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Differential Responses of Bovine Oocytes and Preimplantation Embryos to Heat Shock

J. LANNETT EDWARDS AND PETER J. HANSEN*
Department of Dairy and Poultry Sciences, University of Florida, Gainesville, Florida

ABSTRACT The authors sought to determine whether developmental differences in the magnitude of embryonic mortality caused by heat stress in vivo are caused by changes in resistance of embryos to elevated temperature. In this regard, responses of oocytes, two-cell embryos, four- to eight-cell embryos, and compacted morulae to heat shock were compared. An additional goal was to define further the role of cumulus cells and glutathione in thermoprotection of oocytes. In experiment 1, heat shock (41°C for 12 hr) decreased the number of embryos developing to the blastocyst stage for two-cell (26% vs. 0%) and four- to eight-cell (25% vs. 10%) embryos but did not affect morulae (37% vs. 42%). In experiment 2, exposure of two-cell embryos to 41°C for 12 hr reduced the number of four- to eight-cell embryos present 24 hr after the end of heat shock (88% vs. 62%). In experiment 3, heat shock reduced the number of two-cell embryos developing to blastocyst (49% vs. 8%) but did not affect subsequent development of oocytes when heat shock occurred during the first 12 hr of maturation (46% vs. 41% development to blastocyst); membrane integrity was not altered. In experiment 4, oocytes were cultured with an inhibitor of glutathione synthesis, DL-buthionine-[S,R]-sulfoximine (BSO), for 24 hr and exposed to 41°C for the first 12 hr of maturation. Percentages of blastocysts were 35% (39°C), 18% (41°C), 17% (39°C+BSO), and 11% (41°C+BSO). For experiment 5, oocytes were either denuded or left with cumulus intact and were then radiolabeled with [^{35}S]methionine and [^{35}S]cysteine at 39°C or 41°C for 12 hr. Exposure of oocytes to 41°C for 12 hr reduced overall synthesis of ^{35}S-labeled TCA-precipitable intracellular proteins (18,160 vs. 14,594 dpm/oocyte), whereas presence of cumulus increased synthesis (9,509 vs. 23,246). Analysis by two-dimensional SDS PAGE and fluorography revealed that heat shock protein 68 (HSP68) and two other putative heat shock proteins, P71 and P70, were synthesized by all oocytes regardless of treatment. Heat shock did not alter the synthesis of HSP68 or P71 but decreased amounts of newly synthesized P70. Cumulus cells increased synthesis of P71 and P70. Results indicate there is a biphasic change in resistance to elevations in temperature as oocytes mature, become fertilized, and develop. Resistance declines from the oocyte to the two-cell stage and then increases. Evidence suggests a role for cumulus cells in increasing HSP70 molecules and protein synthesis. Data also indicate a role for glutathione in oocyte function. **Mol Reprod Dev 46:138–145, 1997.** © 1997 Wiley-Liss, Inc.

Key Words: heat shock; embryo; oocyte; heat shock protein 70; glutathione; thermotolerance

INTRODUCTION

It is well documented that mammalian females exposed to heat stress experience increased embryonic mortality (Thatcher and Hansen, 1993). Effects of maternal hyperthermia are most pronounced when experienced between the onset of estrus and insemination (Putney et al., 1989) and during the first few cleavage divisions of the early embryo (Dutt, 1963; Tompkins et al., 1967; Putney et al., 1988; Ealy et al., 1993). Effects of heat stress on embryonic mortality decline as pregnancy proceeds, so that effects are minimal by days 3 to 5 of pregnancy in the ewe (Dutt, 1963) and cow (Ealy et al., 1993) and by day 5 in the pig (Tompkins et al., 1967). Increased embryonic mortality associated with maternal hyperthermia is in part mediated by direct effects of elevated temperature on gametes and early embryos because exposure of embryos to increased culture temperature decreases development (Alliston et al., 1965; Elliot and Ulberg, 1971). In cattle, two-cell embryos were more susceptible to effects of heat shock than morulae (Ealy et al., 1995).

