ABSTRACT

The aim of the present study was to evaluate the effect of heat stress on the developmental competence of bubaline cumulus oocyte complexes (COCs). The in vitro maturation temperature for control group was 38.5 °C for complete 24 hours of maturation. The treatment 1 (T1) and 3 (T3) group oocytes were cultured at 40.5 °C and 41.5 °C respectively, for the first 12 h and at 38.5 °C for rest of the 12 h. However, treatment 2 (T2) and 4 (T4) group oocytes were cultured at 40.5° and 41.5 °C for complete 24 h. For evaluating the developmental competence, COCs were harvested from buffalo ovaries from abattoir and subjected to in vitro maturation, in vitro fertilization and in vitro culture to produce blastocysts. Real time PCR was used for studying the effect of heat stress on the relative expression pattern of candidate genes related to heat shock protein, apoptosis, oxidative stress, developmentally and metabolically important genes between the control and treatment groups. For evaluating the frequency of apoptotic nuclei in control and respective treatment groups, COCs after in vitro maturation and blastocysts produced thereafter were subjected to TUNEL assay. Development of COCs to blastocyst was severely compromised (p<0.001) when matured at 40.5° and 41.5 °C for both exposure periods (12 h and 24 h). It was found that the cleavage rates, blastocyst yield and mean cell number decreased remarkably (p<0.001) in the treatment groups compared to the control group. The relative mRNA expression of heat shock protein (Hsp70.1, 70.2, 70.8, 60, 10 and Hsf-1), pro-apoptotic (caspases-3, -7, -8, Bid and Bax), oxidative stress (iNOS, DnaJ) and endolysosomal proteases (cathepsin B, K, S and Z) related genes was significantly higher (p<0.05) in all the treatment groups compared to the control group. However, the mRNA abundance of anti-apoptotic (Bcl-2, Mcl-1, Bcl-xl), metabolism (Glut1, Glut3 and IGF1R), developmental competence (ZAR1 and BMP15) and mitochondria (MnSOD) related genes was significantly decreased (p <0.05) in the treatment groups compared to control. For evaluating the effect of heat stress on the percentage of apoptotic nuclei, TUNEL assay of COCs indicated significant increase in the frequency of apoptotic nuclei (p <0.05) in the treatment groups viz. T1, T2, T3 and T4 respectively compared to the control group. Significant increase (p <0.05) in the frequency of TUNEL-positive blastomeres was observed in the blastocyst embryos developed from the oocytes matured at 40.5° and 41.5 °C compared to the control group (38.5 °C).The biochemical analysis of COCs, denuded oocytes and cumulus cell mass cultured at 40.5° and 41.5 °C for 12 h and 24 h respectively revealed the significantly higher (p < 0.05) activity of antioxidant enzymes viz. catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase. Results indicated that the production of reactive oxygen species (ROS), lipid peroxides and nitric oxide (NO) was significantly (p < 0.05) higher in the oocytes subjected to heat stress (40.5° and 41.5 °C) during meiotic maturation compared to the COCs matured under standard in vitro culture conditions (38.5 °C).The present study clearly establishes that physiologically relevant elevated temperatures during in vitro meiotic maturation reduce developmental competence of bubaline oocytes thereby affecting the productivity. Therefore, this study clearly depicts that physiologically relevant elevated temperature (40.5 and 41.5 °C) for two time periods (first 12 or complete 24 h) during in vitro maturation reduced the developmental competence of bubaline oocytes and subsequent embryo development, most likely through apoptotic and developmentally regulated mechanisms. Oocyte damage caused by heat shock in vitro is likely to be relevant to the understanding of early embryonic losses in buffalo following heat stress conditions. Therefore, it is concluded that physiologically relevant elevated temperatures have a negative impact on buffalo reproduction.