Animal Reproduction

Abstracts - 37th Annual Meeting of the Association of Embryo Technology in Europe (AETE)

Embryology, developmental biology, and physiology of reproduction

HH5 double-carrier bovine embryos show impaired development through elongation

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Genomics analyses in dairy cows have uncovered deleterious haplotypes (alleles) never found in homozygosity at birth and thereby causing pre-term mortality. Between these deleterious haplotypes, Holstein Haplotype 5 (HH5) consists of a large 138 kb deletion causing the ablation of the gene *TFB1M* (Transcription Factor B1, Mitochondrial). Unfortunately, the developmental period when double carrier embryos (DC, i.e., *TFB1M*-null embryos, homozygous for HH5) arrest their development is unknown. Given that the impact of pregnancy loss varies greatly from early losses (before maternal recognition of pregnancy) to later miscarriages, such information is crucial to evaluate the economic losses associated to the inadvertent cross of single carrier (SC, heterozygous) individuals. To solve that question, we have analysed the development of Day 14 conceptuses collected from 3 superovulated SC cows inseminated with a SC bull. Conceptus were recovered by uterine flushing, fixed in 4% paraformaldehyde and subjected to immunohistochemistry to analyse trophectoderm (CDX2), hypoblast (GATA6) and epiblast (SOX2) development. Following fluorescence microscopy analysis, samples were genotyped by PCR. Mendelian inheritance of the allele was observed in the 25 conceptuses retrieved (5:16:7 for WT:SC:DC). Hypoblast migration was observed in all conceptuses, but a significant impairment in the development of the extra-embryonic membranes (hypoblast and trophectoderm) was evident in DC embryos. The development of such membranes is the main responsible of the increase in conceptus length and the change in shape during elongation. DC conceptuses remained spherical, in contrast to the ovoid or elongated WT (non-carrier) or SC conceptuses, and were significantly smaller (26.2±8.5 vs. 30.5±4.9 vs. 0.7±0.1 mm for WT, SC and DC, respectively, mean±s.e.m., ANOVA $p<0.05$). Embryonic disc was formed in all DC embryos, but its diameter was also significantly reduced compared to WT or SC embryos (319±40 vs. 396±27 vs. 223±13 µm for WT, SC and DC, respectively, mean±s.e.m. ANOVA $p<0.05$). In conclusion, the bovine HH5 DC embryos analyzed arrested their development prior to early conceptus elongation and maternal recognition of pregnancy. Supported by StG-757886 from ERC and PID2020-117501RB-I00 from MINECO.

Keywords: Embryo development, conceptus elongation, haplotype, dairy cows, mitochondria.