Ability of the early embryo to withstand elevations in temperature may be contingent on an increase in cell number or the developmental acquisition of thermoprotective biochemical mechanisms. In most cell types studied thus far, biochemical mechanisms implicated in thermoprotection of cells involve members of the heat shock protein 70 (HSP70) family and antioxidants such as the thiol-containing peptide, glutathione (GSH). HSP70 presumably protects cells from heat shock by refolding damaged proteins and stabilizing ribosomal RNA (Duncan and Hershey, 1989; Nover and Scharf, 1991), whereas GSH is thought to limit the effects of free radicals (Loven, 1988). These molecules may also

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JL Edward's present address: U.S. Department of Agriculture, Agriculture Research Service, Beltsville Agricultural Research Center, Livestock and Poultry Sciences Institute, Gene Evaluation and Mapping Laboratory, Beltsville, MD 20705.
*Correspondence to: Peter J. Hansen, Department of Dairy and Poultry Sciences, University of Florida, Gainesville, FL 32611-0920.
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be involved in acquisition of thermal resistance in embryos. Oocytes were more resistant to heat shock when microinjected with HSP70 mRNA (Hendrey and Kola, 1991). Moreover, addition of GSH to culture medium partially reduced inhibitory effects of heat shock on murine (Arechiga et al., 1994, 1995) and bovine (Ealy et al., 1992) embryos. Inhibition of GSH synthesis blocked induced thermotolerance in mouse embryos (Arechiga et al., 1995). Cumulus cells may also provide thermoprotection to oocytes; inhibition of protein synthesis caused by heat shock was reduced in oocytes encased with cumulus (Edwards and Hansen, 1996). The objective of the present study was to determine whether developmental differences in the magnitude of heat-stress–induced embryonic mortality are caused by changes in resistance of embryos to elevated temperature. We compared responses of oocytes, two-cell embryos, four- to eight-cell embryos, and compacted morulae to heat shock. An additional goal was to define further the role of cumulus cells, HSP70, and GSH in thermoprotection of oocytes.

MATERIALS AND METHODS

Materials

DL-buthionine-[S,R]-sulfoximine (BSO), estradiol, fluorescein diacetate (FDA), heparin, HEPES, hyaluronidase, mineral oil, polyvinyl alcohol, sodium pyruvate, and antibiotics were purchased from Sigma Chemical Company (St. Louis, MO). Tissue culture medium-199 (TCM-199) was purchased from Specialty Media (Lavallette, NJ). Bovine steer serum and heat-treated fetal calf serum (htFCS) were purchased from Pel-Freez (Rogers, AK) and Atlanta Biologicals (Norcross, GA), respectively. Frozen semen from various bulls was obtained from American Breeders Service (Madison, WI) or prepared locally. The CZB medium was prepared as described by Chatot et al. (1989), and CR1aa medium was prepared as described by Rosenkrans et al. (1993). Modified Tyrode's solutions (HEPES-TL, SP-TL, and IVF-TL) were purchased from Specialty Media and used to prepare TALP media as described by Parrish et al. (1986). Sterile saline [0.9% (wt/vol) NaCl] was supplemented with 100,000 IU/L penicillin-G and 100 mg/L streptomycin (Pen/Strep). Pituitary-derived follicle-stimulating hormone (FSH-P) was purchased from Schering (Kenilworth, NJ). Materials purchased from Fisher Scientific (Fair Lawn, NJ) included acrylamide solution (40%), ethidium bromide (EtBr), sodium salicylate, and trichloroacetic acid. Radiograph film was from Fuji (Tokyo, Japan) or Kodak (Rochester, NY). Radiolabeled L-[^{35}S]methionine and L-[^{35}S]cysteine (70% and 15%, respectively; specific activity $>$ 1000 Ci/mmol) was purchased from ICN (Costa Mesa, CA). Ampholines were purchased from Pharmacia (Uppsala, Sweden).

In Vitro Maturation, Fertilization, and Culture of Embryos

Procedures used to produce in vitro matured, fertilized, and cultured embryos were modifications of procedures described previously (Edwards and Hansen, 1996). Cumulus oocyte complexes (COCs) were matured for 22 hr in maturation medium [OMM; TCM-199 supplemented with 10% (v/v) steer serum, 50 μg/ml gentamicin, 0.2 mM sodium pyruvate, 2 μg/ml estradiol, and 20 μg/ml FSH-P; 10 COCs/50 μl] and then fertilized as previously described. Eight to 10 hr after addition of sperm, putative zygotes were vortexed and washed extensively in HEPES-TALP to remove cumulus cells and associated spermatozoa. Putative zygotes were placed in CR1aa (10 zygotes/50 μl) covered with mineral oil and allowed to develop further. On day 5 post-fertilization, microdrops of CR1aa were supplemented with 5 μl of neat htFCS. Cleavage and development to blastocyst were recorded on day 3 and days 7–9 post-insemination, respectively.

