Table 1. Least squares means (± SE) of embryonic development of COC matured in SMM or control

Variable	OPU session	COC recovery	Cleavage (%)	Day 7 blastocyst (%)	Transferable embryos per OPU session
Maturation					
Control	32	10.4 ± 1.1	59.3 ± 5.9[a]	33.4 ± 5.7[a]	2.9 ± 0.6[c]
SMM	32	9.0 ± 1.2	77.0 ± 3.9[b]	60.0 ± 4.1[b]	5.0 ± 0.5[d]

Values without common superscripts in the same column differ ([a,b]$P < 0.01$, [c,d]$P < 0.05$).

292 INHIBITION OF HSP90 AGGRAVATES THE EFFECTS OF HEAT SHOCK ON DEVELOPMENTAL COMPETENCE OF BOVINE OOCYTES

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The heat shock protein 90kDa (HSP90) is a chaperone involved in protein homeostasis under normal and stress conditions. Its inhibition by 17-(allylamino)-17-demethoxygeldanamycin (17AAG, Sigma, St. Louis, MO, USA) for 12 or 24 h during *in vitro* maturation reduces the oocyte's ability to develop after *in vitro* fertilization (Souza *et al.* 2014 Reprod. Fert. Dev. **26**, 197). This study aimed to evaluate the effect of treatment with 17AAG during the heat shock on oocyte developmental competence. Immature bovine COC were randomly allocated in 4 treatments during IVM: control = no heat shock or 17AAG; HS = heat shock (41.5°C) for the first 12 h of IVM; 17AAG = 2 μM 17AAG for the first 12 h of IVM; and 17AAG + HS = 2 μM 17AAG plus heat shock for the first 12 h of IVM. *In vitro* maturation was performed in Nunc plate containing 400 μL of TCM199 medium (Invitrogen, Carlsbad, CA, USA) supplemented with porcine FSH (Hertape Calier, Juatuba, Brazil) and 10% oestrus cow serum under 5% CO_2 in air, 95% humidity, and 38.5°C for 24 h. Semen was processed by Percoll gradient (Nutricell, Campinas, Brazil) and oocytes were *in vitro* fertilized for 20 h with 2×10^6 spermatozoa mL^{-1} under the same IVM atmospheric conditions. Presumptive zygotes were completely denuded in a PBS solution with 0.1% hyaluronidase and then cultured in wells with 500 μL of modified CR2aa medium supplemented with 2.5% fetal calf serum (Nutricell) in an incubator at 38.5°C under 5% CO_2, 5% O_2, 90% N_2, and saturated humidity. Cleavage rate was evaluated 72 h postfertilization and blastocyst rate was evaluated at Day 7 (D7) and 8 (D8). Data from 7 replicates were submitted to analysis of variance and means were compared by Student Newman Keul's test. There was no difference ($P > 0.05$) on cleavage rate among treatments. Heat shock or treatment with 17AAG, both for 12 h of IVM, decreased ($P < 0.05$) the blastocyst rate at D7 and D8 when compared to control but no significant difference between HS and 17AAG treatments was found (Table 1). However, the lowest ($P < 0.05$) blastocyst rate at D7 and D8 was achieved when oocytes were submitted simultaneously to 17AAG and heat shock for 12 h of IVM (17AAG + HS treatment, Table 1). In conclusion, the treatment with 17AAG during IVM worsens the deleterious effect of heat shock on oocyte developmental competence and suggests that HSP90 may also play role on cellular protection during heat shock in bovine oocytes.

Table 1. Cleavage and blastocyst (Bl) rates at D7 and D8 for control, 17AAG, Heat Shock (HS), and 17AAG plus HS treatments

Treatment	*n*	Cleavage (%)	Bl D7 (%)	Bl D8 (%)
Control	315	73.35 ± 4.59	32.67 ± 3.76[a]	33.54 ± 4.08[a]
17AAG	298	60.39 ± 7.85	22.44 ± 2.39[b]	23.01 ± 1.94[b]
HS	281	62.49 ± 5.97	17.00 ± 1.61[b]	17.29 ± 0.96[b]
17AAG + HS	312	52.46 ± 6.16	6.04 ± 2.18[c]	5.84 ± 1.75[c]

[a–c]Values are shown as mean ± s.e.m., and those with different superscript letters in the same column differ ($P < 0.05$).

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293 EFFECT OF DIFFERENT CONCENTRATIONS OF LH, FSH, AND E_2 ON THE MATURATIONAL RATE OF INDIGENOUS SOUTH AFRICAN CATTLE OOCYTES SELECTED BY BRILLIANT CRESYL BLUE STAINING

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In vitro maturation of indigenous African cattle oocytes is a major challenge even though different maturation protocols work successfully in other breeds. The objective of this study was to determine the maturation rate of indigenous South African cattle oocytes following *in vitro* maturation in media supplemented with different concentrations of hormones and selected using brilliant cresyl blue (BCB) staining. Indigenous cattle ovaries were

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