Effects of heat stress on ovarian functions and embryonic development: mechanism and potential strategies to alleviate these effects in dairy cows

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Summary

Reduced reproductive performance of lactating cows during the summer is associated mainly with intensive genetic selection for high milk production, which places a great load on the thermoregulatory mechanism. Today, cooling is the predominant strategy used to alleviate the effects of heat stress. However, it cannot eliminate the decline in reproduction. A better understanding of the mechanism by which heat stress compromises fertility is required to develop new strategies to mitigate its effects. This review summarizes what is known about the multifactorial effect of thermal stress on reproductive functions, which includes alterations in the hypothalamus–pituitary–ovarian axis: gonadotropin secretion, follicular development, steroid production and corpus luteum function. The review provides some new insights into the cellular and molecular responses of oocytes and embryos to elevated temperature, discusses some potential underlying mechanisms, such as nuclear and cytoplasmic maturation, mitochondrial function, apoptotic pathways and oxidative stress, and suggests potential approaches to alleviating these effects. Given the complexity of heat-stress effects on reproduction, comprehensive reproductive management during the summer is suggested, based on: (1) an efficient cooling system as a prerequisite for any other strategies, (2) hormonal treatment targeted to specific subgroups of cows that will benefit from it, rather than the whole herd, and (3) combining two or more strategies in a program, rather than correcting the function of a single aspect of the reproductive system.

Introduction

Reduced reproductive performance of lactating cows during the summer is well-documented. Given the intensive genetic selection for high milk production and the concomitant global climate warming, fertility problems are expected to worsen in the coming years. Heat stress

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is not confined only to hot climates. For example, Japan has experienced unusual summer weather over the past few decades leading to reduced production and reproduction in lactating cows in temperate-climate regions such as Hokaido (Kadokawa *et al*. 2012). Moreover, the effects of heat stress are not limited to the hot months as they carry over to the following cooler months, resulting in long-term effects through the year which inflict heavy economic losses on the dairy industry.

The most common strategy to alleviate the effect of heat stress is to provide shade and evaporative cooling, based on combining sprinkling and ventilation in both the holding pen (before milking) and the feeding area (after milking). In Israel, this approach was successfully implemented 22 years ago in a large dairy herd. Cooling management based on seven 30-min cooling periods a day enabled maintenance of normal body temperature (< 39.0ºC) in cows producing 30 kg milk/day, and conception rates (CRs) similar to those in the winter (Wolfenson *et al*. 1988). However today, intensive cooling consisting of 10 periods for a total of 7 cumulative h/day prevents the decline in milk production in extremely high-yielding cows (>13,000 kg/year) but not that in summer reproduction. The summer-to-winter milk-production ratio in Israel is 0.985, whereas CR is lower by 22 percentage units in the summer vs. winter (Flamenbaum & Galon 2010). Nevertheless, efficient cooling management is a prerequisite for other, additional strategies to improve reproductive responses under heat stress.

This review will discuss the potential mechanism by which heat stress impairs reproductive features, focusing on its deleterious effects on ovarian function and embryonic development. New insights into heat-induced cellular and molecular alterations associated with oocyte maturation and developmental competence are presented. The review summarizes strategies that are known to alleviate the effects of heat stress. It also provides some practical guides for implementing reproductive management during the hot season.

Effect of heat stress on ovarian function

Among the components of the female reproductive tract, the ovary is highly sensitive to thermal stress. Given their lengthy developmental process, the ovarian pool of follicles and their enclosed oocytes have the potential to be exposed to environmental stressors, the most studied being thermal stress. Impaired growth and function of the small antral follicles can be expressed weeks later by compromised functioning of the dominant and preovulatory follicles. These alterations might in turn impair the cascade of events leading to ovulation and formation of the corpus luteum (CL). Moreover, while heat stress can directly affect the developing embryos, it can also affect embryonic development indirectly by attenuating oocyte developmental capacity.

Effects of heat stress on the follicles

The follicular stages that are susceptible to thermal stress have not been precisely defined. Roth *et al*. (2000) suggested that early antral follicles of approximately 0.5–1.0 mm in diameter are sensitive to heat stress. In Gir cows, a 28-day period of heat stress reduced oocyte competence for 105 days (de Torres-Júnior *et al*. 2008), indicating that alterations occur at the small-antral follicle stage. Heat stress impairs the growth of medium-size follicles (6–9 mm), reduces the size of the first- and second-wave dominant follicles (Badinga *et al*. 1993, Wilson *et al*. 1998a,b), and attenuates dominance. These alterations are reflected in an increased number of medium- and large-size follicles, reduced concentration of inhibin and increased follicle-stimulating hormone (FSH) in the plasma (Badinga *et al*. 1993, Wolfenson *et al*. 1995, Roth *et al*. 2000).

