants on frozen-thawed ram semen, studying their effect on ROS levels and lipoperoxidation (LPO). Straws from six males were thawed and pooled, washed and diluted to 25×10^6/ml in TALP-Hepes. The pool was split among: control (CT), dehydroascorbic acid (DA), tempol (TP), rutin (RT) and N-acetyl cysteine (NC), at 1 mM. Samples were incubated at 37°C for 4 h with and without oxidative stress (0.1 mM Fe2+/0.5 mM ascorbate). ROS was evaluated by H2DCFDA fluorescence (flow cytometry), and LPO by malondialdehyde production (nmol/108 cells; BIOXYTECH® MDA-586, Oxis). We performed six replicates, analyzing the data by linear mixed-effects models. Induced ROS increase was prevented by TP ($p < 0.01$) and RT ($p = 0.1$). LPO increased in the presence of the oxidant (0 h: 18 ± 3–4 h: 29 ± 4), but it was reduced by TP (13 ± 2; $p < 0.001$), RT (14 ± 3; $p < 0.001$) and NC (20 ± 3; $p = 0.001$). TP reduced LPO in the tubes not treated with oxidant (CT: 16 ± 4 vs. TP: 10 ± 5; $p = 0.015$). DA showed no protective effects, while TP was the most efficient antioxidant, being a candidate for further testing on cooled and frozen storage of ram semen.

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P175

Effect of α-L fucosidase on *p*-tyrosine phosphorylation of boar sperm

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There is evidence for a fucose-binding protein in boar spermatozoa and for α-L-fucosidase activity in the oviduct. However, no studies are available about the role of α-L-fucosidase on sperm and we have data showing a relationship between *p*-tyrosine phosphorylation pattern on boar sperm and penetration rate. The aim of the present study was to determine the effect of α-L-fucosidase on *p*-tyrosine phosphorylation pattern in boar sperm. Porcine spermatozoa were capacitated by Percoll gradient and incubated in TALP medium for 20 min with 0.169 UI of α-L-fucosidase (SE group) or without enzyme (control group). Different samples were analyzed by indirect inmunofluorescence to assess *p*-tyrosine phosphorylation. Three patterns were determined according to their surface distribution: non-phosphorylated spermatozoa (pattern A), equatorial segment phosphorylated (pattern B) and equatorial segment and head phosphorylated (pattern C). The data were analysed by ANOVA ($p < 0.05$). The results showed that the spermatozoa incubated with enzyme presented the highest value to pattern C (52.5 ± 1.7a vs. 23.1 ± 1.4b). Control group showed the highest rates for pattern B (61.8 ± 1.6a vs. 35.2 ± 1.6b). The pattern A was similar between groups. In conclusion, α-L fucosidase increases *p*-tyrosine phosphorylation on boar sperm and this could explain the higher penetration ability of sperm treated with this enzyme.

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P176

Obstetric procedures in miniature dogs

A Max

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The study evaluated the course of labor, incidence of dystocia and effectiveness of obstetric help in miniature bitches at average age of 3 years (15 months to 6 years). Fifty parturitions (P) of dogs of the following breeds were examined during labor: Chihuahua n = 34; Yorkshire Terrier n = 14; Shih Tzu n = 1; Miniature Schnauzer n = 1. The complete parturition process underlay obstetric supervision. Assistance took place either as routine procedure (n = 38) or in emergency cases (n = 12). Puppies per litter averaged 2.2 (1–5). Dystocia, defined as inability to initiate labor, failure of progression during labor and maternal or fetal compromises occurred in 68%. 67.7% of dystocia were caused by fetomaternal disproportion, while 17.6% were functional disorders, 5.9% were fetal malposition and 8.8% a combination. In 19 cases (38%), primary cesarean section (CS) was performed, in one case (2%) a secondary. Indications for CS included considerable fetomaternal disproportion (n = 14), owner's decision (n = 5) and primary functional disorders (n = 1). The remaining bitches (with or without dystocia) delivered naturally needing manual assistance only (n = 19) or oxytocin injections and manual assistance (n = 11). Total time of fetal expulsion and inter-puppy birth interval averaged 1.6 and 0.5 h respectively. Overall 179 puppies were delivered of which 94.1% were alive. There were no differences in proportion of alive and dead newborns between natural parturition and CS. Obstetric supervision throughout and well-timed intervention during parturition assure efficient delivery and high numbers of live newborns in miniature dogs. Appropriately applied CS and non-invasive treatment have the same efficiency.

P177

Filtration of ram semen through sephadex improves sperm motility throughout refrigeration and incubation

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Artificial Insemination using fresh or refrigerated semen depends on its availability in place and time, since fertility of refrigerated semen decreases very fast is difficult to use it in remote farms. The aim of this work was to see whether filtration through sephadex may improve sperm quality after 24 h of refrigeration. Semen from three Canary rams (21 ejaculates) was diluted using a conventional freezing media without glycerol and subjected to: (i) Filtration through sephadex (F), (ii) Filtration through sephadex plus enrichment by adding new freezing media (FE), (iii) Unfiltered (UF).

Sperm from the three treatments were slowly cooled from 23 to 5°C over 2 h and kept at that temperature for 24 h. Then, sperm were rewarmed to 37°C; one part of the unfiltered spermatozoa was filtered through sephadex (RF), an aliquot from this was enriched as mentioned (RFE). Sperm from all treatments were incubated in a water bath at 37°C for 40 min. Filtration of fresh semen increased sperm motility: UF 78% vs. F 89% ($p < 0.05$). After refrigeration and rewarming, motility of FE (59%) was higher ($p < 0.05$) than those of UF (51%) and F (30%). Filtration of refrigerated unfiltered semen increased sperm motility: from 51% to 73% ($p < 0.05$). After incubation, motility of filtered-enriched spermatozoa (FE 52%, RFE 57%) was higher ($p < 0.05$) than those of unfiltered (UF 42%) and filtered non-enriched spermatozoa (F 15%, RF 33%). In conclusion, filtration and enrichment of ram semen improve sperm motility throughout refrigeration and incubation.

P178

Evaluation of three extenders for chilled canine semen

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With breeding selection for work, sport and beauty, and given the limited gene pools of several breeds in many countries, the international exchange of genetic material is becoming a common practice. The use of chilled semen for artificial insemination is the most economical and practical method of doing it. The most used extender for chilled canine semen is the Tris-citrate-egg yolk extender. However, due to the biologic hazards of egg yolk, several countries have imposed restrictions on its use. The aim of this study was to compare the capacity of storage of dog's semen provided by three extenders: TRIS-egg yolk, milk-Tris 25% and milk-Tris 50%. Nine ejaculates were divided into three aliquots, diluted with one of the extenders and stored at 4–5°C. Extended semen was evaluated daily over 4 days in terms of progressive motility, morphology and membrane integrity. The motility of spermatozoa on the fourth day of conservation was slightly higher in egg yolk-Tris (54.7%) than in milk-Tris 25% (42.8%) or in milk-Tris 50% (40.4%). The percentage of live and normal sperm was also higher in the egg yolk extender (75.8%) than in milk 25% (66.2%) or in milk 50% (67.1%). The evaluation of plasma membrane integrity of spermatozoa showed no significant differences between extenders. The egg yolk-Tris extender seems to be superior for preserving chilled dog semen. However, extenders with milk, in a concentration (V/V) of 25–50%, also showed good results, proving to be potential substitutes for shipment of semen to countries where the entry of egg components is forbidden.

P179

Ram sperm capacitation and refrigeration induce changes in apoptotic markers

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The identification of apoptosis in individual cells depends on different markers because a single apoptosis phenotype may not be typical of all cell types. Although several studies have shown a relationship between apoptosis markers and male infertility, apoptosis in spermatozoa is not fully understood yet. In this study, we determine changes in caspase activation, phosphatidylserine externalization and DNA damage related to capacitation and refrigeration of ram spermatozoa. *In vitro* capacitation was induced by incubating 1.6×10^8 cells/ml for 3 h at 39°C with 5% CO_2 in TALP medium containing 1 mM dibutyryl-cAMP, 1 mM caffeine, 1 mM theophylline, 0.2 μM okadaic acid and 2.5 mM methyl-β-cyclodextrin. Phosphatydilserine translocation (PS, using annexinV/CFDA for simultaneous viability determination), caspase activity (Vybrant FAM Caspase-3 and -7 Assay kit) and DNA damage (TUNEL assay) were evaluated by flow cytometer. The obtained results showed significant increases in caspase activity (36.5% vs. 50.8%), DNA fragmentation (24.5% vs. 39.8%) and decrease in membrane integrity (54.7% vs. 46.5%) in both capacitated and refrigerated sperm samples, although changes in PS translocation were not significant. These findings could help in the development of better ram sperm cryopreservation protocols.

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P180

Sperm-mediated gene transfer in the horse: preliminary results

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Sperm-mediated gene transfer (SMGT) has been used with varying degrees of success in the production of transgenic embryos and adults in different species. The objective of this study was to evaluate the uptake of exogenous DNA by equine spermatozoa and to verify their ability to introduce the transgene into *in vitro* produced embryos. Semen was collected from two selected stallions, and seminal fluid was removed by washing the sperm (four different media were tested: TALP Ca^{2+} free + 0.3% BSA, Stallion Sheat Fluid, Whitten Modified medium, TALP Ca^{2+} free). Spermatozoa (1×10^8/ml) were incubated with 5 μg of exogenous DNA (pEGFP) for 1 h at 16°C. The DNA uptake was assessed by PCR. Immature equine oocytes were collected from slaughterhouse-derived ovaries, *in vitro* matured, and fertilized with transfected spermatozoa by conventional IVF (10×10^6 spermatozoa/ml for 16–18 h) or by intracytoplasmic sperm injection (ICSI) and cultured *in vitro* for 6 days. Cleavage rate was recorded 30–40 h after fertilization and embryonic development was assessed daily. Embryos were analyzed by epifluorescence microscopy using filters specific for the fluorescent protein. TALP Ca^{2+} free was the most suitable medium for equine sperm handling, and sperm internalization of pEGFP was confirmed by PCR. Oocyte maturation rate was about 60%. The only embryo obtained after conventional IVF reached the 4-cell stage, while 20 embryos were obtained after ICSI and one reached the early morula stage and expressed the fluorescent protein. In conclusion SMGT, coupled with ICSI, could led to generation of equine transgenic embryos.

P181

Effects of age and salmonella infection on the expression of toll-like receptors in the chicken epididymis

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Protecting developing and maturing spermatozoa and male reproductive organs from microbial invasion is an emerging aspect of research in reproductive physiology. Infection of epididymis with pathogens can hinder maturation and movement of spermatozoa, resulting in impaired fertility. Toll-like receptors (TLRs) are members of the innate immunity mechanism that play a crucial role in recognizing, detecting and responding to invading pathogens. Although TLRs have been studied in various organs in vertebrates, little is known about their expression and function in the chicken male reproductive tract and specifically in the chicken epididymis. The aim of this study was to investigate the expression of the chicken TLRs in the epididymis, to determine their changes in the expression levels during sexual maturation and to investigate whether TLRs expression in the epididymis was constitutive or induced as a response to Salmonella Enteritidis (SE) infection. RNA was extracted from the epididymis of healthy pubertal, sexually mature and aged birds, as well as from sexually mature SE infected birds. RT-PCR analysis revealed that all TLRs, apart from TLR1-1, were expressed in the epididymis, suggesting a defense mechanism against a broad spectrum of microorganisms. Quantitative real-time PCR analysis revealed that expression of TLRs during sexual maturation appeared to be developmentally regulated. In addition, real-time data revealed a significant up-regulation of TLR5 and 15 in the epididymis of sexually mature SE infected birds compared to healthy birds of the same age. These results suggest that a TLR-mediated immune response mechanism against Salmonella infection, driven by TLR5 and 15, exists in the chicken epididymis.

P182

The modified thermoresistance test is not suitable for fertility prediction of frozen-thawed bull semen

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Test of spermatozoa exhaustion at 37°C in a standardized hypotonic saline (0.5% NaCl) has been used to evaluate ability of sperm to maintain motility. Time of monitoring was extended to the point when any sperm progressive movement stopped in consecutive controls every 30 min. While evaluating 344 different commercial bull's semen doses, we have noticed wide variations regarding potential of thawed semen to keep progressive moving, ranging from 1:00 to 13:30 h (6:08 ± 2:09). There was an association between the percent of live intact ($p < 0.05$), but not with the number of total motile sperm (Student's *t* test) in doses. One of the imported batches of sperm doses contained straws with sperm cells that lived only 1.5 h under the test conditions. In order to check fertility potential of such semen, we have used it for artificial insemination of dairy cows. Both tested straws from the suspicious batch had in average 11.4 millions of motile and 3.6 millions of progressively moving sperm in doses (CASA). From May to October 2010, 278 randomly selected cows were inseminated and conception rate (CR) was 36.3%; with 2.27 services per conception (SC). In 14 heifers, conception rate was 78.57% and SC was 1.36. The average CR and SC in the same period of year during 6 months were 38.97% and SC 2.5 for cows and 53.85% and 1.86 for heifers. Based on these field results, we were able to conclude that this test even for short living semen *in vitro* is not reliable in estimating bulls frozen semen fertility.

P183

Induced acute endometritis by frozen semen insemination in donkey

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Fertility results alter artificial insemination with frozen semen in donkeys are very poor. Endometrial cytology and biopsies on oestrus and post-insemination (PI) stages in four female Catalonian donkeys were taken. Polymorphonuclear cells (PMN) counting on cytology was done according Reilas (2001). Biopsies were evaluated taking into account the Kenney and Doig (1986) classification for mare endometrium. A large amount of polymorphonuclear (PMN) population on the PI cytology samples wer found showing an exacerbating acute inflammatory response to frozen semen. In mares there is a physiological inflammatory response to mating but it had not been studied in donkeys. The response in jennies was similar to that seen in mares that has developed persistent mating-induced endometritis. Besides, all biopsy samples (oestrus and PI) were classified as IIA (slight endometritis), highlighting the presence of eosinophils in the stratum compactum. It differs from mare where the presence of eosinophils is related to anaphylaxis or strange body reaction and it seems to be a characteristic on donkey's healthy endometrium. Moreover, occasionally PMN in the luminal epithelium and stratum compactum in PI biopsies were found confirming the acute endometritis developed by frozen semen. In conclusion, donkey uterus is more sensible than mare uterus to frozen semen. A hardest inflammatory response is induced, making until now almost impossible the success of fertilization by artificial insemination.

P184

Main role of c-Jun N-terminal kinases in stallion spermatozoa

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c-Jun N-terminal Kinases (JNK) are mitogen-activated protein kinases (MAPK) which are responsive to stress stimuli from different origins, acting mainly through mitochondria to control cell survival. We have previously shown that JNK are present in mammalian spermatozoa and are activated in response to different stresses. The aim of our present study was to evaluate the main role of JNK in stallion spermatozoa. With this aim we evaluated the effect of a potent and specific JNK inhibitor (BI-78D3) on sperm function. Specifically we evaluated sperm motility by means of a CASA system and sperm viability, caspase activity and mitochondrial membrane potential (MMP) status by using flow cytometry. Results indicate that the inhibition of JNK activity caused a significant

and dose-dependent decrease in the motility of stallion sperm by 30 min of incubation. This treatment also significantly reduced the MMP which was much slower to the effect on sperm motility (3 h). Contrarily, the inhibition of JNK with BI-78D3 did not display any detectable effect on caspase activity or sperm viability at any concentration tested (25–100 μM). These results indicate that, contrarily to somatic cells, JNK is not primarily involved in the control of cell survival in stallion sperm, at least at sort incubations times (up to 3 h). However, JNK is likely involved in the regulation of mitochondria function and motility, although these two events are likely independent because the kinetics required to achieve both effects are completely different.

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P185

Prolificacy of ewe given hCG or GnRH 2 days after long-term progestagen treatment during seasonal anestrus

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A total of 69 Mehraban ewes, 2–5 years of age (mean body weight 59 kg and BCS 2.51 (1–5) were used. Oestrus was synchronised in all groups using intravaginal progestagen sponges (Chronogest, Intervet, UK) left *in situ* for 14 days. At the time of sponge removal all ewes were treated with eCG (400 i.u. Folligon) intramuscularly. Twenty one of ewes received hCG (250 i.u.; Intramuscular injection, Chorulon) and twenty four of ewes treated with GnRH (4.2 μg; Buserelin; im; Vetocept) 2 days after sponge withdrawal in the treatment groups and twenty four of ewes considered as a control group received no drugs. Then, after injection all ewes were exposed to eight rams of proven fertility for 51 days. The lambing data showed that the significant positive correlation between weight of ewe and lamb birth weight in control and hCG group; while, there was no correlation between these parameters in the GnRH group. The mean (±SD) of single lamb birth weights of the ewe with BCS of 2 and 3 were 4398.5 ± 543.7 and 5000.9 ± 632.1 gr ($p < 0.05$). Single and twin lamb birth weights of the ewe in hCG group (4930 ± 665.5 and 8372.7 ± 382.7) and control group (4388.7 ± 546.4 and 7938.9 ± 453.7) were shown significant difference ($p < 0.05$). Percentage (Number) of fertility rate and twin birth rate for hCG 85.7 (18/21) treatment groups were higher than that of those for control 58.3 (14/24) and GnRH 66.7 (16/24) group during seasonal anestrus ($p < 0.05$). These findings suggest that hCG administration 2 days after long-term progestagen treatment may improve Prolificacy of ewe during seasonal anestrus.

P186

Analysis of the zona pellucida resistance to protease digestion in different species using bovine oviductal fluid

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It was previously reported that the zona pellucida (ZP) of bovine and porcine *in vitro* matured oocytes shows an increased resistance to protease digestion and to sperm entry after incubation in bovine oviductal fluid (bOF). This was named as pre-fertilization zona pellucida hardening. The purpose of this study was to analyse the effect of periovulatory bOF in other species. The ZP resistance to pronase digestion after incubation in bOF was assessed in cow, ewe, goat, hamster, mouse, pig, rabbit and rat ewe oocytes and in human ZPs. Oocytes and ZPs were incubated with undiluted bOF (1 oocyte or ZP/μl)for 30 min. A control group without treatment was used for each species. The ZP dissolution time of each oocyte in pronase solution (0.5% w/v in PBS) was registered. A significant increase in ZP resistance was observed in the cow (220.50 ± 38.50 h), ewe (76.85 ± 32.54 h), goat (42.15 ± 22.13 h), hamster (26.85 ± 10.35 h), pig (98.30 ± 38.56 h) and rabbit (13.95 ± 1.29 h) oocytes; however, no effect was observed in mouse (0.06 ± 0.00 h) and rat (0.04 ± 0.00 h) oocytes or in human (0.03 ± 0.00 h) ZPs. Control groups were digested in a few minutes except in hamster (7.21 ± 3.31 h). These data revealed that bOF has a similar effect on the ZP of most species studied. However, in human, mouse and rat ZP, no effect was produced by bOF suggesting that ZP composition could play an important role in this process.

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P187

Incorporation of the membrane permeable calcium chelator BAPTA-AM does not improve the survival of stallion spermatozoa after freezing and thawing

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Many molecular damages are claimed to be associated with cryopreservation, two of them are the increase in intracellular free calcium and Lipid Peroxidation (LPO). In order to evaluate if the addition of a membrane permeable calcium chelator, alone or in combination with a lipid soluble antioxidant, can improve the outcome of cryopreservation in the equine species, individual ejaculates from six stallions were collected and split into five sub samples and a control. The first two were supplemented with the membrane permeable Ca^{2+} chelator BAPTA-AM at final concentrations of 5 and 10 μM respectively, other aliquots combined the same concentrations of BAPTA-AM with the antioxidant butylated hydroxitoluene (BHT) at a final concentration of 1 mM, the fifth subsample served as control. At thawing ejaculates were assessed for motility and kinematics (computer assisted sperm analysis), membrane and acrosome integrity and mitochondrial membrane potential (flow cytometry and confocal laser microscopy). The treatment had no effect either positive or negative in any of the sperm parameters evaluated, only a tendency ($p = 0.05$) to a lower percentage of live spermatozoa with reacted acrosome after thawing was observed in the samples supplemented with 10 μM BAPTA (2.8 ± 0.96 vs. 0.7 ± 0.96).

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Production of recombinant porcine oviductal glycoprotein-1 (OVGP1)

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The OVGP1 is the major secretory glycoprotein present in the oviductal fluid. This glycoprotein is involved in the gamete interaction, blockage of the polyspermy and embryo development. A high incidence of polyspermy is observed during *in vitro* fertilization (IVF) in the pig. The aim of this study is the production of recombinant OVGP1 that will be used as a component of IVF medium to improve the IVF efficiency. For that, total RNA was isolated from porcine oviduct and cDNA was synthesized with oligo-dT as primer. The complete open reading frame of OVGP1 was amplified by PCR and cloned into pcDNA3.1-6xHIS expression vector by means of the Kpn I and Mun I restriction sites. OVGP-1 construct was expressed in human embryonic kidney cell line (HEK 293T). The production of OVGP-1 protein was analyzed by western-blotting in the cell lysates and in the conditioned media using an anti-OVGP1 polyclonal antibody. Western blot analysis demonstrated the existence of a band in cell lysates and media with an apparent molecular weight of 80 kDa. This molecular weight is lower compare to the native porcine OVGP1 previously reported. This might be due to the different glycosylation pattern of the OVGP1 produce in HEK cells compare to the oviductal cells. In conclusion, porcine OVGP1 was successfully expressed in HEK 293T cells and is secreted to cell culture medium. Recombinant OVGP1 has probably a different glycosylation pattern compare to the native OVGP1. Future experiments will be necessary to analyze the effect of OVGP1 into the control of polyspermy in the IVF experiments.

