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Cellular and molecular basis of therapies to ameliorate effects of heat stress on embryonic development in cattle

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Abstract

Much of the effect of heat stress on establishment and maintenance of pregnancy involves changes in ovarian function and embryonic development that reduce the competence of the oocyte to be fertilized and the resultant embryo to develop. There are three possible therapeutic approaches to manipulate the connection between hyperthermia and cellular responses to elevated temperature to improve fertility during heat stress. Embryo transfer is based on the idea that 1) most effects of heat stress on fertility involve actions during folliculogenesis or on cleavage-stage embryos and 2) the embryo has acquired resistance to elevated temperature by the time it is transferred at the morula or blastocyst stage. The mechanisms for acquisition of thermotolerance involve changes in production of reactive oxygen species in response to heat shock as well as accumulation of antioxidants in the embryo. Synthesis of heat shock proteins may not be the controlling factor for acquisition of thermotolerance because transcript abundance for *HSPA1A* and *HSP90AA1* is higher for the two-cell embryo than morula. Involvement of reactive oxygen species in actions of elevated temperature on embryo survival is indicative that provision of antioxidants to heat-stressed cows could improve fertility. More work is needed but there are indications that pregnancy rates can be improved by feeding supplemental β-carotene or administration of melatonin implants. It is also evident that there are genes that control thermotolerance at the cellular level. Brahman, Nelore and Romosinuano embryos have increased resistance to heat shock as compared to Holstein or Angus embryos. Mutations in the gene for heat shock protein 70 that control resistance of cells to heat shock have been identified in Holsteins. Selection for the desirable alleles of genes conferring cellular thermotolerance could lead to development of strains of cattle whose fertility is resistant to disruption by heat stress. Pursuing these and other therapeutic approaches for reducing consequences of heat stress for livestock species should be a priority because of the prospects for continuing global climate change.

Keywords: antioxidants, cattle, embryo transfer, genetics, heat stress.

Introduction

Mammals can maintain a constant body temperature under a wide range of environmental conditions but they do not function well when body temperature rises above the regulated temperature. This is especially the case for fertility (Hansen, 2009). Females of all mammalian species experience infertility when exposed to a heat stress of sufficient magnitude to cause hyperthermia, as has been demonstrated for cattle, mice, rabbits, sheep and pigs (Dutt, 1964; Tompkins *et al*., 1967; Ulberg and Burfening, 1967; Ealy *et al*., 1993; Matsuzuka *et al*., 2005a, b). In dairy cattle, it has been estimated that conception rate begins to decline when uterine temperature rises about 0.5°C above normal (Gwazdauskas *et al*., 1973). The magnitude of heat stress effects depend upon genetic and physiological adaptations that determine regulation of body temperature. Thus, fertility of *Bos indicus* x *B. taurus* females were less affected by heat stress than fertility of *B. taurus* females (Turner, 1982). Similarly, the increased heat production caused by milk synthesis makes lactating females less able to regulate body temperature (Cole and Hansen, 1993) and more sensitive to the anti-fertility effects of heat stress than non-lactating females (Badinga *et al*., 1985).

Much of the effect of heat stress on establishment and maintenance of pregnancy involves changes in ovarian function and embryonic development that together reduce the competence of the oocyte to be fertilized and the resultant embryo to develop. It is possible to manipulate the connection between hyperthermia and cellular responses to elevated temperature to improve fertility during heat stress. Here will be discussed three therapeutic approaches for doing so and what is known regarding the cellular and molecular basis for their efficacy. One of these three approaches, embryo transfer, has been repeatedly demonstrated to greatly reduce the magnitude of infertility associated with maternal heat stress. The second approach, manipulation of the antioxidant status of the female, has not yet been reduced to practice and has yielded equivocal results in the field. The third approach, selection for genes controlling cellular thermotolerance, could lead to development of lines of cattle with superior genetic resistance to heat stress. The demonstration that there are genetic differences in embryonic resistance to elevated temperature (i.e, heat shock) means that such genes exist but their identity remains largely unknown.

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Embryo transfer - Bypassing damage to the oocyte and cleavage-state embryo

Embryo transfer represents the only method currently available to improve fertility during heat stress that is based on manipulating physiology of the cow. The other methods involve cooling cows to reduce magnitude of heat stress (Flamenbaum and Galon, 2010). Large improvements in fertility during the summer can be achieved with embryo transfer (Putney *et al*., 1989a; Ambrose *et al*., 1999; Drost *et al*., 1999; Al-Katanani *et al*., 2002a; Rodrigues *et al*., 2004; Block *et al*., 2010; Stewart *et al*., 2011; see Fig. 1 for examples). As shown in Fig. 1B, the summer decline in fertility can be largely eliminated. Embryo transfer can be expensive and the cost effectiveness of the procedure depends on maintaining a high pregnancy success using a low-cost embryo (De Vries *et al*., 2011; Ribeiro *et al*., 2012). One way to reduce the cost is to produce embryos *in vitro* using abattoir-derived oocytes. The promise represented by use of *in vitro* produced embryos has been limited by problems with vitrification (Ambrose *et al*., 1999; Drost *et al*., 1999; Al-Katanani *et al*., 2002; Block *et al*., 2010; Stewart *et al*., 2011) and reduced competence of embryos to establish pregnancy as compared to embryos produced *in vivo* (Farin and Farin, 1995; Numabe *et al*., 2000).