Alterations in follicular development are tightly associated with changes in the endocrine milieu. Seasonal studies have reported lower steroid concentrations in the follicular fluid obtained from large follicles, reduced granulosa-cell viability and impaired aromatase activity during the hot season (for review see Wolfenson *et al*. 2000). Follicle pieces obtained from heat-stressed cows secrete lower levels of androstenedione and oestradiol upon gonadotropin stimulation (Bridges *et al*. 2005), most likely due to decreased expression of luteinizing hormone (LH) receptors and reduced follicular response to LH (Ozawa *et al*. 2005). Alterations in steroidogenic capacity carry over from the summer to the autumn, as reflected by relatively low oestradiol content in the follicular fluid in late summer and increased levels throughout the autumn (Roth *et al*. 2001b, Roth *et al*. 2004). This transient effect is highly important because induction of frequent follicular waves during the autumn can enhance this spontaneous improvement (Roth *et al*. 2004). Similarly, the idea that hormonal manipulation of follicle turnover will improve fertility was recently tested by Friedman *et al*. (2011; Fig. 1): induction of three consecutive 9-day follicular waves during the summer and fall improved CR (37 vs. 53% for control and treated cows, respectively) in primiparous but not multiparous cows. Treatment also increased the percentage of primiparous cows that were pregnant

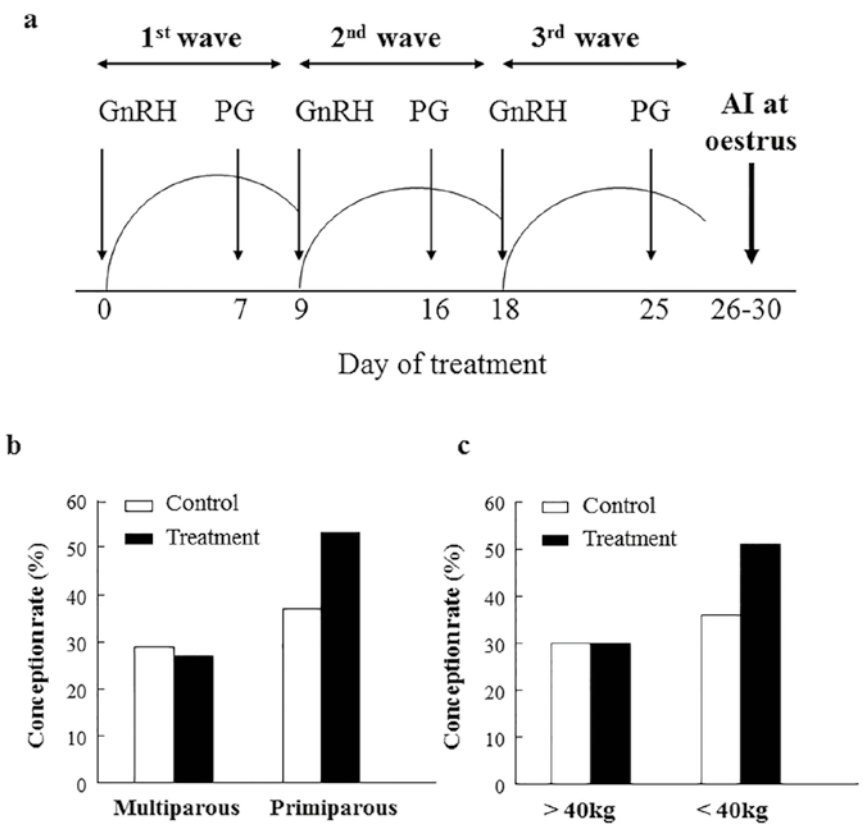

Fig. 1. Follicular turnover to improve fertility of lactating cows during the summer. (a) Schematized treatment protocol: cows 50 to 60 DIM were administered GnRH (day = 0) followed by $PGF2_{\alpha}$ on day 7 to induce three successive 9-day follicular waves. Cows were inseminated at oestrus and pregnancy diagnosis was performed 40 to 50 days postinsemination. Treatment increased conception rates in (b) primiparous cows and (c) cows with milk production < 40 kg. Mean values are presented.

at 120 days in milk (DIM) (51 vs. 65% for control and treated cows, respectively). Treatment was more effective for cows with a high body condition score (BCS) or low somatic cell count (SCC) than for cows with a low BCS or high SCC. As in that study, only 60% of the cows responded to the treatment (i.e., showed oestrus within 5 days of the expected time), fertility could have been improved with timed AI. It seems that hormonal manipulation to induce follicular turnover might be beneficial for some subgroups of cows while utilizing efficient cooling management.