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P189

Single layer centrifugation of cooled semen with androcoll-e improves sperm quality

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Low sperm quality in cooled stallion semen doses can be a cause of low pregnancy rates after artificial insemination (AI). Previously, Single Layer Centrifugation (SLC) with Androcoll-E was shown to improve sperm quality in small volumes of both fresh and cooled semen although sperm yield was reduced if the semen was cooled for 24 h before SLC (Morrell *et al.*, EVJ 2009: 41, 53–58). Can the scaled-up version of SLC, Androcoll-E-Large, be used to improve semen quality in cooled semen doses? Semen doses from 15 stallions (3 per stallion) were cooled and transported to the laboratory overnight. After equilibration at room temperature for 1 h, the semen was prepared with Androcoll-E-Large and the resulting sperm pellets were resuspended in INRA96. Uncentrifuged and SLC-sperm samples were analysed for motility, morphology and chromatin integrity; treatment means were compared by ANOVA. Progressive motility was higher in the SLC samples than in the uncentrifuged samples (60% vs. 52%; $p < 0.001$), normal morphology was higher (70% vs. 65.5%; $p < 0.01$); %DFI was lower (13% vs. 15%; $p < 0.001$). Median motile sperm yield was 50% (range 36–78%). Progressive motility was retained for at least a further 24 h (SLC 46%; controls 33%; $p < 0.001$). SLC with Androcoll-E-Large improves sperm quality in cooled semen doses and may extend their "shelf" life for AI.

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In vitro embryo production using Boran (*Bos indicus*) oocytes in Kenya

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The Boran is a zebu found in the arid and semi arid lands (ASAL) of Kenya. Despite its adaptive superiority to the ASAL's it's considered productively inferior to the Bos taurus. *In vitro* embryo production (IVEP) is a technology with a well known potential to increase genetic progress in cattle. This study evaluated the developmental potential of Boran oocytes in an IVEP system. Three hundred and eight ovaries were collected from 154 Boran cows at slaughter. n = 2658 cumulus oocyte complexes (COC) were recovered by aspiration, selected and graded and n = 2358 used for IVEP process. The COC's were fertilized with pre-tested semen from a proven bull and the resulting zygotes cultured to the blastocyst stage. Cleavage and blastocyst rates were assessed 72 and 196 h post-insemination respectively. This study reports maturation, cleavage and blastocyst rates of 90.80 ± 1.29%, 68.15 ± 2.01% and 27.64 ± 2.28% respectively, for COC's from Boran cows during the IVEP process (values are mean + SEM). This study has set up baseline information for enhanced utilization of the Boran cow as oocyte donors. In conclusion, *in vitro* production of embryos for the Boran cow in Kenya is technically feasible and the Boran would be a good cow for ova harvesting, although further studies may be required to optimize results in this breed.

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P191

Reproductive biology of the Depik Fish Rasbora tawarensis (Pisces: Cyprinidae)

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This study provides a contribution to the knowledge on reproductive biology of Rasbora tawarensis an endemic fishes from Lake laut Tawar, Aceh, Indonesia as well as a basic information for conservation of the species. Monthly sampling was conducted from April 2008 to March 2009. The gonado-somatic index (GSI) varied between 6.65–18.60 in female and 5.14–8.56 for male. GSI of female depik was higher in March, September and December, indicating the onset of reproductive seasons, September being the peak of the reproductive season. The GSI and oocyte size was directly correlated with gonadal development stages. A greater proportion of mature male than female was detected during the study and the sex ratio show

that the number of female was higher than male and the male reached maturity earlier than female. The ovaries were multiple oocyte size classes at every stage of gonadal development, thus R. tawarensis can be classified as a group synchronous spawner or a fractional multiple spawners. The average batch fecundity was 3715.4 ± 893.6 eggs, while the average relative fecundity was 518 ± 95.64 eggs. g-1 body weight. There was a positive linear relationship between batch fecundity and body weight and total length. The spawning frequency of the female was 2–11 days and frequent spawning during the reproductive seasons.

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The effect of preoperative antibiotic or antibiotic + vitamin C administrations on the inflammatory and oxidative state in the rabbits with experimentally induced pyometra

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The reduction of postoperative stress and complications which are frequently observed following the operative treatment of pyometra may be possible by the preoperative antibiotic and antioxidant therapies. Eightteen healthy rabbits were divided into three groups. Pyometra was induced in all of the groups experimentally by the administration of Pasteurella multocida inoculum operatively. Daily USG and clinical examinations performed and progression of the pyometra were confirmed. Beginning on the third day after inoculation Group I recieved enrofloxacin (2.5 mg/kg, s.c.), while Group II received enrofloxacin (2.5 mg/kg, s.c.) + vitamin C (100 mg, s.c.) injections for 3 days. Group III were not treated as the control group. Ovariohysterectomy were performed in all groups. Blood samples were collected before inoculation, on the day of operation, 1st, 3rd and 7th day following the operations. Ceruloplasmin, malondialdehydte, vitamin C, haptoglobin, serum amyloid-A levels were analyzed. Ceruloplasmin levels rised in all groups following inoculation and decreased at 3rd day postoperatively in Group I and II while in Group III it was found significantly higher ($p < 0.05$). Malondialedhyte levels were significantly higher in Group III on the day of operation compared to the treatment groups ($p < 0.05$). Serum Amyloid-A levels rised in all of the groups following inoculation, on the day of operation ($p < 0.05$) which is followed by a gradual decline until the 7th day. These findings encountered in this study may indicate a possible effect of preoperative therapy on the reduction of postoperative stress.

P193

Research regarding PG 600 effect on the level of reproductive indices to gilts

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Worldwide, PG 600 therapy is applied to induce oestrus in gilts that are delayed to install puberty. The observations for this study were made on 967 Camborough gilts, allotted into two groups: control (n = 648) and experimental group (n = 319). In the control group the following parameters were recorded: dynamics of coming into heat for 27 days after transfer in to the hall mount preparation, fecundity and prolificacy. In the experimental group the dynamics of coming into heat for 21 days after transfer was examined. Gilts (experimental group) that did not show oestrus during this time period (108 gilts, 33.86%) were treated with 5 ml PG 600. Thereafter the dynamics of coming into heat for were examined 6 days as well as fecundity and prolificacy. In the next 6 days after administration of PG 600, 75.92% of the gilts came into heat which is more than the 36.91% of the untreated controls ($p < 0.001$). Best response to treatment was recorded in spring and winter (81.81% and 82.6%, respectively). After treatment, the highest rate of gilts in oestrus was found on days 3 and 4 (24.07% and 25.92%, respectively). The average fecundity of the gilts in the experimental group was 88.57%, insignificantly higher than the gilts in the control group: 85.05% ($p < 0.05$). Average prolificacy was 10.32 ± 0.11 piglets/farrowing in the control group and somewhat but not significantly higher in the experimental group: 10.53 ± 0.12 piglets/farrowing ($p < 0.05$). According to the results obtained, we believe that the use of hormonal therapy may be an effective tool to optimize the reproductive function in pigs.

P194

Quality analysis of bovine *in vitro* fertilized embryo co-cultured with oviduct epithelial cells

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The current study was designed to investigate the effect of oviduct epithelial cells on quality of bovine embryos. The ovaries and oviduct were collected from a local slaughterhouse in proper temperature and clean condition. A total number of 377 immature oocytes were recovered from 47 ovaries. Three hundred anf forty three oocytes were cultured of which 239 oocytes were matured *in vitro* and reached to metaphase II stage. All matured oocytes were fertilized in BOFM fertilization media for 18–20 h. Oviduct was disinfected by 70% ethanol, followed by washing with sterile DPBS. Mechanical method of cell recovery was done by scraping using two sterile glass slides. Cell suspension was made with TCM 199 media. Cells werewashed three times and isolation of the cells were done based on gravity, supernatant was discarded and cells were resuspended in TC199 culture media. Zygotes (231) with second polar body formation were treated in three groups, (i) control in synthetic oviduct media, (ii) co-cultured with oviduct epithelial cell and (iii) co-cultured with oviduct epithelial cell supplemented with insulin growth factor. After 8–9 days, embryos at blastocyst stage were stained. Quality of embryos was analyzed as per compact total number of blastomeres. Embryos co-cultured with oviduct epithelial cell showed increased number of blastomere in compare with control group; more over this increase in cell number was more in 3rd group where insulin was supplemented in media. This co-culture system with BOEC may not only offer an excellent model for interaction with embryo and also stimulation by embryo but also additional growth factors in media provides a useful tool for the improvement of quality of embryo for implantation.

P195

Seasonal pregnancy rate and economic benefit of artificial insemination with sexed-sorted sperm in holstein dairy Heifers of Shiraz, Southern Iran

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Seasonal pregnancy rate and economic benefit of sexed-sorted sperm in Holstein dairy heifers of Shiraz industrial farms were evaluated. The insemination data of 200 heifers of five farms including sperm types (sexed or conventional), date and times of insemination were collected from March 2009 to March 2010. Results of the Chi-square test showed that the means of pregnancy rate of conventional sperms (72.2%) were significantly higher than the sexed sperms in heifers (49.2%; $p = 0.001$). The lower fertility rate of sexed sperms insemination was in the winter (36.3%) and was significantly less than the fertility rate in the autumn (73.9%; $p = 0.02$). Using the costs of sperm insemination, treatment and price of selling heifers and young bulls, the costs and benefits of sexed and conventional sperm insemination was calculated. The costs of first and second insemination with sexed sperms in heifers was higher than conventional ones (207.7 $ vs. 77.3 $ and 669 $ vs. 408 $, respectively). The economic benefit of using sexed sperms after two inseminations was 296.1 $ lower than conventional sperms. In conclusion, if the fertility rate of sexed sperm insemination in healthy heifers reach to the 90% of conventional sperm, sexed sperm insemination (2555.4 $) can be more beneficial than conventional one (2323.7 $) after two insemination.

P196

Serum steroid fluctuations during early pregnancy in mares, measured by ultra-high performance liquid chromatography coupled to tandem mass spectrometry

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The mammalian reproductive tract undergoes cyclical changes in morphology and physiology in response to steroid hormones. Ultra-high performance liquid chromatography coupled to tandem mass spectrometry (U-HPLC-MS/MS) is a new method which can be applied to determine steroid levels in blood and tissue. We have determined daily changes in steroid levels in the serum of pregnant mares in order to investigate the effect of these changes on the morphology and function of equine oviduct vesicles grown *in vitro*. Serum collected every 24 h from mares during the first 7 days of pregnancy, was extracted using solid phase extraction and analysed using a validated U-HPLC-MS/MS protocol. Simultaneous measurement of 17α-,17β-estradiol, α-,β-testosterone and 17-OH-progesterone was performed. The limits of detection were 10 pg/ml for 17α-and β-estradiol and 1 pg/ml for α-and β-testosterone and 17-OH-progesterone. Levels ranged between 105–1742 pg/ml for 17-β-hydroxyprogesterone and between 0.13–41 pg/ml and 0–8.27 pg/ml for α- respectively β-testosterone and remained below 10 pg/ml for 17α- and β-estradiol. Levels of 17-OH-progesterone and β-testosterone were significantly ($0.001 < 0.05$) higher from day 4 on. This method is sensitive and selective for the detection and quantification of multiple sex steroids in a single analysis and the results will be applied to study the effects of steroids on oviduct cells *in vitro*.

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P197

Do antioxidant and poli-unsaturated fatty acids treatment of epididymal bull sperm samples improve post-thaw quality?

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A key factor to be studied regarding the use of epididymal sperm is the cryopreservation technique. One reason for the negative impact of cryopreservation is the oxidative stress, which may cause structural damage to biomolecules. The objective this study was to test the addition of decosaexaenoic acid (DHA), an important PUFA, associated to antioxidants (e.g., vitamins E and C, reduced glutathione – GSH, superoxide dismutase – SOD, calatalase, glutathione peroxidase – GPx) to the semen extender, aiming to improve post-thaw semen quality. Samples were collected from the caudae epididymides of testicles collected from abattoirs and cryopreserved. The effect of DHA and antioxidant treatments (in different concentrations and combinations) on semen extender was evaluated performing tests of membrane and acrosome integrities (eosin/nigrosin and fast green/bengal rose stain, respectively), mitochondrial activity (diaminobenzidine stain), DNA integrity (sperm chromatin structure assay – SCSA) and, sperm susceptibility to the oxidative stress (TBARS). Results indicate that due to the treatment with DHA, epididymal sperm became more susceptible to the oxidative stress (control: 178.3 ± 9.5a, 5 μM: 298.1 ± 53.2b, and 10 μM: 514.28 ± 43.34c). However, when DHA (5 μM) was associated to SOD (20 IU/ml), an improvement was found on progressive motility (control: 24.28 ± 4.82 vs. DHA + SOD: 34.43 ± 4.12%; $p < 0.05$). Furthermore, the association between DHA and GSH (5 mM) induced an improvement on membrane and DNA integrities when compared to the control (57.83 ± 5.51 and 6.56 ± 0.64 vs. 38.62 ± 7.15 and 4.79 ± 0.41, respectively; $p < 0.05$); when associated to the Vitamin E, DHA showed poor results on mitochondrial activity. Our data indicates that antioxidant treatment to epididymal sperm samples may depend on the concentration of the antioxidant used, the location of the deleterious influence of the oxidative stress and which ROS are causing such damages. Furthermore, the treatment of DHA associated to an antioxidant treatment, may be an alternative to improve post-thaw quality of semen samples.

P198

Methylation characteristic and developmental potential of guangxi bama minipig (Sus scrofa domestica) cloned embryos from donor cells treated with trichostatin A and 5-aza-2'-deoxycytidine

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The aberrant DNA methylation patterns in somatic cell nuclear transfer (SCNT) embryos usually related to the inefficiency of SCNT. To facilitate nuclear reprogramming, this study was conducted to investigate the effect of treatment of Guangxi Bama minipig donor cells with Trichostatin A (TSA), 5-aza-2′-deoxycytine (5-aza-dC), or combination of TSA and 5-aza-dC prior to nuclear transfer. Analysis showed

no considerable changes on cell cycle status among all groups. The transcription data of DNMT1, DNMT3a, HDAC1, and IGF2 genes in donor cells showed that transcription levels of HDAC1 was significantly decreased after treatment with combination of TSA and 5-aza-dC, along with a significantly increased level of IGF2 ($p < 0.05$). The results of this study also demonstrated that combination of TSA and 5-aza-dC significantly improved the development rates of minipig SCNT embryos to blastocyst, and accompanied by decreased levels of DNA methylation in somatic cells and blastocyst ($p < 0.05$). However, treatment of donor cells with either TSA or 5-aza-dC resulted in no significant effects in blastocyst formation rate and DNA methylation levels ($p > 0.05$). In conclusion, our data suggest that combined TSA with lower concentrations of 5-aza-dC can induce a potent demethylating activity, sequentially improve the blastocysts development ability of Bama minipig SCNT embryos.

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Antibiotic susceptibility for *Staphylococcus aureus* Isolated from bovine subclinical mastitis in Tabriz

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Bovine mastitis due to *Staphylococcus aureus* causes substantial economic losses in dairy industry worldwide. Seen from the epidemiological point of view, it is important to determine the origin of organisms involved in the etiology of the disease. The aim of the current study was to determine the pathogens responsible from subclinical mastitis cases in cows in Tabriz and the pattern of antibiotic sensitivity to several antibiotics used for treatment of mastitis in veterinary practice.
A total of 14 dairy farms were visited and 119 cows were examined for subclinical mastitis. From each milk samples, was plated on blood agar plates. After presumptive identification, the isolates were determined by biochemical tests. Antibiotic susceptibilities of the Staphylococci were determined by the MIC method. *S. aureus* was present in 41.01% of the samples. 100% of isolates of *S. aureus*, were resistant to one or more antimicrobial. Penicillin, ampicillin, amoxicillin and gentamicin resistance occurred in 81.8% and were the most common trait. In the abundance of studies investigating the antibiotic resistance of mastitis pathogens, few reports have noted a subclinical occurrence of antibiotic resistance, meaning *S. aureus* is usually negligible as a mastitis pathogen. We found an unusual high prevalence of *S. aureus* in cases of subclinical mastitis in cows in Tabriz, Iran. Antimicrobial resistance determined in our study was in line with other reports. Further epidemiological monitoring of *S. aureus* strains and the resulting severity of mastitis episodes caused by them in dairy herds is warranted.

P200

Sexual response in stallions exposed to mare urine and p-cresol

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The aim of this pilot study was to investigate whether the stallion can discriminate between oestrous and dioestrous urine and if so, whether it is the chemical substance *p*-cresol that arouses the stallion. In mid August, five breeding stallions were exposed for 5 min to the following substances: oestrous urine (OEU), dioestrous urine (DIU), dioestrous urine with *p*-cresol (DIUP), distilled water (DW) and distilled water with *p*-cresol (DWP), one substance per day and with no simultaneous contact with mares. The behavioural parameters of stallions recorded were number of flehmen, degree of erection (1–3), degree of nose secretion (1–3), duration of smelling and number of 'smelling events'. The no of flehmen was significantly higher during exposure to urine (OEU, DIU, DIUP) than during exposure to water (DW, DWP). For the other behavioural parameters the differences between substances were not significant, however the scoring was found to be higher for the urine substances. A significant positive correlation was found between degree of nose secretion and no of flehmen as well as between degree of nose secretion and degree of erection. The difference between stallions for the behavioural parameters was not significant; however there was a tendency towards interaction between stallion and no of flehmen as well as no of smelling events. A stallion cannot discriminate between oestrus and dioestrus urine and p-cresol tested alone does not elicit sexual response in stallions.

P201

Effect of oligoelements in the reproductive postpartum performances of Holstein Friesian heifers

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To evaluate the administration of oligoelements in the reproductive performance of postpartum nulliparous, twenty-six of Holstein Friesian heifers with 200 (± 21) days of gestation were divided in experimental and control groups. In the animals from the experimental group ($n = 13$) sixty days before partum, two capsules intra-ruminal slow-release with oligoelements were administrated, while in the control group no treatment was performed. Immediately prior to administration, blood was collected from all animals to evaluate the values of Iodine, Manganese, Selenium and Copper as well as progesterone levels. In the day after partum and weekly for 11 weeks, blood was collected to assess progesterone levels. Oligoelements were evaluated in day after partum and after sixty days and determined by atomic absorption spectrophotometry. Progesterone were evaluated by the ELFA technique. Sixty days before calving for the oligoelements, no statistical differences between groups were observed, in which all animals had deficiencies in Selenium, Copper and Iodine. In the day after calving and sixty days after a statistical increase ($p < 0.05$) in serum Copper and Selenium was observed in the experimental group, when compared with values obtained before partum. For the other oligoelements no statistical differences were observed. Concerning reproductive characteristics, the experimental group, on 5 weeks after partum, 70% of the cows were cyclic, while in the control group in the same period, only 33% of animals showed estrus signals. It can be thus concluded that the administration of oligoelements elements, sixty days before partum in heifers, reduces the period of postpartum anestrus, increasing moreover the quality of corpora lutea.

P202

Immunolocalization of CYR61 in dog spermatozoa

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CYR 61 is a cystein rich heparin-binding protein that belongs to the CNN family. Members of this protein family have been involved in a broad range of biological functions related to reproductive sucess such as: cell growth regulation, migration, chemiotaxis and cell adhesion. However, its localization in the gametes has not been described. The aim of this study was to immunolocalize CYR 61 in dog spermatozoa by immunocytochemistry. Sperm rich fraction was collected from four dogs by manual manipulation. Each ejaculate was washed and diluted in PBS. Sperm cells were spotted on poly-L-lysine coated slides. Antigens were retrieved by microwave heating the samples in a citrate buffer solution (pH 6.0). After endogenous peroxidase neutralization and blocking, samples were incubated overnight with anti-CYR 61 antibody (H-78; Santa Cruz Biotechnology, Santa Cruz, CA, USA) in a 1.5% BSA 1:200 solution at 4°C in a humid chamber. Primary antibody binding was detected using a biotinylated secondary goat-antirabbit IgG with avidin peroxidase complexing (Vector Laboratories Burlingame, CA, USA). The reaction was detected by the addition of diaminobenzidine (Dako Cytomation Carpinteria, CA, USA) and counterstained with Harris' hematoxilin. Control samples treated subjected to the same procedure with the omission of the primary antibody were included in each experiment. CYR-61 immunoreactivity was present along the sperm tail but not in the neck nor in the head.

