Anim Reprod, v.9, n.3, p.395-403, Jul./Sept. 2012

Heat stress induced alteration in bovine oocytes: functional and cellular aspects

F.F. Paula-Lopes[1,2,4], R.S. Lima[2,3], P.H.B. Risolia[1,2], J. Ispada[1], M.E.O.A. Assumpção[3], J.A. Visintin[3]

[1]Institute of Environmental Sciences, Chemistry and Pharmacy, Federal University of Sao Paulo, Diadema, SP, Brazil.
[2]Institute of Biosciences, Sao Paulo State University, Botucatu, SP, Brazil.
[3]Department of Animal Reproduction, University of São Paulo, São Paulo, SP, Brazil.

Abstract

High environmental temperatures observed during the hot months of the year reduce fertility in lactating dairy cows. Summer heat stress depression in fertility is a multifactorial problem that affects physiological and cellular functions in several tissues. It has been shown that in addition to compromise follicular development, hormonal secretion, endometrial and embryonic function, heat stress also markedly reduces oocyte developmental ability. Oocyte susceptibility to elevated temperature can be detected during the germinal vesicle (GV) and oocyte maturation periods. *In vivo* (heat stress) and *in vitro* (heat shock) experiments indicated that exposure of bovine oocytes to elevated temperature affects the events required for successful oocyte maturation, fertilization and preimplantation embryonic development. This heat-induced decrease in oocyte function occurs due to a series of cellular alterations that affects nuclear and cytoplasmic compartments of the bovine oocyte. However, thermoprotective molecules such as growth factors and apoptosis inhibitors, which rescue heat-induced oocyte damage and developmental competence, can reverse these cellular changes. Therefore, identification of thermoprotective molecules can be considered as an alternative to modulate the effects of elevated temperature in reproductive function.

Keywords: bovine, fertility, heat stress/shock, oocyte.

Introduction

Effects of heat stress on animal fertility

Heat stress can be defined as the sum of external forces acting on a homeothermic animal to shift body temperature from the resting state (Yousef, 1984), causing physiologic, metabolic, cellular and molecular changes. Bovine are homeothermic animals which regulate internal body temperature by the balance between the amount of metabolic heat produced and heat dissipation to the environment in order to maintain constant body temperature (Hansen, 2004).

Bos indicus animals (Zebu) have greater thermoregulatory ability than *Bos taurus* (European). Such thermoregulatory efficiency of *Bos indicus* cattle is due to lower internal heat production and/or higher heat dissipation to the environment. Thus, these breeds are more resistant to hyperthermia (Adeyemo *et al*., 1979; Bennett *et al*., 1985; Hammond *et al*., 1996, 1998). Genetic selection for high milk yield decreases the thermoregulatory ability in animals exposed to heat stress (Berman *et al*., 1985). Lactating cows are more susceptible to heat stress since high metabolic heat production associated with lactation predispose to hyperthermia. Therefore, the magnitude of the deleterious effects of heat stress on fertility is more pronounced in high-producing dairy cows (Al-Katanani *et al*., 1999), whereas fertility of heifers is usually not affected by heat stress (Badinga *et al*., 1985).

High environmental temperatures observed during the hot months of the year reduce fertility in lactating dairy cows (Dunlap and Vincent, 1971; Badinga *et al*., 1985; Al-Katanani *et al*., 1999; Pires *et al*., 2002). Exposure of lactating cows to high temperature and humidity increases internal body temperature, resulting in heat stress and reduced pregnancy rates. In studies conducted in Florida, conception rates of lactating Brown Swiss, Jersey and Holstein cows decreased from 52 to 32% as maximum air temperature increased from 23.9 to 32.2°C during summer (Badinga *et al*., 1985). Al-Katanani *et al*. (1999) reported that 90-day non-return rate to the first service was lower when air temperature was higher than 20°C on day -10, day of insemination (day 0) and day +10 (Al-Katanani *et al*., 1999). Similarly, in Brazil, pregnancy rates of Holstein cows housed in free stall reduced from 71.2% in the winter to 45.7% in the summer (Pires *et al*., 2002).

Summer heat stress depression in fertility is a multifactorial problem that affects physiological and cellular functions in several tissues. It has been shown that in addition to compromise follicular growth (Badinga *et al*., 1993; Wolfenson *et al*., 1995), hormonal secretion (Wolfenson *et al*., 1995; Roth *et al*., 2000), uterine blood flow (Roman-Ponce *et al*., 1978), endometrial (Malayer *et al*., 1988) and embryonic (Ealy *et al*., 1993; Paula-Lopes and Hansen, 2002b) function, heat stress also causes a great reduction in oocyte developmental potential (Al-Katanani *et al*., 2002; Paula-Lopes *et al*., 2008).

The cellular mechanisms triggered by elevated temperature in bovine oocytes are not well known, as well as the molecular events associated with oocyte

[4]Corresponding author: paula.lopes29@unifesp.br
Phone:+55(11) 3319-3300; Fax: +55(11) 4043-6428
Received: July 3, 2012
Accepted: August 13, 2012

death or survival in response to stress. This review aims to characterize the effects of elevated temperature in bovine oocytes, especially the cellular changes triggered in response to stress. The terminology heat stress and heat shock will be used to describe increased *in vivo* and *in vitro* temperatures, respectively.

