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Cellular responses of oocytes and embryos under thermal stress: hints to molecular signaling

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Abstract

Reduced reproduction in domestic species has long been a problem in tropical and subtropical areas. How to improve thermal resistance of domestic species and their gametes/embryos is centrally important for efficient production in the animal industry. In response to environmental challenges, stressed cells or embryos have a universal heat shock response, which is tightly modulated at the transcriptional and translational levels in the cell. Some of these regulatory pathways may be shared by mammalian oocytes and embryos. When a deleterious *in vitro* heat shock is applied to a mature oocyte, its membrane characteristics, configuration of the chromatin, and meiotic spindle are altered. The developmental competence of the oocytes after fertilization or parthenogenetic activation is also compromised. When heat shock occurs during pregnancy, defective fetal development could happen. However, the teratogenic mechanism is not clear yet. Currently, more information is available on the physiological responses of the cells to heat stress or elevated temperature compared to those of mammalian oocytes and embryos. To better understand the mechanisms of thermal injuries or tolerance, more work on cellular and molecular changes in oocytes and embryos in response to heat shock is necessary. This knowledge would be helpful in resolving or developing strategies to mitigate the low fertility and high embryo mortality of domestic species in the hot seasons.

Keywords: embryos, heat shock proteins, fertility, mitogen-activated protein kinases, oocytes, thermotolerance.

Introduction

Elevated ambient temperature is one of the major factors responsible for reduced fertility in farm animals (Ealy *et al*., 1995). It has been reported that the viability of cow oocytes and embryos is lower during the hot seasons than in the cool seasons (Monty and Racowsky, 1987; AI-Katani *et al*., 2002). This seasonal depression of reproductive performance may be caused by multiple factors, including suboptimal environment or management, as well as age and species-specific sensitivity to those factors (Badinga *et al*., 1985). High effective ambient temperature (HEAT; Baumgartner and Chrisman, 1987) and a combination of high temperature with humidity which causes changes in an animal's physiological core temperature, is one of the major factors responsible for low reproductive performance in domestic animals during the hot seasons. Therefore, improving reproductive efficiency during periods of HEAT becomes a major challenge for animal producers in tropical and subtropical areas.

A close correlation was found between the rectal temperature of cows (39 °C to 42°C) and a higher percentage of abnormal embryos recovered following breeding (Putney *et al*., 1988). However, the molecular and cellular mechanisms or etiology of heat induced cell damage, especially the deleterious effect of HEAT on oocyte or embryo viability, remains poorly understood. Recent studies, applying *in vitro* heat shock to oocytes and embryos, have helped in resolving the mechanism of heat-induced changes in developmental potential or damage to bovine oocytes and embryos. This paper reviews the heat shock response, the regulatory pathways of heat shock protein (HSP) expression, and thermal tolerance of oocytes and embryos. The effects of heat shock on the cellular and molecular changes of cells, oocytes, and embryos are also discussed.

Heat shock proteins and heat shock response

A large group of HSPs are molecular chaperones whose biological role is to maintain the unfolded, newly-synthesized proteins and allow them to traverse biological membranes or different cellular compartments such as the endoplasmic reticulum (ER) and mitochondria. Chaperones also prevent proteins from denaturing and help with renaturing during and after stresses (Ang *et al*., 1991).

Generally, the HSP family can be grouped into five subfamilies based on molecular weight, these include: HSP100, HSP90, HSP70, HSP60, and small heat shock proteins (sHSPs) such as HSP27, αB-crystalline (Morimoto *et al*., 1994) and immunophilins (Bose *et al*., 1996; Duina *et al*., 1996; Pennisi, 1996). In fact, the family of heat shock proteins or molecular chaperones is expanding. Heat shock proteins can be classified into two major groups, the constitutive and the inducible forms. The HSPs, synthesized at a constant rate without stimulation by stress, are termed 'constitutive' HSPs or heat shock cognates (HSC). The other group of the HSP family that can only be induced

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by heat or some other stresses are designated as "inducible" HSPs.

Heat shock proteins are actively transcribed and translated in response to elevated temperatures, usually 5 to 10°C higher than the normal homeostatic temperature of the organism (Maresca and Lindquist, 1991). This phenomenon was first discovered in 1962 when active transcription of Drosophila DNA was observed after heat shock (Ritossa, 1962). Much more attention has been drawn to this area in the last two decades.

At normal temperatures, HSPs are involved in several conventional and critical cellular functions such as protein folding and transportation, re-naturation of protein molecules, stage-specific regulation of development and cellular events, control of the cell cycle, stability of the cytoskeleton, immune responses, and conferring thermotolerance to cells and/or embryos after heat shock. Factors and stresses other than heat shock that could induce the heat shock response and HSP production include growth factors, hormones, reactive oxygen species (ROS), hypoxia, heavy metal ions, amino acid analogues, ethanol, disease, and viral infections (Ananthan *et al*., 1986; Morimoto *et al*., 1992; Fig. 1). In the case of environmental stress and certain patho-physiological stages, each condition acting on the cell leads to the activation of heat shock factors (HSFs) that in turn enter the nucleus and bind to the heat shock elements (HSEs), the DNA binding site for HSFs, in the promoter regions of heat shock genes. This leads to the activation of heat shock gene expression and synthesis of HSPs.

