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# Effect of conceptus size on embryo-maternal communication during early pregnancy in cattle

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Conceptus elongation is an essential prerequisite to maternal recognition of pregnancy and implantation in cattle. During elongation, the trophoblast cells secrete interferon-tau (IFNT), which prevents the upregulation of oxytocin receptors in the endometrium required for prostaglandin-induced luteolysis. Large variation exists in the length of conceptuses recovered on the same day of gestation, which may reflect an inherent lack of developmental competency. For example, larger conceptuses produce more IFNT, but the underlying factors that regulate conceptus-maternal crosstalk between advanced (large) or retarded (small) conceptuses and the endometrium are unknown. Thus, the aim of this study was to interrogate the response of the endometrium to Day 15 conceptuses of different sizes by examining the global transcriptome profiles of uterine explants exposed to large vs small conceptuses. First, 10 grade 1 in vitro produced blastocysts were transferred into synchronized recipient heifers on Day 7 for further development. The resulting conceptuses were recovered on Day 15 by post-mortem uterine flushing. Seven large (mean length  $\pm$  SEM 25.4  $\pm$  5.7 mm) and six small conceptuses (1.8  $\pm$  0.3 mm) were individually placed on top of endometrial explants that had been collected from uteri during the late luteal phase of the estrous cycle, and co-cultured for 6 h in one mL of RPMI media. Additional explants were cultured with media containing 100 ng/mL of recombinant ovine IFNT (IFNT; n=6) to identify endometrial responses dependent and independent of IFNT or in media alone (Control; n=6). Total RNA was isolated from explant cultures and analysed by RNA-Seq. Exposure of endometrium to IFNT, a large conceptus or a small conceptus altered (P < 0.05) expression of 491, 498 and 230 transcripts, respectively, compared with control endometrium. Further, 223 differentially expressed transcripts were common between conceptus-treated and IFNT-treated explants, and classical interferon-stimulated genes (e.g., RSAD2 and ISG15) were amongst the most upregulated transcripts compared to control endometrium. In addition, 369 transcripts were uniquely altered in explants exposed to large conceptuses and IFNT. Of these transcripts, 101 and 100 were specific to large conceptuses and IFNT-treated endometrium, respectively, while 168 were common to both groups. Only 6 of 108 conceptus-induced differentially expressed genes were shared between small- and large conceptuses. Interestingly, 101 transcripts were exclusively regulated by large conceptuses; of these, PCSK1, TNNI3K, MPV17L, and IL17 were the most upregulated and TEPP, CACNAIL, AOP1, and HIP1 the most downregulated. This study provides new knowledge of differences in gene expression in endometrial tissue induced by large and small conceptuses. The results provide a better understanding of the underlying molecular pathways involved in embryo survival and maternal recognition of pregnancy in cattle. Funded by Irish Department of Agriculture, Food and The Marine through the Research Stimulus Fund (Grant number: 13/S/528).