At the current level of embryo transfer technology, the improvement in pregnancy rates over artificial insemination is not a general feature of embryo transfer. In cases where heat stress was not present or cows were not inherently infertile (i.e, repeat-breeder cows; Son *et al*., 2007; Block *et al*., 2010; Canu *et al*., 2010), there was no difference in pregnancy rate between inseminated cows and those receiving embryos (Sartori *et al*., 2006; Rasmussen *et al*., 2013). The lack of advantage for embryo transfer in the absence of heat stress is apparent by examination of Fig. 1B to view the differences between embryo transfer and artificial insemination in the winter.

Embryo transfer improves fertility during heat stress because it bypasses loss of pregnancies caused by damage to the oocyte and preimplantation embryo. To better illustrate the biological basis of the effectiveness of embryo transfer for improving fertility during heat stress, let us consider the timing of effects of heat stress on the oocyte and preimplantation embryo. It is the exploitation of this timing that makes ET effective at improving fertility during heat stress.

Timing of heat stress effects on the oocyte

Heat stress affects embryonic development long before the embryo is formed because it disrupts the process of oogenesis. This consequence of heat stress is indicated by observations that competence of oocytes to be fertilized and/or develop to the blastocyst stage is lower in summer than in winter. This is true both following insemination of cows *in vivo* (Sartori *et al*., 2002) and after oocytes are recovered and subjected to *in vitro* fertilization (Rocha *et al*., 1998; Al-Katanani *et al*., 2002b; Ferreira *et al*., 2011) or chemical activation (Zeron *et al*., 2001). Should fertilization succeed despite damage to the oocyte, the newly formed embryo remains susceptible to damage caused by heat stress. Exposure to heat stress *in vivo* (Putney *et al*., 1988a; Ealy *et al*., 1993) or elevated temperature *in vitro* (Edwards and Hansen, 1997; Sakatani *et al*., 2004, 2012; Eberhardt *et al*., 2009) reduces the competence of the cleavage-stage embryo to develop to the blastocyst stage.

The process of oogenesis is a long one - it takes about 16 weeks for a primordial follicle to grow to the point where it exerts dominance (Webb and Campbell, 2007) - and it is not clear how early in the process heat stress can disrupt oocyte development. In one experiment with Gir cows, a 28-day period of heat stress (achieved by placing cows in environmental chambers) resulted in reduced oocyte competence for *in vitro* fertilization as late as 105 days after the end of heat stress (Torres-Júnior *et al*., 2008). It follows, therefore, that heat stress affects oogenesis at early stages of follicular growth. In another study, Roth *et al*. (2001b) found that exposure of lactating Holstein cows to heat stress of 12 h duration affected androstenedione production of cultured thecal tissue isolated from preovulatory follicles collected 28 days after heat stress.

Sensitivity of the early growing follicle to heat stress is a likely explanation for the fact that oocyte quality is only gradually restored in the autumn and that restoration of oocyte competence for cleavage in the autumn can be hastened by treatments that increase follicular turnover (Roth *et al*., 2001a, 2002).

Disruption of oogenesis could be the result of actions of elevated temperature on the follicle or oocyte or to alterations in the endocrine control of folliculogenesis. Culture of cumulus cells and thecal cells at elevated temperature has sometimes been reported to reduce steroid secretion (Wolfenson *et al*., 1997). Hormones involved in follicular growth and oocyte function whose concentrations in blood are changed by heat stress including luteinizing hormone (Wise *et al*., 1988a; Gilad *et al*., 1993), estradiol-17β (Wolfenson *et al*., 1997), and progesterone (Wolfenson *et al*., 2000). Concentrations of progesterone before ovulation can affect subsequent fertility (Bisinotto *et al*., 2010; Denicol *et al*., 2012), possibly because of actions on oocyte function, and a reduction in circulating progesterone concentrations before ovulation caused by heat stress (Wolfenson *et al*., 2000) could conceivably compromise the oocyte.

The oocyte remains sensitive to heat stress through the period of oocyte maturation. The proportion of embryos recovered from superovulated heifers at day 7 after estrus that exhibited normal morphology was reduced by exposure to heat stress for 10 h beginning at the onset of estrus and before insemination at 15-20 h after the beginning of estrus (Putney *et al*., 1989b). Heat stress can reduce the magnitude of the preovulatory surge

of LH and estradiol-17β (Gwazdauskas *et al*., 1981; Gilad *et al*., 1993). There are also direct effects of elevated temperature on nuclear maturation, spindle formation, cortical granule distribution, free radical formation, mitochondrial function and apoptosis (Payton *et al*., 2004; Roth and Hansen, 2004, 2005; Ju *et al*., 2005; Soto and Smith, 2009; Andreu-Vázquez *et al*., 2010; Nabenishi *et al*., 2012).