Among the potentially adverse effects of heat stress associated with low oestradiol levels are impairment of oestrus duration and intensity, increased incidence of anoestrus, silent ovulation, and reduced number of mounts (Gwazdauskas *et al*. 1981). Poor oestrus detection can be improved by using modern aids such as the Heat-Watch system, a radio-telemetric pressure transducer and pedometric oestrus detection (Nebel *et al*. 2011). Alternatively, utilization of ovulation-synchronization protocols ending with timed AI procedures can improve rate of pregnancies per AI (Moore & Thatcher 2006). Nevertheless, timed AI cannot overcome the negative effects of heat stress on conception, as heat-stress alterations involve impairment in oocyte developmental competence and development of early-cleaving embryos. These might be better overcome by a timed embryo transfer procedure, which bypasses the effects of heat stress on these sensitive developmental stages (Hansen 2013a,b).

Reduced oestradiol concentrations during the follicular phase may also affect the preovulatory LH surge, and disrupt the cascade of events leading to oocyte ovulation. These, in turn, might lead to the development of non-ovulatory follicles and formation of ovarian cysts or alternatively, affect the formation and functioning of the CL. Suppression of pulsatile LH release and the preovulatory LH surge has been reported in hot climates (Wise *et al*. 1988). Heat-stressed cows with low oestradiol concentrations expressed a low-amplitude GnRH-induced LH surge (Gilad *et al*. 1993). Nonetheless, GnRH administration at the onset of oestrus (Ullah *et al*. 1996, Kaim *et al*. 2003) increased CR in mainly primiparous, and less in multiparous heat-stressed cows.

Effect of heat stress on oocyte developmental competence

Mammalian oocytes are arrested at the prophase stage of the first meiotic division and acquire their meiotic competence and fertilization potential in a stepwise manner during follicular development. Perturbations in the physiology of the follicle-enclosed oocyte during the lengthy period of follicular development can potentially lead to an oocyte with reduced competence for fertilization and subsequent development. Oocytes harvested from cows during the summer exhibit reduced ability to develop to the blastocyst stage after in-vitro fertilization (Al-Katanani *et al*. 2002) or chemical activation (Zeron *et al*. 2001). A period of two to three oestrous cycles is required for recovery from summer heat damage and appearance of competent oocytes in the subsequent fall (Roth *et al*. 2001a). A similar form of recovery has been shown in mice, where the effects of induced maternal hyperthermia carried over through three pregnancy cycles (Aroyo *et al*. 2007).

Gendelman *et al*. (2010) reported that the ratio between 2- and 4-cell-stage embryos (42 h postfertilization) was higher in the hot season (May–November) than in the cold season (December–April), indicating a delay in the two first embryonic divisions. As early-cleaving zygotes are more competent to develop to the blastocyst stage than their late-cleaving counterparts, it appears that embryos developed from oocytes collected in the hot season have inferior developmental competence. Moreover, findings indicate that not only the individual ovulated oocyte, but also the ovarian pool of germinal vesicle (GV)-stage oocytes is impaired during heat exposure. Payton *et al*. (2004) examined the effect of heat shock on oocytes held at the GV stage using S-roscovitine, a cell-cycle inhibitor. While exposure to 41ºC did not impair GV breakdown, it reduced the proportion of oocytes that progressed to metaphase II (MII) and those that developed to blastocysts.