P203

Blood flow in the wall of the fsh-superstimulated preovulatory follicles in Santa Inês Ewes

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Ten adult ewes were equally divided into two treatments, according to day of start of FSH treatment (G1: near the beginning of the first wave; G2: last wave). The estrus was synchronized with a CIDR device inserted on Day 0 and remained until D7 and D13 in G1 and G2, respectively. Two doses of the 37.5 μg of D-cloprostenol were administered on D0 and CIDR removal day. There were administered 200 mg of FSHp in eight decreasing doses, starting on D4 and D10 in G1 and G2, respectively. All ewes received 300 IU of eCG on CIDR removal day. Color-Doppler and B-mode ultrasound were performed daily during FSH treatment to evaluate the blood flow in POF wall and to measure POF diameters. Blood flow was classified according to their area [0–3 point score: (0) absence; (1) small; (2) moderate; (3) intense]. Data were expressed as mean ± SD and analyzed by ANOVA (Tukey test; SAS). The diameters of follicles were 4.3 ± 0.5a, 4.7 ± 0.3ab, 5.1 ± 0.3abc, 5.5 ± 0.3bc and 6.3 ± 0.7c mm at day 4, 5, 6, 7 and 8 in G1 and 3.8 ± 0.3ab, 3.6 ± 0.2a, 4.5 ± 0.3abc, 5.1 ± 0.4bc and 6.2 ± 0.1c mm at day 10, 11, 12, 13 and 14 in G2, respectively (abc, $p < 0.05$). The blood flow score were 0.8 ± 0.4a, 1.4 ± 0.5ab, 2 ± 0bc, 2.8 ± 0.4c and 2.75 ± 0.5c in G1 and 0.25 ± 0.5a, 0.25 ± 0.5a, 1.25 ± 0.5b, 1.75 ± 0.5b and 2.8 ± 0.4c in G2 at the same day, respectively (abc, $p < 0.05$). There was difference between groups on second and fourth days of FSH treatment for blood flow score ($p < 0.05$). Blood flow was detected in the POF wall, which demonstrated increased according to the progress of FSH treatment in Santa Inês ewes. *Financial support: FAPESP.*

P204

Long duration of farrowing affects fertility of sows

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Failure to get sows pregnant within a short time after weaning is still a major cause of economic loss in modern swine production. Many factors can be responsible of rebreeding in sows. Very little research is available on factors happening during farrowing, and which could be their effect on the subsequent fertility. Our aim was to explore if parity, piglets born alive, piglets stillborn, body condition, duration of farrowing and weaned piglets are associated with the fertility of the sows at the following breeding in a farm with 440 sows (Yorkshire × Landrace). We recorded farrowings with video cameras, the beginning and the end of parturition were established by reviewing the recordings. Sows ($n = 93$) were of parity 4.7 ± 1.9 and their average back fat 3 weeks prior and at farrowing was 15.5 ± 4.2 mm and 14.3 ± 3.6, respectively. The sows had 11.9 ± 3.2 piglets born alive, 1.0 ± 1.6 stillborn and 9.7 ± 1.5 weaned. The mean duration of farrowing was 283 ± 157 min. After weaning, 11 % of the sows failed to get pregnant at the first insemination ($n = 10$). A logistic regression model was used in order to find significant predictors between rebred sows and successfully pregnant sows. The mean duration of farrowing in the rebred sows was 434 ± 259 min, while in the pregnant sows it was 264 ± 144 min ($p < 0.01$). No significant differences were found in the other parameters observed. In conclusion, longer duration of farrowing significantly increased the risk of rebreeding.

P205

The diagnosis and treatment of reproductive problems during the breeding season in barren arabian mares – a preliminary study

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The aim of the present study was to investigate the reproductive problems and evaluate the treatment success according to histopathological classifications in ten barren Arabian mares. The condition of the uterine and ovarian was evaluated by the ultrasonographical and the gynecological exam procedures. Also, the endometrial swabs, smears and biopsy and blood serum samples were taken for microbiological, cytological, histopathological exam and diagnosis of metabolic diseases, respectively. The histopathological lesions were evaluated as

Category (C) I (n = 2), C IIA (n = 4), C IIB (n = 3) and C III (n = 1) (Kenney and Doig, 1986, Current Therapy in Theriogenology, 723–729). The current treatment approaches to barren mares were applied. Although all mares in C I were conceived, two out of four mares in C IIA were not conceived. Nonpregnant mares in C IIA had the problem of resistant anovulatory follicle together with the Cushing's syndrome, resistant *Klebsiella pneumoniae* infection and intrauterine fluid accumulation. Two out of the three mares in C IIB were not conceived. Cystic uterine structures, purulent contents, *Staphylococcus aureus* + *Escherichia coli* and resistant Candida spp. infection were detected in nonpregnant mares in this category. One mare in C III was pensioned from the stud duty at the instance of enterprise. As a result, five out of nine mares were conceived. It was observed that the intrauterine fluid accumulation and susceptibility to uterine infections was increased; when the histopathological category score was getting increased.

P206

Recovered ovine oocytes by consecutive follicular aspiration

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The aim of this study was to evaluate the quantity and quality of oocytes recovered by consecutive follicular aspiration using videolaparoscopy in hormone stimulated ewes. Nine follicular aspiration sessions, with 7 days interval between procedures, were carried out in six Santa Inês ewes. The estrous cycle of these animals were synchronized by using an intravaginal device of 60 mg of medroxyprogesterone acetate for 6 days with 37.5 μg of D-cloprostenol and 300 UI of eCG injections (IM) being given 24 h prior to progesterone device removal. After synchronization, animals received 80 mg of FSHp and 300 UI of eCG 36 h before follicular aspiration. Follicular aspiration was carried out by videolaparoscopy. The oocytes were evaluated according to cytoplasm homogeneity and the number of cumulus cell layers. The mean number of visualized, aspirated and recovered oocytes were 13.24 ± 2.0, 11.27 ± 3.03 and 5.79 ± 2.3, respectively. A recovery rate of 51.69% was observed. There was no significant difference ($p > 0.05$) between sessions or oocyte quality. Successive sessions of follicular aspiration did not change the number of visualized or aspirated oocytes nor the quantity or quality of these recovered oocytes. In conclusion, follicular aspiration of up to 9 consecutive sessions per week produced viable results with great potential for optimizing assisted reproduction in sheep.

P207

Aspects in reproductive biology of the pointed nose sensory Barb (*Cyclocheilichthys repasson* Bleeker, 1853) in a man-made Lake, Thailand

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The pointed nose sensory barb (*Cyclocheilichthys repasson* Bleeker, 1853) is the dominant species in the Num Oun reservoir, Sakon Nakhon Province, Northeast Thailand. The aim of this study was to examine a gonadosomatic index to estimate the spawning season. Pointed nose sensory barbs (958) were sampled during May 2008–April 2009 from six sampling sites around the reservoir. The length at 50% maturity was estimated by fitting a logarithmic function curve between the proportion of the cumulative frequency of occurrence on matured females and the total length. Fecundity was estimated on the basis of the relationship between fecundity-total length and fecundity-body weight. Based on the sampling length at 50% maturity was 11.2 cm. The average fecundity was at 9149 ± 293 eggs. The relationships in total length (L) and body weight (W) to fecundity (F) were $F = 2.014L^{3.253}$ and $F = 363.798W^{0.951}$, respectively. The results indicate that the spawning season of the fish was throughout the year due to the data the mature females had presented all year round. However, the presence of mature females was greatest between April and August indicating this period is the highest spawning season of this fish in the man-made reservoir. The information suggests that fishing during April and August may interrupt the spawning and cause the reduction of number of fish.

P208

Post-thaw quality of boar semen frozen at low sperm concentration

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Boar spermatozoa are routinely cryopreserved at concentrations of 1000×10^6 sperm/ml. However, this sperm concentration cannot be applied when biotechnologically treated spermatozoa, as sex-sorted sperm are, must be frozen. Our aim was to evaluate the post-thaw functionality of boar semen frozen at a low sperm concentration. Semen samples from three boar (three ejaculates per boar) were divided in three aliquots: (i) frozen at 1000×10^6 sperm/ml, as control; (ii) non-sorted and (iii) sex sorted sperm samples frozen at 20×10^6 sperm/ml. All samples were frozen using the 0.25 ml-straw procedure. Total (TM) and progressive (PM) sperm motility and viability (intact plasma membrane) were evaluated at 5°C (prior to freezing) and 30, 90 and 150 min after thawing. At 5°C, whereas TM was similar ($p > 0.05$) in all aliquots, PM was higher ($p < 0.05$) in C samples. After thawing, TM and PM were higher ($p < 0.05$) in A compared with B (from 90 min post-thawing) and C samples (from 30 min post-thawing). Sperm viability did not differ ($p > 0.05$) among aliquots in any of the evaluation times. In conclusion, boar semen frozen at 20×10^6 sperm/ml using a standard freezing procedure, showed lower post-thaw sperm quality than those frozen at 1000×10^6 sperm/ml. Further studies regarding to the factors that could affect sperm motility (glycerol concentration, cooling rates, etc) should be done to adapt the freezing protocol to low sperm concentrations.

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P209

Superovulation in the mare with commercially available pFSH

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To date, superovulation is still unsatisfactory in the mare. This study aims at assessing commercially available pFSH (Stimufol®, Merial, Belgium) for superovulation in this species. The study was partly conducted during (three cycles/five mares) and out (two cycles/four mares) of the breeding season. The first untreated cycle of each mare served as control group. Mares were short-cycled with 125 mg of cloprostenol 7 days post-ovulation and received 6.25 mg of pFSH IM twice daily for 2 days. Ovulation was induced when a follicle reached 30 mm with either hCG or busereline. Ovaries were scanned daily until induction and then twice daily until all follicles >25 mm had ovulated or disappeared. Numbers of ovulations was recorded for all cycles. Kruskal-Wallis Test was used and significance was established at $p < 0.05$. Ovulation rates for controls were 1.6 and 1.2 during and out of the breeding season respectively and were not statistically different, allowing results to be pooled. Ovulation rates for the treated cycles were 2.9 and 1.5 during and out of the breeding season respectively. The overall ovulation rate of treated cycles was higher than that of controls ($p = 0.025$). Ovulation rate of treated cycles during the breeding season (2.9) was also higher than that of untreated cycles during the same period ($p < 0.01$) but not out of the breeding season. pFSH seems to increase ovulation rates if administered during, but not out, of the breeding season. However, numbers of studied cycles are too small to determine whether this is due to an inadequate regime or if this just reflects individual variation. Further studies on Stimufol® with larger numbers, should be conducted as it might prove inexpensive and easy to superovulate mares.

P210

Immunopresence and enzymatic activity of nitric oxide syntases, cyclooxygenases, and pge2-9-ketoreductase and *in vitro* production of progesterone, PGF2α, and PGE2 in Mediterranean buffalo (*Bubalus bubalis*)

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This study was performed to evaluate the immunopresence and the enzymatic activity of cyclooxygenase 1 (COX1), COX2, PGE2-9-ketoreductase (PGE2-9-K), and nitric oxide synthases (NOSs) and hormone *in vitro* production in buffalo corpora lutea (CL) at early (day 4 diestrus), mid (day 10), and late (day 18) stages. Immunohistochemical data revealed that COX1, -2 and PGE2-9-K were localized in the cytoplasm of luteal cells of all the stages considered. The expression of constitutive and inducible NOS were evidenced in the nuclei and cytoplasm of all the luteal cells as well as in the nuclei of endothelial and stromal cells during all stages studied; these immunosignals increased ($p < 0.01$) during the late stage. Enzymatic results showed that COX1 activity did not changed during diestrus, whereas COX2 and NOS increased ($p < 0.01$) from early to late stage and PGE2-9-K was higher ($p < 0.01$) in late CL. Hormone *in vitro* data displayed that progesterone release was higher ($p < 0.01$) in mid and lower ($p < 0.01$) in early phase, PGF2α synthesis increased ($p < 0.01$) from early to late stage, and PGE2 was higher ($p < 0.01$) during early stage. These results support the idea that COX, NOS and PGE2-9-K regulate buffalo CL life span similarly to findings previously reported in different mammalian species.

P211

Evaluation of trypsin treatment in inactivation of bovine herpesvirus type-1 (BoHV-1) in murine embryos for sanitary control

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Researches about the sanitary quality and control of *in vitro* bovine embryo production (IVP) are being carried out in several countries because of the contaminations that may occur during the production and transfer of embryos. The International Society of Embryo Transfer (IETS) suggests the treatment of embryos with trypsin or antibiotics in alternating washes with culture medium in order to remove and/or inactivate infectious agents that may interfere with the final product. The aim of this study was to evaluate the effectiveness of trypsin treatment in the elimination and/or removal of bovine herpersvirus type-1 (BoHV-1), Colorado strain, in murine embryos. The viral detection was made by n-PCR and cytopathic effect in Madin Darby Bovine Kidney (MDBK) cells. Six-to-8-week-old female mice (Swiss) were superovulated and mated with fertile males of the same strain. After 24 h, the zygotes ($n = 262$) were divided into three groups: control group submited to sequential wash (CSW), the group exposed to the virus (30 μl; title 106.5 vírus/ml) and submited to sequential wash (ESW)and the group exposed to the virus and submited to the trypsin treatment (ETT). All the groups of zygotes and the last sequential wash drops were tested by the n-PCR and inoculated in MDBK cells for cytopathic effect observation. All groups, except for the (CSW) showed positive results for the n-PCR for both zygotes and for the last drops. There was the presence of cytopathic effect in all groups except the (CSW) demonstrating the viability of the virus after treatment. These results demonstrated that trypsin treatment was not effective in BoHV-1 eliminating and/or removal. We concluded that the quality control regulations established by IETS for *in vitro* embryo production could be reviewed and possibly redefined, since there is the potential risk of pathogen transmission by biotechnologies of reproduction.

P212

Seasonal reproductive activity of damascus goats

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Goats exhibit a seasonal variation in their reproductive activity and many differences are observed in the seasonality between different breeds and locations. The onset and length of the

natural breeding season in goats is dependent on a number of factors including latitude and climate, breed, physiological stage, presence of a male, breeding system but mainly the photoperiod. The aim of this experiment was to determine the onset and length of the natural breeding period of Damascus breed goats throughout the year. Ten Damascus goats were used for this experiment. Ovarian activity was assessed from plasma progesterone profiles over a 2 years period. Goats gained puberty during the first year of the experiment. At the age of 10 months 66.6% of the goats presented ovulation. From February until April, only 11.1% continued to ovulate. Thereafter, the anoestrus season without behavioral or ovarian cyclicity took place from mid-April to late September. At the second year of the experiment, ovulatory activity started in September and reached the maximum of 100% in November. This breed demonstrated variations of sexual behavior and gonadic activity during the year, with a non-breeding season that lasts from mid-Spring to the beginning of Autumn. Determination of the natural breeding season is a valuable tool for the improvement of the reproductive capacity of goats. The onset and end of the reproductive activity of Damascus goats should be considered in natural breeding, and artificial insemination.

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P213

Melatonin reduces lipid peroxidation and maintains the mitochondrial membrane potential in Stallion spermatozoa

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Lipid peroxidation has been claimed as a major factor involved in sperm damage during storage. Since melatonin is a potent antioxidant, the aim of the present study was to investigate the effect of melatonin during *in vitro* incubation. Furthermore we investigated the presence of specific melatonin receptors (MT1 and MT2) using polyclonal antibodies. Stallion spermatozoa were incubated up to three hours at 37°C in presence of different concentrations of melatonin (0, 50 pM, 100 pM, 200 pM or 1 μM). At the beginning and at the end of the incubation period, sperm motility (using computer assisted sperm analysis), membrane integrity and permeability, fluidity of the sperm membrane, lipid peroxidation and mitochondrial membrane potential ($\Delta\psi m$) were flow cytometrically evaluated. Melatonin reduced changes in the spermatozoa related to apoptosis (increased sperm membrane permeability and lowered $\Delta\psi m$) ($p < 0.05$). Furthermore lipid peroxidation was dramatically reduced ($p < 0.01$) while no effect was observed on sperm motility or kinematics. Interestingly, melatonin helped maintain a more fluid sperm plasmalemma ($p < 0.05$). Our results clearly show the absence of MT1 and MT2 receptors in the stallion spermatozoa. It is concluded that melatonin is a useful tool to improve the quality of stored stallion sperm, increasing their life span and reducing premature aging, this likely relates to melatonin′santioxidant properties.

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P214

Does reduced glutathione improve post-thaw quality of ovine sperm?

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In sheep the percentage of unsaturated cholesterol in the sperm membrane composition is higher than in other species. This makes it more susceptible to attack by free radicals. Furthermore, cryopreservation may lead to damaged sperm due to oxidative stress. This study aimed to verify if antioxidant reduced glutathione (GSH) protects ovine cryopreserved sperm against oxidative damage. Ejaculates of four rams were cryopreserved using Tris-egg yolk extender supplemented with different concentrations of reduced glutathione (0, 1, 5 or 10 mM). After thawing, samples were evaluated for motility, vigor, acrosome and membrane integrity, mitochondrial activity and chromatin denaturation. Aliquots of each thawed sample were submitted to induce lipid peroxidation, with further measurement of thiobarbituric acid reactive substances (TBARS), an index of oxidative stress. Treatment with GSH decreased the proportion of intact acrosomes; samples treated with 5 mM had a lower percentage of intact membrane cells (9.2 ± 1.3%) when compared to control samples (15.1 ± 2.3) and those treated with 10 mM (18.3 ± 2.1). Control samples were more susceptible to chromatin denaturation when compared to the GSH treated groups (control 12.3 ± 1.54; 1 mM 9.07 ± 1.52; 5 mM 7.85 ± 1.07; 10 mM 6.64 ± 0.62). The addition of GSH offers protection to ovine sperm which is probably limited to chromatin cellular structures. Therefore, the combination of GSH with an extracellular antioxidant would provide an increased protection against damage caused by oxidative stress during cryopreservation.

P215

Effect of glutathione on ovine cryopreserved sperm

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The high susceptibility of sperm to the oxidative stress occurs especially due to high content of poly-unsaturated fatty acids (PUFAs) in its plasma membrane. The PUFAs provide the necessary fluidity to the plasma membrane. However double bonds present in those fatty acids are more susceptible to oxidative stress. Studies in human indicate that cryopreservation may lead to damages to the sperm due to oxidative stress. This study aimed to verify if the antioxidant glutathione (GSH) may protect ovine cryopreserved sperm against damages caused by oxidative stress. Semen samples of four rams were cryopreserved using Tris-egg yolk extender supplemented with different concentrations of reduced glutathione (control, 1, 5 and 10 mM). After thawing, samples were evaluated using conventional (motility and vigor) and functional tests (membrane integrity and mitochondrial activity). Aliquots of each thawed sample were submitted to protocol of induced lipid peroxidation using ascorbate (20 mM) and ferrous sulphate (4 mM), with further measurement of tiobarbituric acid reactive substances (TBARS), index of oxidative stress. No

effect of GSH was observed on variables assessed by conventional tests. GSH decreased the proportion of intact acrosomes. Samples treated with 5 mM GSH showed lower percentage of intact membrane cells when compared to control samples and those treated with 10 mM. The percentage of cells with mitochondrial activity was affected by GSH, but no effect on TBARS. Samples from control group were more susceptible to denaturation of chromatin. In conclusion, the addition of Glutathione (GSH) offers protection to DNA and mitochondrial activity of ovine sperm.

P216

Association between testicular weight and brominated flame retardants in male wild mink (Neovison vison)

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Polybrominated diphenyl ethers (BDEs), or brominated flame retardants, are widely used in both industry and consumer products. They have experimentally been shown to affect the reproductive system in mammals. The purpose of this study was to examine if the reproductive system of wild mink, a high trophic level mammal with a semi-terrestrial lifestyle, could be used for screening of effects of BDEs on wildlife reproduction. Necropsies with examinations of the reproductive organs were performed on 101 male wild mink from Sweden, caught from August to April during 2004–2009. BDEs were analyzed in subcutaneous fat using a gas chromatograph coupled to a mass spectrometer. The effect of the BDEs on selected reproductive traits was analysed in a multiple regression model, including season and age which are factors known to influence reproduction in mink. The model explained up to 76% of the variation. Mean concentration of the sum of BDE was 42.2 ng/g fat (range 2.3–390). The concentration of BDEs affected the weight of the testicles ($p = 0.02$), but not epididymal weight or anogenital distance. The weight of the testicles increased with increasing levels of BDEs. In conclusion, the concentration of BDEs in the environment in Sweden may be sufficiently high to affect the reproductive system in high trophic level wildlife.

P217

Treatment of dairy cow endometritis with different antiseptic solutions – comparison of their efficacy

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Uterine infections still present a significant problem in dairy cows. The percentage of endometritis affected cows may be over 50–90%, especially in herds with a high incidence of retained placenta. Infection can be systemic and demand the use of common systemic or local antibiotics and/or hormones. Treatment with intrauterine antiseptics is preferred as they act locally and are not excreted in the milk. This trial compared effectiveness of 0.2% chlorhexidine solution with the most frequently used uteroantiseptic Lotagen® at concentrations of 1% and 2% for endometritis treatment. Four-hundred Holstein Friesian cows aged from 2 to 8 years were used. The control group ($n = 100$) consisted of cows with no history of retained placenta and uterine infections. Treatment groups contained cows recorded with uterine infections and were treated with 0.2% chlorhexidine ($n = 100$), 1% ($n = 100$) and 2% ($n = 100$) Lotagen® solution. Pregnancy rate (77%) in cows treated with 0.2% chlorhexidine was higher than in the control group (74%) and the groups of cows treated with 1 (71%) and 2% (73%) Lotagen®. Service period in the cows treated with 0.2% chlorhexidine (149.5 ± 58.6 day) was similar to the control group (150.0 ± 45.1 day) but significantly shorter than in cows treated with 1% (175.0 ± 45.9 day) and 2% (176.0 ± 56.5 day) Lotagen® ($p < 0.01$). Insemination index in cows treated with 0.2% chlorhexidine (1.4 ± 0.9) was lower than in the control group (1.5 ± 0.8). When compared to 1% (1.9 ± 0.8) and 2% (1.9 ± 0.9) Lotagen® treated cows, significantly lower insemination indexes were determined in the control group ($p < 0.05$) and with chlorhexidine ($p < 0.01$) treated cows.

P218

Impact of milk somatic cell count on *Escherichia coli* growth *in vitro*

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Bovine mastitis is of major economic impact on global dairy industries. Overshooting *Escherichia coli* (*E. coli*) growth due to impaired neutrophil (PMN) function increases severity of acute mastitis. Besides PMN, soluble antimicrobial factors are secreted into milk during mastitis. Question of the current study was for how long and to what extent immigrating PMN and soluble factors can inhibit *E. coli* growth *in vitro*. In 14 Holstein heifers, with somatic cell counts (SCC) $< 50\,000$/ml a SCC increase ($> 2.5 \times 10^6$) was induced by infusion of 1 μg Lipopolysaccharide (LPS) per quarter. Milk was collected 0, 24, 72 and 240 h after LPS infusion and *E. coli* growth was assessed in whole milk, cell depleted skim milk and lysogeny broth (LB). Whole milk significantly inhibited *E. coli* growth 24 h after LPS infusion ($p < 0.001$). No effect could be seen in cell depleted skim milk. There was a negative correlation ($R = -0.83$, $p < 0.001$) between *E. coli* growth and SCC levels. SCC increase was mainly determined by a PMN influx (90%) as assessed by flow cytometry. In conclusion, PMN transiently act as the main inhibiting factor for *E. coli* growth in mastitis milk *in vitro*. No potential defence mechanisms by soluble antimicrobial factors could be detected after inducing a mild mastitis by intramammary LPS treatment. In summary, the data indicate that not alone the initial abundance of PMN is of key importance to mastitis pathogenesis but a fast and constant immigration to the site of infection is crucial for effective pathogen elimination.

