Animal Reproduction

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Nitric oxide acts as an epigenetic regulator of histone H3K9 acetylation in oviduct cells in luteal and follicular phase of estrous cycle

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Resumo

Reproductive events that occur in the oviduct depend on signaling molecules, e.g. nitric oxide (NO). The NO, a short-lived free radical, has a variety of cellular effects, including alterations in mitochondrial metabolism and participation in reproductive processes such as fertilization and preimplantation embryo development. Recently, NO has also been associated to Histone Deacetylase 2 (HDAC2) function in neurons, resulting in altered pattern of histone acetylation, with consequences to the gene expression. Thus, this study aimed to analyze whether differences in the NO availability in the bovine oviduct epithelial cells (BOEC) lead to changes in the mitochondrial metabolism and in the histone H3K9 acetylation profile (H3K9ac). BOEC were collected from a slaughterhouse from animals at luteal (L) or follicular (F) phases (set by ovarian morphology). In total, 12 animals were selected and divided in three replicates. In each replicate, BOEC of the same estrous cycle phase (n=2 animals/phase) were pooled and cultured in DMEM + 10% FBS (38°C, 5% CO_2, high humid) until 70% confluence, moment when the cells were submitted to the treatments. Cells were treated with S-Nitrosoglutathione (GSNO), a nitric oxide donor, in three concentrations: zero (control), 100 (GSNO100), and 500 µM (GSNO500). Therefore, six groups were analyzed according to the treatment and the estrous cycle phase: control follicular (CF), control luteal (CL), GSNO100F, GSNO100L, GSNO500F, and GSNO500L. The mitochondrial membrane potential (MMP) was analyzed by fluorescent probe and the H3K9ac levels by immunocytochemistry, both analysis were done at 0h (before treatment), 4h, 48h, and 96h after addition of GSNO (0, 100, and 500 µM). Microscopy images were processed by the Fiji package and the data analyzed by the GraphPad Prism software (Kruskal-Wallis test for non-parametric data and Tukey test for parametric data, $p<0.05$). The MMP remained constant in all samples, except for GSNO500F and GSNO500L, which was higher than CF and CL, respectively, at 48h of incubation. The H3K9ac profiles were distinct in each estrous phase. In the F phase, the control group assumed a V shape profile as time passes (high at 0h, low at 4h, lower at 48h, and up at 96h similar to 0h), while the GSNO treatments showed no effect at 4h, but both GSNO100F and GSNO500F were higher than CF at 48h, and GSNO500F were lower than CF at 96h. In the L phase, the profile of the control group was a gradual decrease over time (0h > 4h = 48h > 96h), while the GSNO100L treatment led to higher H3K9ac levels at 4h and 48h than their respectively control groups and the GSNO500L treatment had a later effect increasing the H3K9ac levels at 48h and 96h compared to their respectively control groups. In conclusion, NO acts to increase H3K9ac levels in oviductal cells, which might occur by inhibiting HDACs, however this modulation seems to be dependent on the estrous cycle phase.

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