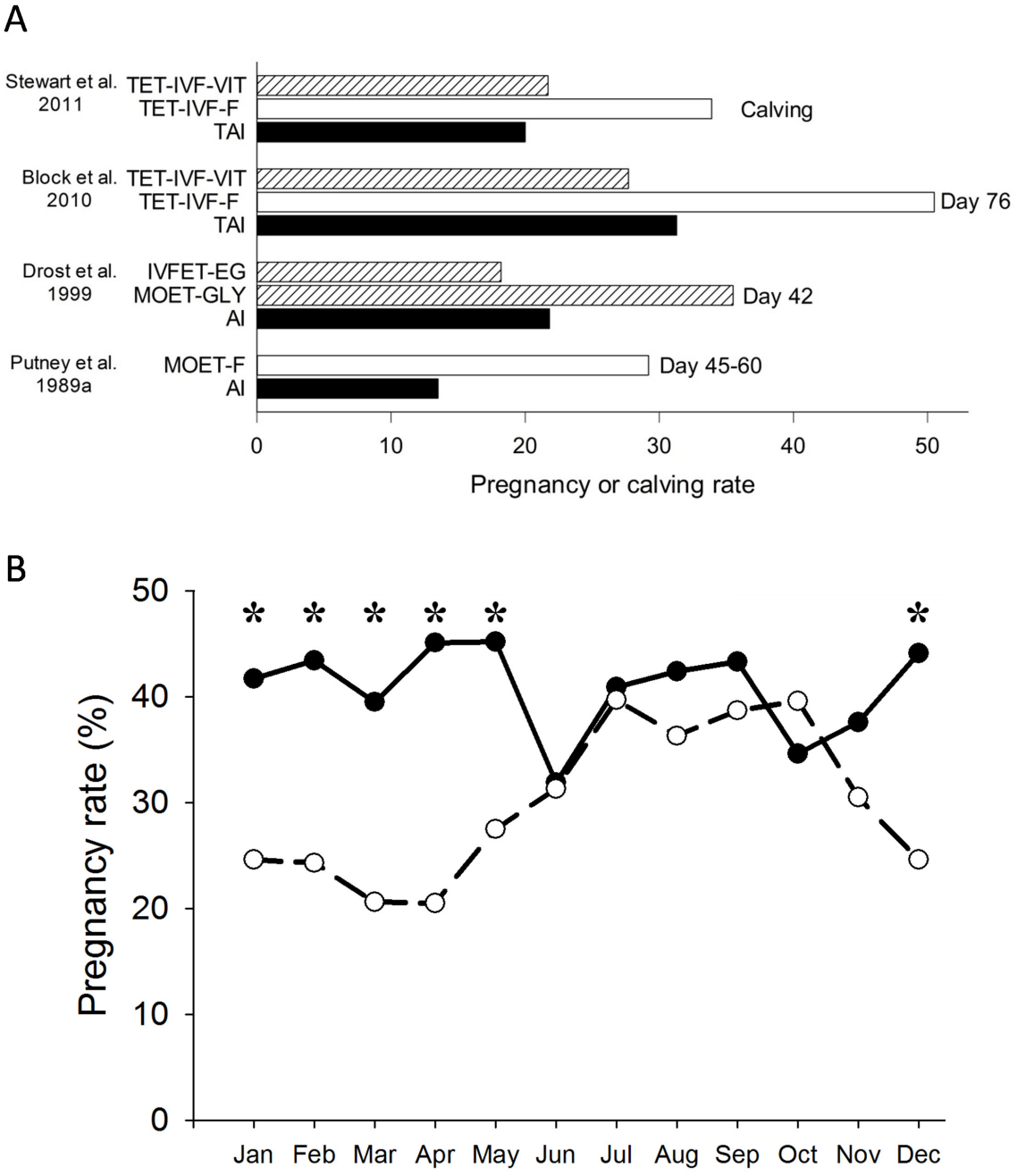

Figure 1. Examples of the effectiveness of embryo transfer for improving fertility in the summer in lactating dairy cows. Data in Panel A are from various experiments in the summer in Florida or Texas. Abbreviations are as follows: AI: artificial insemination; EG, frozen in ethylene glycol; F, fresh; Gly, frozen in glycerol; IVFET, embryo transfer with an in vitro produced embryo; MOET, multiple ovulation embryo transfer; TAI, timed artificial insemination; TET-IVF, timed embryo transfer with an in vitro produced embryo; VIT, vitrified. The numbers in the graph represent the day of gestation at which pregnancy diagnosis was carried out. Note that data from Stewart *et al*. (2011) represent calving rates. Panel B represents data from a commercial dairy in Brazil in which cows were either inseminated (open circles) or received an embryo produced by superovulation (closed circles). Asterisks represent months in which pregnancy rate was different between AI and ET. The data are from Rodrigues *et al*. (2004).

Effects of elevated temperature on the embryo

When first formed, the preimplantation embryo is very susceptible to elevated temperature. Developmental competence of the zygote and two-cell embryo can be compromised by exposure to elevated temperature *in vitro* (Edwards and Hansen, 1997; Sakatani *et al*., 2004, 2012). Disruption of developmental competence involves reduced protein synthesis, swelling of mitochondria, and cytoskeletal changes characterized by movement of organelles towards the center of the blastomere (Edwards *et al*., 1997; Rivera *et al*., 2003). Generation of free radicals occurs in response to culture at elevated temperature, at least in embryos at day 0 and 2 after insemination (Sakatani *et al*., 2004) and oxidative damage to macromolecules in the embryo could compromise development.