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Comparison of ultrasonic (USG) images retrieved with two different (mechanical sector and linear) probes and macroscopic features of bovine reproductive organs: biometric studies

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In veterinary practice, ultrasonography has become an important noninvasive, painless diagnostic tool for evaluating pregnancy diagnosis and reproductive system disorders. This study was designed to compare biometric measurements of the reproductive organs using sector or linear array scanners against macroscopic measurements (post mortem) in 24 cows. Two types of scanners were used: Draminski ANIMALProfi (www.draminski.com): mechanical sector (rectal 3.5/5.0/7.0 MHz; 180°) and linear array (rectal; 7.5 MHz). Corpora lutea (CL) were imaged in 16 ovaries, follicles in 13 ovaries and cysts were found in six ovaries. Endometritis or pyometra was diagnosed in six uteri, and pregnancy (8–10 week) in one uterus. There were no significant differences between images retrieved using sector or linear transducers and macroscopic features. High correlations between post-mortem biometric measurements of examined organs and monitored in conscious animals using sector or liner probes were found: CL ($r^2 = 0.89$, $r^2 = 0.82$), follicles ($r^2 = 0.77$, $r^2 = 0.78$) and the thickness of the uterine wall ($r^2 = 0.93$, $r^2 = 0.81$; respectively, $p < 0.001$). Both, sector and linear probes have proved to be useful clinical and research tools.

P220

Introduction of Valdostana breed in Brazil: comparison in weaning body weight in pure nelore and in different F1 Nelore crossbreed and correlation with pubertal characteristics in F1 heifers

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Body weight at weaning and the following pubertal status were compared in pure Nelore (N) and in different N-F1 crossbreds, born by IATF, in two different farms located in Mato Grosso, Brazil. A total of 806 F1 calves (Valdostana-Valore®, Charolais, Red and Aberdeen Angus) were kept on Brachiaria brizantha grass pasture till weaning and only 608 received additional protein supplementation. All animals were weighed electronically at weaning (7 ± 1 month) and reproductive tracts score was performed in females at 12 months of age. The influence of the breed on the body weight at weaning was analyzed by ANOVA (SAS 9.1, 2000) taking sex and farm into account. The correlations with sexual development were analyzed with the chi-squared method. In Farm I, F1 Valore® females and males were heavier (201 and 207 kg, respectively) at weaning than F1 Red and Aberdeen Angus (186–189 and 174–181 kg $p < 0.05$), Nelore (176 $p < 0.05$ and 196 kg $p > 0.05$) and F1 Charolais (196–202 kg $p > 0.05$). About pubertal status: 55% of Valore® heifers reach puberty at 12 month in Farm I and 10% in Farm II vs. 15% of F1 Charolaise and 7.4% of F1 Red Angus (Farm I) and none in Nelore. F1 Valore® seems to have better weights at weaning and heifers present an earlier pubertal age than other the crossbreeds.

P221

Breed, parity, and cycle season effects on life-time reproductive parameters in bitches

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Breeding and pregnancy statuses as well as proestrus, estrus, and pregnancy durations and seasons, the number of puppy, and cycle interval were complied from German Shepherds (GS, n = 34), Labrador (LD, n = 23), Belgium Malinoa (BM, n = 13) and Pointers (PO, n = 9) during 10 parities to evaluate the effect of breed, parity, and cycle season on reproductive parameters. Data were subjected to the FREQ, CORR, and MIXED Procedures. Parity was negatively correlated with cycle interval ($r = -0.18$) and proestrus duration ($r = -0.09$) as well as the number of puppy ($r = -0.20$), but not correlated with estrus and pregnancy durations. The mean puberty age did not differ by the breed (464.8 ± 26.2 days, $p < 0.30$). Breeding rate was affected by the breed (83.2, 60.1, 71.8, and 72.1% for GS, LD, BM, and PO, respectively, $p < 0.0001$). However, pregnancy rate did not differ across the breeds (74.6%, $p < 0.12$). Pregnancy duration was affected by the breed (63.7, 63.5, 62.2, and 65.2 days for G, L, B, and P, respectively, $p < 0.02$). As the age advanced, pregnancy duration did not change ($p < 0.33$), but the number of puppy decreased linearly from 6.9 at the 1st parity to 5.6 at the 10th parity ($p < 0.03$). The cycle interval varied by the breed (208.2, 215.1, 208.6, and 237.0 days for GS, LD, BM, and PO, respectively, $p < 0.01$), which also decreased linearly from 241.1 to 202.0 as parity increased from 1 to 10 ($p < 0.04$). Proestrus duration was not affected by the breed (8.8 ± 0.3 days, $p < 0.20$). However, estrus duration varied by the breed (9.5, 8.5, 8.0, and 8.2 days for GS, LD, BM, and PO, respectively, $p < 0.001$). In conclusion, at latitude of Turkey reproductive parameters do not change among GS, LD, BM, and PO bitches.

P222

Ovarian real-time blood flow changes in rabbits during PGF2α-induced luteolysis

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The dynamic change of ovarian blood flow was monitored by power Doppler ultrasonography in both oestrous and pseudopregnant rabbits before and after treatments with a luteolytic dose of PGF2α. Pseudopregnancy was induced by 20 IU eCG followed 2 days later by 0.8 μg GnRH (day 0). In oestrous rabbits, PGF2α challenge caused a transitory reduction ($p < 0.05$) of ovarian blood flow, evaluated with the number of pixel recorded, within 20 min. By 50 min after PGF2α, the blood flow returned to basal values, but thereafter it gradually increased to almost two-fold ($p < 0.01$) 90 min

later. At either days 4 or 9 of pseudopregnancy, the ovarian blood flow was two- to three-fold ($p < 0.01$) greater than that found in oestrous does. After PGF2α administration, the haemodynamic changes in blood flow were similar to those observed in oestrous rabbits, but more relevant, being characterized by a transitory, four-fold decrease ($p < 0.01$) at 40 min followed by a rebound peak 90 min later and then by a gradual decrease to approximately 50% of the basal values 12 and 24 h later. Plasma progesterone concentrations declined ($p < 0.01$) within 6 h after PGF2α, only in day 9 pseudopregnant rabbits. These results confirm that the local vascular mechanisms controlling ovarian blood flow are not acutely responsible of the luteolytic effects of PGF2α causing progesterone decline.

P223

Experimental testing of laparoscopic intrauterine insemination in fallow deer (Dama dama) with frozen semen

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The aim of the study was to evaluate the use of laparoscopic intrauterine insemination (LII) in fallow deer females using deep frozen fallow deer semen. Twelve fallow deer females were synchronized during the breeding period using CIDR and i.m. administration of FSH. The LII was performed by deposition of 0.03 ml of thawed fallow deer semen (20×10^6 motile spermatozoa) into the middle of each uterine horn using laparoscope and inseminating pipette, fitted with a 25-gauge needle either 42 or 50 h after CIDR removal. Ovulation was monitored by ultrasonography with an abdominal linear probe as well as by laparoscopic examination. Embryos were recovered at blastocyst stage retrogradually using a Folley catheter. The highest ovulation rates were detected between 60 and 64 h after the CIDR removal. Recovery of embryos was 28% and 37%, fertilization 42% and 60%, unfertilized oocytes 58% and 40%, intact-transferable embryos 27.6% and 33.3% and damaged embryos 72.4% and 66.7% after insemination 42 and 50 h after CIDR removal, respectively. The results showed that the middle of the uterine horn is an acceptable deposition site for the insemination dose (ID). To inseminate 50 h after the CIDR removal seems to be better than 42 h if using LII with frozen-thawed semen. The volume of ID and number of progressively motile sperm cells per ID were sufficient. Therefore, LII can be used successfully in fallow deer and may be implemented to use in controlled reproduction.

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P224

Annexin V binding assay can predict the quality of dog cryopreserved spermatozoa

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Dog spermatozoa are characterized by marked interindividual differences in resistance to freezing. Discovery of a method showing whether the sperm can sustain cryopreservation would help development of novel preservation procedures. The aim of this study was to investigate whether it is possible to predict success rates of cryopreservation on the basis of fresh semen examination. Thirty-nine ejaculates from dogs of different breeds, age and fertilizing potential were evaluated. Semen volume and sperm concentration were assessed, total and progressive sperm motility, viability, acrosomal integrity and detailed morphology analysis was performed. The proportion of sperm with signs of early membrane alterations was estimated using Annexin-V binding. Ejaculates were frozen in Tris-fructose-egg yolk extender. Sperm survival rate was calculated as a sum of averages of respective parameter, i.e. total and progressive motility, viability and acrosomal integrity at 0 and 2 h after thawing, divided by total and progressive motility, viability and acrosomal integrity of fresh sperm × 100. Ejaculates were divided into two groups according to the survival rates after cryopreservation. It was confirmed that the percentage of spermatozoa showing early membrane alterations was higher in the group of ejaculates with a higher survival rate ($p < 0.05$). Sperm survival rates were correlated with percentages of Annexin V positive spermatozoa (−0.577; $p < 0.001$). No relationship between other fresh semen parameters and sperm ability to survive cryopreservation was found.

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P225

Influence of rapid BCS change on fertility rate in Lipizzan Mares

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The aim of the study is to determine the potential influence of rapid BCS change on fertility in Lipizzan mares. A group of Lipizzan mares (n = 32) ey were extremely thin (BCS = 0) and during the a period of starvation had even eaten each other's tails. Their BCS improved quickly after re-alimentation and in Spring started cycling (BCS = 3 in average). Since all were mature pluriparous broadmares (aged 8–19 years) they were all covered. In this study we compared reproductive data from that first re-fed season (spring 2008) with similar group of Lipizzan mares (n = 48) from another studfarm using the same stallions (n = 6). All mares in the other studfarm had BCS around 3 and were also pluriparous aged 8–19 years. They did not experience any significant change of BCS during the trial. We also compared reproductive data from the next year (2009) for both studfarms. There was a significant difference in fertility rate for the first year only. Mares with rapid improvement of BCS had significantly higher fertility rate (according to pregnancy checking and foaling data) compared with the other studfarm (98% vs. 82%, $p < 0.01$), Furthermore, the number of services per cycle and number of cycles per conception were significantly decreased for the group with rapid BSC change (1.3 and 1.2 vs. 2.1 and 2.3). Data for the following year were similar in both stud farms. Clearly rapid and positive BCS change influences fertility rate in Lipizzan horses, but further research is needed to determine the exact mechanism involved.

P226

Pro/acrosin activity analysis along porcine epididymis

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Acrosin is an acrosomal serine proteinase present in spermatid cells and in epididymal and ejaculated spermatozoa as an inactive zymogen proacrosin. It is activated during sperm capacitation being involved in sperm penetration through the zona pellucida. The aim of this experiment was to analyze pro/acrosin activity during epididymal sperm maturation. Undiluted epididymal samples were obtained by cannulation of four epididymal regions of adult fertile boars (proximal and distal caput, corpus and cauda) and washed with PBS at $600 \times g$ for 10 min. Acrosin activity of epididymal fluid was determined spectrophotometrically after incubation with a detergent-substrate solution mixture. Results showed that acrosin activity (μUI acrosin/10^6 spz) of epididymal spermatozoa from proximal (403.7 ± 53.37) and distal (438.5 ± 3.15) caput, corpus (336.8 ± 142.26) and distal cauda (438.38 ± 11.9) did not differ significantly ($p \geq 0.05$). However, previous results demonstrated a change in the isoform content during epididymal maturation. In conclusion, these results demonstrated, for the first time, that acrosin activity is constant throughout the epididymis, despite the variations in acrosin expression along the epididymis.

P227

Characteristics of estrous cycle in "Asinina de Miranda" Donkeys

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Six healthy jennets of "Asinina de Miranda" breed with ages 3.5–9 years old were used to typify the estrous cycle and ovarian events for the breed. The study followed 18 complete cycles in the breeding season, from June to October of 2009 and April to May of 2010, with the animals l on the north of Portugal (41°17′N). The animals were teased daily and examined by ultrasound with a 5 MHz linear probe (Shnezhen Veterinary US scanner), at every other day if in diestrus and every 12 h in estrus. The results presented herein are expressed as mean ± SEM. The length of the estrous cycle was 24.38 ± 0.78 days, with diestrus lasting for 18.20 ± 0.69 days and the estrus lasting for 5.5 ± 0.44 days. The incidence of single ovulation was 83.3%, whilst double ovulation occurred in only 16.7% of the cycles. No differences were found between single and double ovulatory cycles on duration of the aforementioned parameters. Ovulations from the right ovary were more frequent: 65% vs. 35% from the left ovary. In single ovulations, the diameter of the dominant follicle changed from 30.164 ± 1.32 mm at the beginning of estrus to 40.19 ± 1.28 mm before ovulation. For double ovulations, the size of dominants follicles ranged from 21.50 ± 1.14 mm to 34.18 ± 1.59 mm, respectively at the beginning of estrus and before ovulation. The differences observed between single and double ovulations in follicular size at the beginning of estrous and before ovulation were of statistic significance ($p = 0.001$). Comparing to the reported to other donkey breeds, the jennets in this study showed similar average length of the estrous cycle although with slightly smaller follicular size at ovulation.

P228

Equine endometritis bacteria and neutrophil extracellular traps

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Even thought *Streptococcus zooepidemicus* (Szoo) is the main pathologic agent responsible for mare endometritis, others may be involved. Neutrophils (PMN) when in contact with many pathogenic bacteria have the ability to induce extracellular traps (NET) that bind and kill pathogens extracellularly at infection site. Phorbol-myristate-acetate (PMA) is a strong NET inducer while Cytochalasin C (Cyt) is a phagocytosis inhibitor. The aim of this study was to evaluate the *in vitro* capacity of equine PMN to develop NET when stimulated with Szoo, *Escherichia coli* and *Staphylococcus capitis* (Scap) strains obtained from mare endometritis. Equine blood PMN were isolated by Ficoll gradient, plated (2×10^6/ml) and incubated for 1 h. Whenever stimulated, PMN were incubated with 25 or 100 nM PMA for 30 min. Further incubations (1 h for bacteria assays or 1, 2 and 3 h for PMA) were performed, with Cyt (10 μg/ml) or without it, as follows: Control group (PMN); PMN + PMA; PMN + bacteria (2×10^7/ml); PMN + bacteria + PMA. An increase in NET was seen after 3 h incubation of PMN + PMA ($p < 0.05$), regardless PMA concentration. PMA at 100 nM concentration seemed to be cytotoxic. After 1 h incubation, bacteria alone were unable to induce NET. However, Scap + 25 nM PMA and Scap + PMA + Cyt increased PMN NET, with Cyt inhibiting phagocytosis ($p < 0.05$). Stimulated equine PMN have NET formation capacity that might be a mechanism to fight some bacteria endometritis.

P229

Progesterone concentration levels in blood serum of dairy cows with physiological and pathological development of puerperium

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The aim of this study was to find the average maximum progesterone value (P4) in blood serum during the first oestrus of dairy cows related to physiological and pathological development of puerperium. Slovak spotted breed dairy cows ($n = 30$) with milk production more than 7000 kg/years were used and received all the same quality and quantity of nutrition. Blood samples for analysis of P4 were collected every second day from first day after calving. Group A contained cows ($n = 12$) with physiological course of post-partum (PP) period, group B contained cows ($n = 10$) with primary endometritis and group C contained cows ($n = 8$) with retained placenta. The cows of group B and C were treated with antibiotics and uterotonics. Evaluation of the concentrations of P4 in dairy cows during puerperium showed that P4 was low in all cows during the first 3 weeks. In group A

timing of the first progesterone rise was determined on the day 28–30 from 2.92 ± 2.54 nM to 16.28 ± 3.12 nM ($p < 0.01$). The level of P4 was on the day 40 PP 1.57 ± 0.25 nM. In this group a corpus luteum on the ovary was visualized by ultrasonography on the day 20–22 PP. In the group B, was detected timing of the first progesterone rise on the day 34 PP from 3.72 ± 0.32 nM to 17.2 ± 2.89 nM ($p < 0.01$), until the day 40 PP. In the group C, was found timing of the first progesterone rise on the day 36 PP from 5.27 ± 1.6 nM to 15.32 ± 1.72 nM ($p < 0.01$). In both groups (B + C), was presented of corpus luteum on the ovary by ultrasonography on the day 25–28 PP.

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P230

Abnormal protamination in nelore bull semen with proximal cytoplasmic droplets

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The objective of this study was to evaluate DNA protamination on Nelore bull semen with proximal cytoplasmic droplets (PCD) and the effects on semen quality (cytoplasmic membrane, acrosome, mitochondrial function, DNA integrity). Frozen-thawed samples from three ejaculates of eight adult (6.9 ± 2.4 years) bulls with high numbers of PCD (group A; 24.3 ± 10.3%) and ten adult (6.8 ± 2.3 years) bulls with normal values (group B; 0.5 ± 0.3%) were evaluated for sperm concentration, motility, morphology, simultaneous evaluation of acrosome, membrane and mitochondrial potential (FITC-PNA, PI, JC-1) and chromatin integrity (acridine orange – AO). Protamination was evaluated by chromomycin A3 (CMA3) staining using acoustic focusing flow cytometry (Attune™). A total of 10 000 events was accumulated for each measurement. The results showed that the high incidence of PCD affected membrane integrity, acrosome status and mitochondrial function when compared to group B. Nevertheless, group A did not differ from group B concerning concentration (A: 124.8 ± 25.2; B: 116.8 ± 26.4 sptz/ml), motility and DNA integrity on AO test. Group A showed higher levels of protamine deficiency (A: 1.4 ± 0.61; B: 0.6 ± 0.2%) ($p > 0.01$). Further research is essential on the pathway of spermatogenesis and its relationship with morphological defects and DNA packing.

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P231

Effect of omega 3 fatty acids dietary supplementation on fresh and frozen-thawed dog spermatozoa

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A positive effect of dietary supplementation with n:3 polyunsaturated fatty acids such as docosahexanoic acid (DHA) on quality of fresh and cryopreserved semen has been observed in several species. The aim of this work was to evaluate the effect of feeding omega-3 supplemented diets on the quality of fresh and cryopreserved dog spermatozoa. Three mature dogs were fed during 3 months with a commercial dry food (without supplementation group). Ejaculates from dogs were pooled (four replicates) and frozen using the Uppsala system. Percentages of total sperm motility (TSM) and progressive motility (PM) (computer-assisted analysis), and sperm membrane integrity (SMI), evaluated using SYBR-14 and PI, were assessed in fresh and thawed samples (at 30 and 150 min incubation at 37°C) in order to obtain control values of sperm quality. After that period, the same animals received oral supplementation with 20 mg/kg per day DHA and 22 mg/kg per day EPA during 5 months (Omega group) and ejaculates (four replicates) were processed as described above. Values of Mean ± SEM of each group was compared. In fresh semen percentages of TSM and PM were higher ($p < 0.05$) after omega-3 supplementation. Values of TSM and PM were also higher in the frozen-thawed semen in the omega group at 30 and 150 min of incubation. Values of SMI were not influenced by diet supplementation. In conclusion, diet supplementation with omega-3 improve sperm quality in fresh and frozen-thawed canine semen.

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P232

Localization of glycoconjugates in the inner perivitelline layer of japanese quail (Coturnix Japonica)

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In birds, the inner perivitelline layer (IPVL), the homologue of the mammalian zona pellucida, is relatively thin (1–2 μm). It surrounds the polylecithal oocyte after ovulation and plays an important role in the species specific binding of spermatozoa to the egg and in the induction of the acrosome reaction. Whereas the protein components of the IPVL have been well characterized during the last few years, little information of the oligosaccharides of these glycoproteins, which are involved in sperm binding to the IPVL, is available. Therefore, a broad panel of histochemical lectin binding assays together with enzyme elimination and saponification was used to characterize the carbohydrate components of the IPVL during follicular development in the quail ovary. Since the IPVL is not yet developed in primordial follicles, no glycostaining could be detected at this follicular stage. Further, no differences in glycostaining were found in the IPVL of growing previtelline and vitelline follicles. By "glycan mapping", which has been done for the first time in avian IPVL, the sugar residues α-D-Man, β-D-Gal-(1-3)-D-GalNAc, β-D-Gal, α-D-GalNAc + α-DGal, (D-GlcNAc)2NeuNAc, and NeuNAc could be identified. In conclusion, our results support the data of previous studies that N-glycans instead of O-glycans are involved in sperm binding to the IPVL of quail.

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Glucose and fructose as functional modulators of overall dog sperm function

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The main aim of the present work was to test glucose and fructose effects on the phosphorylation levels of proteins

linked to the control of sperm function in two species with different metabolism, dog and boar. Incubation of dog spermatozoa with 10 mM glucose increased serine phosphorylation of cell cycle and signal transduction proteins like cyclins B and E, Cdk2, Cdk6, Cdc6, PYK2, c-kit, Raf-1, TRK and protein phosphatases. Incubation with 10 mM fructose decreased serine phosphorylation levels of cyclins B and D3, Cdk1/Cdc2, Cdk2, Cdk6, Akt, PI3 kinase, ERK1 and PKC. Incubation of boar spermatozoa with glucose or fructose did not have any effect. Given that one important difference between dog and boar spermatozoa is the presence of glucokinase (GK) in dog, GK-transfected COS7 cells were incubated with either 10 mM glucose or 10 mM fructose. Incubation of GK-transfected cells (GKC), but not control cells (CC), with fructose decreased serine phosphorylation of cyclin A (60.1 ± 1.3 arbitrary units [AU] in GKC vs. 100.0 ± 0.8 AU in CC), ERK-2 (78.5 ± 1.6 AU in GKC vs. 100.0 ± 1.6 AU in CC) and Hsp-70 (70.7 ± 1.6 AU in GKC vs. 100.0 ± 3.1 AU in CC). Our results indicate that monosaccharides are signalling compounds in dog spermatozoa after ejaculation through changes in the phosphorylation levels of specific proteins. One of the implied factors is the equilibrium of the total sperm hexokinase activity, in which the presence or absence of GK is relevant.