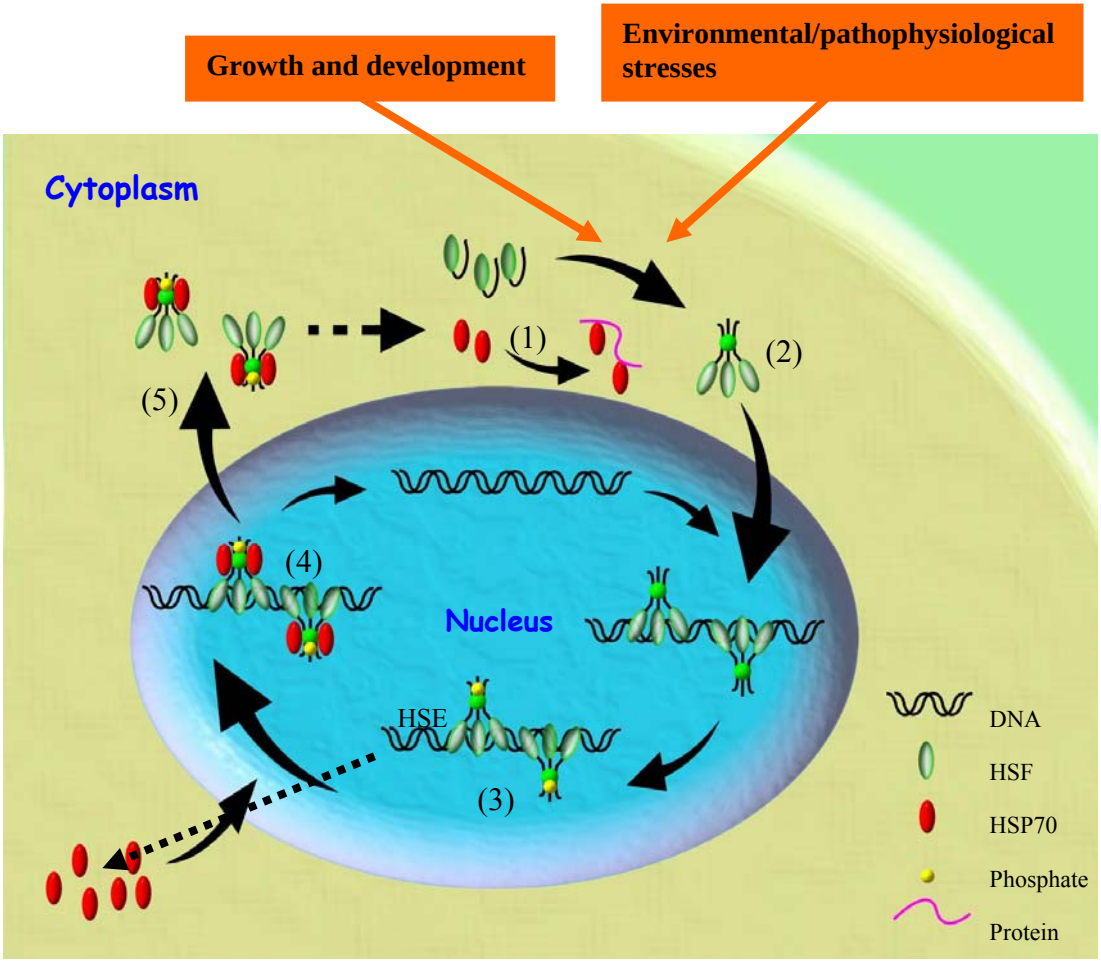

Figure 1. A proposed model for the regulation of heat shock protein (HSP70) synthesis. Representative classes of conditions known to result in the elevated expression of HSP70 are shown. Environmental/pathophysiological stresses including toxic elements and diseases, and non-stressful conditions such as cell growth and development are possible candidates. In the unstressed condition, heat shock factor (HSF) is maintained in a monomeric, non-DNA binding form through its interactions with HSP70 (1). Upon heat shock or other forms of stress, HSF assembles into a trimer (2), binds to specific sequence elements (HSEs) in heat shock gene promoters, and then becomes phosphorylated (3). Transcriptional activation of the heat shock genes leads to increased levels of HSP70 and to formation of an HSF70 complex (4). Finally, HSF dissociates from the DNA (5) and is eventually converted to non-DNA-binding monomers. (Modified from Morimoto *et al.*, 1992; Morimoto, 1993).

