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procedure. One-way ANOVA followed by Duncan's multiple range tests was performed. The significance level was $<0.05$. In total, 446 oocytes were recovered from 60 bitches, with an average of 6.4 oocytes/dog in Group A (from 49 bitches), 16.6 oocytes/dog in Group B (from 5 bitches), and 8.5 oocytes/dog in Group C (from 6 bitches). The oocytes collection rate (number of oocytes per dog) in Group B was higher ($P < 0.05$) than those in the other 2 groups. In conclusion, the results showed that eCG treatment with hCG in early-proestrus-stage bitches can result in a greater number of recovered *in vivo* matured oocytes. This technology could become a useful research tool for canine cloning and ART.

## 323 EFFICIENCY OF CLOPROSTENOL-INDUCED LUTEOLYSIS IN SUPEROVULATED COWS

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The induction of multiple ovulations is a key procedure for *in vivo* embryo production. Many corpora lutea (CL) are developed, resulting in abnormally high progesterone concentrations. Luteolysis induction by prostaglandin $F_{2\alpha}$ and its analogues is well described in cows bearing one or few, but not multiple, CL, as occurs after superovulation. The objective of the first experiment was to compare the efficacy of a single prostaglandin $F_{2\alpha}$ treatment on inducing luteolysis in embryo donors immediately after flushing (D7, $N=24$) or 4 days later (D11, $N=22$). Holstein cows were superovulated with 400 IU of FSH following standard procedures. Embryo flushing was performed 7 days after AI, and cows were randomly allocated into 2 groups to receive either a 0.5 mg of sodium cloprostenol IM treatment immediately after flushing (D7 group) or the same treatment 4 days later (D11 group). Occurrence of luteolysis was monitored by plasma progesterone concentrations (P4), measured by radioimmunoassay in blood samples taken at 4-h intervals. There was no difference in P4 before treatment between D7 and D11 groups ($28.6 \pm 5.2$ *v.* $36.4 \pm 7.4$ ng mL$^{-1}$, respectively; $P > 0.05$). Although cloprostenol caused a remarkable decline in P4 48 h after treatment in both groups ($1.8 \pm 0.3$ and $1.6 \pm 0.4$ ng mL$^{-1}$ for D7 and D11 groups, respectively; $P < 0.05$), P4 continued decreasing in D11 cows thereafter, remaining smaller than 1 ng mL$^{-1}$ up to 196 h after treatment, whereas in D7 cows, there was no further reduction in P4. Luteolysis (P4 $<1$ ng mL$^{-1}$) was observed in all D11 cows, but failed in 11 of 20 (55%) D7 cows, in which P4 increased after the initial cloprostenol-induced decrease. The second experiment compared luteolysis in superovulated *v.* nonsuperovulated cows. Non-superovulated (control group, CG, $N=8$) and superovulated cows (SOV, $N=6$) received a single dose of sodium cloprostenol IM (0.5 mg) on day 11 after oestrus. Morphological and functional luteolysis were monitored daily by ovarian ultrasonography and P4 analysis; also, plasma LH was measured in blood samples taken every 20 min for 1 h, during 5 days. Individual CL size was smaller ($1.8 \pm 0.1$ *v.* $3.5 \pm 0.4$ cm$^2$) but total luteal tissue was greater ($29.8 \pm 7.0$ *v.* $3.5 \pm 0.4$ cm$^2$; $P < 0.05$) in SOV than in CG. A considerable decrease in P4 occurred in both groups 24 h after treatment (from $51.1 \pm 12.9$ to $5.1 \pm 0.9$ ng mL$^{-1}$ in SOV and from $5.9 \pm 0.6$ to $1.1 \pm 0.1$ ng mL$^{-1}$ in CG); however, SOV cows did not reach P4 values similar to CG until 96 h after treatment ($0.9 \pm 0.3$ *v.* $0.4 \pm 0.2$ ng mL$^{-1}$, respectively; $P > 0.05$). There was no difference in initial LH values between SOV and CG ($1.5 \pm 0.1$ and $1.5 \pm 0.1$ ng mL$^{-1}$; $P > 0.05$), but the slower decrease in P4 in the SOV group prevented LH from increasing up to 96 h after luteolysis induction, whereas mean LH values increased ($P < 0.05$) in CG after 24 h. In conclusion, luteolysis failure may occur when cloprostenol is given at the day of flushing (7 days after AI) in superovulated cows. In addition, luteolysis induction on day 11 after SOV is efficient, but the initial high progesterone concentration results in a slower rate of P4 decrease to basal levels.

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*Transgenesis*

## 324 NEGATIVE SELECTION DOES NOT FURTHER INCREASE THE EFFICIENCY OF A PROMOTER TRAP

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Every year more than 100 000 people need organ transplants and in 2009 there were only 20 139 deceased organ donors. Genetically modified swine may be able to fill this unmet clinical need. It is clear that multiple transgenes will be required to make porcine organs or cells compatible with human recipients. Efficiencies in production of xenotransplantation pigs could be gained if multiple transgenes could be sequentially stacked at a single locus. The larger purpose of this project is to evaluate a site-specific recombination system as a tool to sequentially add multiple transgenes to the porcine α galactosyltransferase (α1,3-Gal) locus. One xenotransplantion related transgene, human decay accelerating factor (hDAF), is likely to be a part of any successful project because hDAF can prevent acute rejection by deactivating the complement system. To generate transgenic pigs that express an hDAF transgene that is genetically linked to an α1,3-Gal disruption, an α1,3-gal gene targeting vector was constructed with homologous arms that were 4.8 kb (5′ arm) and 1.8 kb (3′ arm) and were derived from genomic sequence from intron 8 and exon 9 of the α-Gal locus. Within exon 9, an IRES-Neo cassette and a CAG-hDAF cassette were inserted. A Phi-C31 AttB site was also included in the construct to later receive additional transgenes via a site-specific recombination system. This vector (pBB7) is designed to utilise a promoter-trap strategy as a method of enrichment for targeting events. We hypothesised that further enrichment could be obtained by the addition of a negative selectable marker. The goal of the experiment presented here was to evaluate this hypothesis by adding a truncated diphtheria toxin cassette (TdT) to pBB7 as a negative selection directed against random integration events. Two additional plasmids were constructed that harbored TdT, pBB8.1 (single TdT addition) and pBB8.2 (addition of 2 TdT cassettes). Four treatments (Trt) were performed in triplicate: Trt1, pBB7; Trt2, pBB8.1; Trt3, pBB8.2; and Trt4, pBB7 co-transfected with TdT. Targeting efficiencies were 13.9, 11.1, 5.6, and 6.1%, respectively. Successful targeting was assessed by PCR with primers specific for a targeting event. The first pair of primers flanked the 5′ arm of the construct and the neomycin resistance gene cassette. The second pair of

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