Soon after the two-cell stage, the bovine embryo becomes more resistant to elevated temperature. Development of four to eight-cell embryos can be compromised by heat shock but to a lesser extent than for two-cell embryos (Edwards and Hansen, 1997). By the morula stage of development, exposure of cultured embryos to elevated temperatures has little effect on development (Edwards and Hansen, 1997; Sakatani *et al*., 2004; Eberhardt *et al*., 2009; Sakatani *et al*., 2012). *In vivo*, heat stress reduced blastocyst yield from superovulated cows when occurring at day 1 after estrus but not when occurring at day 3, 5, or 7 after estrus (Ealy *et al*., 1993).

Mechanisms for acquisition of thermotolerance

Acquisition of thermotolerance by the bovine embryo is coincident with onset of embryonic genome activation, which occurs at the 8-16 cell stage (Memili and First, 2000). However, there are several lines of evidence to indicate that it is not the capacity to synthesis thermoprotective molecules that make the embryo so sensitive to elevated temperature early in development. Perhaps by virtue of inheritance from the oocyte, steady-state amounts of mRNA for *HSPA1A* (heat shock protein 70), *HSP90AA1* (heat shock protein 90) and *SOD1* (superoxide dismutase) are actually higher in the one-cell or two-cell embryo than the morula (Fear and Hansen, 2011; Sakatani *et al*., 2012; see Fig. 2). New synthesis of heat shock protein 70 in response to elevated temperature can also occur in the two-cell embryo (Edwards *et al*., 1997; Chandolia *et al*., 1999).

Recently, it was shown that exposure of morulae to 40^{o}C did not cause a large increase in expression of genes involved in the heat shock protein response. Transcript abundance following heat shock increased for only 4 of 68 genes associated with the heat shock response (Sakatani *et al*., 2013). Since the main intracellular signal for transcription of heat shock protein genes is denatured protein (Calderwood and Gong, 2012), it is possible that thermotolerant embryos undergo less protein denaturation, and perhaps less cellular damage in general, than thermosensitive embryos. One reason might be a change in the balance between reactive oxygen species (ROS) generation and antioxidant protection. Culture at elevated temperature increased production of ROS at days 0 and 2 after fertilization but not at days 4 and 6 (Sakatani *et al*., 2004). The cytoplasmic antioxidant glutathione is at its lowest during the two to eight-cell stages and increases thereafter (Lim *et al*., 1996).

Rationale for ET during heat stress

Changes in thermosensitivity of the oocyte and embryo to heat shock and what is known about the molecular basis for these changes is illustrated in Fig. 3. As has been outlined in the preceding paragraphs, the oocyte is susceptible early in the process of folliculogenesis and continues to be through the period of oocyte maturation. The embryo also begins its existence in a state that is very susceptible to heat shock. By the 8-16 cell stage, however, the embryo gains resistance to elevated temperature, possibly because of alterations in the balance between ROS production and antioxidant defenses. Given this prolonged period of sensitivity of the oocyte and embryo to heat shock, it is not surprising that cooling cows for short periods of time coincident with ovulation have had only limited impact on fertility (Stott and Wiersma, 1976; Wise *et al*., 1988b; Ealy *et al*., 1994). Hormonal treatments have also been unsuccessful at improving fertility during heat stress (see Hansen, 2011 for review). Their ineffectiveness is also probably tied to the broad period of time in which the oocyte and early embryo are susceptible to disruption by heat shock. Hormonal treatments that might mitigate some effects of heat stress cannot reverse others. For example, treatment with GnRH to turnover follicles could conceivably remove a follicle damaged by heat stress but follicles that emerge from the growing pool after GnRH treatment would also have been damaged by heat stress.

Embryos are typically transferred into recipient females when they have reached the morula or blastocyst stages of development, typically at day 7 after ovulation. Embryos used for embryo transfer have escaped harmful consequences of heat stress on the oocyte and embryo, either because they were collected in the cool season, produced *in vitro*, or represent the fraction of oocytes and embryos capable of continued development after heat shock. Moreover, exposure to elevated temperature while developing in the recipient female is unlikely to affect an embryo selected for transfer because the embryo is at a stage of development that is resistant to elevated temperature. The bypassing of effects of heat stress on the oocyte and embryo combined with the placement of a thermoresistant embryo in the uterus means that pregnancy rates with embryo transfer during heat stress can be equivalent to those after artificial insemination during cool weather (Fig. 1B; Rodrigues *et al*., 2004). Similarly, there was no difference in pregnancy rate in

lactating recipients between summer and winter (Putney *et al*., 1988b; Drost *et al*., 1999; Rodrigues *et al*., 2004; Loureiro *et al*., 2009).

