In Vitro Reminiscence: Uterine Disturbances in Vivo Affect Respective Luminal Epithelial Cells Function in Vitro

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In cattle, adequate programming of uterine function is required to support pregnancy. The endometrium during diestrus and early pregnancy displays cellular responses that are consequences of prior long term and transient stimuli. Here, we tested a novel study paradigm to characterize endometrial cellular memory. Such paradigm consists of three steps: (1) treating cows, (2) collecting endometrial epithelial cells (BUECs) using a cytology brush (cytobrush) from the same cows, and (3) culturing and measuring responses from cow-specific BUECs. The hypothesis is that stimuli given to endometrium in vivo are retained as a cellular memory that remains after BUECs are isolated, cultured, and further stimulated in vitro. Objectives were to measure BUEC proliferation/migration and responsiveness to recombinant bovine interferon-tau (rbIFNT) in vitro: among cows that showed estrus (experiment 1, N=12; Exp1), cows that became or not pregnant to artificial insemination (AI; Exp2, N=21), cows that received (high) or not (normal) supplemental progesterone (P4; Exp3, N=24) and cows that received or not a cyclooxygenase 1/2 inhibitor (Flunixin; Exp4, N=12). Only cows that displayed estrus were included. BUECs were collected four days after estrus via cytobrush. The BUECs were cultured, propagated. Responsiveness to rbIFNT was measured by the expression of interferon stimulated genes (ISGs). Proliferation/migration was assessed by a wound healing assay. Confluent BUECs were mechanically displaced from a straight line running diametrically in the center of the well. Micrographs were taken every 2 hours for 24 hours using light microscopy. The horizontal distance between the two borders created by the displacement was recorded. The time point in which 50% of the horizontal distance was repopulated by BUECs was used for analysis. In Exp1, cows clustered according to the time interval to reach 50% of cellular repopulation. In Cluster A (N=5) 50% cellular repopulation happened 10 hours earlier than for Cluster B (N=7; P=0.001). rbIFNT responsiveness measured by *ISG15* was 11-fold greater on Cluster A than B (P=0.042). Expression of IFNT receptor (*IFNAR1*) was similar between clusters. In Exp2 there was no effect of pregnancy on the time to reach 50% of cellular repopulation. Pregnant cows had a greater proportion (19.5%) of proliferating cells (Ki67 positive) in the border of repopulation than non-pregnant cows (6.8%; P=0.012). BUEC from pregnant cows had 116.6% greater rbIFNT responsiveness than non-pregnant (P=0.09). In Exp3, the

proportion of cows from high-P4 (50%; 6/12) whose BUECs proliferated in culture was lower than normal-P4 (84%; 10/12; P=0.083). P4 did not affect the rbIFNT responsiveness in BUEC (P4*rbIFNT: P=0.3). However, BUECs from high-P4 cows had increased expression of *RSAD2* regardless of rbIFNT treatment (P=0.039). In Exp4, Flunixin in vivo did not affect cellular repopulation in vitro. BUECs from Flunixin treated cows had greater rbIFNT responsiveness (Flunixin*rbIFNT) for classical ISGs (*ISG15; P=0.08* and *RSAD2; P=0.008;* respectively) and showed decreased rbIFNT responsiveness for non-classical ISGs (*CST3; P=0.05* and *CST6;* P=0.03 respectively). In conclusion, physiological and pharmacological stimuli received by the endometrium in vivo were retained as cellular memory in BUECs, persisted in culture, and modulated BUECs proliferation/migration and responsiveness to rbIFNT, which are characteristics associated with fertility in cattle. Long-term implications of these findings include the possibility of establishing in vitro assays to understand endometrial biology and predict the fertility potential of cows. This study is supported by USDA NIFA Award Number 2022-67015-36839.