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Inhibition of methylation with 5-aza-2'-deoxycytidine interferes on development of bovine embryos derived from heat-shocked oocytes

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Epigenetic modifications are required during pre-implantation embryo development but little is known about the effects of heat shock. Chemical agents that modulate epigenetic events can contribute to understand the effect of IMV and heat shock on embryo development. This study evaluated the effect of 5-aza-2'-deoxycytidine (5-aza; Sigma, St. Louis, USA), a DNA methylation inhibitor, on development of bovine embryos derived from oocytes submitted or not to heat shock during IVM. Experiment 1 (NHS – non-heat-shock) evaluated the effect of 5-aza on development of embryos derived from oocytes matured at 38.5°C and 5% CO_2 for 24h, and the Experiment 2 (HS - heat shock) evaluated the effect of 5-aza on development of embryos derived from oocytes matured at 41.5°C for 12h in 6.5% CO_2 followed by 38.5°C for 12h in 5% CO2. After IVM and IVF, denuded presumptive zygotes were cultured with 0 or 10 nM of 5-aza for 24h or 48h in CR2aa plus 2.5% FBS at 38.5°C with 5% CO_2, 5% O_2 and 90% N_2. After that, embryos were cultured in CR2aa plus 2.5% FBS until day eight post-fertilization. The experiments were composed by the following treatments: Exp. 1: NHS (control, without 5-aza; n=391); NHS24h (5-aza for 24h, n=380) and NHS48h (5-aza 48h, n=379); Exp. 2: HS (control without 5-aza; n=329); HS24h (5-aza 24h, n=320) and HS48h (5-aza 48h, n=381). The proportion of embryos that reached the 8-cell stage on day three (D3) post-fertilization was analyzed by chi-square. Total cleavage rate on D3 and blastocyst rate on day eight (D8) were analyzed by ANOVA and means compared by SNK. Values are shown as mean±SEM. The proportion of embryos with 8-cell in the Exp 1 was lower ($P<0.05$) in NHS24h (28.1%) and NHS48h (33.4%) than in the control (NHS: 42,3%). In Exp. 2 HS48h (23.0%) had lower ($P<0.05$) proportion of embryos at 8-cell stage than the control (HS: 34.0%), with no significant difference with HS24h (27.1%). There was no ($P>0.05$) difference in the cleavage rate among treatments in the Exp. 1 and in the Exp. 2. In the Exp. 1 blastocyst rate was lower ($P<0.05$) for NHS48h (15.7 ± 2.9%) than for NHS (32.2 ± 3.4%) and NHS24h (25.8 ± 3.9%) treatments, and in Exp. 2 blastocyst rate was lower ($P<0.05$) for HS24h (9.5 ± 2.2%) and HS48h (11.1 ± 2.4%) than for HS (21.6 ± 3.4%). In conclusion, inhibition of DNA methylation for 48h in embryos derived from non-heat-shocked oocytes influences the production of blastocysts (Exp. 1) whereas that same effect is found with shorter time of embryo exposure to DNA methylation inhibitor (24h) for embryos derived from heat-shocked oocytes (Exp. 2). Those data suggest that embryos derived from heat-shocked oocytes are susceptible to epigenetic modulation but in a different time-dependent manner from those derived from non-heat-shocked oocytes.

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