

ABSTRACT BOOK



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M75 SPATIOTEMPORAL REGULATION OF SPERM-INTERACTING PROTEINS IN THE BOVINE OVIDUCT FLUID

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BACKGROUND-AIM

Interactions between proteins from the oviduct fluid (OF) and spermatozoa (spz) are crucial for the progressive acquisition of sperm fertilizing ability in mammals. However, only few sperm-interacting proteins (SIP) were identified and their regulation during sperm migration toward the fertilization site around ovulation time is currently unknown. Our aim was to identify OF SIP according to the (1) oviduct region (isthmus vs. ampulla) and (2) time relative to ovulation (pre- vs. post-ovulatory).

METHODS

The ipsilateral isthmus and ampulla from adult cows at both stages collected at local slaughterhouse were flushed separately (4 pools of 2-3 cows per region x time condition). Frozen-thawed Percoll-washed bull spz were co-incubated (40.106 spz/mL) with phosphate-buffered saline (controls) or OF flushes (spz-OF) at 3 mg/mL of proteins at 38.5°C for 1h. Sperm and OF proteins were extracted, Lys-C/trypsin-digested (iST kit, PreOmics) and analyzed by nano liquid chromatography coupled with tandem MS (nanoLC-MS/MS). Protein abundance was evaluated by spectral counting and normalized using the Scaffold software. Proteins were considered as SIP when meeting the following criteria: (i) identification in the OF and (ii) specific detection in spz-OF (no detection in controls) or higher detection in spz-OF than in controls (p-value ≤ 0,05) with a minimum fold-change ratio of 3. Prediction of secretion pathways and functions were assessed by Outcyte, SignalP and Metascape online tools.

RESULTS

In total, 2220 proteins were identified among which 228 SIP were detected, with 28% predicted to be secreted and 78% previously reported in oviduct extracellular vesicles. SIP included MYH9, OVGPI and GRP78 as the most abundant ones. The highest number of SIP (170) and specific SIP (30) were found in the pre-ovulatory isthmus, i.e. time and place of the sperm reservoir. Among the 68 SIP shared between conditions, 9 were differentially abundant between regions at a given stage and 7 between stages in a given region. Only 11 SIP have previously reported roles in fertilization and establishment of pregnancy.

CONCLUSIONS

This study provides for the first time an exhaustive list of SIP and highlights important region- and time- specific interactions between bull spz and OF proteins

M76 EVALUATION OF OXIDATIVE STRESS AND PERIPHERAL CONCENTRATIONS OF INFLAMMATORY CYTOKINES IN DAIRY COWS WITH POSTPARTUM METRITIS

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BACKGROUND-AIM

Postpartum (pp) dairy cows suffering from metritis may experience greater oxidative stress (OS) in comparison to healthy herdmates. This OS might occur due to the consequences of dysregulation of systemic inflammation in the early postpartum. This study aims to assess OS indicators and inflammatory cytokines in the serum of pp dairy cows and their use as potential markers for metritis.

METHODS

Twenty-five Holstein cows were weekly blood sampled from 7 ± 2 to 35 ± 2 dpp. Eleven cows were diagnosed with metritis (abnormal vaginal discharge, enlarged uterus, and >39.5°C within 21 dpp). Oxidative stress markers like reactive oxygen metabolites (d-ROM), antioxidants (OXY), and oxidative status index (OSI) (evaluated via photometric determination of plasma thiols) and inflammatory cytokines like pro-inflammatory cytokines (TNF-α and IL-6) and the anti-inflammatory cytokine (IL-10) (all evaluated via ELISA) were analyzed in the serum samples. Statistical analyses were done via ANOVA, accounting for repeated measurements.

RESULTS

Serum concentrations of d-ROMs and OSI were greater in metritis than healthy at day 7 and 14 pp (106 ± 6.26 and 111 ± 6.26 Carratelli Units (UCarr) in metritis vs 81 ± 4.75 and 84 ± 4.69 UCarr in healthy; P < 0.001 and 0.35 ± 0.03 and 0.34 ± 0.03 UCarr in metritis vs 0.20 ± 0.02 and 0.18 ± 0.02 UCarr in healthy; P < 0.001, respectively). The concentration of OXY was lower in metritis than healthy at days 7, 14, 21, 28, and 35 pp (P < 0.05). Serum concentrations of IL-10 were greater in metritis than healthy cows at 21 dpp (53 ± 4.5 and 35 ± 3.4 ng/L; P < 0.001, respectively), whereas no differences (P > 0.05) in TNF-α and IL-6 concentrations could be detected.

CONCLUSIONS

This study showed that cows with metritis experience a greater degree of OS in comparison to healthy cows. However, the serum concentrations of pro-inflammatory cytokines were not different between groups. Metritis cows had greater IL-10 (anti-inflammatory) than healthy at 21 dpp, which may be associated with the uterine healing process. These findings provide new avenues for research for prevention and potential supportive treatments for metritis via the utilization of antioxidants in the feed and immunomodulating agent-based dietary adjuvant therapy.