Developmental Changes in Embryonic Responses to Heat Shock

Within a given replicate, two-cell embryos, four- to eight-cell embryos (28–29 hours after insemination [hpi]), and compacted morulae (day 5) were washed once in HEPES-TALP and cultured in CR1aa (up to 10 embryos/50 μl) at 39°C or 41°C for 12 hr. Following heat shock, embryos were cultured continuously at 39°C. Development to the blastocyst stage was recorded on days 7–9 post-insemination. The experiment was replicated on three to five occasions and included a total of three to seven microdrops per treatment (n = 34–69/treatment).

Differential Effects of Heat Shock on Oocytes and Two-Cell Embryos

COCs were placed in OMM and cultured at 39°C or 41°C for the first 12 hr of maturation. Subsequently, all further steps of maturation (12–22 hr after collection), fertilization, and embryonic development were performed at 39°C. Two-cell embryos produced from COCs maintained at 39°C were collected at 28–29 hpi and cultured at 39°C or 41°C for 12 hr, followed by 39°C. Development to blastocyst stage was recorded. The experiment was replicated using three to six microdrops per treatment on three occasions (n = 124–135 oocytes; n = 90–100 two-cell embryos/treatment).

To determine effects of heat shock on subsequent development of two-cell embryos, embryos were collected at 28–29 hpi and cultured at 39°C or 41°C for 12 hr. At 24 hr following the end of heat shock, the number of embryos at the four-cell stage or greater was recorded. The experiment was replicated on five occasions using three to seven microdrops per treatment (n = 51 embryos).

Membrane Integrity of Oocytes, Two-cell, and Four- to Eight-Cell Embryos Following Heat Shock

Oocytes or two-cell and four- to eight-cell embryos obtained at 28–29 hpi were cultured at 39°C or 41°C for 12 hr followed by 39°C. At 12 hr following the end of heat shock, membrane integrity was assessed using FDA/EtBr to distinguish membrane-intact cells from membrane-permeable cells (Edwards and Hansen, 1996). The experiment was performed one time using

37 and 49 oocytes, 5 and 9 two-cell embryos, and 15 and 16 four- to eight-cell embryos at 39°C and 41°C.

Effects of Heat Shock and Depletion of GSH During Oocyte Maturation

Treatments were arranged in a 2 × 2 factorial with main effects of temperature (39°C vs. 41°C) and BSO (+ or −). Freshly collected COCs were placed in maturation medium (10/microdrop) ± 1 mM BSO. Control oocytes were cultured continuously at 39°C. Heat-shocked oocytes were cultured at 41°C for the first 12 hr of maturation and then cultured for the remaining 10 hr at 39°C. Following maturation, fertilization and culture of embryos, number of cleaved embryos, and blastocysts was recorded on day 3 and days 7–9. This experiment was replicated on two occasions using six to nine microdrops per treatment (n = 138–159/treatment).

Total Intracellular Protein and HSP70 Synthesis in Heat-Shocked Oocytes

Immediately after collection, oocytes were either denuded by vortexing for 7 min in 50 μl HEPES-TALP containing 300 μg/ml hyaluronidase or left with intact cumulus (COCs). COCs and oocytes were then transferred to 50 μl of CZB medium covered with mineral oil and containing 10% FCS and 50 μCi [^{35}S]methionine and [^{35}S]cysteine (20 oocytes/drop). Oocytes were cultured at 39°C or 41°C for 12 hr. Following heat shock, COCs were denuded and checked microscopically to ensure removal of cumulus cells. Both groups of oocytes were separately washed three times in CZB + 0.1% (w/v) polyvinyl alcohol; transferred in a minimum volume to 50 μl of 5 mM K_2CO_3 containing 9.4 M urea, 2% (v/v) Nonidet P-40™ (Shell Oil Co., Houston, TX), and 0.5% (w/v) dithiothreitol; and frozen at −70°C until analysis.

Incorporation of the radiolabel into intracellular proteins was determined by TCA precipitation (Mans and Novelli, 1961). Proteins from solubilized oocytes were analyzed using two-dimensional SDS-PAGE as previously described by Edwards and Hansen (1996). Each gel was loaded with 61,000–77,000 dpm of TCA-precipitable radioactivity and exposed to radiograph film. Within each replicate (n = 4), equal amounts of radioactivity were loaded for control and heat shock treatments, and films were exposed for the same amount of time. Quantitative estimates of radioactivity were obtained using densitometric analysis (Edwards and Hansen, 1996). Intensity of spots corresponding to heat shock protein 68 (HSP68), P71, and P70 were expressed on a per oocyte basis.

