



A193 Embryology, Developmental Biology and Physiology of Reproduction

Effect of epigenetic modulators on development of bovine embryos derived from heat-shocked oocytes

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High temperatures during bovine oocyte maturation have been associated with changes in gene expression and on embryonic chromatin organization. This study aimed to analyze the effect of two epigenetic modulators on the development of embryos derived from oocytes submitted to heat shock: 1) Scriptaid, an inhibitor of histone deacetylase and 2) 5-Aza-2'-deoxycytidine (AZA), an inhibitor of DNA methylation. Bovine oocytes from slaughterhouse ovaries were matured *in vitro* at conventional temperature (38°C) for 24h (group with no heat shock: NHS) or at 41.5°C for 12h followed by 38°C for a further 12h (heat shock group: HS) under the same conditions of the NHS group. Afterwards, the oocytes from both groups were fertilized *in vitro* for 20h and after the end of fertilization the presumptive zygotes were denuded and randomly exposed to 500 nM Scriptaid or 10 nM AZA for 0h or 24h, comprising six treatments: NHS-0h (n=258), NHS+24hScriptaid (n=242), NHS+24hAZA (n=255), HS-0h (n=263), HS+24hScriptaid (n=261) and HS+24hAZA (n=276). Embryos were cultured in CR2aa medium supplemented with 2.5% FBS at 38.5°C with 5% CO₂, 5% O₂, 90% N₂. Five replicates were performed and data (mean±SEM) was analyzed by logistic regression (Pro Logistic, SAS). Cleavage rates at day three and blastocysts rates at day eight post-fertilization were compared among treatments. Higher cleavage rates (P<0.05) were found in all NHS groups (NHS-0h: 67.4±3.8%; NHS+24hAZA: 60.5±5.7%; NHS+24hScriptaid: 76.2±3.3%) when compared to heat shock groups (HS-0h: 53.0±10.6%; HS+24hAZA: 45.3±9.3%; HS+24hScriptaid: 53.2±13.8%). The use of both epigenetic modulators in embryos derived from oocytes with no heat shock (NHS+24hAZA: 22.8±2.6%, NHS+24hScriptaid: 24.8±4.9%) reduced (P <0.05) blastocyst rate when compared with NHS-0h (38.1±5.2%). However, the same effect was not observed when the comparison was performed among embryos derived from oocytes submitted to heat shock. There was no difference (P> 0.05) between HS+24hAZA (16.4±3.1%), HS+24hScriptaid (15.2±3.8%) and HS-0h (17.1±4.5%) treatments. In conclusion, AZA and Scriptaid have a negative effect on the development of embryos derived from oocytes matured *in vitro* under conventional temperature, but that same effect is not observed when embryos are derived from oocytes matured under heat shock conditions. It is suggested that the effect of heat shock during *in vitro* maturation overlaps the effects of AZA or Scriptaid on embryonic development. Further analyzes should be performed to identify possible differences in chromatin organization among treatments. Financial support: CNPq and Fapemig.

SP 7625