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THE EFFECT OF ANTISERUM TO HEAT-SHOCK PROTEINS 70 (HSP 70) ON THE *IN VITRO* DEVELOPMENT OF PORCINE EMBRYOS EXPOSED TO HYPERTHERMIA

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ABSTRACT

We studied the influence of short-term exposure (6 h) to the elevated temperature (41.5°C) on the vitality of porcine embryos with blocked heat-shock proteins 70 (Hsp70). Intrinsic Hsp70 in embryos was blocked by adding the antiserum to Hsp70. The embryos at morula stage were cultured either at standard temperature (37.5 °C) or hyperthermic (41.5 °C) conditions for 6h and post-incubated at 37.5 °C for 24 h. Embryo development to the blastocyst stage, embryo cell number and apoptosis occurrence were examined. Hyperthermia alone did not affect the blastocyst rate, cell number or apoptosis (TUNEL-index) compared to control. However, anti-Hsp70 addition caused significant decreases in cell number and blastocyst rate, whilst TUNEL-index was dramatically increased compared to non-blocked embryos. These results show that brief exposure to hyperthermia does not affect embryo development, whereas the blockage of Hsp70 leads to developmental failure mainly under hyperthermic conditions.

Key words: pig, embryo, hyperthermia, apoptosis, heat shock proteins 70

INTRODUCTION

Heat stress destroys the structure and function of embryonal proteins, induces damages of the cytoskeleton and cell death (apoptosis or necrosis) affecting developmental capacity of embryos. Exposure of bovine embryos to hyperthermia induces the increase in apoptotic cell number (Paula-Lopes and Hansen, 2002). A reason for the embryo developmental arrest due to hyperthermic stress may be disorders in the actin cytoskeleton (Valderrama et al., 1998). Heat stress can be considered as a model situation for the investigation of adaptation reaction of embryos, which is a similar as at other types of stress (cold, oxidative, toxic a. o.), for example, by the production of heat shock proteins (Hansen, 2007).

It is assumed that a resistance of embryos to heat stress is associated with Hsp70 proteins. These proteins have protective function against protein denaturation, caused by elevated temperature. They are assumed to be a factor of embryo resistance to heat stress, as well as to other types of stress. Blockage of Hsp70 during embryo culture led to significant reduction in blastocyst rate (Matwee et al. 2001). Since a mechanism of thermotolerance is still not clear, elucidation of adaptation mechanisms of embryos on heat stress may help to improve developmental ability of embryos *in vitro*. The aim of this study was to determine the role of Hsp70 in the adaptation response of pig embryos to hyperthermia.

We determined the influence of short exposure (6 h) of pig morula stage embryos with blocked intrinsic Hsp70 formation (anti-Hsp70 addition) to elevated temperature (41.5 °C). The embryo development to higher preimplantation stages, cell number and cell death occurrence were evaluated.

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MATERIALS AND METHODS

Slovakian White gilts at the age of 6 months were hormonally synchronized by PMSG (1500 m.j., Sergón, Bioveta, Ivanovice na Hané, Czech Republic) administration followed by HCG injection (1000 m.j., Werfachor, ALVETRA & WERFFT AG, Austria) 72h after PMSG and Supergestran injection (1 ml, Lecirelinum 25 µg/ml, Ferring-Léčiva, Prague, Czech Republic). The insemination was done 24-36 h after the HCG injection. The gilts were slaughtered on day 4 following the HCG administration and the embryos were recovered by the oviduct flushing.

The embryos were divided into two groups: 1) standard temperature (37.5 °C) – control and 2) elevated temperature (41.5 °C). In part of embryos from both groups intrinsic Hsp-70 was blocked by anti-Hsp70 monoclonal antibody (Chemicon International, Temecula, CA, USA, 4 µg/ml). The embryos were cultured in hyperthermic conditions for 6 hours and then post-cultured overnight (16-20h) at 37.5 °C.

Developmental ability was evaluated by recording the rate of blastocysts (Bl), expanded blastocysts (XBl) and hatching/hatched blastocysts (HBl); the embryos at morula stage were considered as developmentally arrested. Total cell number was determined by counting the blastomere nuclei after labelling with the DAPI fluorescent stain. The occurrence of dead (apoptotic or necrotic) cells was determined by TUNEL assay using MEBSTAIN Apoptosis kit Direct (Immunotech, Marseille, France).

Embryo samples were analyzed using Leica fluorescent microscope (Leica Microsystem, Germany) using specific wave-lenght filters. The experiments were performed in three replicates, totally 113 embryos were used.

Data were statistically processes by Chi-square test (embryo development), one-way ANOVA (total cell number, dead cell index) using the SAS program.

RESULTS AND DISCUSSION

Embryo development to higher preimplantation stages (XBl and HBl) was not significantly influenced by elevated temperature (41.37 %) compared to control (53.57 %). Moreover, at higher temperature (41.5 °C) a slightly positive effect was observed. All the embryos advanced in development and 3 of them (10.34%) reached a highest stage - HBl, whilst at standard temperature 3 embryos (10.71%) were arrested at morula stage (Table 1.). Significant changes occurred when the antiserum against Hsp70 (ASHsp) was added to culture medium. In these groups almost half (44.44 %) or most (58.62 %) of the embryos were arrested at morula stage. Only low percentage of embryos (7.41 % and 3.45 % resp.) was developed up to XBl stage and no embryos reached HBl stage.