Statistical Analysis

Data were analyzed by least squares analysis of variance using the General Linear Models procedure of SAS (1989) and are presented as least squares means ± SEM. Each experiment was performed on several different days, using one or more microdrops (replicates) of embryos or oocytes per treatment on each day. All effects were considered fixed. To avoid statistical problems associated with percentage data, numbers of embryos cleaved or developed to the blastocyst stage within a microdrop were used as the dependent variable in statistical models, and total number of embryos or oocytes per drop were used as a covariate to adjust for differences in initial number. To ensure ease of comparison to other developmental data in the literature, data were also analyzed as percentage cleaved or percentage developed to blastocyst (calculated within a microdrop) and are presented this way. Both analyses gave very similar probability estimates; estimates reported in this article are based on analysis of number developing. TCA-precipitable radioactivity was estimated on a pool of oocytes derived from one microdrop; data are expressed on a per oocyte basis. Similarly, the peak volume obtained from densitometric analysis for HSP68, P71, and P70 represents analysis of a pool of oocytes; data were log transformed before statistical analysis. Statistical models included sources of variation due to stage of development or BSO, temperature, and replicate.

RESULTS

Developmental Changes in Embryonic Responses to Heat Shock

Exposure to 41°C for 12 hr greatly reduced the number of two-cell ($P < 0.005$) and four- to eight-cell ($P < 0.02$) embryos that developed to the blastocyst stage (Fig. 1). In contrast, subsequent development of compacted morulae was unaltered by heat shock.

Differential Effects of Heat Shock on Oocytes and Two-Cell Embryos

As shown in Figure 2, deleterious effects of heat shock were not evident when oocytes were exposed to 41°C during the first 12 hr of maturation; however, heat shock greatly reduced the number of two-cell embryos developing to the blastocyst stage (stage × temperature, $P < 0.002$). A second experiment was conducted to define the developmental period when heat shock reduces development of two-cell embryos. Exposure of two-cell embryos to 41°C for 12 hr reduced the number of embryos that were at the four-cell stage or greater 24 hr after the end of heat shock (88% vs. 62%; SEM = 12; $P < 0.01$).

Membrane Integrity of Embryos Following Heat Shock

Heat shock for 12 hr did not alter membrane integrity of oocytes, two-cell embryos, or four- to eight-cell embryos when determined 24 hr after heat shock. All oocytes and embryos examined had intact membranes regardless of treatment.

Effects of Heat Shock and Depletion of GSH During Oocyte Maturation

Exposure of COCs to BSO for 24 hr or to 41°C for the first 12 hr of maturation did not alter the number of cleaved embryos assessed on day 2 post-fertilization (Fig. 3, top). Both BSO and exposure to 41°C decreased

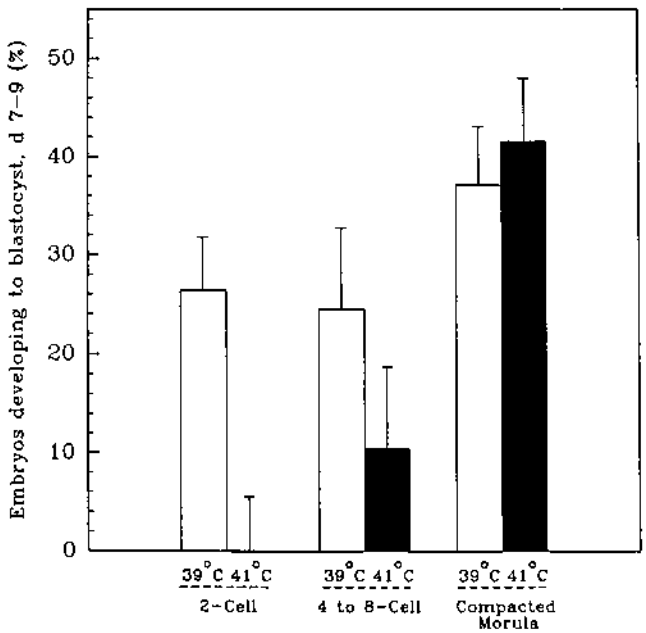

Fig. 1. Percentages of embryos developing to blastocyst following exposure of two-cell embryos, four- to eight-cell embryos, and compacted morulae to 41°C for 12 hr. The total number of embryos per treatment varied from 34 to 69. Exposure of embryos to heat shock reduced the number of two-cell ($P < 0.005$) and four- to eight-cell ($P < 0.02$) embryos developing to blastocyst but did not alter the number of morulae that developed. There were stage × temperature interactions when comparing two-cell versus compacted morula ($P < 0.05$) and when comparing two-cell and four- to eight-cell embryos versus compacted morulae ($P < 0.10$).

