Animal Reproduction

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Folliculogenesis, oogenesis and superovulation

Increased progesterone concentration in follicular fluid due to corpus luteum proximity: Consequences in miRNA biogenesis pathway in bovine granulosa cells

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Resumo

Follicular fluid (FF) is modulated by dynamic changes in progesterone (P4) levels during the different stages of the estrus cycle. Interactions between microRNA (miRNA) expression and changes in P4 levels are reported in several tissues in female reproductive physiology. Nevertheless, the P4 effects on the follicular microenvironment, especially in the miRNA-biogenesis pathway of follicular cells, are poorly understood. Thus, this study hypothesizes that elevated intrafollicular P4 levels caused by corpus luteum proximity alter the miRNA-biogenesis-related genes in granulosa cells. Ovaries (n=3/replicate; 6 replicates) from a local slaughterhouse were collected in pairs and classified as stage 3 of the estrous cycle (according to corpus luteum morphology, related to the middle diestrus; Ireland et al. 1980. J Dairy Sci. 63:155–160) to obtain groups modulated by high or low intrafollicular P4. Small follicles (3-6mm) were aspirated (n~15/ovarie) from ipsilateral or contralateral ovaries to the corpus luteum. The FF was analyzed for P4 concentration (6 replicates; intra and interassay coefficients variations were 9.77% and 21.47%, respectively) and granulosa cells (n~5 pools/replicate; 6 replicates) were collected for mRNA analysis. Total RNA was isolated (TRIzol®; Invitrogen) with an RNA co-precipitant (GlycoBlue®; ThermoFisher Scientific) and treated with DNase (DNaseI, Invitrogen). The cDNA was synthetized (High Capacity; ThermoFisher) and analyzed by RT-qPCR (GoTaqR qPCR Master Mix; Promega) analysis. Seven transcripts related to the miRNA-biogenesis pathway (DROSHA, DICER1, AGO2, DGCR8, XPO5, PRKRA, and TARBP2) were analyzed and normalized by the geometric mean of two endogenous genes (PPIA and ACTB). The relative expression (ipsilateral n=6; contralateral n=4) was calculated using the ΔCt method, and the normalized data were transformed by 2−ΔCt for representation of the relative expression. P4 concentration and relative gene expression data (mean ± SEM) were tested for outliers' presence, normality (Shapiro-Wilk test) and were analyzed by Student's t-test (GraphPad Prism; Software), considering a significance level of 5%. The intrafollicular P4 concentration was higher in follicles localized ipsilateral (high P4 - 348.38 ± 60.26 ng/mL) to the corpus luteum compared to contralateral (low P4 - 91.06 ± 3.99 ng/mL; p=0.0018) group. The results demonstrated that AGO2 transcript was up-regulated in ipsilateral (0.01252 ± 0.00193) when compared to contralateral (0.00633 ± 0.00129) granulosa cells (p=0.0465). In this sense, altered miRNAs-biogenesis machinery is a possible pathway affected by P4, which could impact transcripts and protein levels in granulosa cells. Further analyses are necessary to understand the effects of high and or low intrafollicular progesterone concentration on AGO2 protein levels and granulosa cells as well as miRNAs levels.

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