P234

Preliminary study on the role of α-L-fucosidase on porcine *In vitro* fertilization

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Activity of α-L-fucosidase has been described in the porcine oviduct at the time of ovulation and there is evidence for a fucose-binding protein in boar spermatozoa. We have also shown that α-L-fucosidase inhibitors increase monospermy during porcine *in vitro* fertilization (IVF). The aim of this study was to determine the effect of α-L-fucosidase on porcine *in vitro* fertilization. *In vitro* matured porcine oocytes were incubated for 60 (E1 group, n = 122) or 120 (E2 group, n = 113) min in Talp medium with α-L-fucosidase (0.169 IU) or without α-L-fucosidase (control group, n = 140) and were inseminated with 1×10^5 sperm/ml. Gametes were cocultured for 15 min and oocytes were then transferred to fresh Talp medium (without enzyme), for an additional 16 h. Putative zygotes were fixed and stained to evaluate mean number of sperm bound to ZP (SPZ-ZP), penetration rate (PEN), mean number of sperm per penetrated oocyte (S/O) and monospermy rate (MON). Four replicates were run. The data were analysed by ANOVA ($p < 0.05$). Results showed significant differences for SPZ-ZP data, being 4.9 ± 0.5a, 15.4 ± 1.3b and 11.71 ± 0.93b for control, E1 and E2 groups, respectively; for PEN data, being 69 ± 4a, 93 ± 2b and 89 ± 3b for control, E1 and E2 groups, respectively; for S/O data, being 3.4 ± 0.3a, 7.1 ± 0.6b and 4.0 ± 0.3a for the same groups and for MON data, being 28.9 ± 4.6a, 15.0 ± 3.3b and 10.0 ± 3b, again for the same groups. In conclusion α-L-fucosidase enhances the sperm entry into the oocyte. Further studies are necessary to determine the effect of this enzyme during IVF and on spermatozoa in order to improve porcine IVF.

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P235

Addition of α-L-fucosidase to the porcine *in vitro* fertilization medium increases penetration rates

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Activity for α-L-fucosidase has been described in the porcine oviduct at the time of ovulation and there is evidence for a fucose-binding protein in boar spermatozoa. We have also showed that α-L-fucosidase inhibitors increase monospermy during porcine *in vitro* fertilization (IVF). The aim of this study was to determine the effect of direct addition of α-L-fucosidase to the porcine IVF medium. *In vitro* matured porcine oocytes were incubated for 60 (E1 group) or 120 (E2 group) min in TALP medium with α-L-fucosidase (0.169 IU) or without α-L-fucosidase (control group) and were inseminated with 1×105 sperm/ml. Gametes were cocultured for 15 min and oocytes were then transferred to fresh TALP medium (without enzyme) for additional 16 h. Putative zygotes were fixed and stained to evaluate mean number of sperm bound to ZP (SPZ-ZP), penetration rate (PEN), mean number of sperm per penetrated oocyte(S/O) and monospermy rate (MON). The data were analysed by ANOVA ($p < 0.05$). Results showed significant differences for SPZ-ZP data, being 4.89 ± 0.49a, 15.44 ± 1.29b and 11.71 ± 0.93b for control, E1 and E2 groups, respectively; for PEN data, being 69.29 ± 3.9a, 92.62 ± 2.3b and 88.50 ± 3b for control, E1 and E2 groups, respectively; for S/O data, being 3.36 ± 0.29a, 7.07 ± 0.6b and 4.04 ± 0.26a for the same groups and for MON data, being 28.87 ± 4.6a, 15.04 ± 3.3b and 10.00 ± 3b, again for the same groups. In conclusion α-L-fucosidase enhances the sperm entry into the oocyte. This study confirms the role played by α-L-fucosidase on porcine sperm-oocyte interaction.

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P236

Uterine response and fertility after artificial insemination with frozen-thawed donkey spermatozoa

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Ejaculates of two donkeys were split and frozen in an INRA96® – 2% egg yolk extender with the addition of either 2.2% glycerol (GLY) or 1.4% ethylene glycol (EG). Fertility and post-insemination endometritis were evaluated after artificial insemination (AI) with frozen-thawed semen further diluted either with INRA96® (Groups GLY-INRA and EG-INRA, eight cycles each) or with seminal plasma (Group GLY-SPL, eight cycles). Jennies, aged 8.0 ± 4.5 years, were inseminated twice in each cycle, 20 h apart, with 500×10^6 spermatozoa (250×10^6 from each donkey), at fixed times after induction of ovulation. Uterus was flushed 6 and 10 h after first and second AI, respectively. Cells in the recovered fluid were counted with a Thoma chamber and distinguished as polymorphonuclear (PMN) or other cells in Diff-quik®-stained smears. Pregnancies were diagnosed by ultrasound examinations at 14 and 16 days. Pregnancy rate was 2/8 (25%) in both GLY-INRA and GE-INRA, while it was 5/8 (62.5%) in GLY-SPL, including a twin pregnancy.

PMN concentration was higher after the first AI (median: 341×10^3/ml), compared to the second (median: 128×10^3/ml), and in pregnant jennies (median: 523×10^3/ml), compared to the non-pregnant (median: 199×10^3/ml), while there was no statistical difference between treatments. These results indicate that it is possible to obtain pregnancies both using GLY or EG as a cryoprotectant for donkey semen. The effect of the post-thaw dilution in seminal plasma on fertility needs to be investigated on a larger number of cycles.
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P237

Effect of energy source on *in vitro* embryo development and freezability in cattle

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Most media employed for producing bovine embryos *in vitro* include glucose as an energy source despite putative toxic effects. The aim of this work was to evaluate whether replacing glucose with myo-inositol and citrate during IVC improves *in vitro* embryo development and resistance to cryopreservation. Abattoir-derived oocytes were matured and fertilized *in vitro* using standard procedures. After 20–22 h of gametes co-incubation, zygotes were denuded and cultured in SOF containing either 1.5 mM glucose (n = 604) or 2.77 mM myo-inositol and 0.34 mM citrate (n = 575) for 7 days. Embryos were first incubated in 7.5% EG and 7.5% dimethyl sulfoxide (DMSO) for 3 min, then transferred into 16.5% EG and 16.5% DMSO and 0.5 M sucrose for 25 s before being loaded into the cryotop. Warming was carried out by immerging the cryotop into a 0.25 M sucrose solution and by transferring the embryos into a 0.15 M sucrose for 5 min. Vitrified-warmed embryos were then cultured *in vitro* for further 24 h, after which the embryo survival rate was recorded. Data were analyzed by Chi-Square test. The results of this study showed that myo-inositol-citrate increased blastocyst yield (37.4 vs. 29.5%, respectively; $p < 0.01$). However, blastocysts produced in the medium containing myo-inositol and citrate had a lower survival rate after vitrification-warming than those cultured with glucose (60.4% and 73.6%, respectively; $p < 0.05$). In conclusion, replacement of glucose with myo-inositol and citrate during culture increases blastocyst production without improving embryo quality, i.e. resistance to cryopreservation.

P238

In vitro development of nuclear-transferred pig embryos following use of trichostatin a for epigenetic transformation of both recipient oocytes and nuclear donor somatic cells

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The purpose was to determine the effect of trichostatin A (TSA: non-specific inhibitor of histone deacetylases), applied for epigenomic modulation of not only *ex vivo*-maturing nuclear recipient oocytes, but also cultured foetal fibroblasts on the development of porcine cloned embryos. Cumulus-oocyte complexes (COCs) were matured *in vitro* for 20 h in TC 199 medium supplemented with 5 mIU/ml pFSH, 0.1 IU/ml hMG, 10% FBS, 10% pFF, 5 ng/ml rh-bFGF and 0.6 mM L-cysteine. Subsequently, the COCs were incubated for 22–24 h in the same medium enriched with 80 nM TSA. Before use in the somatic cell nuclear transfer (SCNT), fibroblast cells were simultaneously serum-starved and treated with 50 nM TSA for 24 h. SCNT-derived oocytes were electroactivated, and then cultured up to morula and blastocyst stages (four replicates in total). Among cultured embryos reconstructed with nuclear recipient and donor cells, each of which had been exposed to TSA, the frequencies of uncleaved embryos, dividing embryos (between 2- and 16-cell stages), morulae and blastocysts yielded 9/145 (6.2%), 42/145 (29.0%), 38/145 (26.2%) and 56/145 (38.6%), respectively. In control (TSA-untreated) group, these rates were 29/132 (22.0%), 40/132 (30.3%), 28/132 (21.2%) and 35/132 (26.5%), respectively. In conclusion, increased capability of cloned pig embryos to reach the morula and blastocyst stages appears to result from enhanced efficiency of transcriptional reprogramming for TSA-treated fibroblast cell nuclei in an epigenomically-matured cytoplasm of recipient oocytes also undergoing exposure to TSA.

P239

Cyclic changes in MUC1 localization in the canine endometrium

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MUC1, a polymorphic glycoprotein expressed in the epithelia of diverse organs, including the uterus, has been found in the endometrial glandular and luminal epithelium. In the uterus, mucins form a natural barrier that contributes to local protection against external threats In the uterus, MUC1 activity is controlled mainly by progesterone. In this study, we sought to localize MUC1 in the canine endometrium and to investigate possible changes in the immunostaining intensity during dog estrous cycle. Formaline fixed, samples from canine endometrium (n = 43) were used for immunohistochemistry analysis using a streptavidin-biotin-peroxidase method. The primary antibody (clone MH1 – CT2, AbCam) was used at 1:200. Scoring intensity (weak, moderate or strong) was obtained for each epithelial structure. Epithelial MUC1 immunostaining was observed at all stages of the canine estrous cycle. Regardless of the cycle stage, stronger MUC1 positivity was found in the superficial epithelium (SE) in comparison to the glandular epithelia ($p < 0.001$; Fisher = 36.56), which didn't differ between superficial and deep glands. Moreover, overall highest intensity of immunolabelling was recorded during anestrus, proestrus and estrus ($p < 0.001$; Fisher = 36.26), in particular in the SE, whilst the lowest intensities of immunostaining were found in diestrus. No differences were found between intensity immunoscoring in early and full diestrus. Our results shows that MUC1 location in the canine uterus is similar to the reported in humans for follicular and luteal stages, and also that a decrease in MUC1 content occurs during progesterone-associated stages.

P240

Estrus synchronization during transition period, timed artificial insemination (TAI) and the effect of GnRH administration at the TAI on fertility in lactating goats

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The study was carried out to determine the efficacy of synchronization of estrus with vaginal sponges for 6 (Short Term, ST) or 12 (Long Term, LT) days, TAI 48 h after sponge withdrawal in combination with GnRH administration at TAI on the fertility of lactating goats during the transition period. Research was conducted on 104 goats (2–5 years old). The goats received vaginal sponges containing 30 mg fluorogestone acetate. Additionally, 400 IU PMSG and 0.075 mg cloprostenol were administered at the time of sponge withdrawal. The goats were randomly assigned to ST (n = 52) and LT (n = 52) treatment with vaginal sponges. Two teaser bucks were introduced for estrus detection. Goats were inseminated intracervically with cooled semen (1×10^8 motile cells/ml) 48 h after sponge withdrawal. Both ST and LT groups were divided into two groups as ST1 (n = 24) and ST2 (n = 24), LT1 (n = 22), LT2 (n = 23). ST1 and LT1 groups were left as control, ST2 and LT2 groups received 5 μg buserelin acetate at TAI. The mean interval from sponge removal and the onset of estrus and estrus rates for ST and LT groups were 36.0 ± 1.7 and 38.8 ± 1.1 h and 79.1% and 86.6%, respectively ($p > 0.05$). Pregnancy and twinning rates of the ST1, ST2, LT1 and LT2 groups were 37.5%, 41.6%, 40.9%, 47.8% and 22%, 30%, 11%, 18%, respectively. It was concluded that the TAI could be established by ST and LT sponges applications. Although the pregnancy and twinning rates of the GnRH groups were numerically higher than the others, the difference among the groups was statistically insignificant ($p > 0.05$).

P241

The antioxidative effects of cysteamine, hyaluronan and fetuin on post-thaw semen parameters of Brown-Swiss bulls

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The aim of this study was to compare the effectiveness of different antioxidants (cysteamine, hyaluronan and fetuin) to freeze bull semen. Ejaculates from Brown-Swiss (n = 36) were diluted in seven aliquots with Tris-based extender containing cysteamine (2.5, 7.5 mM), hyaluronan (0.5, 1 mg/ml) and fetuin (5, 10 mg/ml), and an extender containing no antioxidants (control) 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 means of CASA), acrosomal and total abnormalities (evaluated by means of Hancock solution under phase-contrast microscopy) and plasma membrane integrity (evaluated by means of HOST). The use of a Tris based extender supplemented with 2.5 mM cysteamine (55.3 ± 2.2%) and 10 mg/ml fetuin (52.6 ± 2.9%) led to an increase in postthaw motility and significant decreases in acrosomal (4.9 ± 0.3% and 4.3 ± 0.4% respectively) and total abnormalities (13.0 ± 0.7% and 11.7 ± 0.6% respectively) in comparison to other groups ($p < 0.001$). The postthaw progressive motility was significantly better for semen parts diluted in hyaluronan 1 mg/ml and cysteamine 2.5, 7.5 mM compared to other groups. For average path velocity (100.2 ± 6.5 μm/s), curvilinear velocity (160.7 ± 15.4 μm/s) and amplitude of lateral head displacement (6.3 ± 0.5 μm), the highest values were obtained from hyaluronan 1 mg/ml ($p < 0.05$). Except 5 mM fetuin, all treatments significantly increased the HOST (56.4 ± 1.4%) results as compared to the control group ($p < 0.001$). Supplementation with these antioxidants prior to the cryopreservation process protected sperm motility against the cryodamage. Furthermore, future research should focus on the molecular mechanisms of the antioxidative effects of the antioxidants cysteamine, hyaluronan and fetuine during cryopreservation.

P242

Effects of semen extender enriched with vitamin E in chilled canine epididymal spermatozoa

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The aim of the present study was to investigate the protective effects of vitamin E in canine epididymal spermatozoa after 40 h of chilling. Eight experimental units, each consisting of a pool of epididymal spermatozoa from three healthy dogs (total of 24 animals) were analyzed. After orchiectomy, recovered epididymal spermatozoa were pooled and separated in four samples, two were incubated with Tris egg yolk extender (control-CE), while the others were submitted to a Tris egg yolk extender enriched with 0.25 mM/ml of vitamin E (antioxidant-AE). One sample of each extender was immediately evaluated (fresh) while the other was evaluated 40 h after chilling in a cool storage container (Botutainer®). Total motility, vigor, hyposmotic and thermal resistance tests and free radicals quantification were performed in all samples. The results were analyzed by Tukey test, with significance level 5%. In fresh samples, the control group presented motility, vigor and hyposmotic test values of 78, 4, and 71%, respectively. Thus, the enrichment with vitamin E did not affect sperm parameters ($p > 0.05$). In chilled samples enriched with vitamin E, motility (21%), vigor (16%) and hyposmotic test (17%) increased significantly ($p < 0.05$), compared to the control group that presented values of 43% of motility, 2.8 of vigor, and 51% in the hyposmotic test. In conclusion, the extender containing 0.25 mM/ml of vitamin E improved physical characteristics of canine epydidimal spermatozoa after 40 h of chilling.

P243

Influence of isolated bacteria from the bovine uterus on endometrial epithelial cells

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A variety of pathogenic and commensal bacteria are found in the bovine uterus during the puerperium. It is hypothesized

that parts of the bacterial microbiota influence reproductive function. The aim of the study was to isolate pathogenic and commensal bacterial strains from the bovine endometrium. The influence of the isolated bacteria on endometrial cells was evaluated. Samples for bacterial analysis were collected from healthy bovine endometrium postpartum using a cytobrush. Collected samples were used for enrichment and cultivation on selective media. Bacterial colonies of interest were established in pure culture. Isolated bacterial DNA was subjected to PCR using 16S rDNA primers. Resulting amplicons were sequenced. This revealed the presence of Weissella spp., Bacillus spp. and Staphylococcus spp. in the bovine uterus. Bovine endometrial epithelial cells were co-cultured with the isolated bacterial strains in different multiplicities of infection (1, 5 and 10). Endometrial cells cultured without bacteria served as controls. Weissella sp. did not influence viability, monitored by trypan blue staining, and proliferation of endometrial cells. Staphylococcus sp. and Bacillus sp. induced cell death within 3 days. 2, 4 and 6 h after begin of co-cultivation, total RNA was extracted from treated cells and subjected to real-time RT-PCR. All strains induced mRNA expression of inflammatory factors, e.g. Toll-like receptor 6, interleukin 8 and cyclooxygenase 2 with individual differences in endometrial cells compared to controls. These results suggest that the different bacteria interacted with endometrial cells, but showed different influence on the viability.
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P244

Canine semen quality, and ions, albumin and cholesterol in seminal plasma

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The aim was to measure Ca, Na, K, P, Cu, Zn, albumin and cholesterol in seminal plasma of canine ejaculates, since abberations these parameters can decrease sperm motility or cause early capacitation, increased lipid peroxidation and membrane damages. Albumin for instance is essential for cholesterol efflux, a prerequisite for cell adaptability. Semen was collected twice within 1 week from 13 male dogs of different breeds with proven fertility. Sperm motility of the main fraction was measured by computer assisted sperm analysis. Samples were centrifuged ($700 \times g$, 5 min), and the seminal plasma frozen-stored until analyses. Concentrations of ions, albumin and cholesterol were measured by an autoanalyzer for clinical chemistry and flame atomic absorption spectroscopy. Collection of semen twice in 1 week did not cause significant changes in any parameter (n.s.). All concentrations were normal according to literature (x ± SD; Ca 1 ± 4 mM, Na 173 ± 199 mM, K 10.5 ± 3.3 mM, P 0.8 ± 0.5 mM, Cu 6.1 ± 6.4 mg/l, Zn 77.9–71.9 mg/l, albumin 0.26–0.11 g/dl, cholesterol 12.2–29.5 mg/dl). Concentrations of K ($p < 0.01$), P ($p < 0.01$) and Zn ($p < 0.01$) were positively correlated with sperm concentration which varied from 22.9 to 1300×10^6 cells/ml; K ($p < 0.01$), Zn ($p < 0.01$) and Cu ($p < 0.01$) correlated positively with Albumin (Pearson's rank correlation). We conclude that the here measured variations in concentrations did not influence motility.

P245

Calving season influences on energy metabolites trait, body condition scoring and ovarian resumption in high producing dairy cows

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Relation between calving season and metabolic traits, BCS, and ovarian resumption in dairy cows were studied in Holstein cows (n = 45; parity 1–4; Age = 4.4 ± 1.0 years; 11329 ± 1429 kg/305 days milk). Cows were allocated into summer (SG; n = 10), autumn (AG; n = 12), winter (WG; n = 12) and spring (SPG; n = 11) groups. Examination of ovulation and corpus luteum formation as well as blood metabolites [β-hydroxyl butyric acid (BHBA), non estrified fatty acid (NEFA), total cholesterol (T-chol), glucose (GLU), urea (BUN)] and BCS were carried out on a weekly basis from 2nd to 7th week postpartum (pp). Animals resuming ovarian activity within 7 weeks pp were 90% (9/10), 66.7% (8/12), 66.7% (8/12) and 54.5% (6/11) in SG, AG, WG and SPG, respectively. BCS was low ($p < 0.01$) in SG if compared to other groups at different weeks pp. NEFA were elevated ($p < 0.01$) in WG when compared to SG at week 3 (488.8 ± 58.3 vs. 209.1 ± 33.3 μEq/l) and 4 pp (429.4 ± 87.5 vs. 201.8 ± 25.8 μEq/l). T-chol was higher ($p < 0.05$) in SPG (113.1 ± 8.3 mg/dl) than that of SG (96.8 ± 7.3 mg/dl) during W2 pp. GLU was higher ($p < 0.01$) in SPG than other groups during weeks 4, 5, 6 and 7 pp. BUN was higher ($p < 0.01$) in SPG than SG at weeks 2, 3, 4, 5, 6 and 7 pp. Conclusion, calving season influenced BCS, metabolic status and time of ovarian resumption in high milking cows by supressing feed intake during hot season.

P246

Obstetrics doppler sonography in late pregnant sheep complicated with gangrenous mastitis

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Recording vascular pulsatility and resistance from foetal-maternal vessels has become a routine in foetal surveillance. Documenting the blood flow waveform of the umbilical artery (UA) is a common method to monitor foetal health, because it is strongly predictive of an adverse outcome of pregnancy. Also measurement of the FHR is an important indicator of well-being in foetal sheep. The aim of the current case report was to state that the umbilical and placental arteries' Doppler indices and FHR in a foetus in a high risk pregnant Chios ewe. A 4-year-old, late pregnant Chios breed ewe was referred to our clinic with hypothermia, depression, and severely gangrenous mastitis. Transabdominal ultrasound revealed a single live near-term foetus. B-mode and Doppler examinations were performed to evaluate foetal viability and foetal-maternal blood flow. Foetal heart rates (FHR), pulsatility index (PI) and resistance index (RI) were recorded from the UA and placental artery. Parameters were recorded as FHR: 132 beats per minute (bpm), PI: 1.05 and RI: 061. No reverse or absent end-diastolic flow from UA was observed. Thirty minutes after

the first examination, blood flow in the placental artery was evaluated, which was localized on a cotyledon. In this second examination, FHR, PI and RI were measured as 130 bpm, 1.09 and 0.65, respectively. As maternal health deteriorated due to toxaemia, a healthy male lamb (2800 g) was delivered by emergency caesarean section. This case shows that Doppler sonography of foetal lamb umbilical and placental artery can be used to evaluate ovine foetal health despite maternal toxaemia. Further research is needed to unravel the physiological mechanisms involved.

