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Corrigenda

18 COMPARISON BETWEEN THE EFFICIENCY OF 30-MG FLUROGESTONE ACETATE INTRAVAGINAL SPONGE (FGA-30) AND CONTROLLED INTERNAL DRUG RELEASE (CIDR) TO SYNCHRONIZE OESTRUS IN EWES

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In the text, the term ‘Key Square’ should be replaced by ‘Chi Square’.

sequence of one of the peptic peptides was WSEKYGIPGGKAH. The amino acid sequence showed a high homology with tyrosine-protein kinase ZAP-70. These results suggest that boar SG contains mucin-like glycoproteins carrying heavily sialylated *O*-glycans. Additionally, the current study suggests a possibility that some protein components of the boar SG derive from high concentration of the kinase in (dead) sperms.

17 EFFECT OF LOCAL TREATMENT OF SEMINAL VESICULITIS ON THE QUALITY OF EQUINE FRESH SEMEN

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Stallions affected by seminal vesiculitis present history of infertility or subfertility, ejaculatory disturbance, spread of sexually transmitted pathogens, and changes in semen characteristics, leading to reduced semen quality and longevity. The aim of this study was to evaluate the semen quality of stallions with seminal vesiculitis before and after local treatment. Five stallions with a mean age of 12.4 years diagnosed with seminal vesiculitis were used. The identification of the microorganism involved in the pathogenesis of seminal vesiculitis of each animal was performed by bacterial culture of the seminal vesicles flush with Ringer Lactate solution, performed in duplicate at 1-week intervals. After identification of bacteria was performed, there was susceptibility testing to antibiotic (antibiogram) and the appropriate antibiotic was chosen. The local treatment was performed by endoscopy for 10 consecutive days, and this consisted of flushing with Ringer Lactate solution, followed by infusion of the antibiotic selected. The semen analyses were performed before starting the local treatment for seminal vesiculitis (M0), after a week (M1), and after a month (M2) of therapy. Sperm kinetics were performed by computerized method – CASA for the following parameters: percentage of sperm with total motility, progressive motility, and rapid sperm. Analysis of plasma membrane integrity was performed by epi-fluorescence microscopy, using the combination of fluorescent probes carboxyfluorescein diacetate and propidium iodide. Percentage of leukocytes was assessed through evaluation in light optical microscopy of semen smears stained with DiffQuick. The content of nitric oxide (NO) was determined by colourimetric Griess reaction by a spectrophotometer through the concentrations of nitrate (NO_3^-) and nitrite (NO_2^-). To perform the count of colony forming units per millilitre ($CFU\,mL^{-1}$), an aliquot of 0.1 mL of semen was diluted in 9.9 mL of saline. A 0.1-mL aliquot of this sample was plated on Mueller-Hinton agar. The seeded plates were incubated, and the bacterial colonies were counted after 24 h. According to the performed dilution, total colonies identified corresponds to $\times 10\,000\,CFU\,mL^{-1}$. The data were analysed by two-way ANOVA followed by Tukey's test ($P < 0.05$). The values (mean ± standard error) of seminal parameters on M0, M1, and M2 were the following, respectively: sperm kinetics (total motility: 46.5 ± 5.13^a; 75.1 ± 3.42^b; 42.8 ± 5.28^a; progressive motility: 19.3 ± 3.86^a; 33.4 ± 2.39^b; 16.5 ± 2.40^a; rapid sperm: 22.2 ± 1.82^a; 52.2 ± 5.65^b; 22.1 ± 2.62^a); plasma membrane integrity (47.5 ± 4.65^a; 62.9 ± 5.41^b; 39.1 ± 4.32^a); percentage of leukocytes (35.2 ± 2.36^a; 15.1 ± 2.55^b; 36.1 ± 4.04^a); CFU ($119\,980 \times 10^3 \pm 19\,528.0 \times 10^{3a}$; $5375 \times 10^3 \pm 2453.7 \times 10^{3b}$; $65\,850 \times 10^3 \pm 19\,701.0 \times 10^{3ab}$) on fresh semen; and NO content (0.645 ± 0.172^a, 0.117 ± 0.023^b, 0.364 ± 0.110^{ab}) on seminal plasma. The results demonstrate that local treatment after a week leads to an improvement in sperm quality; however, this was not maintained after 1 month of therapy, since the seminal parameters at this time are similar to pretreatment, which can be justified by recurrent disease.