Effects of elevated temperature on oocyte function and developmental competence

In vivo studies

Bovine oocyte function is highly compromised in heat stressed animals (Rocha *et al*., 1998; Al-Katanani *et al*., 2002; Gendelman *et al*., 2010). Susceptibility of bovine oocytes to elevated temperature can be detected at the germinal vesicle (GV)-stage and during oocyte maturation (Fig. 1). Exposure of Holstein cows to heat stress reduced GV oocyte developmental competence as indicated by a reduction in subsequent embryonic development (Rocha *et al*., 1998; Al-Katanani *et al*., 2002). A pioneering study conducted by Putney *et al*. (1989) demonstrated that exposure of Holstein heifers to environmental chamber heat stress of 42°C for 10 h during oocyte maturation decreased the number of normal embryos as compared to control (24ºC; Putney *et al*., 1989).

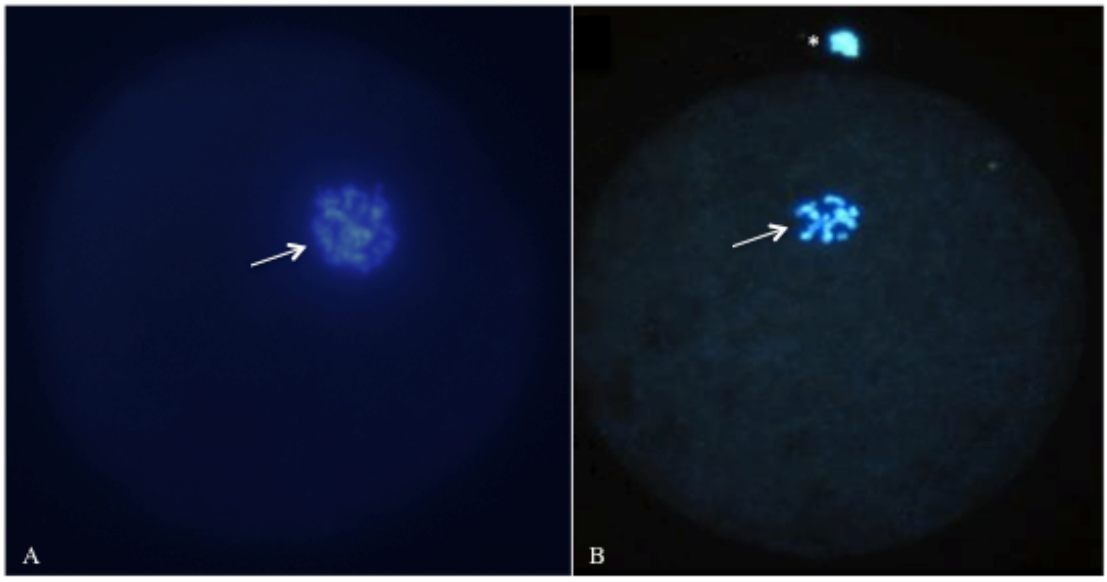

Figure 1. Representative images illustrating bovine GV (A) and MII (B) oocytes stained with Hoechst 33342. Arrows indicate the DNA and asterisk the polar body.

The deleterious effect of heat stress on oocyte function has been well characterized in seasonal studies. Rocha *et al*. (1998) conducted experiments in which *Bos taurus* (Holstein) and *Bos indicus* (Brahman) oocytes collected during hot and cold season were subjected to *in vitro* maturation and fertilization. The percentage of oocytes classified as normal, fertilized and that reached the 8-cell stage was reduced for *Bos taurus* oocytes collected in the hot season as compared to the cold season. Moreover, no embryos reached morula or blastocyst stages. In contrast, developmental potential of *Bos indicus* oocytes was not affected by season (Rocha *et al*., 1998). Similarly, summer seasonal conditions reduced oocyte developmental competence in heifers, repeat-breeder and peak lactation Holstein cows. Such negative effect was more pronounced in repeat-breeder cows (Ferreira *et al*., 2011).

Exposure of dairy cows to summer heat stress reduced conception rates from the summer to early autumn (Badinga *et al*., 1985), suggesting a delayed effect of heat stress on reproductive function. Heat stress caused a seasonal reduction in oocyte competence, which was recovered 2-3 estrous cycles after the end of summer (Roth *et al*., 2001). These findings indicated that heat stress damaged the pool of follicles and oocytes that began their growth during the warm period.

Roth *et al*. (2001) conducted a study to characterize the delayed effect of heat stress and the rescue of oocyte quality by removal of summer damaged follicles. Holstein cows exposed to summer heat stress were subjected to follicular aspiration (OPU) on day 4 of the cycle (control group) or days 4, 7, 11 and 15 of the cycle (treated group) during four estrous cycles in the autumn cooler months. Cleavage and blastocyst rates did not differ between groups for

oocytes collected during the first two estrous cycles. However, cleavage rate and preimplantation embryonic development increased in cycles 3 and 4 of the treated group as compared to control (Roth *et al*., 2001).