The ratio of embryos contained at least one TUNEL-positive (dead) blastomere, was in the range of 82 – 96 %, whilst the differences between groups were not statistically significant (Table 2.).

Table1: Effect of hyperthermia and anti-Hsp70 (ASHsp) on preimplantation embryo development

Groups	No. embryos	Developmental stages		
		Mo (arrested) n (%)	Bl n (%)	XBl + HBl n (%)
37.5 °C	28	3 (10.71)a	10 (35.71)a	15 (53.57)a
37.5°C+ASHsp	27	12 (44.44)b	13 (48.15)b,c	2 (7.41)b
41.5 °C	29	0 (0)c	17 (58.62)b	12 (41.37)a
41.5°C+ASHsp	29	17 (58.62)d	11 (37.93)a,c	1 (3.45)c

a,b,c,d – differences between groups are significant at $p < 0.05$ (Chi-square test)

Table 2: Effect of hyperthermia and anti-Hsp70 (ASHsp) on the apoptotic embryo rate

Groups	Total no. embryos	No. apoptotic embryos	Apoptotic rate %
37.5 °C	28	25	89.29
37.5°C+ASHsp	27	26	96.3
41.5 °C	29	24	82.76
41.5°C+ASHsp	29	27	93.1

Table 3: Effect hyperthermia and anti-Hsp70 (ASHsp) on cell number and TUNEL-index

Groups	No. embryos	Cell number $\bar{x}$ ± SEM	TUNEL-cells/ embryo $\bar{x}$ ± SEM	T- index, % $\bar{x}$ ± SEM
37.5 °C	28	43.29 ± 2.25a	2.32 ± 0.43a	6.45± 1.83a
37.5°C+ASHsp	16	36.63 ± 2.17b	5.93 ± 1.42b	14.79± 3.51b
41.5 °C	29	42.31 ± 2.28a	2.48 ± 0.39a	6.77± 1.24a
41.5°C+ASHsp	20	31.17 ± 1.45b	4.30 ± 1.1b	18.83± 3.81b

a,b – differences between groups are significant at $p < 0.05$ (one-way ANOVA)

Hyperthermia at 41.5°C did not influence total number of blastomeres, number of TUNEL-positive cells per embryo and TUNEL-index compared to standard temperature (Table 3.). However, the addition of anti-Hsp 70 (ASHsp) led to a significant decrease in cell number and an elevation of apoptotic cell number as well as TUNEL-index at both tested temperatures (Table 3.). TUNEL-index was increased two-fold (37.5 °C +ASHsp) or three-fold (41.5 °C+ASHsp) compared to groups with no ASHsp.

It is supposed that a brief exposure to temperatures over 41 °C is less deleterious than a chronic heat stress even at lower temperatures. Our results show that short exposure of embryos to elevated temperature does not impair their development but even contributes to faster advancement up to higher preimplantation stages. It was found in bovine (Ryan et al., 1992), that short impulses of higher temperature (43 °C) stimulated embryo development to hatching stage compared to non-heat-shocked embryos (38 °C). Kojima et al. (1996) in porcine embryos, cultured at 42 °C, recorded higher cell number and embryo diameter compared to control. Developmental disorders in these embryos became evident even upon short incubation (10-60 min) at 43-45.5 °C. These temperatures may be considered as a threshold of thermotolerance in porcine embryos. It seems that this value is different for each animal species. The temperature of 41.5 °C, in our experimental conditions, did not impair pig embryo viability, whereas in bovine embryos Jousan and Hansen (2004) observed increase in apoptosis occurrence and cell number reduction already at 41 °C. In rabbit embryos, the temperature of 42.5°C was defined as a threshold of thermotolerance (Makarevich et al., 2007).

These results show that porcine preimplantation embryos, exposed to hyperthermia for 6 h, are resistant against the temperature of 41.5 °C (Table 1). Thermotolerance of embryos in these conditions is probably associated with the Hsp70 production, because the anti-Hsp 70 addition led to destroying embryo development, which is correspond with the observation of Matwee et al. (2001). In bovine embryos a response to hyperthermia was dependent on the developmental stage (Edwards and Hansen, 1997). The exposure of 2-cell- and 4-8 cell- embryos to 41 °C declined blastocyst yield, whilst morula stage embryos were not heat-stressed. In our experimental conditions at 41.5 °C for 6 h, porcine embryo development, similarly to bovine embryos, was not affected.

In conclusion, the results show that 6 h exposure of embryos to elevated temperature (41.5 °C) did not impair their development, but even accelerated the advancement up to higher preimplantation stages. Blocking of Hsp 70 proteins by the adding anti-Hsp 70 decreased total cell numbers and induced apoptosis, what led to the developmental arrest and degeneration of embryos. These observations suggest an important role of Hsp 70 proteins not only in thermotolerance, but also in other essential processes running in the embryo.

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