Transcriptional and translational regulations of HSPs

The promoter region of human HSP70 gene contains multiple HSEs to which HSFs bind. By binding to the multiple HSE regions, HSFs control the transcriptional activity of both the constitutive and inducible HSP messages which may be autoregulated. A proposed mechanism is that transcription is controlled by the HSFs (Ang *et al*., 1991). The HSFs interact with HSP70 to form an oligopolymer. Therefore, some HSPs are required to suppress the HSF activity. Such a suppression is released when the titration of HSPs decreases due to stress-induced unfolding and denaturation of native proteins (Lindquist and Craig, 1988; Morimoto, 1993; Zou *et l*., 1998). Figure 1 shows a hypothesized model of the HSF cycle, in which the proposed regulation of HSP gene expression is depicted. In the unstressed cell, HSFs are maintained in a non-DNA binding state through interactions with HSP70. Heat shock or other forms of stress activate the phosphorylation of HSFs to form trimeric or oligomeric structures (Morimoto *et al*., 1992; Morimoto, 1993), which can then bind to the HSE in the heat shock promoter region. Once the oligomeric HSFs bind to the HSE, the transcriptional activity is enhanced and the cellular concentration of HSP70 is increased. An excess of HSP70 molecules serves as a negative regulator by interacting with HSFs on the HSE and leading to the formation of the HSP70-HSF complex and dissociation of HSFs, from the DNA binding sites (Morimoto, 1993; Baler *et al*., 1996). Subsequently, transcription is reduced to the basal level in the unstressed cell. A similar auto-regulation model was suggested in prokaryotes (Ang *et al*., 1991). In the non-stressed conditions, DnaK, a homologue molecule of HSP70 in eukaryotes, interacts with or sequesters the HSF of prokaryotes (σ32) to protect it from degradation by the ubiquitin pathway or binding of RNA polymerase. In a heat-stressed condition, DnaK is associated with the damaged or denatured molecules in the cytosol. After dissociation of DnaK, σ32 can bind to the transcription complex and initiate transcription of the heat shock genes.

An intriguing aspect of HSP expression is the selective transcription and translation caused by the heat shock response. Heat shock immediately activates the transcription of heat shock genes and RNA processing and degradation (Yost *et al*., 1990). A study of Drosophila HSP82 revealed a possible regulatory control of HSP gene expression by different introns compared to those of most HSP genes. Inhibition of HSP82 expression by severely elevated ambient temperature was found during the splicing process of mRNA, in which immature HSP82 transcripts accumulated in the cytoplasm without being spliced (Yost *et al*., 1990).

Most eukaryotic HSPs are coded by multiple genes and their expression is regulated by multiple DNA binding domains (Morimoto *et al*., 1992). Since there is only one HSF found in Drosophila, regulation of transcription is much simpler in this organism than in vertebrates. It has been demonstrated that RNA polymerase II (RNAP II) is associated with the heat shock promoter at the transcription start site of the HSP70 gene in the unstressed Drosophila cells (Gilmour and Lis, 1986). Its transcription has actually initiated but then arrested at about 25 RNA nucleotides without further elongation until reactivated by heat stress (Rougvie and Lis, 1988). Therefore, the rate limiting step in transcription is the event subsequent to binding of RNAP II to the promoter region (Lis, 1991). After heat shock, the block of elongation is removed resulting in a rapid transcriptional activation (Morimoto *et al*., 1992).

The effects of HEAT on reproductive performance of animals

Many factors affected by HEAT may contribute to the low reproductive performance of farm animals during hot seasons. Feed intake and digestibility are significantly reduced by heat stress (McGuire *et al*., 1991; Bonnet *et al*., 1997). Poor nutrition during heat stress could impair both the immune system and reproductive performance in cattle (Elrod and Butler, 1993; Morrow-Tesch *et al*., 1994; Franci *et al*., 1996). An elevated ambient temperature can cause changes in an animal's metabolism by increasing blood flow and respiration rate (McGuire *et al*., 1991; Blackshaw and Blackshaw, 1994). Following exposure to heat, it may take 2 to 7 weeks to acclimate cattle (Blackshaw and Blackshaw, 1994). An animal's inability to maintain homeostasis at high temperatures may result in reduced productivity or even death.

Changes of hormonal profiles, such as increased thyroxine and insulin-like growth factors (IGF-II) levels in the circulation of the animals after heat shock, have also been reported (McGuire *et al*., 1991). Reproduction-related endocrine profiles may also be affected by HEAT. Rosenberg *et al*. (1977) reported that progesterone (P4) levels of cycling cows were significantly lower during the summer than during the winter. Gilad *et al*. (1993) found that cows with low blood estrogen levels were more sensitive to an elevation of ambient temperature. The mean concentrations of plasma gonadotropins were also lowered by heat stress. These effects could directly or indirectly modify maternal hormonal profiles and in turn alter the uterine environment (Rosenberg *et al*.,1977; Putney *et al*., 1988; Gilad *et al*., 1993). After 18-h of culture at 43 C°, interferon τ (b-TP1, bovine trophoblast protein-1) and protein synthesis in postimplantation (Day 17) bovine concepti were decreased, and the expression of HSPs and prostaglandin F (PGF2α) secretion were increased in the endometrial tissue at a

temperature of 39 °C (Putney *et al*., 1988).

Decreased reproductive performance, resulting from a hyperthermic environment, is likely due to the direct effect of elevated temperatures on the reproductive tract and/or the developing oocytes or embryos (Elliott *et al*., 1968; Ulberg and Sheean, 1973; Edwards *et al*., 1974). When albino ICR female mice were exposed to 34.5 °C and 65% humidity for 24 h immediately after mating, accumulation of 2-cell stage embryos was found at 54 h after mating. Those which developed to 4-cell stage embryos had few blastomeres capable of synthesizing RNA (failure of [3H] uridine incorporation; Bellve, 1972; 1976).

Hyperthermia can cause defective development including microcephaly, microphthalmia, and an open neural tube in 9- to 10-day-old rat fetuses (Walsh *et al*., 1987). Teratogenic development such as microcephaly resulted when the rat embryos were heat-treated for 7.5 min at 43°C. When the heat shock temperature was decreased to 42°C, no effect on the development was observed, but abnormal development occurred following 20 min of heat treatment (Walsh *et al*., 1987). However, when early head-fold stage rat fetuses (9.5 days after mating) were cultured *in vitro*, the heat shock response could be induced by a 10-min challenge at 41 °C. The severity of the abnormalities was evident relative to the increase of temperature and duration of heat shock (Walsh *et al*., 1987; Fig. 2).