Immediate and delayed effects of severe heat stress (environmental chamber: 38°C and 80% relative humidity (RH) during the day and 30ºC and 80% RH during the night) were also evaluated in *Bos indicus* animals (Gir; Torres-Júnior *et al.*, 2008). Heat stress caused no immediate effects on reproductive function. However, there was a decrease in the proportion of embryos that reached blastocyst and expanded blastocyst stages for oocytes collected after the stress period (Torres-Júnior *et al*., 2008). Although *Bos indicus* breeds are known to be heat tolerant this study indicated that severe heat stress could also damage oocytes. Moreover, it is possible that preantral follicle-enclosed oocytes are more susceptible to heat stress than antral follicle oocytes.

In vitro studies

The direct effect of elevated temperature on GV and matured oocyte has been demonstrated in experiments conducted *in vitro*.

GV-stage oocytes reside in the antral follicle environment for 42 days (Lussier *et al*., 1974) and during this period may be exposed to daily body temperature fluctuations above 40-41°C (Putney *et al.*, 1989; Ealy *et al*., 1993; Wolfenson *et al*., 1993; Rivera and Hansen, 2001) compromising oocyte function prior to maturation.

Development of *in vitro* models to study direct temperature effects on GV oocytes is challenging since removal of the oocyte from antral follicle induces spontaneous meiotic progression to metaphase II (MII) stage (Pincus and Enzmann, 1935). One approach in order to by pass this problem would be to use pharmacological tools such as meiotic inhibitors. Payton *et al*. (2004) evaluated the direct effects of elevated temperature on GV oocytes cultured in the presence of the meiotic inhibitor $p34^{cdc2}$/cyclin dependent kinase (roscovitine-50 µm). Heat shock at 41°C for 6 and 12 h reduced embryonic development to the 8-16 cell stage and blastocyst stage, respectively (Payton *et al*., 2004). Similarly, culture of bovine oocytes in meiotic inhibition medium at 41°C for 14 h did not affect cleavage rate but reduced embryonic development to the blastocyst stage on days 8 and 9 after fertilization (Lima, 2012).

The intensity of stress can be considered as a function of temperature and exposure time. Exposure of bovine oocytes to severe (42-44°C; not physiologically relevant temperature; Edwards and Hansen, 1996; Roth and Hansen, 2004b; Paula-Lopes *et al*., 2008) or moderate (40-41°C) temperatures (Edwards and Hansen, 1996; Roth and Hansen, 2004a; Paula-Lopes *et al*., 2008) during the first 12 h of IVM (0-12 h IVM) blocked or reduced preimplantation embryonic development, respectively. Moderate heat shock (40-41°C) for 0-12 h during IVM reduced the proportion of day 3 cleaved embryos and day 8 blastocyst (Roth and Hansen, 2004a; Paula-Lopes *et al*., 2008). Moderate heat shock (40.5°C and 41.5°C) for a short period of time (30 and 60 min) during oocyte maturation did not affect development to the blastocyst stage (Ju *et al*., 1999). However, exposure of oocytes to severe heat shock of 43°C for short periods of 45 and 60 min during IVM reduced blastocyst and expanded blastocyst rates (Ju *et al*., 1999).

Cellular alterations induced by elevated temperature in bovine oocytes

The mechanisms by which elevated temperature affects oocyte physiology are not completely understood. However, it has been shown that depending upon stress intensity, elevated temperature can cause reversible or irreversible cellular damage in different cell structures and organelles (Ju *et al*., 2005; Roth and Hansen, 2005). Such effects can trigger adaptive and/or cellular death response (Paula-Lopes and Hansen, 2002a). Considering that apoptosis is the major process responsible for the reduction in oocyte number on mammalian reproductive female lifetime (Morita and Tilly, 1999; Tilly, 2001) is possible that this form of cell death has an essential role in oocytes exposed to stress conditions.

Heat-induced cellular damage in bovine oocytes can be observed in biological membranes as well as cytoplasmic and nuclear compartments (Table 1). However, there is evidence that oocyte cytoplasm is more susceptible to the adverse effects of elevated temperature than the nucleus (Shen *et al*., 2010). Nuclear transfer studies in *Bos indicus* and *Bos taurus* oocytes indicated that exposure of donor cell nucleus to heat shock did not affect embryonic development in both *Bos indicus* and *Bos taurus* oocytes. However, exposure of receptor ooplasm to heat shock decreased developmental competence of *Bos taurus* oocytes (Shen *et al*., 2010).

Table 1. Cellular alterations induced by elevated temperature in GV-stage and maturing oocytes.