A word of caution is appropriate. There are two papers with lactating Holsteins that would suggest that, at least under certain circumstances, heat stress can compromise pregnancy establishment in embryo transfer recipients. Working in Brazil with embryos produced by superovulation, Vasconcelos *et al*. (2006) found that rectal temperature at the time of transfer was inversely related to pregnancy rate at days 25 and 46 of gestation, and positively related to pregnancy loss between those times. Also, Block and Hansen (2007) found seasonal variation in pregnancy rate in Florida using embryos produced *in vitro*. The pregnancy rate at day 45 of gestation was 28% in the cool season and 18% in the warm season. The seasonal effect could be abolished, however, if embryos had been produced in culture in the presence of insulin-like growth factor 1 (IGF-1). In that case, pregnancy rates were 23% in the cool season and 49% in the warm season. Treatment with IGF-1 can make embryos resistant to heat shock (Jousan and Hansen, 2007) and there might be variation between embryos in the degree of thermotolerance at the blastocyst stage.

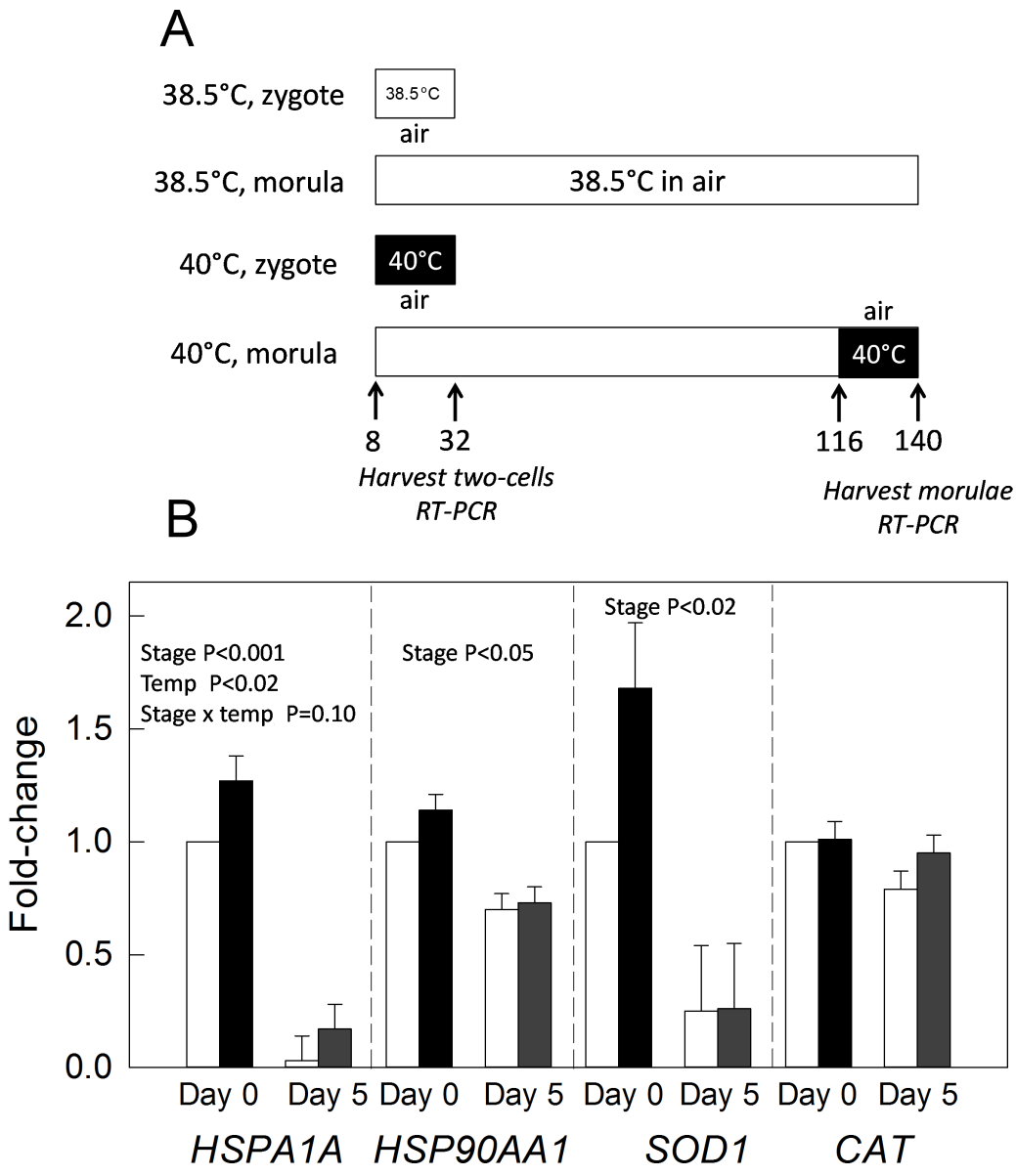

Figure 2. Effects of heat shock on expression of genes involved in cellular thermotolerance in preimplantation bovine embryos. Embryos were collected at the zygote (day 0) or morula stage (day 5) and cultured for 24 h at 38.5°C (open bar) or 40°C (black bar; Panel A). Thus, day 0 embryos had reached the two-cell stage at the time embryos were collected for analysis of RNA. Gene expression is presented in Panel B. The figure is reproduced from *Journal of Dairy Science*; Sakatani *et al*. (2012) with permission.