Similarly, preculturing with 75 μM 3-isobutyl-1-methylxanthine (IBMX) maintained meiotic arrest by elevating cAMP concentrations in the oocyte. Exposing IBMX-blocked oocytes to heat shock (41.2ºC) reduced the proportion of oocytes that developed to the blastocyst stage, similar to the reduction reported for seasonal thermal stress (Gendelman & Roth 2012a). The same study provided new insight into the molecular alterations resulting from heat shock in the GV-stage oocytes. Both seasonal and induced thermal stress impaired the expression of maternal transcripts (*MOS*, *GDF9*, *POU5F1* and *GAPDH*) involved in oocyte maturation and early embryonic development (Fig. 2a). Although it was the GV-stage oocyte that was exposed to the thermal stress, the effect of heat stress on *POU5F1* expression first appeared in MII-stage oocytes and further in embryos, before (i.e. 2-, 4-, and 8-cell stages) and after (i.e. 8–16-cell stage) embryonic genome activation (Fig. 2b). The maternal mRNA stored in the oocyte is critical during the period of oocyte maturation, fertilization, and first embryonic divisions, until the embryonic genome becomes fully functional.

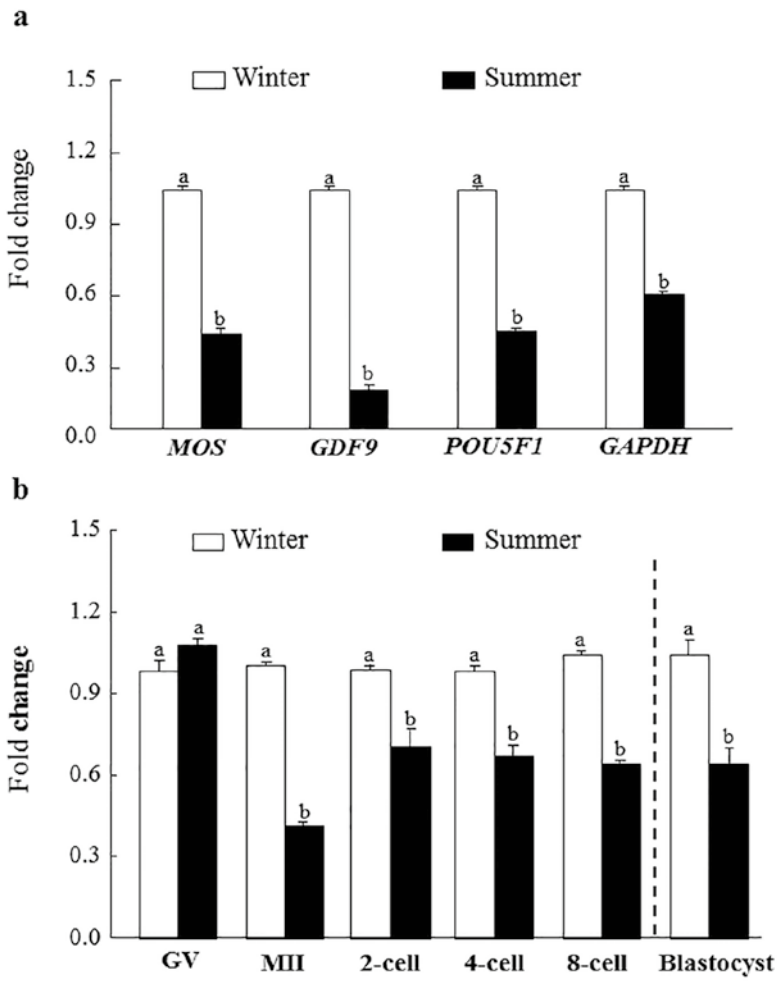

Fig. 2. Seasonal variations in transcript levels in oocytes and preimplantation embryos. (a) Transcript levels of *MOS*, *GDF9*, *POU5F1*, and *GAPDH* in MII-stage oocytes collected in hot and cold seasons. (b) Transcript level of *POU5F1* in GV- and MII-stage oocytes and in cleaved embryos before (2-, 4- and 8-cell) and after (blastocyst) embryonic genome activation. Data from real-time PCR, presented as means ± SEM; $P < 0.05$.

The level of POU5F1 governs embryo fate and a critical amount of POU5F1 protein is required to sustain embryonic stem cells (Niwa *et al*. 2000). Thus, the low expression of *POU5F1* found in embryos during the hot season could explain their low developmental competence.

Given the substantial damage caused by heat stress to the ovarian pool of oocytes, enhanced removal of the damaged follicles has been suggested. Frequent follicle aspirations by an ovum pick-up procedure improved the morphology and developmental competence of oocytes aspirated in the fall (Roth *et al*. 2001a). Hormonal treatment with FSH increased the number of growing follicles (Friedman *et al*. 2010) and the proportion of grade-1 oocytes and those cleaved to the 2-cell stage (Roth *et al*. 2002). Similarly, short-term administration of bovine somatotropin (bST) increased the proportion of grade-1 oocytes, but it did not improve cleavage rate or blastocyst formation (Roth *et al*. 2002).

