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Inhibition of HSP90 associated to heat shock during *in vitro* maturation of bovine oocytes alters the relative amount of transcripts in 8-cell stage embryos

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HSP90 is a protein involved in cellular homeostasis and its inhibition during maturation reduces the oocyte developmental competence (Souza et al. 2015, Reprod. Fertil. Dev. 27:235). The present study investigated the relative amount of heat shock (*HSF1, HSP90, HSP40*) and totipotency (OCT4) transcripts in bovine embryos at 8-cell stage derived from oocytes exposed to an inhibitor of HSP90 (17AAG; 17-allylamino-17-demetoxigeldanamicina; Sigma, St Louis, EUA) associated to heat shock (HS) during *in vitro* maturation (IVM). Cumulus-oocyte complexes (COC) were allocated in four groups during IVM: Control - without both heat shock and 17AAG; HS - heat shock (41.5ºC) for the first 12h of IVM; 17AAG - 2µM of 17AAG for the first 12h of IVM, and 17AAG+HS - 2µM of 17AAG plus heat shock for the first 12h of IVM. *In vitro* maturation was performed in Nunc plates, containing 400µL of TCM199 medium (Invitrogen, Carlsbarg, USA) supplemented with porcine FSH (pFSH - Pluset, Lab. Callier, Espanha) and 10% estrus cow serum, and incubated under 5% CO_2, 95% humidity and 38.5ºC for 24h. The heat shock was performed under 7% CO_2, 95% humidity at 41.5°C. After maturation, oocytes were *in vitro* fertilized for 20h with $2x10^6$ spermatozoa/mL. The presumptive zygotes were cultured in four-wells plate with 500 µL of modified CR2aa medium supplemented with 2.5% FCS (Nutricell, Campinas, Brasil) in an incubator at 38.5ºC under 5% CO_2, 5% O_2, 90% N_2 and saturated humidity for 52h. Eight-cell stage embryos were washed three times in PBS plus 0.1% polyvinyl alcohol and then rapidly frozen in liquid nitrogen, and stored at -80°C. Three pools of 10 embryos per group where used for total RNA extraction with RNeasy Micro Kit (Quiagen,Valencia, CA, USA) and reverse transcribed using the SuperScript III First-Strand Synthesis Supermix (Invitrogen, Carlsbad, CA, USA). Relative quantification was performed by Comparative Ct quantification ($2^{-\Delta\Delta Ct}$) method relative to the sample with the highest delta Ct value in the control group (calibrator sample) and was based on primer efficiency. Analysis was performed by mixed model using the Proc Mixed command in the SAS 9.0 software. $P<0.052$ was considered significant and the relative amount values are presented as mean ± S.E.M. The relative amount of *HSF1* transcripts was higher ($p<0.052$) in 17AAG group than in Control and 17AAG+HS groups but similar to HS group. Higher ($p<0.03$) amount of *HSP90* transcripts was found in 17AAG+HS group than Control and HS groups, but similar to 17AAG group. No difference was found for *HSP40* and *OCT4* transcripts among groups. Those data show that despite inhibition of HSP90 during IVM can affect the expression of *HSF1* in 8-cells embryos, it does not have the same effect on expression of *HSP90*. In contrast, inhibition of HSP90 associated to heat shock influences the *HSP90* expression but has no effect on *HSF1* transcript. In conclusion, relative expression of genes in 8-cell stage embryos is influenced by the inhibition of HSP90 and heat shock during *in vitro* maturation of bovine oocytes. Financial support: CNPq, FAPEMIG and FAPES.