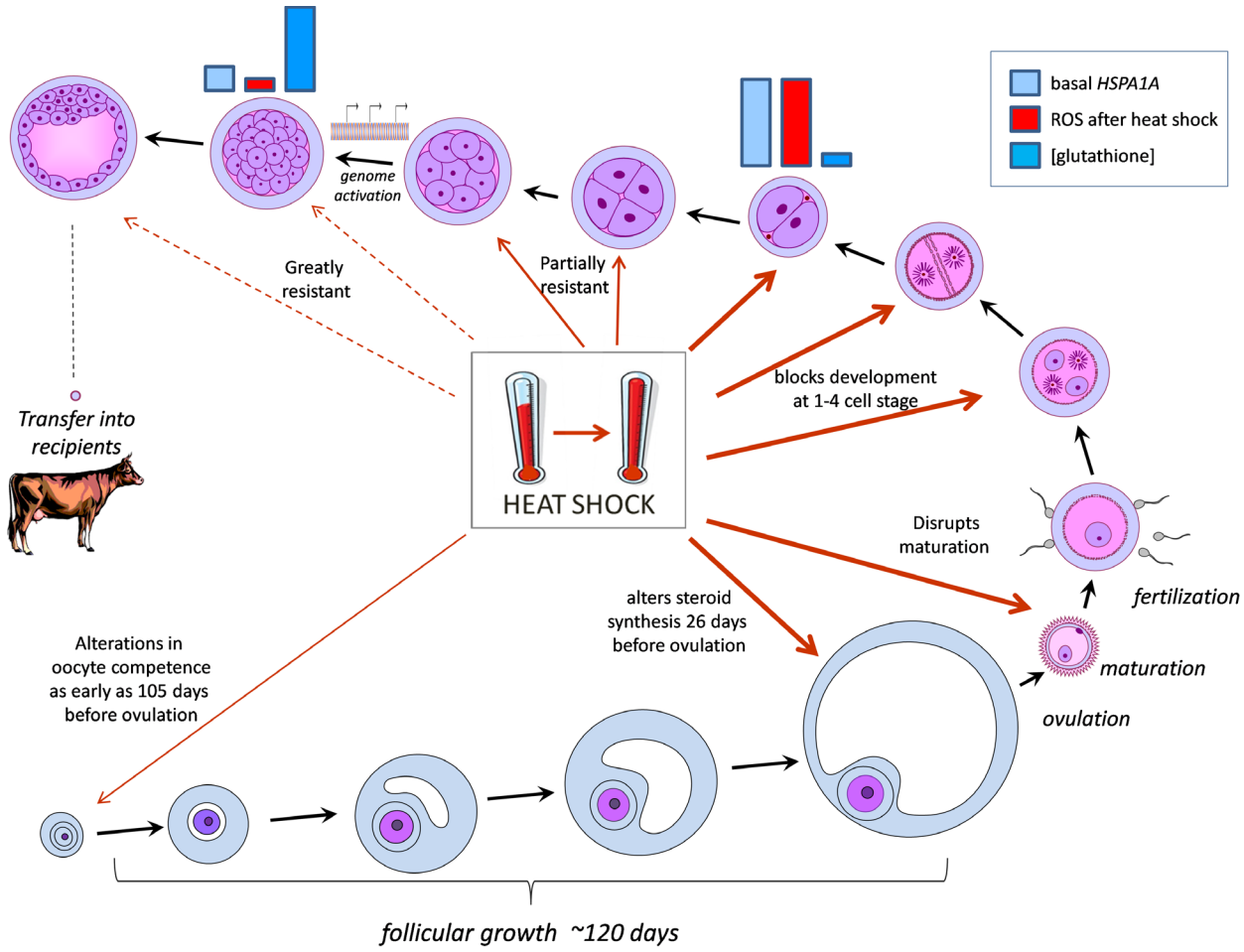

Figure 3. Diagram illustrating the timing of heat shock effects on events leading to blastocyst formation. Heat shock can affect oocyte competence for fertilization and development during follicular development. It is not known how early in the process of folliculogenesis that heat stress is disruptive to oocyte development but results from one paper suggests actions could occur as early as 105 days before ovulation. Oocyte maturation is also compromised by heat shock. The early cleavage-stage embryo remains susceptible to heat shock but thermal tolerance increases by the morula stage. Acquisition of thermotolerance occurs coincident with activation of the embryonic genome. Nonetheless, transcript abundance for some key cytoprotective molecules, including *HSPA1A*, is actually higher at the two-cell stage than at the morula stage. In contrast, the embryo is more susceptible to damage by reactive oxygen species (ROS) early in development. The production of ROS in response to heat shock is greater at the two-cell stage than in the morula. Furthermore, intracellular concentrations of the antioxidant glutathione are low at this stage. Finally, note that by the time an embryo is selected for embryo transfer, typically at the blastocyst stage, it has bypassed damage caused by heat shock during follicular growth or early embryonic development and has also acquired biochemical systems that protect it from elevated temperature. As a result, effects of heat stress in embryo transfer recipients is reduced or absent as compared to cows bred by natural or artificial insemination.