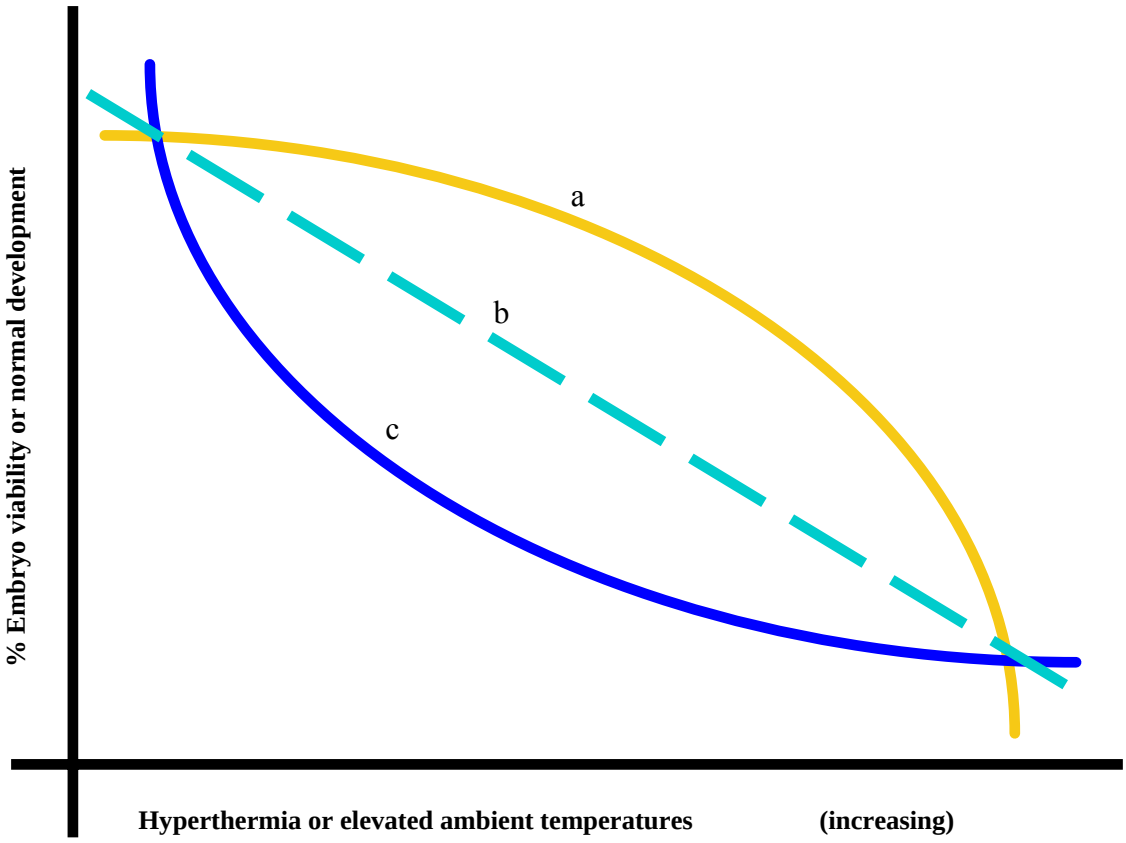

Figure 2. The conceptual illustration of the impact of ambient temperatures on the viability of mammalian embryos. The relationships between these two variables are presented as three possible regression curves (a, b, c).

Thermotolerance of oocytes and embryos

Elevated ambient temperatures may directly impair maturing and preovulatory oocytes. Mammalian oocytes and pre-implantation embryos are known to be sensitive to heat stress. The most vulnerable stages are during ovulation, fertilization, within 2 days after fertilization, and at the first cleavage division (Zavy, 1994; Ealy *et al*., 1995). Heat shocking bovine oocytes for 1 h at 40 to 42°C had no deleterious effects on subsequent blastocyst formation after *in vitro* fertilization (Ju *et al*., 1999). However, blastocyst formation rate decreases when heat shock is sustained for 12 h at 41°C (Edwards and Hansen, 1997). When the temperature increases to 43°C, the developmental competence of the treated oocytes is severely reduced following a 45-min exposure (Ju *et al*., 1999). For 2-cell bovine embryos, a 3-h heat shock at 40°C has no effect

on further development, but developmental competence decreases when heated at 41°C to 42°C for a longer period of time (Ealy *et al*., 1995; Tseng *et al*., 2004). This effect has been reported in porcine embryos (Ju *et al*., 2004).

Ulberg and Burfening (1967) investigated the relationship between the rectal temperature at the time of mating and the embryo survival or pregnancy rate in sheep and dairy cows. A negative relationship was found between embryo survival to term and pregnancy rate and increased rectal temperatures (37.5 to 40 °C). When bovine embryos were recovered on Day 7 after insemination, a positive correlation between rectal temperatures and percentage of retarded or abnormal embryos was found (Putney *et al*., 1988). Also, the survival and implantation rates of early embryos of maternally stressed mice have a negative regression coefficient with elevated rectal temperatures (39 to 41 °C; Elliott and Ulberg, 1971). It seems clear that the effect of hyperthermic conditions on the viability and developmental competence of oocytes and embryos depends upon both the temperature and the duration of exposure (Fig. 2).

