Animal Reproduction

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Folliculogenesis, oogenesis and superovulation

Increased intrafollicular concentration of β-hydroxybutyrate (BHBA) affects follicular growth but does not compromise the ovulatory cascade in cattle

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Resumo

Metabolic stress conditions caused by negative energy balance (NEB) have been associated with reduced fertility in cows. β-hydroxybutyrate (BHBA) is the main circulating ketone body during NEB, which accumulates within follicular fluid.The aim of this study was to evaluate the effects of BHBA on follicle growth and ovulatory mechanisms in cattle. Two experiments were performed. In Experiment 1, to assess the effect of increased BHBA concentration on follicular development, cows had the emergence of a new follicular wave induced by using a P4-releasing intravaginal device (IVD; Primer, Tecnopec, Brazil; 1 g P4) and an intramuscular injection of 2 mg EB (Gonadiol, Zoetis, Brazil; D0). In D4, P4 device was removed and PGF2α analog (500 µg cloprostenol, Estron, Agener União Saúde Animal, Brazil) was administered intramuscularly. The ovaries were monitored daily using transrectal ultrasonography procedures. When the follicles reached a diameter of 8-9 mm, the animals were intrafollicularly injected with BHBA (15 mM; n = 8) or PBS (control; n = 8). Follicular growth was monitored for 72 h, ovulation rate and follicle diameter at ovulation were monitored for 120 h. In Experiment 2, the effect of high concentration of BHBA on the granulosa cells of preovulatory follicle was assessed to study the ovulatory mechanism. Cows had the emergence of a new follicular wave induced as described above. On D9, the follicles with diameter ≥ 12 mm were injected intrafollicularly with BHBA (15 mM; n = 4) or PBS (n = 5). The cows were ovariectomized after 6 h of BHBA injection. After ovariectomy, granulosa cell was collected for evaluation of relative abundance of mRNA transcripts. Differences between follicular sizes were compared between groups by mixed models for repeated data. The effect of treatments on ovulation rate was analyzed by chi-square test. Differences in the relative abundance of mRNA transcripts were analyzed by one-way ANOVA. At 72 h after intrafollicular injection, there was a decrease in follicular diameter in BHBA group (7.7 ± 1.6 mm) compared to the control (11.5 ± 0.6 mm, P = 0.02). Furthermore, follicle growth rate was reduced post-treatment with BHBA in comparison to the control group (P < 0.03). There was no difference between groups in ovulation rate, however, the diameter of the follicles that ovulated were 12.2 ± 0.46 mm and 10.4 ± 0.30 mm for the control and BHBA groups, respectively (P < 0.01). However, the BHBA intrafollicular injection in follicles with ≥ 12 mm did not affect the relative abundance of genes involved in the ovulatory cascade (ADAM 17, AREG, EREG, PTGS2) and steroidogenesis (CYP19A1, 3BHSD, STAR) between groups. In conclusion, the increase in intrafollicular concentrations of BHBA affects follicular growth but it does not seem to compromise the ovulatory cascade in bovine granulosa cells.

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