ARE HEAT SHOCK PROTEINS ACTING AS MODULATORS OF PRE-IMPLANTATION MOUSE EMBRYO DEVELOPMENT AND APOPTOSIS?
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Objective: Intracellular induction of heat shock proteins (HSPs) in response to various stresses coincides with a state of increased resistance to subsequent cellular damage and apoptosis. The higher incidence of apoptosis is detrimental to blastocyst formation and leads to preimplantation embryo death. The objective of the present study was to determine the effects of HSP 60 and 70 inhibition on preimplantation mouse embryo development and apoptosis.

Design: Mouse embryos cultured in the presence of anti-HSP antibody and the incidence of apoptosis in embryos was determined using confocal microscopy.

Materials/Methods: Two-cell mouse embryo were cultured for 72h in human tubal fluid (HTF) media containing 10% serum substitute supplement (SSS) along with 100 µg/mL of monoclonal antibodies to HSP60 (Group I, n = 51) or HSP70 (Group II, n = 46). Simultaneously cultured embryos in HTF-SSS were used as control group (Group I, n = 71). Since the monoclonal anti HSP antibodies belong to IgG1 subclass, HTF-SSS supplemented with purified monoclonal mouse IgG1 was used as a control immunoglobulin (Group Ia, n = 54). Total blastomere count per embryo was determined by staining with bisbenzimide (Hoechst 33258). A terminal transferase-mediated dUPT end labeling (TUNEL) assay was used to determine apoptosis in blastocysts using the confocal microscopy. To distinguish apoptosis from necrosis, embryos were stained with propidium iodide (PI) before the TUNEL assay.

Results: Blastocyst development rate (BDR) was comparable between two control groups (92% and 89%, respectively). Addition of the anti-HSP60 and 70 antibodies to culture media significantly decreased the BDR (39%, p <0.0001; and 15%, p <0.0001). Compared to control embryos, total cell number per blastocyst (median (25%, 75% inter-quartile range) was significantly lower in the presence of anti-HSP60 (p = 0.002) and anti-HSP70 (p <0.0001). Percentage of cells undergoing apoptosis was significantly higher in presence of anti-HSP60 (12.8%, p <0.0001) and anti-HSP70 (13.4%, p <0.0001) compared to control embryos. BDR for embryos cultured in presence of anti-HSP70 was significantly lower compared to embryos cultured in presence of anti-HSP60 (p = 0.04).

Conclusions: The developmental alterations observed following culture of mouse embryos in presence of anti-HSP antibodies provides evidence for the role of heat shock proteins especially HSP70 on mouse embryo development and incidence of apoptosis.

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