Both somatotropin and IGF-I receptors are expressed in ovarian follicles and immature oocytes (Longergan *et al*. 2000). Under thermoneutral conditions, low doses (325 mg) of bST during the pre- and peri-implantation periods (days 0 and 14 post insemination) reduced embryonic losses and improved fertility (Ribeiro *et al*. 2013). Exposure of dairy cows to heat stress decreased plasma IGF-I concentration in association with reduced oocyte quality (de Rensis & Scaramuzzi 2003). It is therefore reasonable to assume that bST administration to increase IGF-I might mitigate the deleterious effects of heat stress. However, a single injection of bST (500 mg) at insemination did not improve fertility of hyperthermic cows (Bell *et al*. 2008). In-vitro studies have reported that physiological doses (12.5 ng/mL) of IGF-I reduce the deleterious effect of heat shock on GV-stage oocytes and improve their cellular and developmental competence (Paula-Lopes *et al*. 2013). On the other hand, non-physiological doses of IGF-I (100 ng/mL) did not improve (Paula-Lopes *et al*. 2013), and even had a negative effect (Zhandi *et al*. 2009) on developmental competence. While its effect on the oocyte is not clear-cut, IGF-I has been shown to reduce the negative effects of heat shock on 5-day-old embryos. Moreover, transferring in-vitro-derived embryos treated with IGF-I resulted in a higher pregnancy rate in the summer (Jousan & Hansen 2007).

Effect of heat stress on nuclear and cytoplasmic maturation

Thermal stress does not affect only the maternal mRNA; it also compromises other cellular characteristics of oocytes. Through maturation, the oocytes undergo multinuclear and cytoplasmic events, all associated with oocyte developmental competence (Eppig 1996). These include reorganization of the cytoskeletal filaments, resumption of meiosis, and redistribution of cytoplasmic organelles such as mitochondria, cortical granules and endoplasmic reticulum (Ferrieria *et al*. 2009; Yamada & Isaji 2011). Exposing bovine oocytes to heat shock during maturation impairs both microtubulin and microfilaments, which are involved in both nuclear and cytoplasmic organelle transport. As a result, most heat-shocked oocytes that fail to undergo maturation and fertilization are arrested at the MI to MII stages (Roth & Hansen 2005). Heat-shock induced perturbations of the spindle apparatus in bovine (Ju *et al*. 2005, Roth & Hansen 2005), porcine (Ju & Tseng 2004) and parthenogenetically activated bovine oocytes (Tseng *et al*. 2004). With respect to cytoplasmic events, heat shock impaired cortical granule translocation (Payton *et al*. 2004) and mitochondrial distribution in oocytes (Gendelman & Roth 2012b) and 2-cell-stage embryos (Rivera *et al*. 2004). Cortical granule relocation is the mechanism underlying the elimination of polyspermy, whereas maternal mitochondrial storage is the primary energy source for embryo growth to the blastocyst stage. Taken together, these alterations might lead to fertilization failure.

Mitochondrial dysfunction in heat-shocked oocytes

Mitochondrial activity and membrane potential are key indicators of cellular viability. Mitochondrial membrane potential reflects the pumping of hydrogen ions across the inner membrane via the processes of electron transport and oxidative phosphorylation. Exposing GV-stage and maturing oocytes to heat shock reduces oocytes' mitochondrial activity and mitochondrial membrane potential in cumulus cells (Paula-Lopes *et al*. 2012). Similarly, the proportion of oocytes with high mitochondrial membrane potential is relatively low during the summer (Gendelman & Roth 2012b). New evidence for season-induced alterations in the expressions of both nuclear (*SDHD* and *ATP5B*) and mitochondrial (*MT-ND2*, *MT-CYB*, *COX2*) genes has been recently provided by Gendelman and Roth (2012b). These genes are crucial in the oxidative-phosphorylation process and therefore, alterations in their expression might impair the number of active components in the electron transport chain and in turn lead to reduced ATP levels. Supporting this assumption, oocyte maturation with 50 μM CoQ10 a ubiquitous free-radical scavenger and a key component of the mitochondrial respiratory chain, increased the proportion of polarized mitochondria and enhanced the proportion of embryos that developed to the blastocyst stage in the fall, to a level similar to that achieved in the winter (Gendelman & Roth 2012b; Fig. 3).