Development of thermotolerance of embryos

Thermal sensitivity of mammalian cells may be affected by many factors such as environmental pH (Overgaar, 1976; Gerwech, 1977), stage of the cell cycle (Westra and Dewey, 1971; Bhuyan *et al*., 1977), presence of blood serum (Hahn, 1974), and concentration of cellular polyunsaturated fatty acids (Hidvegi *et al*., 1980; Guffy *et al*., 1982). When the environmental pH drops to 6.5 to 6.7, thermal resistance of the murine cell line L1A2 is reduced and is even more so in Chinese hamster ovary (CHO) cells (Nielsen and Overgaard, 1979; Gerwech, 1982). In CHO cells, the S-phase is found to be a more sensitive stage to thermal stress during which the inhibition of DNA repairing enzymatic activity (DNA polymerase β in CHO cells) can occur (Denman *et al*., 1982). Absence of serum in culture medium enhances cell lysis during or after heat shock (Hahn, 1974). Similarly, thermotolerance of oocytes and embryos may be regulated or enhanced by a variety of endogenous or exognous factors including developmental stage-specific gene expression, the heat shock response, protection provided by intracellular/extracellular antioxidant and antioxidative defense systems, and perhaps the genetic modification of the animals.

Developmental-regulated thermal tolerance

As described previously, the stages of development sensitive to elevated ambient temperatures are the preovulatory and the mature oocyte (Curci *et al*., 1987), as well as the embryos in early cleavage stages. The thermotolerance of preimplantation embryos increases in further developmental stages of embryos in swine (Omtvedt *et al*., 1967), sheep (Dutt, 1961), cattle (Ealy *et al*., 1993; Ealy and Hansen, 1994), mice (Ealy and Hansen, 1994), and rabbits (Alliston and Ulberg, 1965). Ealy *et al*. (1993) evaluated thermotolerance of cattle embryos by introducing heat shock to the animal on Days 0, 1, 3, 5, and 7 after insemination followed by examination of viability and stage of embryos retrieved from the uterus on Day 8. Starting from Day 3, embryos acquired a greater heat resistance with the progression of developmental stages. A substantial increase in thermotolerance is acquired on Day 4 in bovine embryos derived from *in vitro* fertilization (Ju *et al*., 1999). The thermal resistance appears to be biphasic in nature declining from the preovulatory stage to 2-cell embryo stage and then increasing beyond that (Edwards and Hansen, 1997).

Heat-induced thermotolerance

The heat shock response is a universal response to increased temperature in both prokaryotes and eukaryotes in (Craig and Gross, 1991; Georgopoulos and Welch, 1993). Previous exposure to mildly elevated temperatures induces the expression and accumulation of heat shock proteins. As a result, the resistance to a second severe elevation in temperature can be enhanced in almost all organisms. Similarly, in mouse and bovine *in vivo* produced embryos, a mild temperature increase (40°C, 1 h) induces thermotolerance in the embryos and increases their ability to survive after a subsequent severe challenge (42 to 43°C for 1 to 2 h; Ealy and Hansen, 1994; Paula-Lope and Hansen, 2002). However, a similar study was conducted using *in vitro* produced bovine embryos, but no increased thermotolerance was observed (Ju *et al*.,1999).

Embryos, from the post-implantation period to neurulation (the early organogenesis stage), were also found to be vulnerable to hyperthermia. As mentioned previously, when 9- to 10-day-old rat fetuses were challenged with teratogenic heat shock (43 °C for 5 to 7.5 min), fetal abnormalities were observed in all fetuses. A chronic elevation of temperature (40 to 41 °C for 48 h) also retarded embryonic growth and viability both in rats and cattle (Walsh *et al*., 1987; Ryan *et al*., 1992), and microcephaly was observed in the 12- to 13-day-old rat fetuses after such a treatment. In contrast, a mild, non-teratogenic heat stress (42°C, 10 min) caused embryos to acquire thermotolerance after a 15- min recovery at 38.5 °C (Walsh *et al*., 1987). This response may be mediated by the synthesis of HSPs. However, many reports have suggested that thermotolerance was not induced during HSP synthesis. Clearly, HSPs may not be sufficient by themselves to confer thermotolerance in post-implantation embryos in mice (Edwards *et al*., 1995), rats (Harris *et al*., 1991), and possibly in some other species. Although HSPs play a central role in maintaining cellular homeostasis and

development of thermotolerance (Nover, 1991), cells can acquire heat resistance without HSP synthesis. When cycloheximide, a protein synthesis inhibitor, was used to inhibit HSP synthesis in heat-treated CHO cells, superoxide dismutase (SOD) activity and thermotolerance increased (Loven *et al*., 1985) indicating a positive role of SOD in the development of thermotolerance.