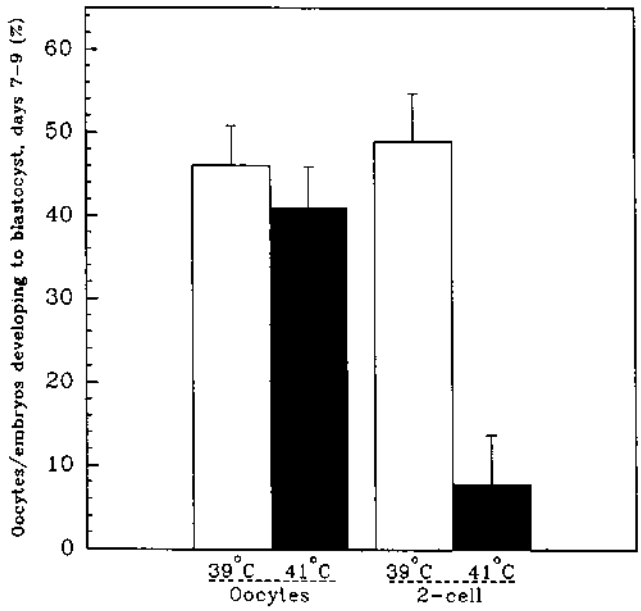

Fig. 2. Percentages of oocytes and two-cell embryos developing to blastocyst following exposure to 41°C for 12 hr. The total number of oocytes per group ranged from 124 to 135, and the total number of two-cell embryos per group ranged from 90 to 100. Heat shock decreased the number of two-cell embryos developing to the blastocyst stage but did not alter the development of oocytes (stage × temperature, $P < 0.002$).

the number of oocytes developing to the blastocyst stage by days 7–9 following fertilization (Fig. 3, bottom). The decrease in development caused by heat shock was less for BSO because development was already low (temperature × BSO, $P < 0.05$).

Total Protein and HSP70 Synthesis in Heat-Shocked Oocytes

The total amount of intracellular radiolabeled protein synthesized by the oocyte was affected by temperature ($P < 0.09$) and association with intact cumulus ($P < 0.0001$; Fig. 4). Exposure of denuded oocytes to 41°C for 12 hr reduced synthesis of intracellular proteins. In contrast, when cumulus remained encased around the oocyte, heat shock did not alter the amount of radiolabeled intracellular protein synthesized by the oocyte.

Representative profiles of intracellular proteins synthesized de novo by oocytes at 39°C and 41°C are presented in Figure 4. Qualitatively, radiolabeled protein synthetic patterns did not differ for oocytes cultured with or without cumulus. Three proteins believed to belong to the HSP70 family were identified based on their similarity to HSP70 proteins previously described in bovine oocytes and two-cell embryos (Edwards and Hansen, 1996). These proteins were HSP68, whose synthesis in two-cell embryos is increased by heat shock, and P71 and P70, whose pI and M_r are indicative that they are HSP70. Radiolabeled HSP68, P71, and P70 were detectable in all oocytes regardless of treatment (Fig. 4). Results of densitometric analysis indicated that heat shock did not alter the synthesis of HSP68 or P71 but decreased synthesis of P70 ($P < 0.001$). De novo synthesis of P71 and P70 was dependent on the presence of intact cumulus cells associated with the oocyte because denuding before radiolabeling reduced amounts of P71 ($P < 0.06$) and P70 ($P < 0.0001$). Amounts of HSP68 also tended to be reduced by denuding, but this effect was not significant.

DISCUSSION

These results indicate that the oocyte and early embryo undergo a biphasic developmental pattern of resistance to heat shock, with fertilization and cleavage to the two-cell stage being associated with increased thermal sensitivity. This is followed by restoration of resistance to elevated temperatures by the morula stage of development. These results imply that infertility caused by heat stress is due, at least in part, to effects of elevated temperatures on the embryo. They also imply that the decline in deleterious effects of maternal heat stress as pregnancy proceeds (Dutt, 1963; Tompkins et al., 1967; Ealy et al., 1993) is related to differences in the thermal sensitivity of the embryo.