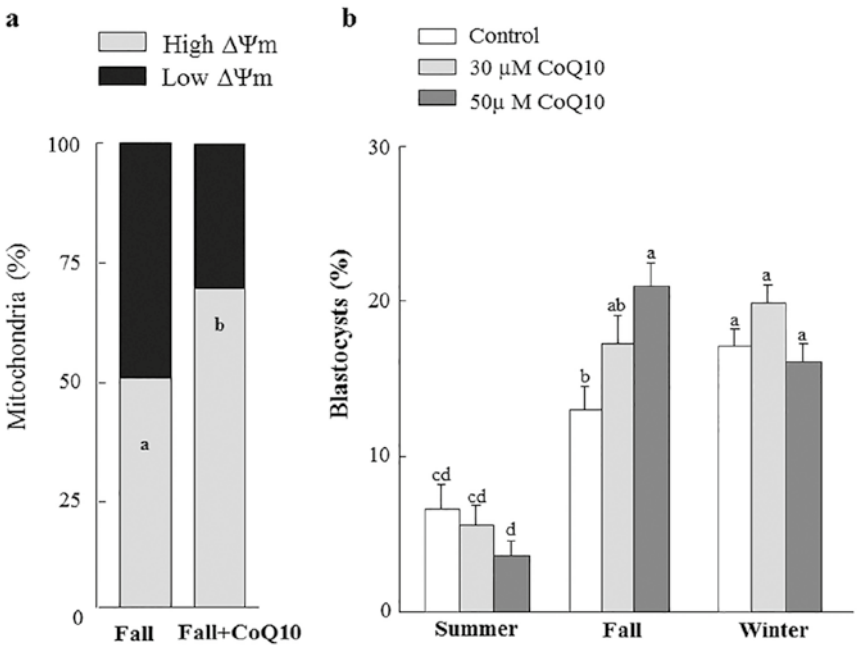

Fig. 3. Effect of CoQ10, a ubiquitous free-radical scavenger, on mitochondrial function and developmental competence of oocytes collected in the fall. (a) Proportion of high-polarized mitochondrial membranes (Δψm) in MII-stage oocytes. (b) Proportion of oocytes developed to 8-day blastocyst. Data are presented as means ± SEM; $P < 0.05$.

Heat-shock-induced apoptosis in oocytes

Heat-induced alterations in mitochondrial functioning are associated with the activation of apoptotic cascades through intrinsic mitochondrial pathways. Exposing oocytes to 41ºC

during maturation decreased the proportion of oocytes with low mitochondrial membrane potential and TUNEL-positive chromatin (Soto & Smith 2009). Similarly, heat shock during maturation increased the proportion of oocytes expressing high activity of group II caspases (i.e., caspases 2, 3 and 7) and nuclear apoptotic fragmentation (Roth & Hansen 2004a,b). The mitochondrial apoptotic pathway involves cell-membrane alterations, such as phosphatidylserine externalization at an early phase of the apoptotic cascade (Lahorte *et al.* 2004) and activation of the sphingomyelin pathway, resulting in DNA damage (Hannun & Luberto 2000). Kalo and Roth (2011) linked heat-induced alterations in the oocyte phospholipid membrane and oocyte developmental competence. Alterations included hydrolysis of membrane sphingomyelin, ceramide generation and phosphatidylserine externalization (Annexin-V assay). Roth and Hansen (2004b) reported that the anti-apoptotic molecule S1P blocks the effect of heat shock on bovine oocytes, by modulating the ceramide–S1P balance. Specific inhibitors—fumonisin B1 to block dihydroceramide synthase and desipramine hydrochloride to block acid sphingomyelinase—were used to inhibit ceramide formation through de-novo and hydrolytic pathways, respectively (Kalo & Roth 2011). Use of these inhibitors alleviated, to some extent, the effect of heat shock and support the notion that thermal-stress-induced apoptosis via ceramide formation is functionally related to the reduced developmental competence of bovine oocytes.