Cellular compartment	Structure	Alterations		References
		GV-stage oocytes	Maturing oocytes	
Cytoplasm	Actin filaments	----------	Reduction in peri-cellular and trans-zonal actin polymerization.	(Ju and Tseng, 2004; Tseng *et al*., 2004; Roth and Hansen, 2005).
	Microtubules	----------	Microtubule disorganization.	(Ju and Tseng, 2004; Tseng *et al*., 2004; Ju *et al*., 2005; Roth and Hansen, 2005).
	Cortical granules (CG)	Increased CG translocation to oolemma.	Increased CG translocation to oolemma.	(Payton *et al*., 2004; Edwards *et al*., 2005).
	Mitochondria	Reduction in mitochondrial activity.	Reduction in mitochondrial activity.	(Ispada *et al*., 2011; Lima, 2012).
	Proteins	Reduction in total protein synthesis.	Reduction in total protein synthesis.	(Curci *et al*., 1987; Edwards and Hansen, 1996; Edwards and Hansen, 1997).
		Group II caspase activity was not affected.	Increased group II caspase activity.	(Roth and Hansen, 2004a; Lima, 2012).
Nucleus	Meiotic spindle	Reduction in meiotic maturation (MII stage).	Reduction in meiotic maturation (MII stage).	(Payton *et al*., 2004; Roth and Hansen, 2005; Paula-Lopes *et al*., 2008).
		----------	Reduction in meiotic spindle size.	(Ju *et al*., 2005).
	DNA	Increased DNA-fragmentation (TUNEL-positive).	Increased DNA-fragmentation (TUNEL-positive).	(Roth and Hansen, 2004a; Ispada *et al*., 2010; Lima, 2012).
	RNA	Amount of total RNA was not affected.	Amount of poly (A) RNA was not affected.	(Payton *et al*., 2011; Gendelman and Roth, 2012).
Membrane	Lipids	Increased saturated fatty acid.	----------	(Zeron *et al*., 2001).
		----------	Phosphatidylserine translocation to outer leaflet of the plasma membrane (Annexin-V).	(Kalo and Roth, 2011).

Cytoplasmic alterations

Cytoskeletal alterations are among the heat-induced cytoplasmic changes that occur in bovine oocytes. Elevated temperature affects microtubules and microfilaments organization (Ju and Tseng, 2004; Tseng *et al*., 2004; Ju *et al*., 2005; Roth and Hansen, 2005). These alterations compromise the transport and distribution of cytoplasmic organelles as well as chromosomes separation during fertilization and cleavage (Tseng *et al*., 2004; Ju *et al*., 2005).

The pericellular actin ring and trans-zonal actin processes were decreased in oocytes matured at 41 (Roth and Hansen, 2005) and 41.5°C (Tseng *et al*., 2004). Actin microfilaments are responsible for cortical granule translocation to the cortical region of the oocyte during maturation (Wessel *et al*., 2002). While GV oocytes show cortical granules aggregates (type I), GV breakdown promotes cortical granules dispersion and translocation to the oolemma (type III). Exposure of GV (Payton *et al*., 2004) and maturing (Edwards *et al*., 2005) oocytes to 41°C increased the proportion of oocytes that had type III cortical granules distribution, suggesting that heat shock hastened cytoplasmic maturation kinetics and induced oocyte aging. In contrast, the percentage of mice oocytes carrying incomplete cortical granules migration during IVM was higher in oocytes matured at 40°C as compared to 37°C (Wang *et al*., 2009).

High temperature affects the organized αβ-tubulin heterodimer structure. Exposure of oocytes to elevated temperature caused meiotic spindle disorganization so that metaphase I and II microtubules become deformed and chromosomes are unaligned (Tseng *et al*., 2004; Roth and Hansen, 2005). There was also a reduction in meiotic spindle size as the duration of the heat shock at 42°C increased from 1 to 4 h (Ju *et al*., 2005). Changes in size and morphology of the meiotic spindle indicated that heat shock affected microtubule polymerization and depolymerization. Such changes probably compromise chromosome separation during fertilization and subsequent embryonic division.

Microtubules disruption also affects cytoplasmic organelles transport such as oocyte mitochondrial distribution (Sun *et al*., 2001). Mitochondria are also targets to the deleterious effects induced by high temperature. Exposure of GV-stage (Lima, 2012) and maturing (Ispada *et al*., 2011) bovine oocytes to heat shock reduced oocyte mitochondrial activity. Similarly, exposure of high and low quality cumulus-oocyte complexes (COCs) to heat shock reduced cumulus cells mitochondrial membrane potential regardless of COCs quality (Paula-Lopes *et al*., 2010).

Changes on oocyte mitochondrial activity may be associated with activation of apoptotic cascade. It has been shown that exposure of oocytes to heat shock during IVM increased the proportion of oocytes with high group II caspase (caspases 2, 3 and 7) enzyme activity (Roth and Hansen, 2004a), however such increase was not observed in GV-stage oocytes.

Nuclear alterations

Depression of nuclear maturation is one of the changes caused by heat shock in bovine oocytes. Exposure of GV-stage (Payton *et al*., 2004) or maturing oocytes (Roth and Hansen, 2005; Paula-Lopes *et al*., 2008) to 41°C heat shock decreased the proportion of oocytes that reached MII stage following IVM. In these experiments, heat shock blocked meiotic progression by increasing the proportion of metaphase I (MI) oocytes. However, in one study, heat shock at 41°C accelerated nuclear maturation kinetics increasing the proportion of MII oocytes after 16-18 h of maturation (Edwards *et al*., 2005).