Responses of oocytes exposed to 41°C varied according to the experiment being conducted. Edwards and Hansen (1996) reported that exposure of oocytes to 41°C for

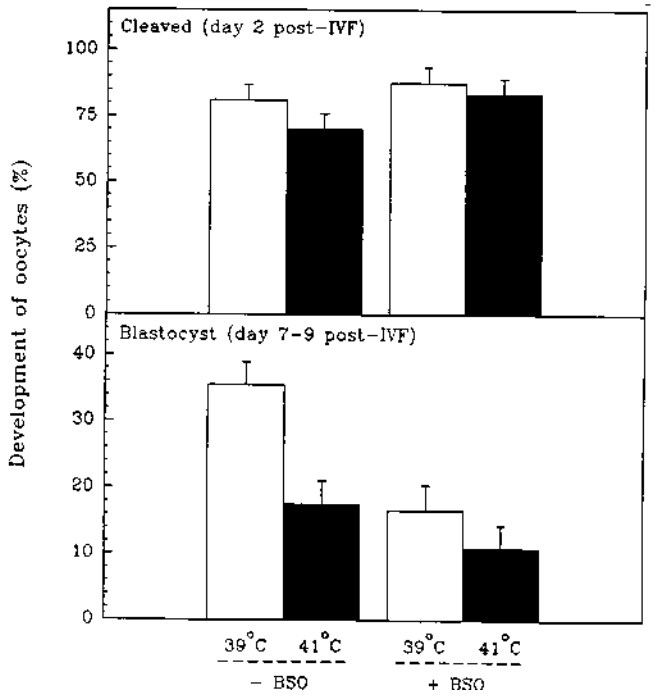

Fig. 3. Effects of BSO and heat shock during oocyte maturation on subsequent development. The total number of oocytes cultured per group ranged from 138 to 159. Neither heat shock nor BSO altered the number of oocytes that had cleaved by day 2 post-IVF (top). Culture of oocytes with BSO or at 41°C decreased the number of oocytes developing to the blastocyst stage by days 7–9 following fertilization (temperature × BSO, $P < 0.05$; bottom).

12 hr reduced subsequent development of oocytes to blastocysts (30% vs. 10% for oocytes cultured at 39°C and 41°C, respectively). Similar findings were noted in one experiment of the present study (35% vs. 18%; Fig. 3). However, there was no effect of elevated temperature in a second experiment (46% vs. 41%; Fig. 2). Disparity of results may reflect variation in the quality of oocytes obtained from the abattoir or subtle differences in culture technique or conditions. In any case, the two-cell embryo is more sensitive to heat shock than the oocyte because when ascertained across all studies, reduction in development caused by heat shock of oocytes generally was of a lower magnitude than for the two-cell embryo (26% vs. 0% in one experiment and 49% vs. 8% in a second experiment). In vivo, the oocyte may be also be resistant to heat stress; Woody and Ulberg (1964) found that pregnancy rates following insemination were not different between ewes receiving oocytes from heat-stressed donors compared with those receiving oocytes from nonstressed donors.

The effects of heat shock on the two-cell embryo occur very early in development; heat shock reduced the number of embryos that reached the four-cell stage. Similar findings have been reported in the mouse (Elliott and Ulberg, 1971). The loss of thermal resistance associated with fertilization and cleavage is likely the result of biochemical changes in the embryo that lead to the depletion of intracellular concentrations of themoprotective molecules. Indeed, HSP70 and GSH have been implicated in cellular resistance to heat shock (Mitchell et al., 1983; Loven, 1988; Riabowol et al., 1988; Hendrey and Kola, 1991; Nover and Scharf, 1991; Arechiga et al., 1995) and are present in higher amounts in the mouse oocyte compared with the two-cell embryo (Manejwala et al., 1991; Gardiner and Reed, 1994). Thus, although oocytes cannot synthesize increased amounts of HSP68 in response to heat shock (Edwards and Hansen, 1996; present paper), most probably because they are transcriptionally inactive, they may have higher basal amounts than two-cell embryos because of prestored mRNA or protein.

