84 Actions of DKK1 on the bovine embryo during the morula-to-blastocyst stage of development on pregnancy outcomes and placental hormone secretion after embryo transfer

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Dickkopf-1 (DKK1) is an embryokine expressed in the bovine endometrium that has been reported to act on the bovine embryo between Days 5 and 7 of development to increase the number of trophectoderm cells in the blastocyst and to improve survival after embryo transfer (ET). Three experiments were conducted to further examine effects of DKK1 on embryo survival and circulating concentrations of pregnancy-associated proteins (PAG) and placental lactogen. Beef oocytes retrieved from slaughterhouse ovaries were fertilised with Y-sorted (experiments 1 and 3) or X-sorted (experiment 2) Angus semen. Cultured embryos were treated at Day 5 after fertilisation with either vehicle (V) or 100 ng mL^{-1} recombinant human DKK1. At Day 7.5, blastocysts were selected and transferred to either beef (Exp 1 and 2) or dairy recipients (Exp 3). Either a single embryo (Exp 1 and 3) or two embryos (Exp 2; both ipsilateral to the corpus luteum) were transferred. Data were analysed by the GLIMMIX and GLM procedures of SAS (SAS Institute Inc.). Treatment did not affect pregnancy rate at Day 30 of gestation, calving rate or pregnancy loss ($P > 0.05$). Values (V vs. DKK1) for pregnancy rate at Day 30 were 46.2% (24/52) vs. 43.1% (22/51) (Exp 1); 53.3% (16/30) vs. 57.0% (45/79) (Exp 2); and 32.8% (21/64) vs. 26.7% (16/60) (Exp 3). Pregnancy loss from Day 30 to 60 of gestation was 8.3% (2/24) vs. 9.1% (2/22) (Exp 1); 6.3% (1/16) vs. 15.6% (7/45) (Exp 2); and 28.6% (6/21) vs. 18.8% (3/16) (Exp 3). Calving rate was 40.4% (21/52) vs. 37.3% (19/51) (Exp 1) and 23.4% (15/64) vs. 20.0% (12/60) (Exp 3). Cows have not yet calved for Exp 2. For Exp 1, PAG concentrations at Day 160 were lower for V than for DKK1 (12.7 ± 1.6 ng mL^{-1} vs. 16.3 ± 0.9 ng mL^{-1}; $P = 0.051$). Treatment did not affect PAG concentration at Day 264 or concentrations of placental lactogen at Days 160 or 264. For Exp 3, there was no effect of treatment on PAG concentration at Day 36 or 64 of gestation. In conclusion, there was no evidence that DKK1 treatment enhances pregnancy outcomes. Effects on PAG concentrations implies that DKK1 causes long-term changes in placental function. Further work will be performed to determine postnatal changes associated with DKK1 treatment.

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85 Anti-Müllerian hormone in Holstein heifers and reproductive performance after fixed-time embryo transfer

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Anti-Müllerian hormone (AMH) has been associated with improved embryo production and fertility outcomes after AI in cattle. Its association with fertility in embryo transfer recipients, however, has not been explored. The aim of this study was to investigate the relationship between circulating AMH and reproductive performance of recipient heifers. Holstein heifers ($n = 750$), 15.7 ± 0.1 months of age, with moderate body condition score (3.2 ± 0.1; scale 1 to 5) were synchronised using a modified 5-day CO-Synch protocol as follows: Day −8: an intravaginal progesterone (P4) implant (CIDR®, 1.38 g P4, Zoetis) was inserted; Day −3: CIDR was removed followed by administration of prostaglandin $F_{2\alpha}$ analogue (PGF; 500 μg cloprostenol, Parnell); Day 0: administration of gonadotrophin-releasing hormone (GnRH; 100 μg gonadorelin, Parnell). On Day −3, all heifers received an Estrotec® patch, which was checked for evidence of mounting activity on Day 0. On Day 5, heifers were examined by ultrasonography to determine the presence and size of the corpus luteum (CL). On Day 7 ± 1, heifers with a CL received an embryo, and pregnancy was determined using ultrasonography 30 and 60 days after GnRH. Blood samples were collected on Day −8 from all heifers and on Day 5 from a subset ($n = 222$) of heifers, for AMH (Bovine AMH ELISA, AnshLabs®) and P4 (ImmuChem™ RIA; MP Biomedicals) measurements, respectively. Heifers were classified into low (129.0 ± 2.7 pg mL^{-1}; $n = 248$), medium (258.1 ± 2.5 pg mL^{-1}; $n = 247$), and high (554.4 ± 22.8 pg mL^{-1}; $n = 255$) AMH based on tercile distribution. Data were evaluated using generalised linear mixed models (SAS 9.4; SAS Institute Inc.). Corpus luteum volume tended ($P = 0.08$) to be larger in high AMH heifers (3669 ± 133 mm^3) than in low (3320 ± 112 mm^3) and medium (3384 ± 132 mm^3) AMH heifers. Conversely, serum P4 was not different ($P = 0.59$) between heifers with low (2.2 ± 0.1 ng mL^{-1}), medium (2.0 ± 0.1 ng mL^{-1}), or high (2.0 ± 0.1 ng mL^{-1}) AMH. There was no effect of AMH group on the percentage of heifers that expressed oestrus ($P = 0.13$) nor in the recipient utilisation rate (transferred/treated; $P = 0.87$; Table 1). Pregnancies per embryo transfer (P/ET) at Days 30 and 60 were not different between AMH groups ($P > 0.50$; Table 1). Similarly, pregnancy loss between Days 30 and 60 was not different between AMH groups ($P = 0.20$; Table 1). As a result, pregnancies per treated (P/treated) heifer at 30 and 60 days were not different ($P > 0.50$) between AMH groups (Table 1). In conclusion, the overall reproductive performance of recipient heifers submitted to fixed-time embryo transfer is not associated with circulating AMH concentration.