Another nuclear change observed in oocytes subjected to heat shock is the DNA fragmentation characteristic of apoptosis. Heat shock during the first 12-14 h IVM increased the proportion of TUNEL-positive oocytes (Roth and Hansen, 2004a; Ispada *et al*., 2010).

Experiments conducted with crossbred *Bos indicus* oocytes indicated that moderate heat shock (41°C) during the first 12 h of IVM reduced oocyte developmental competence. Such heat shock reduced nuclear maturation, cleavage and blastocyst rates (Paula-Lopes *et al*., 2008). However, there was no heat shock effect on the proportion of oocytes in late apoptosis stages as determined by plasma membrane permeability alterations (carbocyanine nucleic acid staining; Paula-Lopes *et al*., 2008). Exposure of bovine oocytes to severe heat shock (44°C) for 12 h also reduced nuclear maturation similarly to the observed response to moderate heat shock. However, there was an increase in the proportion of oocytes positive for apoptosis and necrosis as well as blockage of cleavage and embryonic development to the blastocyst stage (Paula-Lopes *et al*., 2008) suggesting that severe heat shock fully impaired *Bos indicus* oocyte developmental competence.

Thermoprotective factors on bovine oocytes

Heat stress in lactating dairy cows has great economic impact in warm climate dairy industry. However there are few strategies available to prevent this problem. Environmental modifications such as fans and sprinklers allow only a partial improvement in herd reproductive efficiency resulting in high costs to the dairy farm (Hansen *et al*., 1992; Hansen, 2004). Although such environmental changes reduce cow body temperature and increase milk yield, the effects on summer-related infertility are limited. Thus, development of alternatives to prevent the negative effects of heat stress on reproduction of dairy cows is of

major importance in hot climates.

Several approaches to mitigate the deleterious effects of heat stress in animal breeding have been previously described (Hansen *et al*., 1992, 2001; Hansen and Aréchiga, 1999). Thus, the focus of this topic is to present thermoprotective molecules that act by minimizing heat-induced cellular and functional damage in bovine oocytes.

The deleterious effects of heat shock in bovine oocytes may be regulated by growth factors such as insulin-like growth factor I (IGF-I). IGF-I exerts autocrine, paracrine and endocrine actions in cellular metabolism, proliferation, growth and differentiation (Voss and Rosenfeld, 1992; Baker *et al*., 1993; Yakar *et al*., 1999). It has been shown that exposure of dairy cows to heat stress decreased serum IGF-I levels leading to impairment of oocyte quality (De Rensis and Scaramuzzi, 2003).

Recent studies indicated that IGF-I plays a themoprotective role in GV-stage and maturing oocytes. Exposure of GV oocytes to heat shock at 41°C reduced mitochondrial activity, increased the percentage of apoptotic oocytes and reduced cleavage and blastocyst rates (Lima, 2012). However, addition of 12.5 ng/ml IGF-I reduced these deleterious effects of heat shock rescuing oocyte functions. Similarly, 100 ng/ml IGF-I during IVM reversed the detrimental effects of heat shock on oocyte mitochondrial activity and heat-induced apoptosis (Ispada *et al*., 2010, 2011). However, in these studies the concentration of 100 ng/ml IGF-I during IVM was unable to rescue the negative effects of heat shock on blastocyst development (Risolia *et al*., 2011).

Heat-induced oocyte DNA fragmentation (TUNEL positive) can also be reversed by supplementation of IVM medium with molecules that inhibit the apoptotic cascade, such as group II caspase inhibitor z-DEVD-fmk (Roth and Hansen, 2004a). This molecule inhibits the negative effects of heat shock on cleavage and blastocyst rates (Roth and Hansen, 2004a).

Another apoptosis inhibitor molecule, sphingosine-1-phosphate (S1P), is an sphingolipid metabolite that blocks the pro-apoptotic effects of ceramide (Roth and Hansen, 2004a). Addition of S1P to the maturation medium blocked the deleterious effects of heat shock on meiotic progression, apoptosis and increased the proportion of heat-shocked oocytes that cleaved and reached blastocyst stage (Roth and Hansen, 2004a).

Summary

High microenvironmental temperature of the reproductive tract compromises follicular dynamics, hormonal secretion, oocyte and embryonic function. Oocytes at the GV-stage and maturation period undergo cellular changes induced by elevated temperature. These changes reduce the efficiency of physiological processes involved in oocyte growth and maturation, fertilization and preimplantation embryonic development.

Bovine oocyte cellular damage caused by elevated temperature can be detected in different cellular compartments such as oocyte cytoplasmic and nuclear regions. The oocyte cytoskeleton is highly affected by high temperature. Heat-induced disruption of microtubules and microfilaments alters the distribution of cellular structures such as cortical granules and mitochondria, besides affecting chromosome segregation during fertilization and cell division. Heat shock also increases oocyte DNA fragmentation and decreases mitochondrial activity suggesting activation of the heat-induced mitochondrial apoptotic pathway in bovine oocytes.

Several approaches have been employed to alleviate the low fertility associated with heat stress. Recently, molecules such as IGF-I, caspase inhibitors and sphyngolipid S1P were identified as thermoprotective factors in bovine oocytes. These factors rescued several cellular functions damaged by high temperatures and increased oocyte developmental competence. Therefore, identification and characterization of cellular thermoprotective molecules can be considered as an alternative to modulate the effects of elevated temperature in reproductive function.