Cumulus cells may also provide protection to the oocyte from elevated temperatures. If so, loss of cumulus following fertilization and cleavage may increase thermal sensitivity of two-cell embryos. Present data are consistent with previous evidence that association of cumulus cells with the oocyte during maturation enhances the protein synthetic capability of the oocyte (Chian and Sirard, 1995; Edwards and Hansen, 1996). Also, removal of the cumulus decreased synthesis of P71 and P70, and this may make the oocyte more susceptible to heat stress. It is also possible that cumulus cells transfer GSH or amino acid precursors of GSH to the oocyte through gap junctions. Caution must be exercised when interpreting results of cumulus removal experiments because of the possibility of mechanical damage to the oocyte.

There was some evidence that embryos begin to regain thermoresistance as early as the four- to eight-cell stage of development. There was a nonsignificant tendency for heat shock to be less deleterious to development for these embryos than for two-cell embryos (26% vs. 0% for two-cell embryos and 25% vs. 10% for four- to eight-cell embryos). Certainly by the morula stage, embryos have acquired increased thermal resistance. Similar findings have been reported by Ealy et al. (1995). There are at least two possibilities for why embryos become more thermotolerant as development proceeds. One possibility is that increased cell number allows a larger embryo to survive the loss of a fraction of its cells. If one assumes hypothetically that the effect of heat shock is to alter the function of 50% of the blastomeres, a two-cell embryo would be left with only one blastomere to form a viable embryo, whereas a morula or blastocyst would have 30–50+ viable blastomeres to continue in development. Increased cell number is also associated with increased survival after loss of blastomeres following embryo splitting (Williams et al., 1984). A second possibility is that embryos acquire biochemical mechanisms for thermoprotection during development. Bovine embryos can increase synthesis of HSP68 in response to heat shock as early as the two-cell stage (Edwards and Hansen, 1996). However, there may be developmental differences in the magnitude of heat-induced HSP68 synthesis or in the amplitude of other heat shock proteins. Early cleavage stage mouse

