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Effects of prolonged exposure of mouse embryos to elevated temperatures on embryonic developmental competence

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Abstract To investigate effects of heat stress on developmental competence, in-vitro fertilized zygotes were incubated at different temperatures until 96 h post human chorionic gonadotrophin (HCG). Under severe and moderate conditions (41°C and 40°C), most embryos did not overcome the 2-cell block. In long-term mild heat stress (39°C until 96 h post HCG), cleavage and blastocyst formation were comparable to non-heat-stress control, but the number of live pups per transferred embryo and mean litter size were significantly affected ($P < 0.05$) in the mild-heat-stress group (19.4%, and 5.1 ± 0.4, respectively), compared with control (41.7% and 8.3 ± 0.3, respectively). To elucidate the different competence, gene expression was examined and the numbers of inner cell mass (ICM) and trophectoderm (TE) cells were counted. Aberrant expression of genes for embryonic viability and trophoblast differentiation in the mild-heat-stressed blastocysts was found. Moreover, the expanded blastocysts in the heat-stressed group and the control had a ICM:TE ratio of 1:2.47 and 1:2.96 with average total cell numbers of 59.21 ± 2.38 and 72.79 ± 2.40, respectively ($P < 0.05$), indicating lower cell numbers in TE. These findings underscore that prevention of heat stress in early embryos is important for maintaining embryo viability embryos during pregnancy.

KEYWORDS: cell number, developmental competence, embryo development, gene expression, heat stress

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Introduction

It is well known that competence of embryo development is affected by various culture conditions, including temperature and composition of medium and oxygen tension, which are optimized or formulated to have similar physiological and biochemical conditions in reproductive tracts (Cooke et al., 2002; Fischer and Bavister, 1993; Summers and Biggers, 2003). In particular, heat-induced stress adversely affects reproductive efficiency in domestic animals and humans (Ivell, 2007; Jordan, 2003; Rensis and Scaramuzzi, 2003).

Although the need for research had been raised regarding effects of hot climates on fertility many years ago (Meleney, 1964), most studies so far have examined preimplantation embryonic development, effects of in-vitro culture conditions, genotype resistance to elevated temperature, spermatogenesis and genes associated with heat shock under relatively mild temperatures (approximately 2°C higher than normal body temperatures) or short-term heat-shock conditions (i.e. 12 to 24 h), (Barros et al., 2006; Hansen, 2009; Paul et al., 2008; Paula-Lopes et al., 2013; Sharpe, 2010). In domestic animals and mouse studies, elevated temperature affects oocyte maturation and embryonic development because oocytes/early cleaving embryos prior to zygotic gene activation are more vulnerable to heat stress, and the heat-stressed oocytes/embryos show impaired capacity to implant and to carry a pregnancy to term (Ealy and Hansen, 1994; Edwards and Hansen, 1997; Elliott and Ulberg, 1971; Monty Jr and Racowsky, 1987; Ulberg and Burfenin, 1967). More interestingly, early cleaving embryos exposed to a first short-term mild heat stress can develop to the blastocyst after the second more severe heat stress that was relatively longer; however, if not exposed to the first heat stress, the majority of embryos die, suggesting that preimplantation embryos can acquire thermotolerance by a heat shock memory that induces defence systems for embryo viability (Jia et al., 2010; Mirkes et al., 1999).

In the present study, we examined the association between in-vitro culture of zygotes at different temperatures and preimplantation development, and also compared the developmental competence of embryos exposed to heat stress with control embryos after the embryos were transferred to surrogates. In addition, in order to elucidate differences in developmental potential between heat-stressed and control embryos, we analysed relative transcript expression levels of genes responsible for embryo viability by using quantitative real time PCR (qRT-PCR), and trophectoderm (TE) and inner cell mass (ICM) cell numbers and the ICM:TE ratio by differential staining of blastocysts.

Materials and methods

Reagents

All chemicals were purchased from Sigma-Aldrich unless otherwise stated.

Collection of oocyte and IVF

All animal procedures were performed under Home Office project licence, according to UK Home Office and institutional guidelines and with prior approval from the University of Nottingham Animal Welfare and Ethical Review Body(Licence No. PPL40/3443, 24 Aug 2010). For collection of in-vivo-matured (IVO) oocytes and IVF, B6D2/F1 female mice aged 6–8 weeks were superovulated by an i.p. injection of 5 IU of pregnant mare serum gonadotrophin (PMSG), followed by an i.p. injection of 5 IU of HCG 48 h later. Cumulus–oocyte–complexes (COC) were collected 14–15 h later from ampullar regions of the oviducts. IVF was conducted as previously described (Vasudevan et al., 2010). In brief, capacitated spermatozoa that were collected from 10- to 12-week-old B6D2/F1 males and incubated for 60 min at 37°C with 5% CO_2 in a humidified incubator were added to the IVF medium (MEM-alpha supplemented with 0.4% BSA) containing COC. The inseminated oocytes were further incubated for 5 h at 37°C with 5% CO_2 in a humidified incubator. Subsequently cumulus cells and adhered spermatozoa were removed by repeating pipetting prior to transferring to culture medium.

Embryo culture, induced heat stress and embryo transfer

The in-vitro fertilized zygotes were cultured in modified potassium simplex optimized medium (KSOM; EMD Millipore, Billerica, MA, USA) under mineral oil at 37°C in a humidified atmosphere of 5% O_2, 5% CO_2, 90% N_2. For a long-term mild condition, the 1-cell zygotes were incubated in modified KSOM medium at 39°C in a humidified atmosphere of 5% O_2, 5% CO_2 and 90% N_2 until 96 h post HCG for blastocysts, and for a short-term mild heat stress, the fertilized embryos were incubated under the same condition for 8 h until 24 h post HCG. In addition, the fertilized zygotes were incubated under the moderate and severe condition, 40°C and 41°C, respectively (summarized in **Figure 1A**).

Long-term mild-heat-stressed blastocysts, control blastocysts (at 96 h post HCG), or short-term mild-heat-stressed 2-cell embryos were transferred to the oviduct of Day 1 pseudo-pregnant females mated to vasectomized males of proven sterility. Twelve to twenty embryos were equally transferred to the both oviducts of each female.

Quantitative real-time PCR

Total RNA isolated from three pools of 10 blastocysts per treatment were directly converted to cDNA using the FastLane cell cDNA kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Blastocyst embryos were selected on the basis of their morphological appearance (diameter and cavitation) at 96 h post HCG and then were lysed with 8 μl FCP buffer, followed by elimination of genomic DNA by gDNA Wipeout. The whole amount of treated lysate was directly used for reverse transcription at 42°C for 45 min. The resulting cDNA was then diluted 1:5 in PCR grade water (Roche Diagnostics, Mannheim, Germany) before analysis. Quantitative real-time PCR (qRT-PCR) was conducted as previously described (Amarnath et al., 2011a). qRT-PCR was carried out on LightCycler 480 (Roche Diagnostics) using