Acknowledgments

Research was supported by FAPESP Research Grant Award No. 2007/53323-0 and CNPq Research Grant Award No. 478558/2008-2.

References

Adeyemo O, Heath E, Adadevoh BK, Steinbach J, Olaloku EA. 1979. Some physiological and behavioral responses in *Bos indicus* and *Bos taurus* heifers acclimatized to the hot humid seasonal equatorial climate. *Int J Biometeorol*, 23:231-241.

Al-Katanani YM, Webb DW, Hansen PJ. 1999. Factors affecting seasonal variation in 90 day non-return rate to first service in lactating Holstein cows in a hot climate. *J Dairy Sci*, 82:2611-2615.

Al-Katanani YM, Paula-Lopes FF, Hansen PJ. 2002. Effect of season and exposure to heat stress on oocyte competence in Holstein cows. *J Dairy Sci*, 85:390-396.

Badinga L, Collier RJ, Thatcher WW, Wilcox CJ. 1985. Effects of climatic and management factors on conception rate of dairy cattle in subtropical environment. *J Dairy Sci*, 68:78-85.

Badinga L, Thatcher WW, Diaz T, Drost M, Wolfenson D. 1993. Effect of environmental heat stress on follicular development and steriodogenesis in lactating Holstein cows. *Theriogenology*, 39:797-810.

Baker J, Liu JP, Robertson EJ, Efstratiadis A. 1993. Role of insulin-like growth factors in embryonic and postnatal growth. *Cell*, 75:73-82.

Bennett IL, Finch VA, Holmes CR. 1985. Time spent in shade and its relationship with physiological factors of thermoregulation in three breeds of cattle. *Appl Anim Behav Sci*, 13:227-236.

Berman A, Folman Y, Kaim M, Mamen M, Herz Z, Wolfenson D, Arieli A, Graber Y. 1985. Upper critical temperatures and forced ventilation effects for high-yielding dairy cows in a subtropical environment. *J Dairy Sci*, 68:1488-1495.

Curci A, Bevilacqua A, Mangia F. 1987. Lack of heat-shock response in preovulatory mouse oocytes. *Dev Biol*, 123:154-160.

De Rensis F, Scaramuzzi RJ. 2003. Heat stress and seasonal effects on reproduction in the dairy cow - a review. *Theriogenology*, 60:1139-1151.

Dunlap SE, Vincent CK. 1971. Influence of postbreeding thermal stress on conception rate in beef cattle. *J Anim Sci*, 32:1216-1218.

Ealy AD, Drost M, Hansen PJ. 1993. Developmental changes in embryonic resistence to adverse effects of maternal heat stress in cows. *J Dairy Sci*, 76:2899-2905.

Edwards JL, Hansen PJ. 1996. Elevated temperature increases heat shock protein 70 synthesis in bovine two-cell embryos and compromises function of maturing oocytes. *Biol Reprod*, 55:340-346.

Edwards JL, Hansen PJ. 1997. Differential responses of bovine oocytes and preimplantation embryos to heat shock. Mol Reprod Dev, 46:138-145.

Edwards JL, Saxton AM, Lawrence JL, Payton RR, Dunlap JR. 2005. Exposure to a physiologically relevant elevated temperature hastens in vitro maturation in bovine oocytes. *J Dairy Sci*, 88:4326-4333.

Ferreira RM, Ayres H, Chiaratti MR, Ferraz ML, Araújo AB, Rodrigues CA, Watanabe YF, Vireque AA, Joaquim DC, Smith LC, Meirelles FV, Baruselli PS. 2011. The low fertility of repeat-breeder cows during summer heat stress is related to a low oocyte competence to develop into blastocysts. *J Dairy Sci*, 94:2383-2392.

Gendelman M, Aroyo A, Yavin S, Roth Z. 2010. Seasonal effects on gene expression, cleavage timing, and developmental competence of bovine preimplantation embryos. *Reproduction*, 140:73-82.

Gendelman M, Roth Z. 2012. Seasonal effect on germinal vesicle-stage bovine oocytes is further expressed by alterations in transcript levels in the developing embryos associated with reduced developmental competence. *Biol Reprod*, 86:1-9.

Hammond AC, Olson TA, Chase Jr CC, Bowers EJ, Randel RD, Murphy CN, Vogt DW, Tewolde A. 1996. Heat tolerance in two tropically adapted Bos taurus breeds, Senepol and Romosinuano, compared with Brahman, Angus, and Hereford cattle in Florida. *J Anim Sci*, 74:295-303.

Hammond AC, Chase Jr CC, Bowers EJ, Olson TA, Randel RD. 1998. Heat tolerance in Tuli-, Senepol-, and Brahman-sired F1 Angus heifers in Florida. *J Anim Sci*, 76:1568-1577.

Hansen PJ, Thatcher WW, Ealy AD. 1992. Methods for reducing effects of heat stress on pregnancy. *In*: Van Horn HH, Wilcox CJ (Ed.). *Large Dairy Herd Management*. Champaign, IL: American Dairy Science Association. pp. 116.

