236 EFFECT OF HEAT STRESS DURING OOCYTE MATURATION ON GENE EXPRESSION OF *IN VITRO*-FERTILIZED AND PARTHENOGENETIC BOVINE BLASTOCYSTS

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Abstract

Heat stress has been shown to have detrimental effects (41°C) during the first 12 h of *in vitro* maturation on bovine embryo development (Edwards JL and Hansen PJ 1996 Biol. Reprod. 55, 341-346). However, little is known about the effect on gene expression of *in vitro*-fertilized andparthenogenetic bovine embryos. This study evaluated the gene expression of *in vitro*-fertilized andparthenogenetic blastocysts derived fromheat-stressed oocytes. The transcripts evaluated were associated with genes encoding proteins involved in blastocoel formation [aquaporin (Aqp) 3 and Na+/K+-ATPase alpha 1; Watson AJ and Barcroft LC 2001 Frontiers Biosci. 6:d708-730] and cell viability (Bax and Peroxiredoxin 1; Van Delft MF and Huang DCS 2006 Cell Res. 16, 203-213; RaguS *etal*. 2007 PNAS104, 9747-9752). Oocytes were *in vitro* matured for 12 h at 41°C followed by 12 h at 38.5°C (heat-stressed oocytes; HS) or for 24 h at 38.5°C (non-heat-stressed oocytes; NHS) under 5% CO^2. Heat-stressed and NHS oocytes were *in vitro* fertilized with Holstein sperm (HS-IVF and NHS-IVF subgroups, respectively) or activated with ionomycin and 6-DMAP (HS-PART and NHS-PART subgroups, respectively). Presumptive zygotes were cultured in CR2aa medium under 5% CO^2, 5% O^2, and 90% N^2 at 38.5°C. Embryos at blastocyst stage with same quality grade for all subgroups were obtained from 3 different replicates and distributed in pools of 10 embryos for relative quantification of the target transcripts. RNA extraction and reverse transcription were performed and cDNA quantified by real-time PCR. Transcripts of H2a gene were used as endogenous control, and statistical analysis was performed by pair-wise, fixed reallocation randomization test. Gene expression comparisons were performed between HS-IVF and NHS-IVF, HS-PART and NHS-PART, NHS-PART and NHS-IVF, and HS-PART and HS-IVF subgroups. Blastocyst rate is shown as mean ± SEM and relative expression as *n*-fold. The heat stress on oocytes during *in vitro* maturation decreased ($P < 0.05$, ANOVA) the development of presumptive zygotes to blastocyst stage at Day 8 for *in vitro*-fertilized (19.9 ± 2.9% and 10.5 ± 2.0% for NHS-IVF and HS-IVF, respectively) and parthenogenetic (33.0 ± 1.8% and 22.8 ± 2.8% for NHS-PART and HS-PART, respectively) embryos. Embryos from the HS-IVF subgroup showed less ($P < 0.01$) expression of Na+/K+-ATPase alpha 1 (0.67-fold) than did NHS-IVF embryos, whereas no difference was found for others genes. Embryos from the HS-PART subgroup showed less ($P < 0.01$) expression of Aqp 3 (0.77-fold) and greater ($P < 0.05$) expression of Bax (1.40-fold) than did NHS-PART embryos. Expression of Aqp 3 was up-regulated ($P < 0.01$) in NHS-PART (1.42-fold) embryos when compared with NHS-IVF ones, whereas expression of Na+/K+-ATPase alpha 1 (1.42-fold), Bax (1.67-fold), and Peroxiredoxin 1 (1.40-fold) were up-regulated ($P < 0.05$) in HS-PART embryos when compared with HS-IVF embryos. In conclusion, heat stress on oocytes during *in vitro* maturation can affect the amount of transcripts of *in vitro*-fertilized andparthenogenetic blastocysts, suggesting a residual effect on gene expression of bovine embryos.

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