Processes and Pathways Associated with In Vivo derived and In Vitro Produced Male and Female Bovine Conceptus Proteins

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Early pregnancy in cattle involves a protracted period of development whereby the conceptus remains unattached to the endometrium for nearly three weeks. During this time, the conceptus and endometrium secrete factors that reciprocally modulate their activities such as conceptus elongation, immune tolerance, and subsequent attachment. Studies suggest that bovine conceptus sex [male (MC) or female conceptus (FC)] and origin [*in vitro* produced (IVP) or *in vivo* derived (IVD)] impact the endometrial transcriptome via conceptus secreted proteins (CSP). Previously, we identified IVD and IVP-CSP that may impact the endometrial transcriptome. Here, we aimed to identify CSP and associated processes or pathways specific to conceptus origin within sex. To achieve this, Angus-Holstein heifers underwent estrus synchronization and were divided into one of two groups: 1) bred by artificial insemination on Day 0 to produce IVD conceptuses or 2) underwent IVP embryo transfer on Day 7 to produce temporally and sire-matched IVP conceptuses. On Day 16, IVD and IVP conceptuses were flushed from heifer uteri. The conceptuses were then used to develop the following treatments in 1 mL of RPMI medium: 1) culture medium only (base medium; n=7), 2) IVD conceptus only, or 3) IVP conceptus only. After 12 h, conceptuses were sexed using PCR and media from the treatments were collected and analyzed for protein content by LC-MS/MS. Proteomic data were analyzed to identify origin specific proteins within sex [IVP-MC (n=3) vs IVD-MC (n=4) and IVP-FC (n=2) vs IVD-FC (n=3)]. Compared to base medium, culture medium from IVD-MC had fewer DAP than the IVP-MC (564 and 838, respectively, $P<0.05$). Of the DAP, 262 and 535 were specific to IVD-MC and IVP-MC, respectively. Processes or pathways associated with the upregulated IVD-MC specific DAP (191) were related to complement activation whereas the upregulated IVP-MC specific DAP (490) were related to protein translation ($P<0.0001$; FDR<0.05). Compared to the base medium, culture medium from IVD-FC had more DAP than the IVP-FC (912 and 759, respectively, $P<0.05$). Of the DAP, 531 and 378 were specific to IVD-FC and IVP-FC, respectively. Processes or pathways associated with upregulated IVD-FC specific DAP (435) were related to glycolysis whereas upregulated IVP-FC specific DAP (317) were related to microtubule-based processes ($P<0.0001$; FDR<0.001). In conclusion, compared to IVD conceptuses of the same sex, IVP-MC may have increased translation and protein secretion whereas IVP-FC may have reduced glycolytic activity. This may partially explain why IVP male and female conceptuses are more susceptible to reproductive failure compared to those that are

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