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PW1263 - Anti-sense identification of gene expression in live bovine embryos

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We have investigated the application of a commercial anti-sense labelling system as a means of identifying gene products in live bovine pre-implantation embryos.

Bovine embryos were produced by IVF. On day 6, morulae were allocated to 2 groups. Group 1 were cultured in media containing 400pM Cy-3 fluorophore to assess embryo uptake of an un-targeted label. Group 2 were cultured in media containing 400pM Cy-3/beta-actin anti-sense probe, to assess ability to specifically label an abundant mRNA. Incubation times ranged from 16-72h. Visualisation of fluorescence used either a TRITC or G-2A (Nikon) filter system. In order to overcome embryo autofluorescence interference in the 480-650 nm wavelengths (FITC/TRITC), images acquired using a FITC and TRITC filter set were overlayed, and colocalisation was excluded using the RG2B Colocalisation plug-in for ImageJ.

Label uptake did not occur until blastocyst stage, then increased with subsequent developmental stages. Early expanded blastocysts showed labelling in a few peripheral cells. This increased to most peripheral cells prior to hatching, and all cells took up label after hatching. This labelling pattern was similar for both groups, as was label intensity. For hatched blastocysts label identification could be easily distinguished from autofluorescence. For expanded blastocysts it was necessary to exclude autofluorescence in order to accurately visualise the label. Exposure for > 48 h to the un-targeted label did not appear to have any detrimental effect on embryo development as far as hatching. However, > 48 h exposure to the beta-actin anti-sense label appeared to halt embryo development at the late blastocyst/early hatching stage.

This labelling system may have potential for localisation of gene expression in hatched embryos. However, restricted label uptake in blastocysts precludes localisation studies at earlier stages of development. As a diagnostic tool, this technique would have potential for identifying specific genes of interest. Only a few cells would need to be labelled in order to afford a diagnosis. This would, however, require that the target be expressed ubiquitously, and in sufficient copy number to allow visualisation. Anti-sense activity of the label must be taken into account when designing probes for diagnosis. Anti-sense labels bind to RNA, and have the potential to block RNA activity. This may have detrimental effects on embryo development, as appeared to happen with continued exposure to the beta-actin anti-sense label. This might be avoided by restricting diagnosis to early expanded blastocysts, in which label uptake is limited to a few cells. Confining the label to 2-3 cells may protect the remaining embryo from the inhibitory effects of anti-sense strands. Therefore we suggest if the detrimental effect of anti-sense strands were limited, and the target chosen appropriately, this system could be used to select embryos based on presence or absence of a single gene product.

PW1264 - Production of dairy embryos: influence of donor breed and crossbreeding strategy

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In vitro embryo production-IVEP industry has been shifting the focus on animal breeding programs to large scale production of commercially valuable crossbreds such as Gir-Holstein F1. Profit of the industry depends on scale and efficiency rather than on use of highly valuable donors. Previous studies demonstrated that zebu donors produce more oocytes per OPU session compared with European breeds. However, quite less zebu donors of high genetic merit are available, comparing to breeds such as Holstein. This study tested the effects of breed and predicted impact of donor selection on the efficiency of dairy F1 embryo production, based on analysis of data from an IVEP commercial laboratory. Cumulus oocyte complexes (n=37,492) were collected by transvaginal ultrasound-guided OPU from Gir (n=1,497) and Holstein (n=631) donors. Embryos