The amount of lipid in the ruminant oocyte is relatively high and consists of 50% triacylglycerol, 20% phospholipid, 20% cholesterol and 10% free fatty acids (McEvoy *et al.* 2000). Thus, lipid metabolism, in particular that of sphingomyelins, might offer a number of opportunities for modifying oocyte responses to heat shock. In particular, ceramide can be produced de novo from condensation of serine with palmitoyl-CoA (Jenkins *et al.* 2002) and therefore, dietary manipulations that affect the membrane fatty acid composition or reduce ceramide formation might improve oocyte quality. Zeron *et al.* (2001) reported that oocyte membrane composition is affected by season, and is characterized by decreased polyunsaturated fatty acids during the summer. Feeding ewes with polyunsaturated fatty acids increased the proportion of high-quality oocytes in that season (Zeron *et al.* 2002). Similarly, in-vitro supplementation of palm oil Ca-LCFA increased the proportion of oocytes developing to blastocysts (Fouladi-Nashta *et al.* 2007). Nevertheless, dietary fatty acids administered during the summer did not have any effect on in-vitro developmental competence of oocytes aspirated from lactating cows (Bilby *et al.* 2006).

Hyperthermia-induced oxidative stress

Hyperthermia-induced oxidative stress is suggested to be one of the processes by which environmental stress disrupts reproductive performance in mammals. Ozawa *et al.* (2002) reported that exposing zygotes to heat shock, either directly or through maternal hyperthermia, causes early embryonic loss and is associated with reduced levels of the cytosolic antioxidant glutathione (GSH). Elevated body temperature increases intracellular reactive oxygen species (ROS) in bovine oocytes (Nabenishi *et al.* 2012) and preimplantation embryos (Sakatani *et al.* 2004), and decreases GSH concentration in mouse embryos (Matsuzuka *et al.* 2004). The balance between pro- and antioxidants plays a pivotal role in the follicle: spontaneous resumption of meiosis is inhibited by antioxidants and may be induced by an increase in ROS (Takami *et al.* 1999). On the other hand, high ROS levels within the follicle are associated with increased cytoplasmic defects and abnormal chromosomal segregation (Van Blerkom *et al.* 1997). Therefore, it is reasonable to assume that heat-induced perturbations of this balance are involved in disruption of the developmental competence of follicle-enclosed oocytes. Moreover,

administration of exogenous antioxidants has been suggested to overcome the adverse effects of heat stress on fertility. Nevertheless, vitamins A, E and C, provided either as nutritional supplements or intramuscularly, did not have any beneficial effect in heat-stressed cows (Ealy *et al*. 1994, Aréchiga *et al*. 1998, Paula-Lopes *et al*. 2003). Similarly, supplementation of vitamin E to the culture medium failed to improve in-vitro-derived bovine embryos under heat shock (Ealy *et al*. 1994, 1995, Paula-Lopes *et al*. 2003). On the other hand, in-vitro administration of anthocyanin and dithiothreitol was reported to protect embryos from heat shock (Sakatani *et al*. 2007, Castro e Paula & Hansen 2008). Aréchiga *et al*. (1998) reported that feeding supplemental β-carotene for at least 90 days beginning at ~15 days after calving increased the proportion of cows that were pregnant at 120 days postpartum by 14 percentage units, suggesting that long-term rather than periodic administration is needed.

Using a mouse model, Roth *et al*. (2008) showed that in-vivo administration of the antioxidant epigallocatechin gallate (EGCG), the most abundant flavonoid component of green tea, can moderate some of the deleterious effects induced by maternal hyperthermia. Pretreatment with EGCG at a dose of 100 mg/kg body weight increased the proportion of early cleaved embryos and the percentage of blastocysts that formed and hatched. In addition, pretreatment with EGCG reduced the apoptotic status of preimplantation embryos, most likely as a result of its antioxidative, antiapoptotic and/or membrane-stabilizing activities within the antral follicles. Matsuzuka *et al*. (2005) showed that administration of melatonin, another potent ROS scavenger, to heat-stressed mice alleviates hyperthermia-induced early embryonic death. A recent study reported that treatment of dairy cows with melatonin implants before calving improved their reproductive performance in the summer as it reduced days open and the number of AIs per pregnancy (Garcia-Ispierto *et al*. 2013). Taken together, although oxidative stress appears to be involved in the mechanism by which hyperthermia disrupts the oocyte, use of exogenous antioxidants to manipulate the animal's oxidative status has not yet been implemented in practice.