Experience with use of antioxidants to protect embryos from heat shock

Exposure of maturating oocytes (Nabenishi *et al*., 2012) and early cleavage-stage embryos increases production of ROS (Sakatani *et al*., 2004). This action of heat shock is probably a reflection of the increase in cellular metabolism caused by elevated temperature so that reactions that generate free radicals are increased. *In vivo*, as well, there is one report indicating increased oxidative stress associated with heat stress in Holstein cows during the transition period (Bernabucci *et al*., 2002). Results such as these suggest that it might be possible to enhance antioxidant defenses and thereby reduce effects of heat stress on fertility. Unfortunately, most attempts to do so have failed including injections of vitamin E with or without selenium (Ealy *et al*., 1994; Paula-Lopes *et al*., 2003a) and injection of β-carotene (Aréchiga *et al*., 1998b). One reason may be that periodic administration of antioxidants may not be sufficient to

continuously protect the oocyte and embryo from ROS induced by elevated temperature. Fertility was improved by antioxidant supplementation in the one study in which the antioxidant was fed. In particular, Aréchiga *et al*. (1998a) found that feeding cows supplemental β-carotene at a rate of 400 mg/day from about day 15 after calving increased the proportion of cows that were pregnant at 90 days postpartum during the summer but not during the winter. There was no effect of treatment on pregnancy rate at first service so the supplemental β-carotene either improved fertility after first service or estrus detection rate. Recently, Garcia-Ispierto *et al*. (2012) found that administration of melatonin implants beginning at 220 days of gestation to cows during the summer reduced interval to conception in the subsequent postpartum period and decreased the incidence of cows experiencing >3 breedings per conception. Melatonin has antioxidant properties in the follicle (Tamura *et al*., 2013) and had earlier been found to reduce effects of heat stress in mice (Matsuzuka *et al*., 2005b).

Free radical chemistry is complex. Some antioxidants function in the water-soluble fraction of the cell and others in the lipid-soluble fraction. Moreover, molecules that act as antioxidants in certain conditions can function as prooxidants in others (Gutteridge and Halliwell, 2010). We know little about the distribution of antioxidants in the reproductive tract and it may be that some are more likely to enter the oocyte or embryo than others. Two antioxidants have been reported to protect embryos from heat shock in culture (anthocyananin; Sakatani *et al*., 2007), and dithiothreitol (Castro e Paula and Hansen, 2008), whereas vitamin E (Paula-Lopes *et al*., 2003a), glutathione (Ealy *et al*., 1995), and glutathione ester (Ealy *et al*., 1995) were not thermoprotective. There is not yet enough known about the approach to antioxidant supplementation that is the most likely to be effective for improving fertility during heat stress. The recent encouraging results with melatonin (Garcia-Ispierto *et al*., 2012) deserve further research, including whether fertility-promoting effects are seen in the absence of heat stress.

Genetic selection for genes that confer cellular thermoprotection

It has long been known that there are genes in cattle that contribute to maintainance of body temperature during heat stress. Thus, certain breeds of beef (Hammond *et al*., 1996) and dairy cattle (Srikandakumar and Johnson, 2004) are better able to regulate body temperature during heat stress than others. Even in the Holstein, rectal temperature during heat stress is heritable, with estimates of 0.17 (Dikmen *et al*., 2012). Thus, genetic improvement in resistance to heat stress is possible using genetic selection or crossbreeding.

There are also breed differences in cellular responses to elevated temperature. Nelore, Brahman, and Romosinuano embryos are more resistant to the disruptive effects of elevated temperature on development than Angus or Holstein embryos (Fig. 4). In addition, previous exposure to heat shock tended to reduce ability of Angus blastocysts to establish pregnancy after transfer into recipients whereas there was no effect for Nelore embryos (Silva *et al*., 2013). Breed differences in thermotolerance have also been observed in endometrium (Malayer and Hansen, 1990) and lymphocytes (Kamwanja *et al*., 1994; Paula-Lopes *et al*., 2003b).

In crossbred embryos, it was the breed of oocyte and not breed of spermatozoa that determined whether embryos exhibited increased thermotolerance (Block *et al*., 2002; Satrapa *et al*., 2011). For example, embryos produced by fertilization of Brahman oocytes with Angus semen were more resistant to heat shock than embryos produced by fertilization of Holstein oocytes with Angus semen (Block *et al*., 2002). In the same experiment, there were no differences in thermotolerance of Brahman x Holstein and Angus x Holstein embryos. One explanation for this phenomenon is that transcripts that accumulate in the oocyte are responsible for thermotolerance later in development. Alternatively, the genes conferring cellular thermotolerance are paternally imprinted.

An important unanswered question is whether breed differences exist in resistance of the oocyte or early embryo to heat shock. The silencing of transcription in these cells may not allow for expression of genetic differences in thermotolerance. However, if the transcripts that accumulate in the oocyte are responsible for differences in thermotolerance, genetic differences are likely to occur at early stages of development. Transcript abundance for *HSPA1A* and *HSP90AA1* is higher in two-cell embryos than in morulae (Fear and Hansen, 2011; Sakatani *et al*., 2012).