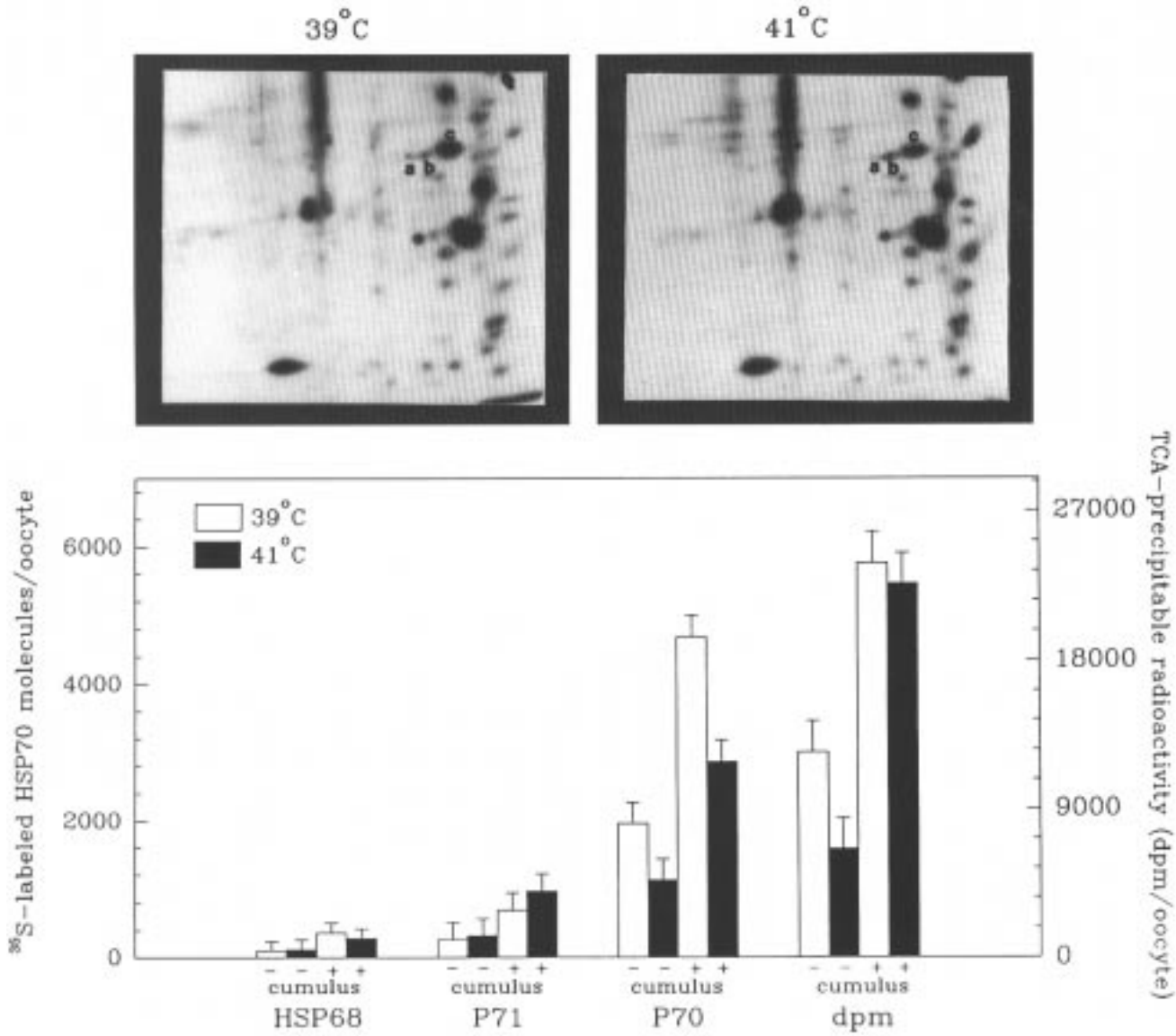

Fig. 4. Effects of heat shock and removal of cumulus on patterns of intracellular proteins synthesized by oocytes. The top panels are representative fluorographs showing [^{35}S]-labeled proteins produced by oocytes cultured at either 39°C or 41°C for 12 hr. This particular example is from oocytes cultured with cumulus intact and then denuded before analysis by TCA-precipitation and 2-D SDS PAGE. The pattern of proteins was qualitatively similar for oocytes cultured without cumulus. Synthesis of HSP68, P71, and P70 are labeled a, b, and c, respectively. The bottom graph shows results of densitometric analysis of HSP68, P71, P70, and the total amount of TCA-precipitable radioactivity synthesized during culture by oocytes cultured with cumulus intact (+) or denuded (-) before treatment. Results are least squares means ± SEM of four replicates. Heat shock did not alter the synthesis of HSP68 or P71 but decreased amounts of newly synthesized P70 ($P < 0.001$) and the total amounts of radiolabeled intracellular protein that were synthesized by oocytes ($P < 0.09$). Presence of cumulus cells increased synthesis of P71 and P70 ($P < 0.06$ and $P < 0.0001$, respectively) and the total amount of radiolabeled protein synthesis ($P < 0.0001$).

embryos have a limited capacity to synthesize GSH compared with blastocysts (Gardiner and Reed, 1995), and thermoprotective actions of GSH have been well documented in many cell types (Mitchell et al., 1983; Loyen, 1988), includingearly embryos (Ealy et al., 1992; Arechiga et al., 1994,1995).

The role of GSH in oocyte resistance to heat shock was evaluated by testing effects of BSO, a specific inhibitor of GSH synthesis (Griffith and Meister, 1979), on oocyte resistance to heat shock. A clear role for GSH in oocyte or early embryonic function was demonstrated because BSO reduced the number of oocytes capable of forming blastocysts. Additional support for this idea comes from an experiment in which cysteamine, which increases intracellular GSH (de Matos et al., 1995), increased the number of oocytes developing to the blastocyst stage following fertilization. Similar effects of BSO, as found in the present study for oocytes, have

been reported for six- to eight-cell bovine embryos (Takahashi et al., 1993). GSH is probably important for the oocyte because of its roles in scavenging free radicals (Loven, 1988), sperm nuclear decondensation (Calvin et al., 1986), and pronuclear formation (Yoshida et al., 1993). Although GSH was clearly important for the oocyte, the general reduction in oocyte developmental competence caused by BSO made it less clear whether depletion of GSH made oocytes more sensitive to heat shock. Perhaps, treatment with BSO did not increase the severity of heat shock because overall effects of BSO at both 39°C and 41°C were severe.

In conclusion, there is a biphasic change in resistance to elevation in temperature as oocytes mature, become fertilized, and develop. Resistance declines from the oocyte to the two-cell embryo and then increases. Evidence suggests a role for cumulus cells in providing protection from heat shock; this effect may be mediated by allowing for increased synthesis of HSP70 and other proteins. Additionally, results support a role for GSH in oocyte function. Mechanisms responsible for increased resistance as two-cell embryos proceed to the morula stage are still undefined. The fact that heat inducibility of HSP68 occurs as early as the two-cell stage (Edwards and Hansen, 1996) indicates that other factors in addition to HSP70 may be involved.

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