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FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION**

Livestock-Forest integrated system attenuates deleterious heat stress effects in bovine oocytes

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The objective of this study was to analyze molecular markers associated with heat stress during the tropical summer in oocytes from Girolando and Holstein heifers kept in an integrated system (IS) or a conventional Full Sun (FS) system. The study was conducted between November and February and the analyses were made at the same time for all groups. Based on temperature-humidity index (THI) data, we found an intense heat stress condition during the experiment. IS used was a prototype modeled by Embrapa Dairy Cattle (*Urochloa decumbens*; 200 eucalypt trees per hectare; basal area (area of the cross-section of trees at chest height) of 1.33 m² ha⁻¹) and FS was composed of a similar pasture without trees. Dairy heifers (16 Girolando 3/4 Holstein and ¼ Gir and 16 Holstein) were allocated in four experimental groups: Girolando IS (8), Girolando FS (8), Holstein IS (8) and Holstein FS (8) (1.8 animal units/ha). Vaginal temperature was assessed using iBotton data loggers every 15 minutes during 2-4 days in 15-day intervals along the experiment, and the number of hours vaginal temperature exceeded 39.1°C and maximum vaginal temperature were compared among groups. Oocytes were obtained on random day of the estrus cycle by OPU at the beginning of experiment (D0), and after 30 (D30) and 60 (D60) days. Oocytes were denuded with hyaluronidase, fixed in 4% PFA and immunostained for caspase3 and IGFBP2, oocyte quality markers previously validated by our group (de Silva, M. O. et al. 2022, *Reprod Dom Ani* 57(9), 980-988. <https://doi.org/10.1111/rda.14164>). Results were transformed by Johnson and means were compared among groups in each breed using ANOVA and Tukey posttest. IVF-grade and oocyte recovery rates were compared among groups in each breed using Fisher Exact test. T Test was used to compare the number of hours above 39.1°C and maximum vaginal temperature. Girolando and Holstein heifers showed an increased ($p < 0.001$) number of hours above 39.1°C when kept at FS (14.1±5.6) compared to IS (11.2±4.9). Meanwhile, Holstein (14.8±5.4) heifers also showed an increased number of hours above 39.1°C compared to Girolando (10.7±5.1). Oocyte diameter decreased during summer in FS and IS groups, but more drastically in FS for Girolando, which was lower than IS at D60 (100.66±1.10 vs 81.93±1.53*). Caspase 3 and IGFBP2 proteins, involved in apoptosis and IGF negative regulation, were increased ($p < 0.05$) from D0 to D30 and D60 in FS but not in ILF oocytes. Caspase was higher in FS oocytes at D30 in Girolando (154.42±1.91 vs 175.70±2.05*) and Holstein (126.97±6.77 vs 141.54±1.00*) and D60 in Girolando (144.08±1.21 vs 163.86±1.33*). IGFBP2 was increased in Girolando FS in comparison to ILF, at D30 (140.46±3.49 vs 166.28±3.2*) and D60 (119.79±1.53 vs 136.81±1.64). In conclusion, ILF system attenuated maternal hyperthermia and its effects on oocytes through caspase-3 and IGFBP2 associated pathways for both Holstein and Girolando heifers.