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The Periovulatory Endocrine Milieu Affects the Metabolomic Profile of the Oviductal Fluid in Beef Cows

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Oviductal secretions regulate the environment in which sperm storage and capacitation, fertilization, and early embryo development occur; however, molecular control of oviduct receptivity to the embryo is poorly understood. A model for receptivity based on the manipulation of the size of the pre-ovulatory follicle (POF) was used to compare oviductal fluid (OvF) composition on day 4 of the estrous cycle. The central hypothesis was that the size of POF modulates the periovulatory endocrine milieu and affects the composition of the OvF. Cycling, non-lactating, multiparous Nelore cows were presynchronized prior to receiving cloprostenol (large follicle [LF] group) or not (small follicle [SF] group), along with a progesterone (P4) device on Day (D) -10. Devices were withdrawn and cloprostenol administered 42–60 h (LF) or 30-36 h (SF) before GnRH agonist treatment (D0). As a result, greater proestrus estrogen concentrations, corpora lutea and early diestrus progesterone concentrations were also observed in LF group in comparison to SF group. Four days after GnRH-induced ovulation, the oviduct was dissected and lumen was flushed using 2 mL of sterile PBS to obtain OvF. The OvF was centrifuged to remove cells and debris. Next, the supernatant was frozen in liquid Nitrogen, and stored at -80°C for further analysis. Quantitative mass spectrometry was used to determine the concentration of 21 amino acids (AA), 21 biogenic amines (BA), 40 acylcarnitines (AC), 76 phosphatidylcholines (PC), 14 lysophosphatidylcholines (LP), 15 sphingomyelins (SM), 29 hexoses (HX), and 17 prostaglandins and related compounds (PGC). Multivariate analyses using the software MetaboAnalyst 3.0 were performed to identify which metabolites better explained the separation of experimental groups and which could be potentially used as markers of receptivity. Analytes with 50% or more of missing data were excluded from analyses. Partial Least Squares Discriminant Analysis (PLS-DA) was used to create a scores plot between the two groups and to identify the most important explanatory variables. The PLS-DA showed that the overall metabolite profiles of the LF-LCL and SF-SCL groups were significantly different and that samples from each group were divided clearly into two non-overlapping clusters. The most influential variables to separate the two groups included AAs, PCs, LPs and arachidonic acid. These results were further confirmed by univariate analyses. There were statistical differences in the concentration of 31 metabolites ($P \leq 0.05$) between groups. We concluded that the composition of the OvF is different between cows with contrasting receptivity and fertility status. Although further studies and analyses are needed, it could be assumed that molecules in OvF presenting different concentrations between groups can be used as biomarkers of receptivity. Additionally, it will be critical to identify the function of each of these compounds during early embryo development and to evaluate their potential use as supplements for in vitro production of embryos. The authors thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq): AMGD grant number 150844/2017-4 and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP): MB grant number 2011/03226-4.