P247

Cloning of DNA sequences encoding RSVP14, RSVP20 and RSVP22 proteins from ovine seminal plasma

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Previously, we reported that three ram seminal plasma proteins specifically secreted by the seminal vesicles and called RSVP14, RSVP20 and RSVP22, are partially able to protect sperm against cold-shock. Obtaining these proteins *in-vitro* would allow us to formulate better cryopreservation diluents. In this study, we have developed a new protocol for the cloning of DNA sequences encoding RSVP14, RSVP20 and RSVP22 for the achievement of the *in vitro* expression of these particular proteins. RNA extracted from the seminal vesicles of Rasa Aragonesa rams was used to obtain cDNA by retrotranscription. The specific sequences were amplified by PCR. Primers were designed for each sequence according to the FlexiVector System (Promega). Sequencing confirmed that cycling conditions of 94°C 1 min; (94°C – 45 s; 54°C – 45 s; 72°C – 1 min) × 30; 72°C-5 min for RSVP20 and RSVP22 are the most appropriate conditions to obtain the desired sequences. RSVP14 can only be amplified using a touchdown PCR protocol. PCR products were ligated to plasmids which were used to bacterial transformation. After bacterial clone selection plasmid DNA was purified by a maxiprep system. Protein expression will be made in a cell-free protein expression system TNT T7 System (Promega).

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P248

Relationship between placental retention and the peripartum leukocyte counts in high-producing dairy cows

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Retained placenta (RP) is a frequent postpartum disorder that can impair uterine involution, predispose to endometritis and affect subsequent fertility. In order to better understand peripartum immunity changes in animals that retained the placenta, leukocyte counts of high-producing Holstein-Friesian dairy cows (93 normal and 9 RP) were studied. Six blood samples were collected every 2 weeks during the last 2 months of gestation and the 1st postpartum one. Total and differential leukocyte counts were automatically analysed using HEMAVET®. Blood counts were statistically analysed by repeated measures General Linear Model ANOVA in regard to presence or absence of RP. Total leukocyte count (TLC, mean ± SD) in cows with RP increased between weeks 4 and 2 prepartum (7.2 ± 1.5–$8.1 \pm 2.8 \times 10^3$ cells/μl, respectively) then decreased, till weeks 2 and 4 pospartum (5.7 ± 2.3–$5.4 \pm 1.8 \times 10^3$ cells/μl, respectively) ($p = 0.015$; within-subject effect). Meanwhile, in normal cows TLC slightly changed during the same period ($7.07 \pm 1.96 \times 10^3$ cells/μl; mean ± SD for week 4 pre- till week 4 post-partum). The prepartum total leukocyte's peak suggests that a higher prepartum immunological-response is related to a higher risk of placental retention. While, lower postpartum TLC numbers should indicate their redirection towards the uterus. The clinical relevance of the prepartum TLC peak is that it could be a useful predictor of the occurrence of Placental Retention.

P249

Development and characterization of a simian virus 40 T-ag antigen immortalized feline endometrial stromal cell line

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An ideal *in vitro* cell model for immuno-endocrine studies would be one, in which cells could propagate without losing their functional characteristics. The major objective of this study was to create an immortalized feline endometrial stromal cell line. The stromal cells were separated enzymatically from the endometrium of cats. Then the cells were seeded in a culture dish and after 24 h transfected by lipofection with plasmid expressing a Simian Virus (SV-40 T-ag). In order to determine effects of sex steroids on prostaglandin secretion, cells at a Passage (P) 10, P15 and P25 were treated with sex steroids for 72 or 72 + 24 h. Progesterone treatment (72 h) or progesterone (72 h) followed by estradiol (24 h) treatment increased PGE2 secretion by the stromal cells at the P10, P15 and P25 ($p < 0.05$). The selected stromal cells could be propagated until at least P25 without losing the expression of estrogen and progesterone receptor mRNA keeping phenotype typical for the primary cell culture. The cells were positive for mesenchyme-specific vimentin, which is a specific stromal cell marker. The immortalized stromal cells possess similar physiological properties as that cultured in primary culture and can be use to study basic immune-endocrinological mechanisms in feline uterus *in vitro*.

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P250

Transcription of Toll-Like receptors in the canine endometrium during the oestrous cycle

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Toll-like receptors (TLRs) play an essential role in the innate immune system by initiating and directing immune response to pathogens. We have shown that TLR2 and TLR4 gene

transcription was higher in canine *E. coli* pyometra than in normal diestrus uteri. However, TLRs transcription during the oestrous cycle was not reported. Here, we evaluated the presence of TLRs 1–7 and 9 by RT-PCR (n = 11 uteri) and we quantified TLR2 and TLR4 mRNA expression by real time PCR, in normal endometrium (n = 25 uteri) during the oestrous cycle. Uteri were collected during routine ovariohysterectomy and the stage of the oestrous cycle determined by the observation of ovarian structures, vaginal cytology and measurement of plasma progesterone concentrations. TLRs 1–7 and 9 mRNA were expressed in canine endometrium throughout the oestrous cycle. TLR2 and TLR4 transcription was higher ($p < 0.05$) at the end of diestrus than on all other stages, which might be associated to the high macrophage content at this stage. TLR2 mRNA expression was lower ($p < 0.05$) on early diestrus than on proestrus and anestrus. TLR4 mRNA expression was lower ($p < 0.05$) on estrus and first half of diestrus than on anestrus. We suggest that the low expression of TLR2 and TLR4 observed at early diestrus, when progesterone concentrations are peaking, may impair antimicrobial defences and could potentially be associated with the increased susceptibility to pyometra observed at this stage.

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P251

Effect post-thaw antioxidant treatment on quality of goat cryopreserved sperm

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Studies indicate that cryopreservation may increase oxidative stress in semen and further that fresh goat sperm is highly susceptible to the attack of hydrogen peroxide. The aim of the present study was to evaluate the efficiency of catalase, an important hydrogen peroxide scavenger, to improve post-thaw quality in cryopreserved goat semen. Fresh samples showing subjective motility higher than 70% were used in this experiment. Ejaculates of five adult male goats (2–3 years old) were collected and cryopreserved with Tris-egg yolk-citrate-glycerol extender. After thawing, samples were incubated (2 h, 37°C) with 0, 60, 120 and 240 UI/ml of catalase. Samples were then analyzed for motility; mitochondrial activity; membrane integrity; acrosome integrity; DNA fragmentation and the measurement of thiobarbituric acid reactive substances (TBARS), an index of lipid peroxidation. Results showed that catalase treatment after thawing played a role on improving mitochondrial activity. Samples treated with 240 UI/ml showed lower percentage of sperm showing low mitochondrial activity when compared to samples treated with 0 and 120 UI/ml of catalase (6.5 ± 2.3, 17.2 ± 3.5 and 10.0 ± 1.3%, respectively). However, no effect of catalase was observed on any of the other variables studied. Results indicate that catalase, despite its beneficial effect on mitochondrial activity, does not positively influence sperm quality after thawing. Possibly, the treatment with catalase would be more effective if performed before cryopreservation. Also, it is possible that the use of different antioxidants would provide better results.

P252

Characterization of glycoconjugates in the canine zona pellucida

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The zona pellucida (ZP) is an extracellular glycoprotein matrix that surrounds the oocyte and early embryo. It plays an important role during folliculogenesis, ovulation, fertilization and embryo transport. The ZP is a heavily glycosylated structure and the oligosaccharide side chains of the ZP-proteins mediate the species-specific binding of capacitated spermatozoa. Bouin-fixed canine ovarian tissue sections were probed with a panel of fluorescein-iso-thiocyanate (FITC) or biotin-labeled lectins (ConA, LCA, RCA-I, PNA, DBA, SBA, WGA, s-WGA, UEA, MAA-I, PHA-E, PHA-L, SNA, VAA). Additionally, several galectins (gal-1, gal-3, gal-8, gal-9) were used to sense fine structural features of the oligosaccharides. In the forming zona of activated primary follicles, GlcNAc and Galβ(1-4)-GlcNAc were present. In secondary and antral follicles using glycan mapping a broader spectrum of sugar residues (α-D-Mannose, β-D-Gal-(1-3)D-GalNAc, β-D-Gal, β-Gal(1-4)GlcNac α-D-GalNAc, (D-GlcNAc)2NeuNAc, and NeuNAc) was found. In conclusion, the expression and distribution of carbohydrate residues in the canine ZP show characteristic changes during folliculogenesis and may prepare the oocyte for a successful fertilization.

P253

Decline in apoptosis occurrence among porcine cloned embryos produced using seudophysiological activation of oocytes reconstructed with adult cutaneous fibroblast cell nuclei

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The objective of our study was to examine the effect of pseudophysiological activation (PPA) of fibroblast cell nuclear-transferred (NT) pig oocytes on the embryo developmental outcome and the initiation of apoptosis processes in the cells of the blastocysts generated. The PPA was achieved by electrofusion of NT oocytes with the cytoplasts isolated from *in vivo*-derived rabbit zygotes. In a control group, reconstituted oocytes underwent simultaneous fusion and electrical activation (SF-EA). Cloned embryos were cultured for 144–168 h up to morula/blastocyst stages. The blastocysts were evaluated intra vitam with the use of diagnostic conjugate of annexin V and eGFP protein for the presence of proapoptotic changes in the cell plasma membrane. The PPA resulted in higher morula and blastocyst formation rates (169/265; 63.8% and 71/265; 26.8%) than the SF-EA (164/314; 52.2% and 57/314; 18.2%, respectively). Moreover, lower percentage of blastocysts, in which annexin V-eGFP-positive (i.e., apoptotic) cells were detected, was obtained from NT oocytes stimulated via the PPA (19.7%; 14/71) than from those subjected to the SF-EA (28.1%; 16/57). In conclusion, the PPA turned out to be relatively efficient strategy for the *in vitro* production of porcine cloned blastocysts of higher quality as compared to those created using the SF-EA protocol.

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P254

Ultrasonographic evaluation of uterine involution following induction of abortion in the Bitch

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This study was performed on six pregnant bitches in their third trimester of pregnancy. Pregnancy was terminated in these bitches by medical interventions, using ultrasound-guided induced cardiac arrest in the fetuses. Ultrasonographic examination of the reproductive tract were carried out after the day of complete abortion up to complete uterine involution, in which uterine dimensions were unchanged within two consecutive evaluations. Uterine shape, size, and echogenicity as well as its wall layering were evaluated in ultrasonographic evaluations, and mean values were measured both in placental and interplacental regions. The ultrasound images of the uterine wall revealed six different layers, on the basis of its echogenicity. Wall layering was very explicit and distinct within the first week after abortion and lost its distinction in the course of uterine involution. At the day after abortion, placental regions measured approximately twice the size of the uterus in interplacental areas. In the first week, the mean values of the placental and interplacental thickness were 2.11 and 1.12 cm respectively, measured in uterine horns. After the third week, the placental and interplacental regions lost their distinction and their thickness. In the last 2 weeks, uterine horns were almost equal in dimension in every part. The time of complete involution varied amongst these six different bitches from 6th to 13th week after the induction of abortion.

P255

Actin distribution and tyrosine phosphorylation in sex-sorted bull spermatozoa

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Sexing semen has reached a high commercial level in bovine, even if fertility after sorting is still variable because of stresses due to the process. This study was aimed at evaluating actin rearrangement and protein tyrosine phosphorylation (TP) in sexed spermatozoa, as compared to freshly ejaculated, capacitated and acrosome reacted sperm, in order to determine possible capacitation-like changes. As for TP, sexed spermatozoa showed two main patterns: cells positive in both acrosome and equatorial subsegment (EQSS) (49.3 ± 10.3%, mean ± SEM, five replicates) and cells with acrosome immunoreactivity (43.6 ± 11%). The remaining population was equally divided into EQSS positive and negative spermatozoa. This condition is in-between the fresh (77.2 ± 12.6% acrosome positive) and capacitated (84.9 ± 7.4% acrosome and EQSS positive) spermatozoa pattern distribution. As for actin, three different patterns (F, C and R, typical of fresh, capacitated and acrosome reacted cells, respectively) were observed. In fresh cells, F 92.7 ± 0.4%, C 5 ± 0.4%, R 2.3 ± 0.8%; in capacitated cells, F 47.7 ± 2.4%; C 44.4 ± 2.1%; R 7.9 ± 0.6%; in acrosome reacted cells, F 5.2 ± 0.5, C 55.8 ± 5.8%, R 39 ± 5.5%. Sex-sorting determined a capacitation-like distribution, with an increase of C pattern: F 28 ± 9%, C 67 ± 11%, R 5 ± 2%. In conclusion, sex sorting in bull sperm cells seems to induce capacitation-like changes that could be responsible for reducing semen quality; other studies on possible functional modifications could be useful to improve sexed semen performance.
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P256

Relationships between crystallization of cervical mucus, sperm survival in this mucus and selected reproduction results in dairy cows

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The objective of this study was to determine the relationship between cervical mucus crystallization in dairy cows with reproduction problems (n = 369), sperm motility in this mucus during 30, 60 and 90 min of the cervical mucus survival test, and selected reproduction results (days to first insemination, open days, number of nseminations per pregnancy, and pregnancy rate). The cervical mucus was drained with a sterile pipette by the recto-vaginal method at the time of insemination. The samples were transferred to the laboratory at a temperature of 4°C within 2 h. The arborisation test (crystallization) of the cervical mucus for evaluation of insemination suitability and the test of sperm survival in cervical mucus were performed. The cervical mucus crystallization was related to the pregnancy rate ($p < 0.05 - p < 0.001$). The best conception result (65.41%) was determined in the case of fern-like crystallization of mucus samples collected at the time of insemination, i.e., in the best stage of estrus. The differences in the pregnancy rate in the case of other types of mucus crystallization were from 19.72% to 46.53%. Cows with fern-like crystallization of their mucus had the shortest open days period (162.51 days; $p > 0.05$) had. Cervical mucus crystallization affected the results of the cervical mucus survival test. The highest motility of sperm after 60 and 90 min was detected in moss–fern (15.01% and 8.16%) and fern-like crystallization (13.24% and 7.71%) withstatistical significance ($p < 0.05$).
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P257

Single layer centrifugation can improve poor quality frozen stallion ejaculates for AI

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It is still not possible to freeze all stallion ejaculates successfully. To see if Single Layer Centrifugation (SLC) with Androcoll-E can be used to improve sperm quality in substandard batches of frozen stallion semen for AI, batches (7) of sub-standard frozen semen were studied, with post-thaw progressive motility of <35%. Eight straws from each batch were thawed by immersion in circulating warm water (37°C) for 30 s. The contents of the straws were mixed and extended

to a sperm concentration of 100×10^6/ml with INRA96 before layering on top of 15 ml Androcoll-E-Large in a 50-ml Falcon tube. After centrifuging for 20 min at $300 \times g$, the supernatant was removed and the sperm pellet was resuspended in fresh INRA96. The sperm concentration was adjusted to $< 50 \times 106$/ml. Computerized sperm motility analysis (CASA) and measurement of viability with Nucleocounter SP-100 were performed on both uncentrifuged samples and the SLC samples. Each batch was tested three times, with means being compared by ANOVA. Progressive motility (PM) and viability were generally greater in SLC samples than in the corresponding uncentrifuged samples (differences for PM +1.6% to +31.3%, $p < 0.001$; for viability −2.2% to +17.9%; $p < 0.01$). In all cases PM was >35% after SLC. SLC with Androcoll-E Large can be used to select the most motile and viable spermatozoa from frozen-thawed semen samples to improve sperm quality for AI, thus rescuing sub-standard batches of frozen semen.

P258

Establishment of an *in vitro* method for single bovine oocyte and embryo culture

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The aim of the study was to establish an *in vitro* system allowing the embryo production from immature bovine oocytes in an individually manner. This single *in vitro* embryo production (s-IVP) enables us to associate the blastocyst outcome to the original oocyte and the follicular origin. It is also useful if only single oocytes from different individuals are available. The maturation, fertilization and embryo culture was performed individually in microdrops of different sizes (10 and 20 µl) compared with a conventional group culture (control). Oocytes showing compact cumulus were used after a single follicle aspiration from ovaries collected at slaughterhouse. There were no significant differences in the level of oocytes reaching metaphase II after maturation (10 µl: 77.9%; 20 µl: 85.0% and control: 77.6%). Further, fertilization resulted in similar cleavage rates between the groups (10 µl: 83.3%; 20 µl: 81.2% and control: 84.7%). At day 8 after IVF the control showed a slightly elevated development to embryos (31.6%) compared to single culture (10 µl: 23.5%; 20 µl: 25.0%). The number of nuclei determined after Hoechst 33258 staining was increased ($p < 0.05$) in the control (82.7) in contrast to s-IVP (10 µl: 66.8; 20 µl: 62.1). Regardless, there are comparable efficiencies between individual and group culture regarding the developmental competence. In conclusion, the study demonstrates that s-IVP is a useful method to obtain embryos from individual matured, fertilized and cultured oocytes.

P259

Equine endometrium secretory function modulation by ovarian steroids

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The equine endometrium undergoes physiological changes throughout the estrous cycle in order to facilitate the establishment and maintenance of pregnancy. An *in vitro* model, with isolated equine endometrial cells (a mixed culture system), allowed us to study how ovarian steroid hormones progesterone (P4) and estradiol (E2) can influence prostaglandins E2 (PGE2) and PGF2α secretion, as well as cell viability, during follicular (FP, n = 5) and mid luteal phase (MLP, n = 5). Both epithelial and stromal cells were enzymatically isolated from uterine horns. Positive controls (tumor necrosis factor – TNFα and oxytocin – OT) were used in order to assess cell culture model adequacy. After 24 h stimulation with: (i) medium without factors; (ii) P4 (10^{-7}M); (iii) E2 ($10-^{9}$M) or (iv) P4 + E2, prostaglandins were quantified and cell viability assessed. Secretion of PGE2 was significantly increased after stimulation with positive controls TNF and OT, during both phases ($p < 0.05$), while PGF2α was increased after incubation with TNF and OT in the FP ($p < 0.05$) and OT in the MLP ($p < 0.001$). Stimulation of MLP cells with E2 and P4 + E2 augmented PGF2α production ($p < 0.05$). Cells from MLP had a higher viability after conditioning with TNF, OT and P4 ($p < 0.05$). The present results evidence that, while TNF and OT stimulate PG secretion in both phases, ovarian steroid hormones only appear to stimulate the luteolytic PGF2α by MLP endometrium. This suggests that endometrial secretory function and cell viability in the mare may be influenced by ovarian steroids, endometrial OT and TNF.

P260

Bull sperm motility on percoll selected fraction and its relationship with *in vitro* fertility

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In order to develop a model for predicting *in vitro* fertility on the basis of the characterization of bull motile fraction selected by Percoll, a study of a total of 14 experiments was made. *In vitro* matured oocytes from bovine ovaries were fertilized as described Morató *et al*., 2008 (*Mol. Reprod. Dev*. 75; 191–201). Briefly, motile spermatozoa were obtained by centrifuging frozen-thawed sperm on a discontinuous Percoll density gradient (45/90%) for 8 min at $700 \times g$ at room temperature. The pellet collected was washed and centrifuged again at $100 \times g$ for 5 min and diluted in fertilization medium to give a final concentration 2×10^6 spermatozoa/ml. A 250 µl aliquot of this suspension was added to fertilization wells and co-incubated with oocytes for 22 h. Other 50 µl aliquot was taken and analyzed by a computer-assisted sperm analysis system (CASA). Cleavage and blastocyst rates were recorded at 48 hpi and 7 days pi, respectively. Considering the present results preliminary, the stepwise multiple regression analysis seems to indicate that Linear Velocity and Straightness coefficient are the most determinant motion characteristic ($p < 0.05$) on the IVF outcome. Moreover, motility data were analyzed with the clustering procedure FASTCLUS, dividing the whole motile sperm population in four separate subpopulations, with significant differences in its distribution ($p < 0.001$). Our results show that separate subpopulations of spermatozoa coexist in Percoll fraction, having to analyze still its potential relationship with IVF fertility.

P261

Effects of various milk yield and body condition score indices with the commencement of luteal activity in postpartum high producing dairy cows

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The aim of the present study was to investigate the relation between different indices of milk yield and body condition score (BCS) with the commencement of the luteal activity (C-LA) during the postpartum period in high producing dairy cows. The milk yield indices including 1st week yield, difference and ratio of increase in yield between the 1st and the peak week, peak week, peak milk yield and area under the curve of milk yield (A-BCS) from 1st to 9th week postpartum. The BCS indices including; BCS indices Loss of BCS (L-BCS), area under the chart of BCS (A-BCS), variation of BCS (V-BCS), and the slope of the trend line of the chart of BCS (S-BCS). Seventy one multiparous healthy (free of detectable reproductive disorders) Holstein dairy cows (mean peak milk yield = 56.7 ± 7.4 kg) were used in the present study. Blood samples were also collected twice weekly to measure serum progesterone (P4) concentrations. The BCS was monitored weekly after calving. Cows with serum P4 concentrations ≥1 ng/ml on at least 2 consecutive blood samplings were considered to have commenced luteal activity. The C-LA was observed in 51 out of 71 cows (71.8%) earlier than 45 days postpartum, while 20 out of 71 cows (28.2%) showed the C-LA later than 45 days postpartum. The results of the Analysis of Variance showed that among different indices defined for the milk yield and BCS, the higher milk yield at the peak and lower A-BCS were significantly ($p < 0.05$) resulted in delayed commencement of luteal activity in clinically healthy high producing dairy cows.

