Animal Reproduction

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EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Antioxidant status of oocytes from crossbred dairy cows with different antral follicle counts

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The antral follicular count (AFC) in cows has been linked to oocyte quality. This study aimed to evaluate oocyte quality in cows from different genetic groups with high or low AFC and to determine whether oocyte quality correlates with antioxidant status. Twenty-two crossbred Holstein × Gyr lactating cows, primiparous and multiparous, were assigned to groups: G1 (1/2 + 5/8 HG, n = 9) and G2 (3/4 + 7/8 HG, n = 13). Within each group, cows were classified as having low (G1: n = 6; G2: n = 8) or high AFC (G1: n = 3; G2: n = 5). Before each OPU session, follicular wave emergence was synchronized using a progesterone-releasing intravaginal device, estradiol benzoate, and sodium cloprostenol (D0). Five OPU sessions were performed on D5 for COCs recovery, and three sessions were conducted on D9 to collect follicular fluid (FF) from the dominant follicle. Viable COCs were subjected to IVM for 22–24 hours. Mature COCs were then denuded and stained with fluorescent dyes to assess mitochondrial activity (Mito-Tracker Red CMxRos) and reactive oxygen species (ROS) production (2',7'-Dichlorofluorescin diacetate). Fluorescence intensity was measured using Image J software. Additionally, relative activities of antioxidant enzymes (glutathione peroxidase [GPx], superoxide dismutase [SOD], and catalase) were measured in FF using the Bradford assay. Data were analyzed using the GLIMMIX procedure (SAS v9.4), considering cow and OPU as random effects, genetic group and AFC within group as fixed effects, and body condition score, milk yield, and days in milk as covariates. Least square means were compared using the F-test, with significance set at $P < 0.05$. AFC was lower in low- compared with high-AFC cows (G1: 15.54±1.53 vs 26.81±3.61 and G2: 11.39±1.05 vs 24.60±2.53; $P<0.01$). The number of recovered COCs was lower for low- than for high-AFC cows (G1: 5.49±01.00 vs 10.43±2.59 and G2: 2.65±0.48 vs 8.08±1.55; $P<0.05$). Viable COCs did not differ between G1 (65.33±5.56%) and G2 (69.63±7.27%) or between low- and high-AFC cows ($P>0.05$). Mitochondrial activity and ROS production were higher in oocytes from low-AFC G1 cows compared to high-AFC G1 cows (mitochondrial activity: 19.56±6.17 vs. 7.11±2.76 AU; ROS: 38.98±10.59 vs. 5.04±1.68 AU; $P<0.05$). Similarly, in G2 cows, ROS levels were higher in oocytes from low- versus high-AFC animals (26.52±8.19 vs. 9.01±2.36 AU; $P < 0.01$). Relative GPx activity in FF was lower in G1 than in G2 cows (0.0185±0.0016 vs. 0.0258±0.0018 U/mg protein/mL FF; $P<0.001$) but did not differ between low- and high-AFC cows ($P>0.05$). SOD and catalase activities in FF did not vary between groups or AFC categories ($P>0.05$). In this study, AFC was not associated with oocyte morphological quality in either genetic group. However, increased mitochondrial activity and/or ROS levels without corresponding changes in FF antioxidant enzyme activity suggest an oxidative stress condition in oocytes from low-AFC cows.

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