Activation of the oxidative defense system

Hyperthermia is considered to cause oxidative stress in cells and tissues by generating the superoxide anion (O_2^-) or hydrogen peroxide (H_2O_2) (Fisher *et al*., 1991). In physiological conditions, the superoxide anion is degraded to H_2O_2 by SOD in the cytosol and mitochondria. Hydrogen peroxide is then removed by either catalase or glutathione (GSH) peroxidase and GSH transferase. When somatic cells, such as CHO cells and ovarian carcinoma cells, are exposed to heat shock, the SOD activity is increased in response to the stress (Loven *et al*., 1985). Glutathione is a tripeptide derived from glycine, glutamate, and cysteine and is one of the redox buffers in living cells in which it serves as a free radical scavenger catalyzed by GSH peroxidase to remove peroxides (Lehninger *et al*., 1994). Without these enzymes, cellular injuries can occur in the forms of chromatin damage, lipid peroxidation of membranes, altered cytoskeletal structures, and other detrimental changes (Loven, 1988). In thermal-tolerant cells, GSH levels are higher than in thermal intolerant strains indicating that hyperthermia can activate the antioxidative defense system and enzyme synthesis.

Oocytes and embryos are exposed to an oxidative environment or suboptimal environment during *in vivo* development. When rat embryos were in hyperthermic conditions, the GSH level was increased compared to that of the control group (Harris *et al*., 1991). *In vitro* culture systems are also an oxidative environment for oocytes and embryos (20% oxygen tension), compared to *in vivo* environments. The *in vivo* environment contains only 1.5 to 8.7% oxygen tension in the uterine tube and the uterus in several species including hamsters, rabbits, and rhesus monkeys (Fischer and Bavister, 1993). These low levels of oxygen are far less than that of the regular *in vitro* culture system. Therefore, when GSH was added to the culture medium, maturation and development of oocytes and embryos were improved in mice (Arechiga *et al*., 1995) and in cattle (Ealy *et al*., 1993). When Lbuthionine- S, R-sulfoximine (BSO), a GSH synthesis inhibitor, was used in culture, the thermotolerance of embryos decreased without affecting HSP or its mRNA synthesis both in mouse (Arechiga *et al*., 1995) and rat post-implantation embryos (Harris *et al*., 1991). These reports suggest that a similar redox system might exist in the oocyte and embryos parallel to the protective role of heat-induced thermotolerance.

Genetic manipulation

Thermotolerance of organisms can be altered using recently developed biotechnology. An HSP70 gene knock-out yeast or an anti-HSP70 antibody injected cell line has less thermal resistance than the wild type (Riabowol *et al*., 1988). Similarly, injection of anti-HSP70 mRNA or constitutive expression of HSP70 enhanced thermotolerance of rat fibroblasts (Angelidis *et al*., 1991; Li *et al*., 1991). Conversely, microinjection of HSP70 antibodies increased thermosensitivity of rat embryo fibroblasts (Riabowol *et al*., 1988). Microinjection of HSP mRNA was first reported to be effective in enhancing thermotolerance of mouse oocytes (Hendrey and Kola, 1991). Over-expression of the glutathione peroxidase gene in mice as well as HSP27 in hamsters (Hout *et al*., 1991) also contributes to increased thermotolerance and resistance to oxidative stress. Transgenic approaches and cloning of heat resistant genes or breeds may be one of the potential strategies to improve thermotolerance in domestic species in the future.

Cellular changes and possible molecular signaling of oocytes/embryos after heat shock

Alterations of the chromatin and cytoskeleton

Baumgartner and Chrisman (1981) reported a differential condensation of chromatin of mouse oocytes derived from heat-stressed females. Our recent study also showed that direct *in vitro* heat shock at 41 to 42°C for 2 to 4 h resulted in aggregation or condensation of metaphase chromosomes in both cattle and porcine oocytes (Fig. 3; Tseng *et al*., 2002; Ju and Tseng, 2004). In some cases, the chromatin of the matured oocytes separated into several groups after severe heat shock (Fig. 3B). The reason for the condensation and separation of the chromatin is not clear yet, but it might be due to the alteration of spindle microtubules. Although only limited information is available, previous studies demonstrated that reduced ambient temperature also altered the morphology of meiotic spindles (Aman and Parks, 1994; Wang *et al*., 2001). Exposure of metaphase mouse oocytes to a temperature of 25°C induces spindle disassembly within 10 to 20 min and the spindle is incompletely reassembled at 37°C, when observed by Polscope (Sun *et al*., 2004).