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were produced in vitro by a single laboratory. Oocytes were fertilized with X-sorted semen from Gir or Holstein bulls, resulting in the following breeding groups (dam x sire): G1-HolsteinXHolstein (n=427); G2-GirXGir (n=239); G3-HolsteinXGir (n=204); G4-GirXHolstein (n=1,258). OPU and IVEP outcomes were examined for the effects of dam breed and breeding groups and IVEP efficiency was analyzed among donors ranked in quartiles according to their total oocyte production. Analyses were performed using the PROC GLM of the SAS software. As expected, Gir donors produced more total, viable, and grade I oocytes, as well as higher percentage of viable oocytes compared with Holstein (19.4±0.4 vs 13.4±0.4; 10.3±0.2 vs 7.3±0.3; 1.0±0.0 vs 0.4±0.1; and 53.2±0.4% vs 50.6±0.8%, Gir and Holstein, respectively; P<0.0001). Groups G2 and G4 produced more embryos than groups with G1 and G3 (2.1±0.2, 4.2±0.3, 2.5±0.2, and 3.9±0.1; G1,G2,G3, and G4, respectively; P<0.0001). Embryo production was affected by sire breed, with similar results between the two F1 groups (G3 and G4; P>0.05), but different between purebred groups (G1 and G2; P<0.001) (28.1±1.5%c; 40.6±2.0%a; 31.4±2.2%bc; and 37.1±0.8%ab for G1,G2,G3 and G4, respectively). In both breeds, continuous outcomes increased (P<0.0001) according to the donor's rank in quartiles of total COC recovered. Viable oocytes (6.7±0.1 to 19.7±0.4; quartiles 1 vs 4, respectively), grade I oocytes (0.1±0.0 to 2.3±0.1), and embryos (1.5±0.1 to 7.2±0.3) in Gir, and viable oocytes (2.0±0.1 to 15.9±0.8) grade I oocytes (0.1±0.0 to 1.0±0.2), and embryos (0.6±0.1 to 4.6±0.4) in Holstein donors. Neither breeds affected cleavage or blastocyst rates among quartiles (P>0.05). In conclusion, a greater oocyte production by Gir donors lead to a greater number of in vitro embryos, but breed of the sire led the F1 embryo production efficiency to rates similar between Holstein and Gir donors. Moreover, the selection of donors according to oocyte production in both breeds will mostly improve embryo production, without affecting IVEP efficiency. This strategy may therefore be used to optimize F1 embryo production.

PW1265 - Apoptotic rate of bovine embryos cultured in medium with different concentrations of antioxidant extracted from Lippia origanoides oil

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The *in vitro* production of bovine embryos (IVEP) is a biotechnology alternative to increase the genetic improvement of the herd. However, the culture conditions that are commonly used may cause damage to embryos, such as oxidative stress. Seeking to improve the culture media and reduce apoptosis of embryonic cells, various antioxidants have been added in the medium. This study aims to evaluate the apoptotic index in IVEP, using the extracted antioxidant oil *Lippia origanoides* in maturation and culture media. Ovaries were used slaughterhouse cattle, transported in saline 0.9% NaCl and there 38.0°C to the laboratory. The follicular aspiration was performed with a needle (12Gx40) coupled to a 5ml disposable syringe, and follicular fluid was placed in 15mL tubes for 10 minutes for sedimentation of oocytes. The supernatant was removed and the excess was washed in TCM-HEPES 199. We selected only those oocytes degrees 1 and 2, according to the analysis of complex cumulus oophorus cells and quality cytoplasm. The oocytes were washed in:TCM bicarbonate supplemented with 10% fetal bovine serum, 22mg/ml sodium pyruvate, 50ug/ml gentamicin sulfate, 5µg/ml of LH, 1µg/ml of FSH, 10ug/ml estradiol and 2.5mg/ml of *Lippia origanoides* antioxidant and then were incubated in atmosphere with 5% CO2, 38.5°C for 22 to 24 hours. Then, the oocytes were inseminated with $1x10^6$ sperm *in vitro* fertilization medium and incubated in a humidified atmosphere with 5% CO2, 38.5°C for 18 to 20 hours. After this period, the oocytes were denuded and directed to five treatments containing m-SOF (modified) without adding antioxidant (T1), medium supplemented with 50uM/ml cysteamine (T2) and medium supplemented with 2.5; 5.0 and 1ug/ml of antioxidant *Lippia origanoides* (T3, T4 and T5, respectively). The Shapiro-Wilk test was used to assess the normality of continuous variables. Statistical analysis was performed using the ANOVA (post hoc Tukey) and Kruskal-Wallis. The significance level was set at p<0.05. The number of embryonic cells T1 was 163.27 ± 11.68, higher than those found in T3, T4 and T5 (91.44 ± 7.20, 112.36 ± 9.77, 114.57 ± 9.59, respectively) (p <0.05), similar to T2 (130.67 ± 14.33) (p>0.05). Apoptosis rate had the highest value in T1 (10.01 ± 1.44) and T3 (8.41 ± 2.49) (p> 0.05). Treatments T2, T4 and T5 showed the lowest values (4.54 ± 1.06, 3.44 ± 1.10, 5.52 ± 0.91, respectively) (p>0.05), differing from the T1 treatments and T3 (p<0.05). The blastocyst rate was 40.4; 28.6;24.1;30.7

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ABSTRACT BOOK

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