Hansen PJ, Aréchiga CF. 1999. Strategies for managing reproduction in the heat stressed dairy cow. *J Dairy Sci*, 82:36-50.

Hansen PJ, Drost M, Rivera RM, Paula-Lopes FF, Al-Katanani YM, Krininger III CE, Chase Jr CC. 2001. Adverse impact of heat stress on embryo production: causes and strategies for mitigation. *Theriogenology*, 55:91-103.

Hansen PJ. 2004. Physiological and cellular adaptations of Zebu cattle to thermal stress. Anim Reprod Sci, 82-83:349-360.

Ispada J, Lima RS, Risolia PHB, Andrade ES, Assumpção MEOA, Visintin JA, Paula-Lopes FF. 2010. The role of insulin-like growth factor-I on inhibition of heat-induced apoptosis in bovine oocytes. *Anim Reprod*, 7:333. (abstract).

Ispada J, Lima RS, Risolia PHB, Assumpcao MEOA, Visintin JA, Paula-Lopes FF. 2011. Insulin-like growth factor-I exerts a thermoprotective role on mitochondrial function of bovine oocytes exposed to heat shock. *Reprod Fertil Dev*, 24:209. (abstract).

Ju J, Jiang S, Tseng JK, Parks JE, Yang X. 2005. Heat shock reduces developmental competence and alters spindle configuration of bovine oocytes. *Theriogenology*, 64:1677-1689.

Ju JC, Parks JE, Yang X. 1999. Thermotolerance of IVM-derived bovine oocytes and embryos after short-term heat shock. *Mol Reprod Dev*, 53:336-340.

Ju JC, Tseng J-K. 2004. Nuclear and cytoskeletal alterations of in vitro matured porcine oocytes under hyperthermia. *Mol Reprod Dev*, 68:125-133.

Kalo D, Roth Z. 2011. Involvement of the sphingolipid ceramide in heat-shock-induced apoptosis of bovine oocytes. *Reprod Fertil Dev*, 23:876-888.

Lima RS. 2012. *O papel do fator de crescimento semelhante à insulina-I sobre os efeitos deletérios do choque térmico em oócitos bovinos no estádio de vesícula germinativa* [in Portuguese]. Botucatu, Brazil: UNESP. Thesis.

Lussier JG, Matton P, Dufour JJ. 1974. Growth rates of follicles in the ovary of the cow. *J Reprod Fertil*, 81:301-307.

Malayer JR, Hansen PJ, Buhi WC. 1988. Effect of day of oestrus cycle, side of the reproductive tract and heat shock on in-vitro protein secretion by bovine endometrium. *J Reprod Fertil*, 84:567-578.

Morita Y, Tilly JL. 1999. Oocyte apoptosis: like sand through an hourglass. *Dev Biol*, 213:1-17.

Paula-Lopes FF, Hansen PJ. 2002a. Apoptosis is an adaptative response in bovine preimplantation embryos that facilitates survival after heat shock. *Biochem Biophys Res Commun*, 295:37-42.

Paula-Lopes FF, Hansen PJ. 2002b. Heat shock-induced apoptosis in preimplantation bovine embryos is a developmentally regulated phenomenon. *Biol Reprod*, 66:1169-1177.

Paula-Lopes FF, Milazzotto M, Assumpcao MEOA, Visintin JA. 2008. Heat shock-induced damage in bovine oocytes. *Reprod Fertil Dev*, 43:208. (abstract).

Paula-Lopes FF, Mendes CM, Risolia PHB, Goncalves JSA, Feitosa WB, Assumpcao MEOA, Visintin JA. 2010. Cumulus cells response to heat shock and insulin-like growth factor-I. *Reprod Fertil Dev*, 22:333. (abstract).

Payton RR, Romar R, Coy P, Saxton AM, Lawrence JL, Edwards JL. 2004. Susceptibility of bovine germinal vesicle-stage oocytes from antral follicles to direct effects of heat stress in vitro. *Biol Reprod*, 71:1303-1308.

Payton RR, Rispoli LA, Saxton AM, Edwards JL. 2011. Impact of heat stress exposure during meiotic maturation on oocyte, surrounding cumulus cell, and embryo RNA populations. *J Reprod Dev*, 57:481-491.

Pincus G, Enzmann EV. 1935. The comparative behavior of mammalian eggs in vivo and in vitro. I. The activation of ovarian eggs. *J Exp Med*, 62:665-675.

Pires MFA, Ferreira AM, Saturnino HM, Teodoro RL. 2002. Taxa de gestação em fêmeas da raça Holandesa confinadas em free stall, no verão e inverno. *Arq Bras Med Vet Zootec*, 54:57-63.

Putney DJ, Mullins S, Thatcher WW, Drost M, Gross TS. 1989. Embryonic development in superovulated dairy cattle exposed to elevated ambient temperatures between the onset of estrus and insemination. *Anim Reprod Sci*, 19:37-51.