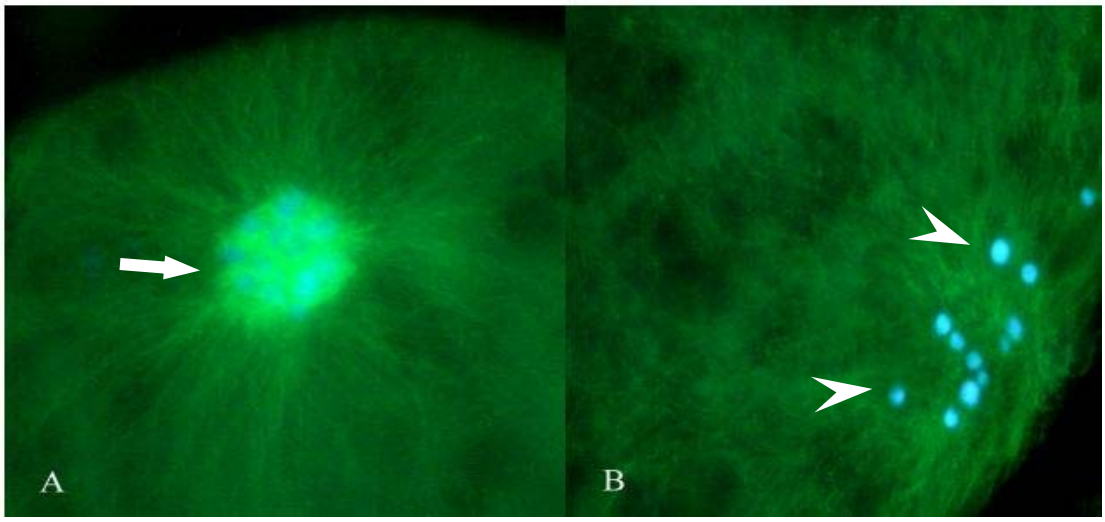

Figure 3. Morphological changes of the chromatin and the spindle in porcine oocytes during heat shock condition (1000×). (A) Disassembly of the spindle (arrow) was observed in the matured porcine oocyte after 2 h of heat shock at 41.5 0C. (B) The chromosomes condensed and separated into groups (arrow heads) accompanied by complete depolymerization of the spindle microtubules following a prolonged heat shock treatment (4 h) at 41.5 0C. (Tseng *et al*., 2002).

Detrimental and irreversible effects of elevated ambient temperatures on the cytoskeleton and meiotic spindle of mammalian oocytes have been reported. When cattle and porcine oocytes are exposed to elevated temperatures, depolymerization of the spindle is prominent and the microfilament density is changed (Tseng *et al*., 2004; Ju *et al*., 2005). It is likely that the changes in chromatin configuration of the ooytes are attributable, atleast in part, to the alteration in size and morphology of the meiotic spindle after heat shock treatment.

Apoptosis of the oocytes/embryos after heat shock

Apoptosis, or programmed cell death, is one of the important processes for normal development and might play a critical role in eliminating cells that are abnormal, damaged, or misplaced during mammalian embryonic development (Jacobson *et al*., 1997; Hardy, 1999; Meier *et al*., 2000). Apoptosis, identified by TUNEL and caspase activity (Paula-Lopes and Hansen, 2002; Roth and Hansen, 2004a), was observed in preimplantation bovine embryos subjected to 41 to 42°C heat shock in a time-dependent manner.

It is known that induction of thermotolerance with a parallel increase of HSPs inhibited heat-induced apoptosis in many cell lines (Mosser and Martin, 1992; Buzzard *et al*., 1998; Mosser *et al*., 2000). Cleavage of caspase 3 polymerase is also inhibited in HSP70-expressing cells (Gabai *et al*., 1997; Buzzard *et al*., 1998) indicating that HSPs can block the apoptotic processes of the cells. Although the apoptotic phenomena of cattle embryos (Roth and Hansen, 2004a;b, 2005) have been reported as mentioned previously, little information is available on the expression of HSPs and apoptosis, and the precise mechanisms of HSP signaling pathway during *in vivo* thermal stresses of oocytes and embryos are still largely unknown.

There have been many studies on the molecular signaling of cells in response to heat shock. Cellular stresses such as osmotic shock, arsenite, and heat shock could act as a signal to activate one of the MAPK pathways through upstream molecules such as Raf-1, Braf, mos and MAPK kinase kinase (MEKK; Moriguchi *et al*., 1996). Nevertheless, the evidence suggests that heat shock and other stresses activate their downstream molecules through a different and, perhaps, a specific kinase cascade (Dorion and Landry, 2002; Zhang and Liu, 2002).

Other MAPK homologues or its subfamily of kinases, Jun kinase (JNK) and reactivated kinase (RK) or p38 (Rouse *et al*., 1994), are stress-activated protein kinases (SAPKs), which relay heat shock and oxidative stress signals in mammalian cells (Fig. 4). Reactivated kinase and classical MAPK (p42 and p44) molecules share about 50% homology in their amino acid sequence. The upstream molecule to RK is RK kinase (RKK), which is activated by cytokines and cellular stressors including arsenite, osmolarity, or heat shock. The kinase that activates RKK, possibly RKK kinase, has not been identified (Rouse *et al*., 1994; Guay *et al*., 1997). The molecule downstream of RK, MAPK activated protein kinase-2 (MAPKAP-2), can phosphorylate small heat shock proteins HSP25 and/or HSP27 (Stokoe *et al*., 1992; Cuenda *et al*., 1995), and in turn, stabilize or repair the microfilament structure of cells (Lavoie *et al*., 1995; Guay *et al*., 1997). When the concentration of HSP27 is elevated extraordinarily, it could cause abnormal actin polymerization, extensive

cell blebbing, or apoptosis (Deschesnes *et al*., 2001; Fig. 4). Therefore, RK appears to be separated from the classical MAPK cascade in signaling a heat stress that might eventually lead to apoptosis. More work is required to further clarify this notion. To our knowledge, little information about the signaling pathways of these kinases in oocytes and embryos is available. It would be an interesting line of research for further understanding the physiologic responses of gametes and embryos under thermal stress.

