38.5°C for 12 h (heat-shock: HS group). Oocytes were IVF for 20 h and the denuded presumptive zygotes from NHS or HS groups were cultured with 0 (nontreated controls) or 10 nM of 5-aza for 24 h or 48 h in CR2aa plus 2.5% FCS at 38.5°C with 5% CO_2, 5% O_2 and 90% N_2. Embryos with 4–7 cells at 44 h post-insemination (hpi) and embryos with 8–16 cells at 68 hpi were fixed in 4% paraformaldehyde and stained with anti-mouse HP1β first antibody, then immunofluorescence was evaluated by confocal microscopy (Leica TCS SP5II) and images were processed by ImageJ 1.49 (NIH, Bethesda, MD, USA). Fluorescence of nuclei and of background area (fluorescence/unit area) were measured and then the corrected relative fluorescence per nucleus was calculated. We analysed 129 and 149 nuclei at 44 hpi from 29 and 34 embryos as well as 268 and 182 nuclei at 68 hpi from 37 and 22 embryos of the NHS and HS groups, respectively, obtained from 3 replicates. Data underwent log-transformation and was analysed by ANOVA, and means compared by Student-Newman-Keuls. Embryos with 8–16 cells derived from NHS oocytes and treated with 5-aza for 24 h or for 48 h had nuclei with lower HP1 fluorescence than their respective NHS (nontreated) control ($P < 0.01$). In contrast, 8–16-cells embryos derived from HS and treated with 5-aza displayed nuclei with the same HP1 fluorescence of their respective HS control ($P > 0.05$). When embryos derived from HS and NHS (nontreated) control groups were compared, higher HP1 fluorescence was found in those with 4–7 cells of HS group ($P < 0.05$); however, embryos with 8–16 cells displayed similar HP1 fluorescence between both NHS and HS control groups ($P > 0.05$). There was no difference on HP1 fluorescence between nuclei of embryos with 4–7 cells treated with 5-aza for 24 h and control (nontreated) in both HS and NHS groups. These data suggest that embryos derived from heat-shocked oocytes can accumulate more heterochromatin at earlier stages than those from non-heat-shocked oocytes and that the effect of DNA methylation inhibition by 5-aza on embryo heterochromatin can vary accordingly to the exposure of the oocyte to heat shock during *in vitro* maturation.

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168 EFFECT OF CYCLIC ADENOSINE MONOPHOSPHATE MODULATORS DURING OOCYTE *IN VITRO* MATURATION ON BOVINE EMBRYOS (Gyr × HOLSTEIN) QUALITY

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In vitro embryo production (IVP) is an important tool for cattle breeding. Brazilian dairy systems are based on Gyr × Holstein crossbreds, which integrates adaptability to tropical conditions and milk production. Quality determines the oocyte proportion that will develop to blastocyst stage, and although the lipid content is important in oocyte development, a high concentration in embryos is associated with cryotolerance reduction, making this a relevant issue for IVP systems. The *in vitro* maturation system (IVM) simulated physiological oocyte maturation (SPOM) mimics the physiological maturation events by using cyclic adenosine monophosphate (cAMP) modulators, which promote the increase of oocyte competence. Among the modulators, Forskolin has lipolytic properties. The aim of this study was to evaluate the effect of the SPOM system (Albuz 2010 Hum. Reprod. **25**, 12) on bovine embryos (Gyr × Holstein) regarding their total number of cells (TNC) and lipid content. Oocytes were obtained by ovum pick-up from Gyr cows in 5 replications. After selection, they were randomly divided into 2 groups: SPOM (S) and control (C). The IVM lasted 24 h for group C (TCM 199 medium without FBS) in culture oven at 38.5°C, 5% CO_2 in atmospheric air and high humidity. In the SPOM system, oocytes were in pre-IVM [TCM 199 medium + 100 μM Forskolin + 500 μM 3-isobutyl-1-methylxanthine (IBMX)] for 2 h and followed for extended IVM (TCM 199 medium + 20 μM cilostamide) for 28 h under the same conditions as control group. After IVM, oocytes were fertilised with semen from a single Holstein bull that was prepared by Percoll gradient method in Fert-TALP medium (Bioklone® Animal Reproduction, São Paulo, Brazil) for 22 h and transfered to culture droplets, where they remained for 7 days ($n = 10$–13 per group). The lipid content analysis was performed by staining with Oil red and the stained area fraction of each embryo was measured using software ImageJ (NIH, Bethesda, MD, USA). The TNC was measured after being stained with Hoechst 33342 and results were analysed by Student's *t*-test in Instat GraphPad program, with a 5% significance level. There was no significant difference ($P > 0.05$) between embryos from both groups on TNC (group S: 88.9 ± 28.0^{A}; group C: 101.6 ± 29.1^{a}) and lipid content (group S: $0.93 \pm 12{:}18^{A}$; group C: ±0.15 to 0.96) analysis. Some studies have shown there is a beneficial effect on embryo quality when using this system; however, our results demonstrated that there was no effect on total number of cells using our conditions. Some authors have also demonstrated a reduction in embryo lipid content using Forskolin during *in vitro* culture. Our results suggest that the time of Forskolin exposure was not enough to ensure lipolytic action on the structures produced from oocytes (Gyr) treated in pre-IVM. It was concluded that the SPOM system had no effect on TNC and lipid content of Gyr/Holstein embryos.

