A147 OPU-IVP and ET

# Effect of HSP90 inibitor on developmental competence of bovine oocytes

**E.D. Souza[1], F. Bernades Eugênio Paula[2], C.C.R. Quintão[2], J.H.M. Viana[2], L.G.B. Siqueira[2], L.T. Iguma[2], B.C. Carvalho[2], I.D. Louro[1], L.S.A. Camargo[2]**

[1]Universidade Federal do Espírito Santo, Vitória, ES, Brasil; [2]EMBRAPA Gado de Leite, Juiz de Fora, MG, Brasil.

**Keywords:** 17AAG, heat shock, maturation *in vitro*.

The heat shock protein 90kda (HSP90) is a cytoprotective chaperone that influences the maturation of *Xenopus* oocytes (Nebreda and Ferby, 2000. Curr Opin Cell Biol 12:666-675). Its effect can be repressed by inhibitors as 17-(allylamino)-17-demethoxygeldanamycin (17AAG, Sigma, St. Louis, USA). This study aimed to evaluate the effect of 17AAG concentration and exposure time during *in vitro* maturation in order to identify a possible HSP90 requirement for oocyte developmental competence. Immature oocytes aspirated from ovaries obtained from slaughterhouse were selected and randomly allocated in a factorial experiment design with three 17AAG concentrations (0, 1 and 2μM) and two-exposure time (12 and 24h) during *in vitro* maturation. The maturation was performed in Nunc plate containing 400μL of TCM199 medium (Invitrogen, Carlsbarg, USA) supplemented with porcine FSH (pFSH - Pluset, Lab. Callier, Espanha) and 10% estrus cow serum, and incubated at 38.5ºC under 5% $CO_2$ and 95% humidity for 24h. Oocytes were *in vitro* fertilized (IVF) for 20h and incubated under the same maturation conditions. Semen was processed by Percoll gradient and a fertilizing dose of $2x10^6$spermatozoa/mL was used. After IVF, the presumptive zygotes were denuded in a solution of PBS plus 1% hialuronidase and then cultured in wells 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% $CO_2$ and saturated humidity for 8 days. Cleavage was evaluated on day three (D3) and blastocysts were evaluated on day seven (D7) and on day (D8) post-fertilization. Data from nine replicates (n=1836 oocytes) were analyzed by Generalized Linear Model procedure of SAS software (version 9.1) considering effect of exposure time, 17AAG concentration and interaction, and means were compared by Student Newman Keuls test. Values are shown as mean ± SEM. Regarding to exposure time, there was no difference for cleavage and blastocysts rates in D7 and D8 between 12h and 24 hours. Blastocyst rates of 2μM 17AAG group were decreased on D7 (18.6±2.2%; $P<0.02$) and on D8 (20.4±2.2%; $P<0.01$) when compared to 0μM (29.2±2.5% and 34.0±3.3% for D7 and D8, respectively), whereas 1μM produced intermediary blastocyst rates (25.6±2.7% and 27.9±3.1% for D7 and D8, respectively). There was no interaction ($P>0.05$) between concentration and exposure time. In conclusion, inhibition of HSP90 by 17AAG decreases oocyte developmental competence and suggests that this protein is also required for maturation of bovine oocytes.

**Financial support**: CNPq 473484/2011-0, FAPEMIG and FAPES.