Identification of the genes controlling cellular thermotolerance or of genetic markers linked to those genes could lead to selection of cattle possessing embryos with increased resistance to disruption by elevated temperature. To date, only one such genetic marker has been identified. Basiricò *et al*. (2011) studied relationship between two SNPs in the 5' untranslated region of the heat shock protein 70 gene and resistance of peripheral blood mononuclear cells from lactating Holsteins to exposure to 43°C for 1 h *in vitro*. Both SNPs affected viability following heat shock. Moreover, the allele that was associated with increased survival also resulted in increased expression of the HSP70.1 gene. It is worth noting that both of these SNPs were related to calving percentage in seasonal-calving Brahman-influenced cows (Rosenkrans *et al*., 2010).

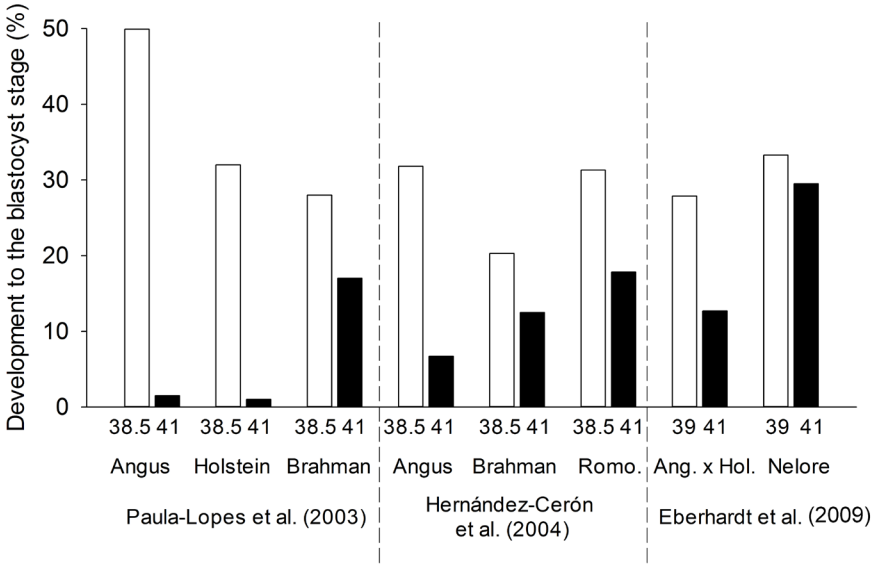

Figure 4. Breed effects on ability of embryos to develop to the blastocyst stage after exposure to heat shock. Embryos were either cultured continuously at either 38.5 or 39°C or were exposed to a heat shock of 41°C on either day 4 (Hernández-Cerón *et al*., 2004; Eberhardt *et al*., 2009) or day 5 (Paula-Lopes *et al*., 2003b) after insemination. The proportion of embryos that became blastocysts was determined. Ang.= Angus, Hol.= Holstein, Romo.= Romosinuano.

Where do we go from here?

It is crucial that we do go somewhere because all indications are that global climate change will have serious effects on agricultural production in the next 40 years or so (Battisti and Naylor, 2009). With respect to cattle reproduction, it is fortunate that there are prospects for using technology to bypass effects of heat stress on the oocyte and embryo by use of embryo transfer and by increasing resistance of the cow and its embryo to heat shock through nutritional or genetic means. Of these three strategies, embryo transfer is the only one that has been reduced to practice. To become a practical solution for large numbers of farms, additional efforts should be made to reduce the costs of producing a pregnancy by embryo transfer, either by improving efficiency of embryo production or by enhancing the competence of the embryo to develop into a healthy calf. An embryo used for embryo transfer can also be made more valuable by use of sexed semen (Rasmussen *et al*., 2012), and by genomic testing of the embryo before transfer (Moghaddaszadeh-Ahrabi *et al*., 2012).

There are encouraging indications that chronic administration of antioxidants such as β-carotene (Aréchiga *et al*., 1998a) and melatonin (Garcia-Ispierto *et al*., 2012) can improve fertility. Additional research into development of practical delivery systems is warranted. It may also be fruitful to evaluation fertility-promoting effects of less-well known antioxidants that exist in nature because they may have different properties than the more commonly-studied antioxidants. Two of these, the anthocyanins found in sweet potato (Sakatani *et al*., 2007) and epigallocatechingallate found in green tea (Roth *et al*., 2008) have been reported to protect the embryo (anthocyanins) or oocyte (epigallocatechingallate) from elevated temperature.

Perhaps the most promising way to mitigate the problem of heat stress is to change cattle genetically so that they are better able to regulate body temperature during heat stress and so that oocytes and embryos are better able to cope with elevations in body temperature that do occur during heat stress. The most practical way to do this in the past was by crossbreeding but this practice resulted into the introduction of undesirable genes as well as desirable ones into cattle populations used for production. SNPs for rectal temperature during heat stress have been identified in Holsteins (Dikmen *et al*., 2013) as well as SNPs for cellular resistance to heat shock (Basiricò *et al*., 2011). Further advances in livestock genomics, including incorporation of whole genome sequencing into selection schemes (Hayes *et al*., 2013), should make it easier to identify and select alleles conferring thermotolerance at the whole animal and cellular level. Moreover, it will be possible to use molecular tools like TALENs (Joung and Sander, 2013) to perform genome editing to change the sequence of specific genes and introduce favorable alleles into cattle populations. Some of these alleles could represent new mutations not existing in nature that improve thermotolerance and other traits of importance in a warming world.

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