P262

Effect of conservation method on the motility of ram epididymal spermatozoa stored at 5°C

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Post-mortem collection of epididymal spermatozoa allows the conservation of genetic material that would otherwise be lost. This procedure is useful when males die accidentally or for germplasm collection from wild populations. We have tested the effect of conservation (in the epididymis or storing the sperm in a tube) on the kinematic parameters of ram epididymal spermatozoa. Testes were collected from six adult males after slaughter and stored at 5°C. The sperm mass was obtained from the cauda epididymes at 0, 24 and 48 h by cuts. At 0 h, the sample was passed to a tube, which was also kept at 5°C. Samples were analyzed for motility (CASA), obtaining total motility (TM), VAP, STR and ALH. The effect of storage time on these variables was analyzed using linear mixed-effect models. Motility parameters decreased with time (TM: 58 ± 6%, VAP: 53 ± 9 μm/s, STR: 80 ± 2% and ALH: 2.2 ± 0.1 μm at 0 h, to 30 ± 5%, 36 ± 3 μm/s, 71 ± 2% and 2.1 ± 0.1 μm at 48 h). Samples kept higher kinematic parameters ($p < 0.05$) in the epididymis than in the tube (VAP: 47 ± 9 vs. 33 ± 2 μm/s at 24 h, 40 ± 5 vs. 28 ± 3 μm/s at 48 h; STR: 75 ± 3 vs. 68 ± 2% at 24 h, 73 ± 3 vs. 66 ± 4% at 48 h; ALH: 2.5 ± 0.1 vs. 1.9 ± 0.1 μm at 24 h, 2.3 ± 0.2 vs. 1.8 ± 0.1 μm at 48 h). Therefore, storing post-mortem sperm samples within the epididymes is a better strategy than extracting the sperm mass and storing it for later use.

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P263

Role of the cAMP acting through a protein kinase a-independent pathway in boar spermatozoa

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Apart from the intracellular signaling pathway mediated by the cAMP through the PKA in sperm physiology, it has been discovered that cAMP exerts its functions through AMP-activated guanine nucleotide exchange factors (Epac1/2). We aimed to study the identification and the role of Epac1/2 on boar spermatozoa using a specific analog of an Epac-selective cAMP (8-pCPT-2′-O-Me-cAMP). Samples were washed and resuspended in non-capacitating (TBM) or capacitating medium (TCM) and incubated for 1 h at 37°C in presence or absence of Me-cAMP. Acrosome reaction was induced in TCM in presenceof ionophore (A23187). Tyrosine phosphorylation of proteins resolved by SDS-PAGEwas studied using a specific antibody (4G10). Membrane fluidity and acrosome reaction were estimated by using specific dyes (merocyanine/Yopro and PNA/IP respective) by flow cytometry. Finally, motility was assessed by Computer-Aided System Analysis. Phosphorylation of p32 was not modified in TCM in presence of Me-cAMP. However, p32 significantly decreased when acrosome reaction was performed in presence of this analog, compared with TCM + A23187 alone. Motility was completely inhibited after addition of A23187, and was significantly reverted when the analog was present. Membrane fluidity was not modified at any condition. However, acrosome reaction was significantly inhibited when Me-cAMP was added. In conclusion, the results of the present work are suggesting a role for the intracellular signaling mediated by Epac1/2 in the acrosome reaction in boar spermatozoa.

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P264

Fertility of dairy cows with cystic ovarian disease after GnRH administration

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The effect of the old and simple treatments of cystic ovarian disease (COD) on fertility needs to be evaluated in the modern dairy cow for the purposes of comparison with the more complicated new methods consisting of several handlings. The data are a field material collected from fertility control visits done by one experienced veterinarian. The material included 210 COD cases treated with gonadotropin releasing hormone (GnRH), a vast majority with 20 μg of buserelin (Receptal 4 μg/ml, Intervet International B.V., Boxmeer, The Netherlands). The cases were diagnosed as COD if a follicle of >25 mm was found in the absence of a CL. The cases <7 weeks pp were diagnosed as COD only, if the cow had

clinical signs of COD as well. The type of a cyst was not classified but the thickness of the cyst wall never exceeded 3 mm. The cases were treated at 60 ± 43 days post partum (pp), 54% of which at 20–49 days pp. Few cases were rejected owing to slaughter after reasons other than infertility. Nearly all (98.5%) animals were inseminated after the treatment. The 1st AI was performed on average 35 days, in animals treated >49 days pp, 27 days, after the treatment. Eventually, 92.4% of the cases got pregnant, and the pregnancy rate after the 1st AI was 45.9%. The time from treatment to conception was 72 ± 63 days on average, with a median of 56 days. Scrutinizing the cases treated 20–49 days and >49 days pp separately, the average time space remained almost unchanged, but the median was 47 and 61 days, respectively. Generally, GnRH seems to be a good choice especially in cases where there is no hurry with conception.

P265

Influence of gold nanoparticles on the fertilising capacity of bovine spermatozoa *In vitro*

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Gold nanoparticles (AuNP) show a great potential in biomedical imaging or drug delivery. But before any widespread application commences, there should be a clear understanding of their toxicity. Especially reprotoxic effects have not been focussed on sufficiently. Therefore, using the bovine model this study investigated whether exposure to AuNP reflects on the fertilising capacity of spermatozoa. Sperm were collected from a fertile Holstein-Frisian bull, washed and diluted to 100×10^6 sperm/ml in TRIS-Buffer and subsequently incubated for 120 min with AuNP in concentrations of 5 and 50 μM Au. The used particles were generated by laser and employed either ligand-free or conjugated with a thiol-modified 18mer oligonucleotide. Additionally, a negative control was run. After incubation spermatozoa were prepared via the 'swim up' method and added to *in vitro* matured oocytes. After 19 h the oocytes were fixed and examined for pronucleus formation. Data was analysed using ANOVA. There was no impact of AuNP on sperm fertilisation capacity if the particles were conjugated with oligonucleotides. However, ligand-free AuNP in a concentration of 50 μM Au led to a significant drop in fertility by 53.4 ± 10.4% ($p < 0.05$) compared to the negative control. The concentration of 5 μM Au also showed the tendency to hamper fertilisation, leading to a drop of 18.2 ± 11.0%, although without showing significance. The results imply a negative effect of AuNP on sperm functionality, but also highlight the importance of surface chemistry for their biocompatibility.

P266

Study of the reproductive system of ewes subjected to consecutive follicular aspirations

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The aim of this project was to analyse if consecutive follicular aspiration causes damage to the internal reproductive system; uterine body, horns and tube and ovaries decreasing oocyte production by ewes that received ovarian stimulation. Six Santa Ines ewes had their estrus synchronized with short protocol medroxyprogesterone acetate based. Ovarian stimulation was induced by single injection of 80 mg FSHp and 300 IU of eCG 36 h prior to intervention. The procedure were performed using three laparoscopic portals and a 16G catheter attached to a simple lumen aspiration system for ovarian puncture. Animals were subjected to nine sessions of ovum pick-up with a 7-day interval between procedures. During each session adhesions, fibrosis and other damages to the reproductive system were recorded. After the last session ovaries were collected by videoassisted ovaryectomy and analysed macroscopically and histologically. Histological analysis was performed using the Hematoxilin and Heosin and Masson's Trichrome staining. The staining intensity was classified visually into absent (score 0), weak (1), moderate (2) and strong (3). Histological assays were compared using the Wilcoxon Signed Rank test. No significant damage to the internal reproductive system or to the ovaries, during the procedures or after ovaryectomy, was observed (score 0). It can be concluded that repeated sessions of follicular aspiration within a short interval do not cause significant reproductive damage in Santa Ines ewes.

P267

Vitrification of immature bovine oocytes: effect of using different cryoprotectants and culture conditions

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The present study aimed to test different cryoprotectants on vitrification of immature bovine oocytes and to evaluate the effect of granulosa cells as a co-culture monolayer on *in vitro* developmental competence of the embryos. Immature oocytes (n = 983) were vitrified using one of the two cryoprotectants, dimethylsulphoxide (DMSO, 1.5 M) or 1.2 propanediol (PROH, 1.5 M). Post thawing immature oocytes were matured in TCM-199 medium and some of the matured oocytes were examined for their nuclear maturation. Data were analyzed by one-way ANOVA and significant differences among groups were tested by LSD. There was no difference in the number of oocytes at Metaphase II (MII) between the two vitrification groups (45 and 41% for PROH and DMSO, respectively). For PROH group, the culture conditions did not affect ($p \leq 0.05$) either the cleavage rate (71.6 ± 1.4, 71.4 ± 7.1% with co-culture and without culture, respectively) or the developed embryo rate (morula, early blastocyst or blastocyst stages): 61.7 ± 7.9, 59.9 ± 9.4% with co-culture and without culture, respectively. The cleavage rate in DMSO group was higher when using co-culture than without culture system (78.1 ± 8.2 and 69.7 ± 2.9%, respectively). The developed embryo rate was significantly lower using co-culture system (49 ± 4.3%) than without culture system (67.7 ± 4.3%). There were no differences between the two cryoprotectants. However, PROH as a cryoprotectant showed better embryonic developmental rate ($p \leq 0.05$) compared with DMSO when co-culture system was used.

P268

Laboratory boar semen assessment in Spain

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This work shows the results of a survey of semen analysis methodology used in the boar artificial insemination stations (AIS) in Spain. The survey was answered by 21 AIS (58.3% of all registered in Spain). The volume and concentration was evaluated in all of the ejaculated collected in the AIS; the volume analysis was performed by weighing, whereas the most used technique in the concentration evaluation was the photometry (81.0% of the AIS). All AIS perform some evaluation of the sperm motility of all ejaculated collected. 85.7% of the AIS evaluated the massal motility, and 100% of AIS analysed the individual motility by a subjective assessment; additionally in the 33% of the AIS performed a CASA of individual motility. Sperm morphology was analysed in all the ejaculates in the 52.4% of the AIS, in the rest the frequency of analyse over the ejaculates of the same boar was monthly. The acrosome integrity analysis was performed in the 85.7% of the AIS, and its frequency was monthly. The most used technique for morphology and acrosome integrity analysis was the phase-contrast microscopy assessment. HOST and integrity test of the spermatozoa membrane was carried out in the 9.5% and 4.8% of AIS, respectively; the frequency of those analysis was monthly. Finally, microbiological test was performed in 85.7% of the AIS, and their frequency was mostly monthly. In conclusion, the Spanish boar AIS employed the simply techniques in the all day work, whereas the complex techniques have less penetration and are used with less frequency.

P269

Interaction between bovine embryos and co-cultured luteal cells in two *in vitro* culture systems: effects on embryo development

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Enriched luteal cell populations were obtained from early (Days 1–6) bovine CLs and frozen in liquid nitrogen. Day 2 (Day 0 = IVF) cleaved bovine embryos were *in vitro* cultured in SOFaa + 5% serum in a atmosphere of 90% N_2, 5% CO_2 and 5% O_2 until Day 7. In experiment 1, cleaved embryos (n = 900) were randomly allocated to one of four *in vitro* culture groups in 4-well dishes: (i) embryos; (ii) embryos co-cultured with thawed luteal cells; (iii) embryos, oil overlay; (iv) embryos co-cultured with thawed luteal cells, oil overlay. Luteal cell co-culture and/or oil overlay increased ($p < 0.001$) total blastocyst output and proportion of quality grade I + II blastocysts ($p < 0.05$). Oil overlay advanced ($p < 0.05$) embryo development. In experiment 2, cleaved embryos (n = 1000) were randomly allocated to two *in vitro* culture groups in slide chambers: (i) embryos; (ii) embryos co-cultured with thawed luteal cells. Luteal cell co-culture increased total blastocyst output ($p < 0.05$) and quality grade I + II blastocysts ($p < 0.00001$) but had no effect on rate of development and embryo mean cell number. In conclusion, frozen-thawed luteal cells cultured in a semi-defined embryo culture system were able to maintain viability and exert an embryotrophic effect. Co-culture and oil overlay had a non-additive positive effect on embryo development. The slide chamber system is suitable for embryo culture without oil overlay, which can be of interest for studying steroid production by embryos and cells. *Funded by FCT, PTDC/CVT/65690/2006.*

P270

The comparison of iranian holstein bull sperm freezing ability using two commercial extender: Bioxcell and AndroMed

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The aim of this study was to investigate the effect of two commercial extenders without egg yolk: Bioxcell and AndroMed on freezing ability in Iranian Holstein's bull. Semen was collected from eight bulls (two ejaculated/bull; 16 samples) in Iranian Progeny Test Center for cattle by using an artificial vagina. Semen were pooled and allocated to the semen extenders in two equal sections. Freezing was conducted using imv semi-automatic equipments. The percentage of progressive motility (PPM) and viability (VI) of these samples were evaluated before freezing (at 37°C) and after thawing. The effect of extender on the PPM and VI after thawing was significant. Mean PPM and VI were high ($p < 0.01$) in Bioxcell (PPM: 48/20 ± 1/5, VI: 58/25 ± 0/25) than Andromed (PPM: 41/00 ± 1/5, VI: 50/19 ± 0/25). Results suggested extender Bioxcell compared with extender AndroMed was more efficient to maintain sperm quality during freezing and thawing processing.

P271

Leptin and ghrelin on mare corpus luteum secretory function

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The metabolic hormones ghrelin and leptin have been shown to participate in reproductive physiology regulation. Thus, the objectives were (i) to assess gene expression of ghrelin, leptin and their receptors; and (ii) the role of these hormones on secretory function (progesterone-P4; prostaglandins-PG) by the equine corpus luteum (CL). Tissues were collected from early luteal phase CL (Early-CL, n = 6) and mid luteal phase (Mid-CL; n = 6) and late luteal phase (Late-CL; n = 6), for PCR analysis and explants culture (Early and Mid-CL). Luteal tissue was exposed to: (i) media without hormones – control; (ii) leptin (20 or 200 ng/ml); (iii) ghrelin (100 ng/ml); or (iv) leptin + ghrelin (20 ng/ml + 100 ng/ml; 200 ng/ml + 100 ng/ml). Even though leptin receptors, ghrelin and its receptor were expressed throughout the luteal phase, leptin mRNA expression was inexistent. No treatment effect was observed on P4. However, the lowest leptin dose stimulated PGE2 production ($p < 0.05$), while leptin + ghrelin (20 + 100 ng/ml) increased PGE2 only by Early-CL ($p < 0.01$). In Mid-CL, when luteal growth is maximized, PGF2α secretion

was increased by ghrelin (100 ng/ml) and leptin + ghrelin (20 + 100 ng/ml) ($p < 0.001$). Interestingly, these data suggest that leptin, from a source other than the CL, acting on its specific receptor on the mare CL, might have a luteotrophic action. Nevertheless, *in situ* produced ghrelin might play a luteolytic role through its specific receptor.

P272

Analysis of bovine sperm DNA protamination using acoustic focusing flow cytometry and fluorescence microscopy

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The aim of this study was to compare two techniques – acoustic focusing flow cytometry (FC) and fluorescent microscopy (FM) – to analyze protamine deficiency in bovine semen accessed by chromomycin A3 (CMA3). Frozen-thawed semen from three ejaculates of nine bulls (four *Bos indicus* and five *Bos Taurus*) was evaluated and showed normal values for: motility, morphology, simultaneous evaluation of acrosome, membrane and mitochondrial potential (FITC-PNA, PI, JC-1) and chromatin integrity (acridine orange – AO). Samples were prepared and stained with CMA3. Sperm cells (n = 500) were classified as green (negative) and bright green (positive – protamine deficiency) at fluorescent microscopy (Olympus BX61: 460–490 excitation; 510 emission). A total of 10 000 events were accumulated for each measurement at flow cytometry (Attune™) equipped with a 488 laser, 620 nm LP and 575/24 nm emission. DNA protamination on fluorescent microscopy (0.25 ± 0.14%) and flow cytometry (0.54 ± 0.08%) showed no statistical difference (t-test; $p > 0.05$), although fluorescent microscopy evaluation revealed higher value for coefficient of variation (157.1) than flow cytometry (44.5). Acoustic focusing flow cytometry can be a reliable technique for assessing sperm quality and protamine deficiency by CMA3. Bovine spermatozoa seem to have lower percentages of impaired protamination, nevertheless further research on deficient protamination and its implications on fertility is necessary.

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P273

Effect of dietary protein on superovulation response and embryo quality in ewes: preliminary study

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Aim was to evaluate superovulatory response and embryo quality in ewes exposed to superovulation program after feeding with ration of different amounts of crude protein. Ewes were fed a ration containing 12% (P12, n = 30), 15% (P15, n = 28), and 18% (P18, n = 30) of protein starting 25 days before beginning of synchronization protocol until day of flushing. Ewes were treated with intravaginal progesterone sponge for 12 days and were administered reducing doses of FSH. After sponge removal, ewes in estrus were mated with fertility-proven rams and given 750 IU hCG. Ovarian response was assessed by laparoscopy 7 days after mating and uterus was flushed for embryo collection. Total number of corpora lutea (CL) and large unovulated follicles > 3 were accepted as superstimulation (SS) whereas total number of CL on both ovaries > 3 were determined as superovulation (SO). Ratio of SS and SO were not different significantly among P12, P15, and P18 (63–50%, 79–75%, and 63–60%, respectively). Average number of CL did not differ due to protein feeding. Embryo quality was assessed according IETS manual and embryos were classified between Grade 1 being the best and Grade 4 being the worst quality. The group fed with 12% protein had highest number of Grade 1 embryos while P18 yielded more Grade 4 embryos. The results suggest that dietary protein had no effect on success of superstimulation of ewes. However, embryo quality was directly influenced, which indicates embryo development was negatively affected with increasing amounts of protein in diet in ewes.

P274

Dynamics of *Coxiella burnetii* antibodies in high producing dairy cows in northeastern spain

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Q fever is a zoonosis caused by *Coxiella burnetii*, an obligate intracellular gram negative bacterium endemic worldwide. There are many mammal reservoirs of the bacterium, but the most commonly identified sources of human infection are domestic ruminants. Infection in these species is mainly asymptomatic but if clinical signs are present, reproductive disorders are the most frequently reported. In cattle, late abortion and infertility are the main clinical manifestations. The aim of this study was to assess *C. burnetii* antibodies in 478 high-producing dairy cows. Serological analyses were performed using a commercial indirect ELISA kit LSIVET. *C. burnetii* prevalence in the herd was 51% in 2009 and 49.4% in 2010. The results showed that in 65%, 10.5% and 24.5% of animals the titer remained stable in the same group, decreased and increased during the study period, respectively. Twenty four animals (5%) seroconverted, while 31 (6.5%) became seronegative. Bulk tank milk analyses by RT-PCR in the 2 years of study confirmed a high bacterial excretion (> 10 000 bacteria/ml). A group of ten cows were selected from the herd: Five seropositive and five seronegative animals. These cows were sampled individually and provided a total of eight milk and ten vaginal fluid samples. All of the fluid and 50% of milk samples were PCR negative. No excretion of C. burnetii into vaginal fluid was found. The results suggest a high stability of antibodies and the bacterium shedding by milk, and a broad distribution of the infection.

P275

Detection of single nucleotide polymorphism (SNP) in TATA box of the lactoferrine gene in dairy cows and its relationship with uterine infections using RFLP-PCR method

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Identification of resistant genotypes to uterine infection is important. Lactoferrine is one of the major antibacterial compounds in the normal uterine discharge. Identification of the resistance genotypes to uterine infections in dairy herds will reduce cost of treatment. Preliminary studies conducted to

identify polymorphism in the gene promoter of lactoferrine in different areas, demonstrate lactoferrine gene of importance as a genetic resistance marker for some infectious diseases. This study conducted to identify single nucleotide polymorphism (SNP) in the TATAbox (−28) of the lactoferrine gene using CRS-RFLP-PCR method. We also investigated the relationship between SNP identified in this area with the uterine infection. Blood samples were collected from 74 multiparous female Holstein cows from an industrial farm with an identified history of uterine infection. A total of 74 cows were divided based on the history of diseases into two groups: cows with a history of uterine infection (n = 43), and cows without history of uterine infections (n = 31). The results revealed a higher percentage (77.3%) of cows with homozygote mutation (−28:CC) had a history of uterine infection; while, a higher percentage (65.2%) of cows without mutation (−28:AA) had no uterine infection ($p < 0.05$). Further studies will be required to determine critical SNPs in lactoferrin gene and status of the risk of uterine infection in cows.

P276

Associations of cytological endometritis with ovarian function in dairy cows

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To quantify the prevalence of cytological endometritis (CE) according to ovarian function, milk samples from 130 multiparous Estonian Holstein cows were taken twice weekly for progesterone (P4) measurement by EIA. P4 profiles were categorized as follows: normal ovarian function (interval from calving to first luteal response (P4 > 5 ng/ml) up to 50 days PP followed by regular cyclicity), delayed ovulation (DOV), persistent corpus luteum type 1 (PCL1; P4 > 5 ng/ml for ≥19 days during the 1st cycle PP) and type 2 (PCL2; P4 > 5 ng/ml for ≥19 days during the 2nd or subsequent cycle PP), and cessation of luteal activity (CLA). Uterine cytology samples were collected at 40 days PP. The cytological criterion was set at >8% of neutrophils as the threshold indicator of endometrial inflammation. Group differencesand the type of the P4 profile were tested using Fisher's exact test. DOV was present in 35.4%, PCL1 in 10%, PCL2 in 4.6%, and CLA in 5.4% of the profiles. CE was present in 30.8% of the cows. The prevalence of CE in cows with normal ovarian function was 15.5%. The prevalence of CE was higher in cows with DOV (41.3%; OR = 3.8; p = 0.004), PCL1 (53.4%; OR = 6.4; p = 0.007), and CLA (57.1%; OR = 7.3; p = 0.025) compared to the cows with normal ovarian function. the prevalence of CE in cows with PCL2 was no different (16.7%; OR = 1.1; p = 1) from the cows with normal ovarian function.
The prevalence of CE was higher in cows with DOV, PCL1 and CLA compared to cows with normal ovarian function.