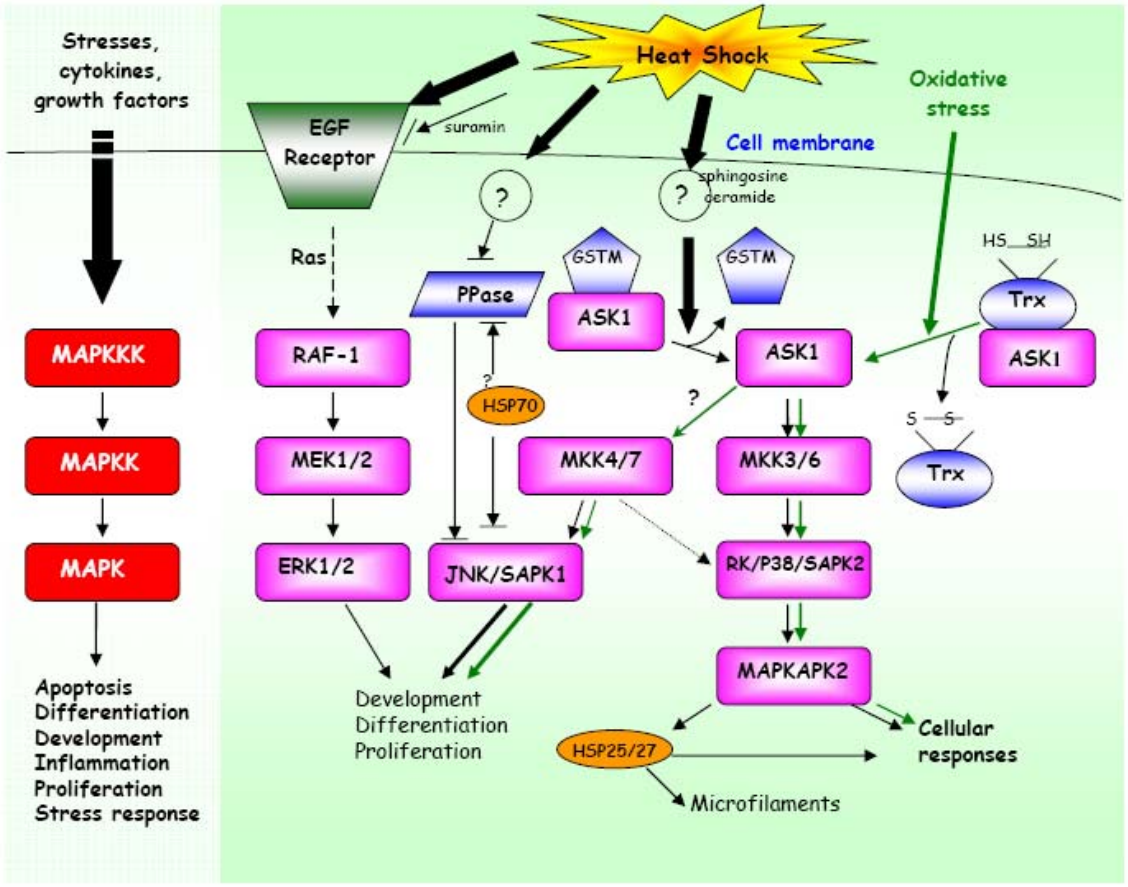

Figure 4. Proposed mechanisms for the activation of the mitogen-activated kinase (MAPK) pathways by heat shock in mammalian cells. Heat shock could activate ERK, JNK/SAPK1, or p38/SAPK2 signaling pathways and eventually trigger different cellular responses including cell proliferation, differentiation, inflammation, or apoptosis. These signaling cascades are required to be elucidated in mammalian oocytes and embryos. ASK: apoptosis signalregulating kinase; ERK1/2: extracellular signal-regulated protein kinase 1/2; GSTM: glutathione S-transferase Mu1; HSP25/27: heat shock protein 25 or 27; RK: reactivated kinase; JNK: Jun kinase; SAPK: stress-activated protein kinase; MAPKAPK2: MAPK activated protein kinase 2; MAPKKK: MAPK kinase kianse; Trx: thioredoxin. Green arrows represent possible different pathways from black arrows. (Modified from Dorion and Landry, 2002; Zhang and Liu, 2002).

In conclusion, increasing reproductive efficiency under elevated ambient temperatures is a high priority for the animal industry in tropical and subtropical areas. Enhancement of thermotolerance of oocytes and embryos appears to be one of the solutions to this problem. However, based on the current understanding, thermotolerance of the oocyte and the embryo may not be simply correlated with the synthesis of HSPs. Decreased development of oocytes and/or embryos subjected to heat stress could be caused, at least in part, by heat shock-induced apoptosis and alterations of the chromatin and spindle microtubules. Heat-elevated free radicals in the oocyte or embryos may also contribute to their low developmental competence (Hansen *et al*., 2001; Rivera and Hansen, 2001). Some other factors, such as anti-oxidative defense system, uterine environment and circulating hormonal profile of the animal are also involved. In addition, heat shock may cause calcium ion elevation in the cell (Stevenson *et al*., 1986) and could activate a large proportion of mature mouse oocytes (Komar, 1973). It is still premature to speculate if intracellular

Ca^{2+} elevation leads to apoptosis of the oocytes and embryos and the link between heat shock and oocyte activation could be through MAPK cascades. However, to resolve the mechanisms of thermotolerance in oocytes and embryos, it would be pivotal to identify whether the heat shock-induced Ca^{2+} elevation and MAPK signaling pathways occur in mammalian oocytes and embryos.

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