18 COMPARISON BETWEEN THE EFFICIENCY OF 30-MG FLUROGESTONE ACETATE INTRAVAGINAL SPONGE (FGA-30) AND CONTROLLED INTERNAL DRUG RELEASE (CIDR) TO SYNCHRONIZE OESTRUS IN EWES

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The aim of the present study was comparing between the efficiency of FGA-30 (intravaginal polyurethane sponges impregnated with 30 mg of flurogestone acetate; Synchropart®, Ceva Sante, Animale, France) and EAZI-Breed™ CIDR® (an inert silicone elastomer impregnated with 0.3 g of natural progesterone; Pfizer Animal Health, Hamilton, New Zealand) to synchronize oestrus in ewes. Three hundred twenty multiparous ewes of 2 native breeds (Naimi and Najdi) were equally and randomly allotted into 2 groups: Group A (FGA-30, $n = 160$) and Group B (CIDR®, $n = 160$). Both methods were inserted intravaginally for 14 days with intramuscular administration of 600 IU of eCG (Synchropart® Ceva Sante, Animale, France) at withdrawal time. Retention, vaginal discharge, and drawstring breakage rates were calculated at withdrawal time. The standing oestrous was detected using a vasectomized ram starting 24 h after progestagen withdrawal and repeated every 12 h (24 h, 36 h, 48 h, 60 h, and 72 h). The blood samples were collected at the time of progestagen withdrawal, after 24 h, and after 48 h (at time of AI). Follicular stimulating hormone, LH, oestradiol (E2), and progesterone (P4) serum concentrations were measured using commercial ELISA kits and micro-titrimetric plates. Laparoscopic insemination was performed 48 h after progestagen withdrawal. Pregnancy was diagnosed by ultrasonography at day 23 after insemination and confirmed at Day 35 and 60. Number of fetuses was recorded and confirmed at lambing. Pregnancy rate, fecundity percentage (litter size percentage), and prolificacy percentage (lambing rate) were calculated. The SAS programme was used for all analyses. Data were expressed in percentages except hormone levels, which were expressed as the mean ± standard error. Comparisons among groups were evaluated using Key Square in all measured parameters except hormone levels, which were evaluated using an analysis of variance (ANOVA) test. A difference was considered significant at $P < 0.05$. The results revealed that retention rate was insignificantly different between the two groups. Drawstring breakage was observed only in FGA-30 and was absent in CIDR (9.33% *v.* 0). Moreover, vaginal discharge rate was significantly higher in FGA-30. Oestrus response was significantly higher in CIDR at 24 h and 48 h after progestagen withdrawal. Oestradiol and progesterone serum levels were significantly higher in CIDR, whereas LH and FSH serum levels showed insignificant differences. Pregnancy rate, twining rate, prolificacy, and fecundity were

significantly higher in CIDR (75.71%, 33.96%, 1.34 and 1.01, respectively). These results show that although FGA and CIDR devices are efficient in synchronizing oestrus in ewes, CIDR provides higher oestrus response rate, pregnancy rate, twining rate, prolificacy, and fecundity. Consequently, the use of CIDR is recommended.