P277

Effect of deferoxamine mesylate on freezability of blood supplemented canine semen

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Detrimental effects of blood contamination on total and progressive motility of frozen-thawed canine spermatozoa, caused by the release of hemoglobin during freezing have been previously reported. This study investigated the effects of deferoxamine mesylate (DFO, Desferal®, Novartis Pharma, Belgium), an iron chelating agent, on freezability. Semen of 5–6 Beagle dogs was pooled and frozen using a two-step dilution method. Semen was divided in eight groups before adding the second extender. To the first four groups, no blood was added; groups 1–4 contained increasing concentrations (0, 1, 10 and 50 μl/ml) of DFO. Groups 5–8 each contained 30 μl/ml of blood and increasing concentrations of DFO (0–10 μl/ml). Post-thawing motility, viability (eosine-nigrosine; SYBR-14-Propidium Iodide), morphology (eosine-nigrosine) and acrosomal status (Pisum Sativum Agglutinine) were assessed. The experiment was repeated five times. No significant differences were observed in sperm morphology, viability and acrosomal status among the eight different groups. Blood admixture was detrimental on total ($p < 0.05$) and progressive motility ($p = 0.08$). However, this effect is not as marked as in previous reports where blood was added just after semen collection. Erythrocytes were centrifuged and equilibrated with the semen, possibly causing a weakening of their membranes. In our experiment, DFO adjunction did not alter blood-free semen quality nor did it improve it in the presence of blood. Our study shows the innocuity of DFO on semen and suggests centrifugation is an important step explaining toxicity of blood on semen. More experiments are needed to further investigate how DFO can improve canine semen freezability.

P278

Effect of heat stress and dietary restriction on sperm and plasma oxidative status parameters in rats

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Heat stress is an important stress factor on metabolism. Heat-induced stress increases free radical generation. It is reported that dietary restriction (DR) decreases the detrimental effects of free radicals. The objective of the study was to determine the effects of heat stress and DR on plasma total oxidant status (TOS), total antioxidant status (TAS) and spermatological parameters including motility, volume, concentration, acrosome and membrane integrity in rats. Sexually mature 40 male Sprague Dawley rats (10 weeks old) were evenly divided into four groups according to environmental temperature (room temperature; 22–23°C and high temperature; 37–39°C) and to dietary regime (*ad libitum* and 40% dietary restriction of the daily food). At the end of the ninth week of the study, the rats were euthanized and their cauda epididymides were excised. Each epididymides was dissected and placed in a 35 mm dish containing PBS medium supplemented with 3 mg/ml BSA. The sperm concentrations were adjusted to 50×10^6 spermatozoa/ml. The plasma membrane and acrosome integrity were assessed using propidiumiodide PI/SYBR-14 and fluorescein isothiocyanate-conjugated peanut agglutinin (Arachishypogaea; FITC-PNA). The data were analyzed by one-way ANOVA followed by Tukey test. Differences were considered significant when $p < 0.05$. The motility, volume, acrosome and membrane integrity of sperm were significantly negatively affected by heat stress ($p < 0.05$) but weren't affected by DR ($p > 0.05$). The sperm concentration was different only in room temperature-*ad libitum* group ($p < 0.05$). Neither heat stress nor DR affected TAS and TOS values. In conclusion, these results suggest that while rat sperm are sensitive to heat stress, DR have not significant effect on sperm parameters.

P279

The Effect of peripheral T4 blood level on the outcome of sheep embryo transfer (ET) programs

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Thyroid activity is considered crucial to sustain the reproductive performance in domestic animals. Marked seasonal variation in thyroid activity and thyroid hormone blood concentration has been reported by several others. These hormone variations are particularly important in the free-ranging and grazing animals, e.g. goat and sheep. The objective of our study was to investigate the possible effect of T4 blood level on the outcome of superovulation (induced by FSH-Ovagen®) + Embryo transfer (ET) in sheep. Blood samples were taken from merino donor and recipient ewes (n = 32) three times during ET program (d0: at the time of artificial insemination (AI) of donor ewes/heat detection of recipient ewes, d2: at the beginning of fasting before surgery, d4: at the time of embryo flushing/ET) for measuring the peripheral blood level of T4. Statistical analyses were performed using SPSS 12.0 and T-method. In donor ewes, significant connection was found between the number of corpora lutea ($p = 0.007$) and embryos obtained ($p = 0.006$) and the T4 blood level. Moreover, the peripheral blood level of T4 was significantly lower in the pregnant recipients too. According to our results, T4 has an important role in the superovulatory response of donor ewes and after ET in the embryo development in recipient sheep. Further investigations are needed to find out the exact role of T4 in the success of sheep ET programs.

P280

A novel mutation that causes stillbirths in cattle

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Of the pregnancies sired by one Finnish Ayrshire bull, 42.6% (318 calves) ended in stillbirths or abortions after 7 months of pregnancy. The stillborn calves were small and their lungs were not inflated. High-density single nucleotide polymorphism assay (Illumina BovineSNP50 BeadChip) was used to genotype DNA samples of the bull and 26 descendants: five stillborn calves, 13 live offspring and eight foetuses. These results were analysed with half-sib linkage analysis (GridQTL) and founded area was fine mapped with sequencing. The copy number of the area was studied with SybrGreen detection chemistry (Applied Biosystems). Expression of the candidate genes was studied with reverse transcriptase PCR on the brain and cotyledon tissue of the foetuses. Relatives of the bull were tested with a PCR-based assay. In the BTA18 was a 12.4 Mb associated region that contained a 110 kb microdeletion. The deletion removes a part of the non-protein coding MER1 repeat containing imprinted transcript 1 gene (MIMT1). None of the foetuses that inherited the deletion expressed MIMT1 in either tissue. The deletion is probably a de novo mutation in the bull. The mutation was also found in two of the live offspring. The deletion is semi-lethal with a mortality rate of 85%.

P281

Total prostatectomy in papillary prostatic adenocarcinoma in dog

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Prostatic cancer represents 5% of canine prostatic diseases. Adenocarcinoma is an androgen independent cancer with prostatic ductal epithelium origin. Neutered dogs have 2.83–4.3% increased risk compared to intact dogs, due to reduction of basal glandular cells androgen-dependent in parenchyma and basal ductal cells androgen-independent proliferation. Here we report on a 15 year old neutered Dachshund that presented with dysuria, haematuria, tenesmus, prostatomegaly and pain at palpation, trombocitosis, leukocytosis and severe *E. coli* cystitis. Ultrasonography and radiography showed a prostate with 8 cm diameter, irregular margins, heterogeneous parenchymal echotexture, cavitations, calcifications, severe colon compression and nodules in the urinary bladder. Retrograde urethrocystography showed positive contrast to prostatic parenchyma. The fine needle aspirations confirmed the diagnosis of prostatic adenocarcinoma. Total prostatectomy was performed which involved urethral anastomosis by castor oil polyurethane membrane and biopsy of urinary bladder. Histopathologic exam revealed neoplastic cell proliferation of glandular epithelium, cellular pleomorphism, mitoses, papillary projections and urinary bladder metastasis. The animal had an excellent postoperative recovery. Chemotherapy with carboplatin was performed. Nine months after surgery, metastases were detected in iliac, sublumbar, pelvic lymph nodes and lumbar vertebrae, leading to the decision to euthanise the animal. We conclude that total prostatectomy in the case of prostatic ademocarcinoma improves patient's quality of life and survival, but has limitations as an effective treatment, proving the need of prostatic markers research for early diagnosis of prostatic cancer.

P282

Application of economic optimisation in conservation of sheep genetic resources: a prototype model

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The benefits of conserving animal genetic resources depend on the increased adaptive capacity in response to change that such preservation in a genome resource bank (GRB) offers beyond that of alternatives. Greater GRB size and diversity increases benefits but also costs. Operational plans to establish GRBs have attributes of typical management problems. The aim of this work was to use an economic framework to measure and so minimise initial collection costs. A financial objective was set to minimise cost of collecting and storing Cheviot sheep semen and embryos and it had to be achieved within financial, logistical and biological constraints. These included quantity of semen and embryos to be stored, collecting and storing efficiencies, breed population, geographical distribution of flocks (travel constraint) and number of possible donor farms.

The objective was achieved through an activity set bounded by the constraints. Modelled activities were: travel to farms, on-farm preparatory (e.g. MOET), collection procedures and initial storage of genetic materials. Linear programming (LP) determined the optimal plan in terms of farms to visit and number of visits per farm. Population and geographical data for 63 Cheviot flocks (Carson *et al*., 2009; *Livestock Sci,* 123:288–99), published collection and storage efficiencies plus commercial costings were used. After 100 iterations of the model (nine farms per iteration) mean ± SE minimised cost of collecting and storing 1000 embryos and 500 units of semen was estimated at £30634 ± 16. In conclusion, this LP successfully identified optimal plans for a GRB.

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Primiparous midlactating vs. multiparous periparturient cows: comparison of selected blood parameters

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Dairy cows experience a higher risk for metabolic and infectious diseases post partum (p.p.) than midlactating cows. Furthermore these diseases occur more frequently in multiparous cows leading to high culling rates. Reduced capacities in immune defence mechanisms due to hormonal changes as well as metabolic reprogramming in combination with a negative energy balance are responsible factors. It has been frequently reported that around parturition immune cells are functionally impaired but there is hardly information about reference values for blood cells at this particular period. Aim of this pilot survey was to investigate differences in common blood parameters between primiparous cows (n = 52) 115 ± 43 days p.p. and multiparous cows (n = 11) 60–120 min after parturition. Red and white blood cell counts were analyzed and data were compared using t-test. After parturition we found significantly higher values for segmented neutrophils ($p < 0.001$), monocytes ($p < 0.029$), hemoglobin and packed cell volume ($p < 0.0001$), the latter indicating a reported hemoconcentration. However there were lower levels found for total leukocytes and banded neutrophils ($p < 0.001$), lymphocytes ($p < 0.0001$), eosinophilic granulocytes ($p < 0.005$) and thrombocytes ($p < 0.02$) in periparturient cows. It remains unclear whether these differences might account for a higher risk of diseases thus further studies with larger numbers and clinical records are needed to evaluate potential biomarkers for cows at risk. Furthermore species specific reference values for blood parameters should be advanced considering age and stage of lactation.

P284

The expression of LHR mRNA was induced by the intracervical application of FSH or PGE1 analogue in the cervix of goats (*Capra hircus*) at the oestrus

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Intracervical application of PGE1 analogue (Misoprostol) and FSH increases cervical relaxation at oestrus in goats. Plasma gonadotrophins (LH, FSH) are also said to have receptors in the cervix and are implicated in cervical relaxation during the periovulatory period. The aim of this study was to investigate if intracervical application of FSH or PGE1 increases cervical relaxation in goats by their effect on LHR mRNA expression. Oestrus was synchronised in 20 Thai goats using progestagen pessaries and PMSG. Intra-cervical hormone was applied at 24 or 48 h after the pessary removal: Group 1; controls, Group 2; FSH 2 mg at 48 h, Group 3; FSH 2 mg at 24 h and PGE 11 mg at 48 h, Group 4; PGE 11 mg at 48 h. Cervices were collected at 54 h after sponge removal and divided transversely into three regions (vaginal, mid and uterine) and stored at −20°C. The LHR mRNA expression was determined by RT-PCR using β-actin as reference. Data on the relative expression levels of LHR mRNA were analysed by ANOVA. LHR mRNA expression following application of FSH or PGE1 on their own or their combination was higher ($p < 0.05$) than in control group without any difference between the hormones. LHR mRNA expression was also higher ($p < 0.05$) in the uterine and vaginal ends than the mid region. These results demonstrated that intra-cervical application of FSH or PGE1 increases LHR mRNA expression. They confirm that LH may have the role in the cervical relaxation at oestrus in the goat and its mRNA can be induced by the application of FSH and PGE1 analogue.

P285

Reproductive biology of green catfish (*Hemibagrus nemurus*) in the Chi River, Thailand

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The green catfish, *Hemibagrus nemurus,* is widely distributed in Thailand, being an important commercial fish species. This study was based on wild-caught green catfish from the Chi river, Northeast Thailand, (16°11′3″N, 103°18′4″E). The experiment was carried out from October 2009 to September 2010 to evaluate the spawning season and other aspects of its reproductive biology. Four hundred and forty three fish were obtained, of which 196 were males, 112 non-ovigerous females, and 135 ovigerous females. The mean body length and body weight of female and male green catfish sampled were 20.33 and 20.40 cm, and 72.27 and 70.99 g, respectively. The sex ratio did not vary significantly from 1:1 throughout the year. The gonadosomatic index (GSI) of the female ranged from 0.92% to 13.45%, and the highest GSI as well as the highest proportion of mature fish were recorded from April–September. Fecundity of green catfish ranged from 288 to 70 322 eggs with the average 13 873 ± 1194 eggs/fish for females of 16 to 35 cm in length, and 31 to 321 g in total body weight, respectively. Additionally, the length at 50% maturity (L_m50%) was 19.5 cm. The length-fecundity relationship was $F = 0.00000017\ L^{8.160}$. The weight-fecundity relationship was $F = 0.055\ W^{2.799}$. Therefore the spawning season period is between April and September with the length and weight of fish being no different from the rest of the year. Due to the over fishing in the Chi river, the information suggests avoiding fishing during this time of year to allow the fish uninterrupted spawning.

P286

Uterine infection confounds the effects of negative energy balance on the liver GH-IGF1 axis

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Dairy cows experience NEB in the first weeks postpartum during which the GH-IGF axis becomes uncoupled and peripheral IGF concentrations are low. Concurrently, bacteria contaminate the uterus causing infection. Experiments in rats show that bacterial products affect liver GH and IGF1 expression. Thus, the aim of this experiment was to investigate the relationship between NEB, uterine infection and liver gene expression. Fifty-three Holstein-Friesian cows were monitored from calving until day 35 postpartum. Uterine health was monitored by vaginal mucus assessment and energy status calculated using standard equations. Liver tissue was biopsied on day 35 and gene expression analysed by real-time PCR. When all animals were analysed together NEB was significantly related to reduced expression of liver GHR1a ($p < 0.01$) and IGF1 ($p < 0.001$) mRNA. However, when animals with uterine infection were removed from the dataset this relationship ceased to exist ($p = 0.44$ and 0.33 for IGF1 and GHR1a, respectively). Indeed, IGF1 mRNA expression was reduced in animals with uterine infection compared with clean animals (862 ± 77 vs. 1132 ± 135 units, $p < 0.05$), as was GHR1a (730 ± 54 vs. 975 ± 170 units, $p < 0.05$). The results demonstrate that uterine infection reduces liver gene expression in the postpartum dairy cow. Furthermore, there was no relationship between energy balance and liver IGF1 and GHR1a mRNA expression in animals that did not suffer from clinical uterine disease.

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P287

Relation between postpartum uterine pathogenic bacteria and follicular growth in dairy cattle in Iran

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In cattle the first postpartum dominant follicle grows slower and produces less oestrogen in animals with high numbers of bacteria contaminating the uterine lumen. The present study examined the relationship between pathogenic bacteria in the postpartum uterine lumen and follicle growth. Swabs were collected from the uterine lumen of cattle on day 7 postpartum. Bacterial growth was scored semi-quantitatively and animals categorized into two groups: group A with high numbers of pathogens (more than 10 colonies per swab) and group B with low numbers (<10 colonies per swab). Treatment did not follow for either group. Ovarian structures were monitored by daily transrectal ultrasonography and blood samples collected for progesterone measurement. In animals with high numbers of uterine pathogens on day 7, the diameter of the first postpartum dominant follicle was smaller than in the group with few pathogens. Average follicle growth in high and low pathogen cows was 0.14 ± 0.06 and 0.48 ± 0.1 mm/daily, respectively. It was also observed that 78.9% of cows with high numbers and 65.2% of cows with low numbers of pathogens had started to cycle and had follicular waves on day 14 after parturition. Altogether, uterine contamination affects follicular growth in the postpartum period and causes a delay in resumption ovarian cycles in dairy cattle.

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cDNA cloning of porcine E-cadherin and its expression profile in porcine early parthenotes

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E-cadherin is essential for normal compaction and blastulation in the process of embryo development in mammals. The aims of this study were to clone the complete cDNA sequence of porcine E-cadherin gene and detect the gene expression in different developmental stages of porcine early parthenotes. After cloned the E-cadherin gene from porcine oviduct using rapid amplification of cDNA ends (RACE) method, the sequence analysis revealed that porcine E-cadherin gene complete cDNA nucleotide sequence was 4283 bp including 2652 bp of open reading frame (ORF), 105 bp of 5′ untranslated region (UTR) and 1526 bp of 3′ UTR, and the ORF encoded a deduced protein precursor molecular of 97 kDa with 883 amino acids residues. The precursor protein including signal peptide, extracellular region, membrane-spanning region and cytoplasmic region had a single transmembrane structure, and its extracellular region had HAV motif and some Ca2+ binding locus. The porcine E-cadherin showed high homologous with cattle (89%), horse (87%), dog (86%), human (84%), chimpanzee (83%) and mouse (83%). The results from RT-PCR and real-time PCR showed that E-cadherin gene could be expressed in immature and mature oocytes, and early parthenotes (2-, 4-, 8-cell embryos, morula, blastocysts), and the expression showed a diminishing trend. This is the first report of cloning and analysis of porcine E-cadherin cDNA thus provides critical imformation for further investigation of its functions in porcine embryo development.

P289

Radioimmunoassay of fecal progestins can be used to diagnose ovarian disturbance in beef cows

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The aims of this study were to compare fecal progestin concentration between cows with cessation of ovarian cycle and regular cycling cows, and to assess its application to diagnose ovarian disturbance. Twelve brangus cows were subjected to matched blood and fecal sampling, and ovarian ultrasonography twice a week for a period of 3 months. The concentration of progesterone (P4) in plasma and progestins in the fecal samples extract were determined by using a P4 radioimmunoassay (RIA) (DSL-3900, USA). Based on plasma P4 assay and ovarian ultrasonogram, eight of the cows had cessation of ovarian cycle during the course of the study while the other four presented regular cycle. There was a significant positive correlation between plasma P4 and fecal progestin

profile ($r = 0.52$) indicating physiological validity of the assay method. The mean ± SE of fecal progestin concentration in cows with cessation of cyclicity (129.3 ± 34.2 ng/g) was significantly ($p < 0.01$) lower than cows with regular cycle (306.3 ± 51.6 ng/g). Fecal progestin concentration was < 344 ng/g of feces (cut-off value) when plasma P4 was < 1 ng/ml for > 14 days that indicates cessation of ovarian cycle. In conclusion, cessation of ovarian cycle in cows can be diagnosed based on fecal progestin concentration when it remains below 344 ng/g for at least 14 days.

P290

Ascorbic acid effects on *in vitro* maturation of bovine oocytes with or without cumulus cell

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Ascorbic acid has long been associated with fertility. This study was designed to determine the effects of ascorbic acid (Vitamin C) on *in vitro* maturation of cow oocytes with or without cumulus cells. The oocytes of antral follicles, 2–8 mm in diameter, were aspired; then oocytes with at least three layers of cumulus cells (COCs) were selected and washed four times in HEPES-TCM-199 supplemented with 5% fetal bovine serum and 1% penicillin/streptomycin. Then 5–10 COCs were subjected to each droplet of maturation medium and incubated at 38.5°C, 5% CO_2 and 95% humidity with different levels of ascorbic acid (0, 150, 200, 250, and 300 μM/ml) for 24 h. Maturation medium was bicarbonate-buffered TCM-199 supplemented with 10% fetal bovine serum, 0.1 IU/ml human menopausal gonadotropin, 1 μg/ml estradiol and 1% penicillin/ streptomycin. Results showed cumulus expansion did not affect by different concentrations of ascorbic acid ($p < 0.05$). Ascorbic acidwith 250 and 300 μM/ml in oocytes with cumulus and 250 μM/ml without cumulus was significantly increased nuclear maturation of bovine oocytes compared with control group ($p < 0.05$). So, this study showed that ascorbic acid with cumulus cells enhanced bovine oocytes *in vitro* maturation.

P291

Interrelation of milk SCC, PMN and milk constituents in primiparous holstein cows

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Early diagnosis of intramammary infection is a crucial factor considering food quality and safety and for effective treatment of diseased cows. Recent development of automated milking systems has even increased the need for appropriate biomarkers, which can be measured online and help identifying cows with mastitis. The somatic cell count in milk (SCC) has been utilized as a biomarker for udder health over decades and a low SCC is a top on the list objective in the dairy breeding industry. Aim of the study was to evaluate to what extent SCC correlates with milk polymorphonuclear neutrophilic granulocytes (PMN), fat, protein and lactose content. Since milk constituents significantly vary depending on breed, age and lactation state only pure bred primiparous Holstein cows in midlactation (average 115 days in milk) were included in the study. In total 1692 quarter milk samples were analyzed. We could show a strong correlation between milk SCC and PMN ($R = 0.98$; $p < 0.001$) as assessed by flow cytometry. There was no correlation between SCC and milk fat ($R = 0.11$) or protein ($R = 0.23$) content. However milk lactose showed a moderate negative correlation ($R = -0.50$; $p < 0.001$) with SCC. Milk PMN and lactose are candidate biomarkers for udder health. Data indicate that detectable alterations happen simultaneously with increased SCC levels thus there is no clear advantage over the current method. Future studies should evaluate to what extent a combined detection of udder health parameters can improve the diagnostic value of online monitoring during milking.