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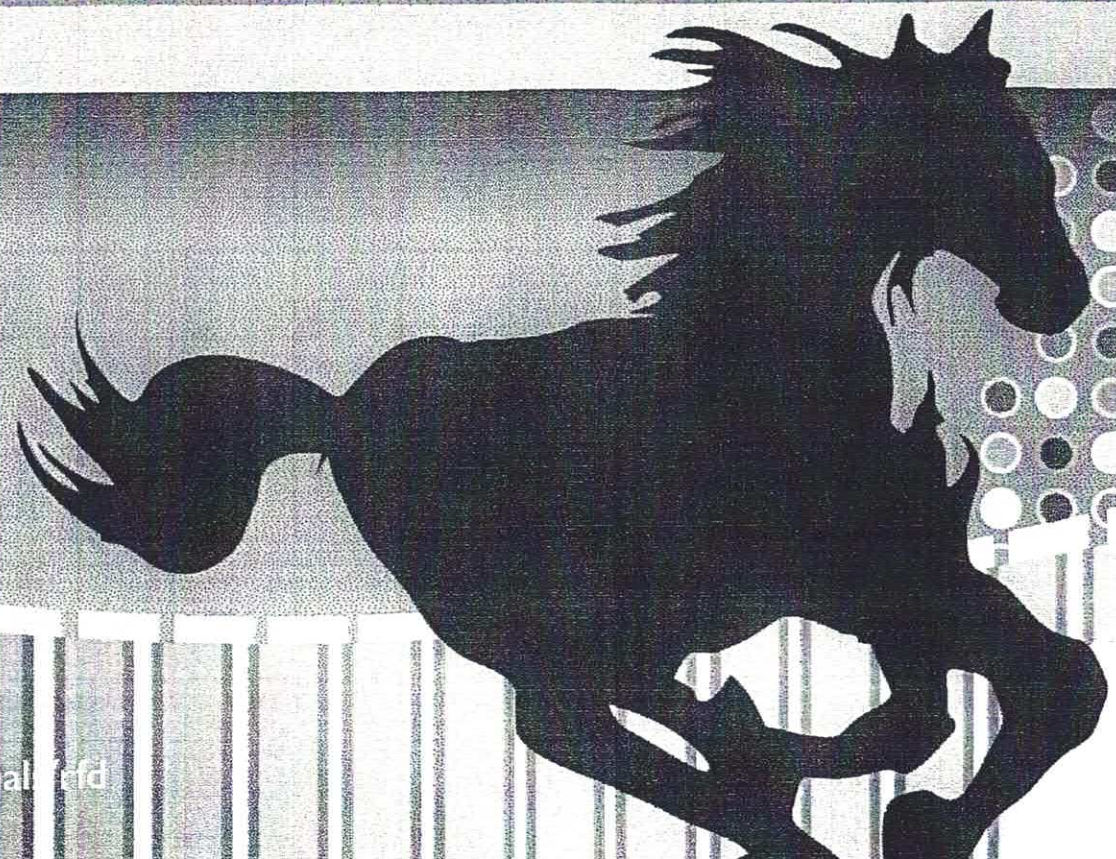

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