Cloning/Nuclear Transfer

19 GENOME-WIDE ANALYSIS OF DNA METHYLATION IN CLONES AND NONCLONES OF TWO DIFFERENT BREEDS: HOLSTEIN AND JAPANESE BLACK

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Epigenetic marks, and especially DNA methylation, are at the interplay of both environmental and genetic factors. By facilitating the metabolic adaptation of highly selected rent animals to their environment, DNA methylation could contribute to the phenotypic differences observed between breeds. The aim of this study was to assess to which extent the methylome of 2 specialised cattle breeds – the dairy breed Holstein and the beef breed Japanese Black – could show some variability. We focused on the liver, which has a central role in metabolism and is therefore most susceptible to be affected by genetic and environmental variations. For each breed, both cloned and noncloned animals were included in the study. We used 9 adult Holstein cows aged from 5 to 15 years (5 healthy clones generated from ear skin fibroblasts of 4 genotypes, 2 cell donors obtained by AI and 2 other AI controls of unrelated genotypes, and 11 Japanese Black cows aged from 4 to 10 years (5 healthy clones generated from cumulus cells of one genotype and 6 AI controls of unrelated genotypes). The Holstein breed and Japanese Black breed were therefore represented by 6 and 7 genotypes, respectively. Liver samples were snap-frozen after slaughtering, and genomic DNA was extracted. To identify methylated regions, we used immunoprecipitation of methylated DNA followed by hybridization on a bovine promoter microarray (MeDIP-chip). The microarray targets the upstream region (−2000 to +1360 bp) of 21 416 genes (UMD3.1 assembly). After normalization of the data, enriched probes were identified using ChIPmix (Martin-Magniette *et al*. 2008). Results of exploratory analysis, including correlation clustering and principal component analysis, show a clear separation between the two breeds. A statistical test based on differences in the proportion of the enriched probes was used to identify differentially methylated regions (DMR) related to cloning and breed (Spatstat R package; http://www.spatstat.org/spatstat/). Only a restricted number of cloning-related DMR could be found (240). Interestingly, most of these DMR showed no overlap between Holstein and Japanese Black animals, maybe reflecting the different origin of the somatic cells used for cloning (fibroblasts *v.* cumulus cells). In contrast, we identified an important number of breed-related DMR (3642). These DMR were significantly enriched in genes involved in placental development and lactation, suggesting an adaptation of the two breeds to the different metabolic demand during gestation. Whether these epigenetic differences rely on environmental variations or genetic polymorphism remains to be elucidated.

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20 NUCLEAR TRANSFER ALTERS EXPRESSION AND HISTONE MODIFICATIONS OF THE IMPRINTED GENE *PHLDA2* IN THE BOVINE PLACENTA

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Proper implantation and placental formation are crucial for the continuity of mammalian species. Embryonic and placental developments are under intense genetic and epigenetic control, such as the regulation of differentiation of pluripotent cells into highly specialised fetal and placental cells. In the present study the objectives were to evaluate expression and epigenetic control of the imprinted gene *PHLDA2*, a maternally expressed gene that appears to be a regulator of placental growth, in cotyledonary and inter-cotyledonary tissues of bovine placentas on Day 60 of pregnancies produced by embryo transfer (ET; $n = 3$), *in vitro* fertilization (IVF; $n = 5$), and nuclear transfer (NT; $n = 6$), by real time PCR (qPCR). *In vitro* culture of IVF and NT embryos was performed in SOF medium supplemented with 2.5% fetal bovine serum, at 39°C in a humidified atmosphere of 5% CO_2 and 5% O_2 for 7 days. For evaluation of gene expression, gene-specific standard curves were used, and results were analysed as a ratio to 2 separate housekeeping controls (*GAPDH* and β-actin). Chromatin immunoprecipitation followed by qPCR (ChIP-qPCR; precipitated/total input DNA) was also performed on the proximal promoter region of *PHLDA2*, with antibodies against H3K4me2 (permissive histone modification) and H3K9me2 (inhibitory histone modification) in these samples. Products of the ChIP-qPCR for *PHLDA2* were digested with a restriction enzyme (*AciI*) that recognises a specific sequence of the maternal allele (*Bos indicus*), separating it visually on a gel, from the paternal allele (*Bos taurus*). Digestion