Risolia PHB, Lima RS, Ispada J, Assumpcao MEOA, Visintin JA, Paula-Lopes FF. 2011. The role of insulin-like growth factor-I on developmental competence of bovine oocytes exposed to heat shock. *Biol Reprod*, 44:79-79. (abstract).

Rivera RM, Hansen PJ. 2001. Development of cultured bovine embryos after exposure to high temperatures in the physiological range. *Reproduction*, 121:107-115.

Rocha A, Randel RD, Broussard JR, Lim JM, Blair RM, Roussel JD, Godke RA, Hansel W. 1998. High environmental temperature and humidity decrease oocyte quality in Bos taurus but not in Bos indicus cows. *Theriogenology*, 49:657-665.

Roman-Ponce H, Thatcher WW, Canton D, Barron DH, Wilcox CJ. 1978. Thermal stress effects on uterine blood flow in dairy cows. *J Anim Sci*, 46:175-180.

Roth Z, Meidan R, Braw-Tal R, Wolfenson D. 2000. Immediate and delayed effects of heat stress on follicular development and its association with plasma FSH and inhibin concentration in cows. *J Reprod Fertil*, 120:83-90.

Roth Z, Arav A, Boar A, Zeron Y, Wolfenson D. 2001. Improvement of quality of oocyte collected in the autumn by enhanced removal of impaired follicles from previously heat-stressed cows. *Reproduction*, 122:737-744.

Roth Z, Hansen PJ. 2004a. Involvement of apoptosis in disruption of developmental competence of bovine oocytes by heat shock during maturation. *Biol Reprod*, 71:1898-1906.

Roth Z, Hansen PJ. 2004b. Sphingosine 1-phosphate protects bovine oocytes from heat shock during maturation. *Biol Reprod*, 71:2072-2078.

Roth Z, Hansen PJ. 2005. Disruption of nuclear maturation and rearrangement of cytoskeletal elements in bovine oocytes exposed to heat shock during maturation. *Reproduction*, 129:235-244.

Shen PC, Lee JW, Cheng WTK, Su HY, Lee SN, Liu BT, Wang CH, Chen LR, Ju JC. 2010. Differential thermal sensitivity between the recipient ooplasm and the donor nucleus in Holstein and Taiwan native yellow cattle. *Theriogenology*, 74:1587- 1595.

Sun QY, Wu GM, Lai L, Park KW, Cabot R, Cheong HT, Day BN, Prather RS, Schatten H. 2001. Translocation of active mitochondria during pig oocyte maturation, fertilization and early embryo development in vitro. *Reproduction*, 122:155-163.

Tilly JL. 2001. Commuting the death sentence: how oocyte strive to survive. *Nature Rev Mol Cell Biol*, 2:838-848.

Torres-Júnior JR, Pires MF, Sá WF, Ferreira MA, Viana JH, Camargo LS, Ramos AA, Folhadella IM, Polisseni J, Freitas C, Clemente CA, Sá Filho MF, Paula-Lopes FF, Baruselli PS. 2008. Effect of maternal heat-stress on follicular growth and oocyte competence in *Bos indicus* cattle. *Theriogenology*, 69:155-166.

Tseng JK, Chen CH, Chou PC, Yeh SP, Ju JC. 2004. Influences of follicular size on parthenogenetic activation and in vitro heat shock on the cytoskeleton in cattle oocytes. *Reprod Domest Anim*, 39:146-153.

Voss JW, Rosenfeld MG. 1992. Anterior pituitary development: short tales from dwarf mice. *Cell*, 70:527-530.

Wang J, Sui H, Miao D, Liu N, Zhou P, Ge L, Tan J. 2009. Effects of heat stress during in vitro maturation on cytoplasmic versus nuclear components of mouse oocytes. *Reproduction*, 137:181-189.

Wessel GM, Conner SD, Berg L. 2002. Cortical granule translocation is microfilament mediated and linked to meiotic maturation in the sea urchin oocyte. *Development*, 129:4315-4325.

Wolfenson D, Bartol FF, Badinga L, Barros CM, Marple DN, Cummings K, Wolfe D, Lucy M C, Spencer TE, **Thatcher WW**. 1993. Secretion of PGF2a and oxytocin during hyperthermia in cyclic and pregnant heifers. *Theriogenology*, 39:1129-1141.

Wolfenson D, Thatcher WW, Badinga L, Savio JD, Meidan R, Lew BJ, Braw-Tal R, Berman A. 1995. Effect of heat stress on follicular development during the estrous cycle in lactating dairy cattle. *Biol Reprod*, 52:1106-1113.

Yakar S, Liu JL, Stannard B, Butler A, Accili D, Sauer B, Leroith D. 1999. Normal growth and development in the absence of hepatic insulin-like growth factor I. *Proc Natl Acad Sci*, 96:324-7329, 1999.

Yousef MK. 1984. Stress physiology: definition and terminology. In: Yousef MK (Ed.) *Stress Physiology in Livestock*. Boca Raton, FL: CRC Press.

Zeron Y, Ocheretny A, Kedar O, Borochov A, Sklan D, Arav A, Abe H. 2001. Seasonal changes in bovine fertility: relation to developmental competence of oocytes, membrane properties and fatty acid composition of follicles. *Reproduction*, 121:447-454.