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

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INTRODUCTION

Several studies showed that, when comparing cows that ovulate large (LF-LCL) vs. small follicles (SF-SCL), they process differences in their endometrial and oviductal receptivity to the embryo and therefore, in their fertility after artificial insemination. In previous studies, we showed that oviductal gene expression, morphology, and extracellular remodeling process are also different in those two groups. Therefore, our main objective was to compare the composition of the oviductal fluid (OF) between LF-LCL and SF-SCL cows.

METHODOLOGY

Cycling, non-lactating, multiparous Nellore cows (n=41) were randomly divided into two groups: LF-LCL and SF-SCL and submitted to a hormonal manipulation protocol, as shown in figure 1. As a result, greater proestrus estrogen concentrations, corpora lutea, and early diestrus progesterone concentrations were obtained in LF-LCL group in comparison to SF-SCL group. Four days after GnRH-induced ovulation animals were slaughtered. The oviduct ipsilateral to CL was dissected and lumen was flushed using 2 ml of sterile PBS to obtain OF. The OF was centrifuged, frozen in liquid Nitrogen, and stored at -80°C for further analysis.

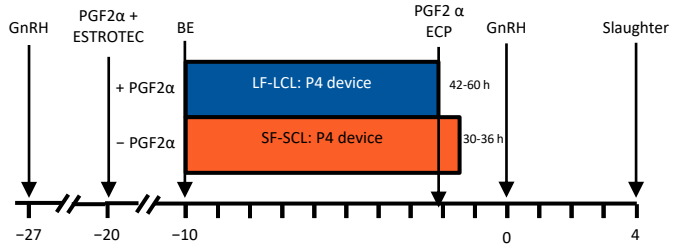

Figure 1. Hormonal manipulation protocol used in the present study. BE, injection of 2mg of estradiol benzoate (Sincrodiol; Ourofino, Cravinhos, SP, Brazil); GnRH, injection of 0.01 mg of buserelin acetate (Sincroforte; Ourofino); P4 device, P4-releasing device containing 1 g of P4 (Sincrogest; Ourofino); PGF2α, injection of 0.5 mg of sodium cloprostenol (Sincrocio; Ourofino); Slaughter, endpoint for sample collection.

On D4 cows were slaughtered. OF was recovered by washing the oviductal lumen with 2 ml of sterile PBS. After centrifugation (7000 g, 10 min, 4°C), OF was frozen in liquid Nitrogen, and storage at -80°C for further analyses. Quantitative metabolomic analysis was conducted in a private company (Biocrates INC; Insbruck, Austria). Quantitative mass spectrometry was used to determine the concentration of 223 metabolites.

METHODOLOGY (Cont.)

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 30% or more of missing data were excluded from analyses. Next, univariate analyses of the metabolite composition were performed using Proc Mixed (SAS 9.3 Institute Inc., Cary, NC, USA, 2003).

RESULTS

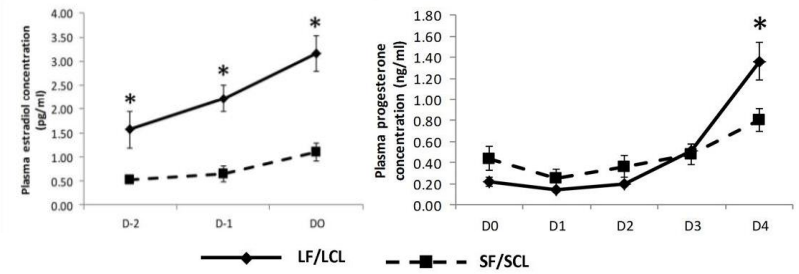

Figure 2: Plasma concentrations (mean ± SEM) of estradiol (left) and progesterone (right) of cows in the LF/LCL group (n = 13) and in the SF/SCL group (n = 8). Within a given Day, significantly different means (P < 0.05) were indicated by an asterisk (*). LF/LCL, large follicle-large CL; SF/SCL, small follicle-small CL.

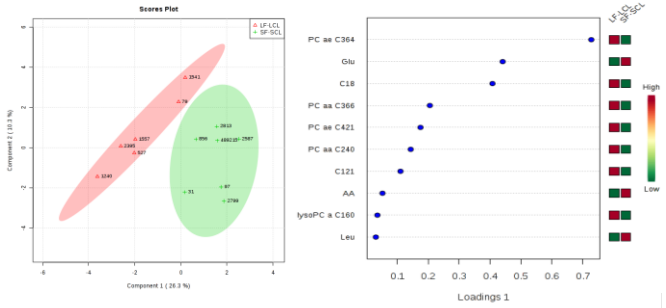

Figure 3. Multivariate analyses: discriminant metabolomic signature in the OF. PLS-DA score plot of the metabolic profiles in the oviductal fluid of the LF-LCL and SF-SCL groups (Left). Important variables identified by PLS-DA (Right); N=14; 172 were included in the analyses; R2 = 0.54; Accuracy = 0.61; Q2 = 0.16.

The most influential variables to separate the two groups included: Glutamate, Leucine, four phosphatidylcholines, three lysophosphatidylcholines, and arachidonic acid.

RESULTS (Cont.)

Univariate analyses further confirmed these results. There were statistical differences in the concentration of 31 metabolites (P ≤ 0.05) between groups.

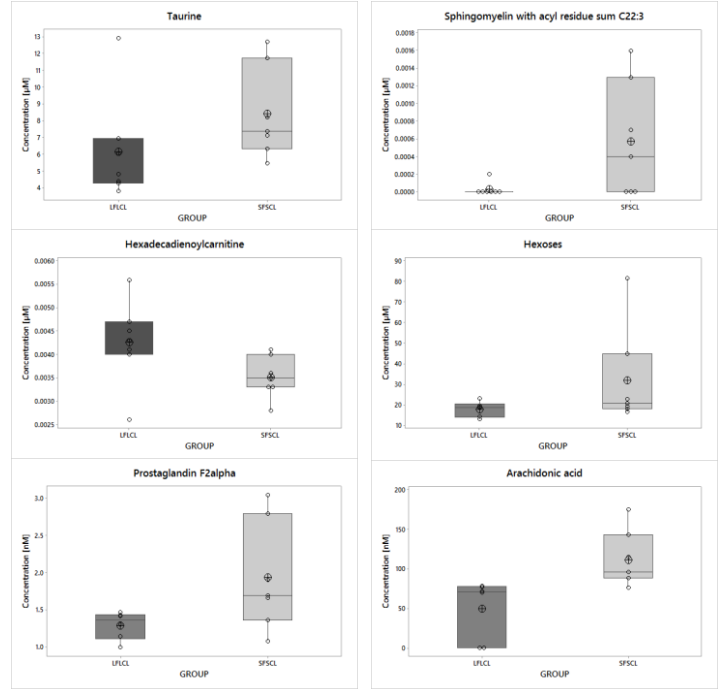

Figure 3. Box-and-wisker plot graphs of metabolites concentration (µM) values in OF collected from cows in the LF-LCL and SF-SCL groups. Due to the lack of space, only some of the metabolites presenting concentrations significantly different between groups (P ≤ 0.01) are shown. Individual values (circles), the mean value (cross circle) and the boxes and wiskers represent the interquartile amplitude.

CONCLUSION

We concluded that the composition of the OFL is different between cows with contrasting receptivity and fertility status. Further studies should be conducted to evaluate the physiological role of this compounds during embryo transport and development.