Heat stress and early embryonic development

Much of the effect of heat stress involves changes in ovarian function. However, studies indicate that preimplantaion embryos are also sensitive to elevated temperatures in a stage-dependent manner. Embryos at early developmental stages are more susceptible to thermal stress and then become more resistant at later developmental stages (Hansen 2007a,b). Exposing cows to elevated temperatures between onset of oestrus and insemination (Putney *et al*. 1988) or on day 1 after oestrus (but not on days 3, 5 or 7) decreased the development and viability of embryos on day 8 (Ealy *et al*. 1993). Similarly, induction of heat shock blocked the development of 2-cell-stage embryos, had a moderate effect on 4- to 8-cell-stage embryos and only a limited effect on the morulae (Hansen 2007a). A change in the balance between ROS generation and GSH level has been recently suggested to underlie acquisition of embryonic thermotolerance (Hansen 2013b).

Embryo-transfer procedures can bypass the thermosensitive developmental stages (for review see Hansen 2013b). Various studies have reported that embryo transfer during the summer results in pregnancy rates similar to those achieved with AI or embryo transfer in the winter. One major limitation of this approach is the poor survival of embryos following freezing. The percentage of pregnancies during the hot season was greater for cows receiving in-vitro-derived fresh, but not frozen, embryos (Ambrose *et al*. 1999, Drost *et al*. 1999). On the other hand, transferring in-vivo-derived frozen–thawed embryos increased pregnancy rates for recipient cows relative

to artificially inseminated ones (Putney *et al.* 1989). However, when the recipient could not maintain normal body temperature, pregnancy rate following embryo transfer was compromised (Vasconcelos *et al.* 2006). Use of an efficient cooling system to maintain normothermia in the recipient cows is thus highly important as it might alleviate the deleterious effects of heat stress on the uterus and plasma progesterone concentrations.

Effect of heat stress on the CL and progesterone level

Formation of a functioning CL following ovulation is obligatory for pregnancy maintenance. Thus, heat-induced alteration in luteal function and reduced progesterone (P_4) concentration in the circulation is another mechanism suggested to reduce embryo survival and to increase early embryonic loss. Plasma P_4 concentrations during the summer are lower than those during the spring or winter (Howell *et al.* 1994, Wolfenson *et al.* 2002). Chronic seasonal exposure has a deleterious carryover effect on follicular function that leads to the formation of a suboptimal CL and low P_4 concentration in the plasma (Wolfenson *et al.* 2000). An in-vitro study suggested that luteal insufficiency is the result, at least in part, of previous exposure of the preovulatory follicle to thermal stress and decreased production of P_4 by follicular cells, in particular theca cells (Wolfenson *et al.* 2002).

As a delayed increase in postovulatory P_4 and low concentrations during the preimplantation stages can lead to early embryonic losses (Mann & Lamming 2001), various studies have attempted to moderate the deleterious effects of heat stress on luteal function by inducing an accessory CL and excess luteal tissue. Administration of human chorionic gonadotropin (hCG) on day 5 or 6 post-AI did not affect CR (Schmitt *et al.* 1996) or increased it by 13 percentage units (Beltran & Vasconcelos 2008). On the other hand, administration of GnRH between days 5 and 15 post-AI increased conception rate by approximately 15 percentage units (Willard *et al.* 2003; López-Gatius *et al.* 2006), whereas others found no improvement in summer fertility (Franco *et al.* 2006). Differences among studies could be related to variations in the day of hCG or GnRH administration or to severity and duration of the thermal stress.

Supplementation of exogenous P_4 at the very early stages of pregnancy has the potential to increase embryo survival and improve conception in cows. Under normothermal conditions, P_4 supplementation during the early (days 5 to 9), but not late (days 12 to 16) luteal phase increased trophoblast length and Interferon-τ production on day 16 postfertilization (Mann *et al.* 2006). Friedman *et al.* (2012) recently reported that administration of a controlled intravaginal drug-releasing (CIDR) device on day 5 $\pm$ 1 post-AI for 13 days had a substantial effect on CR in the summer when the device was inserted on day 4, relative to days 5 or 6 (43, 39 and 34%, respectively). Importantly, cows with low BCS at peak lactation, or those diagnosed with postpartum reproductive disorders, or both, gained significant benefit from the CIDR implant (Fig. 4). It should be noted that cows were maintained under normothermic to temporary mild hyperthermic conditions.

Conclusion

In-vitro and in-vivo studies explored the multifactorial effects of heat stress on ovarian function (Fig. 5). These effects include alterations in follicular growth, steroid production and hormonal secretion, and reduction in oocyte and embryonic developmental competence. Cooling is a prerequisite for any additional strategy and should be performed over long periods of time. Similarly, periodic administration of antioxidant is not efficient at protecting the follicle and

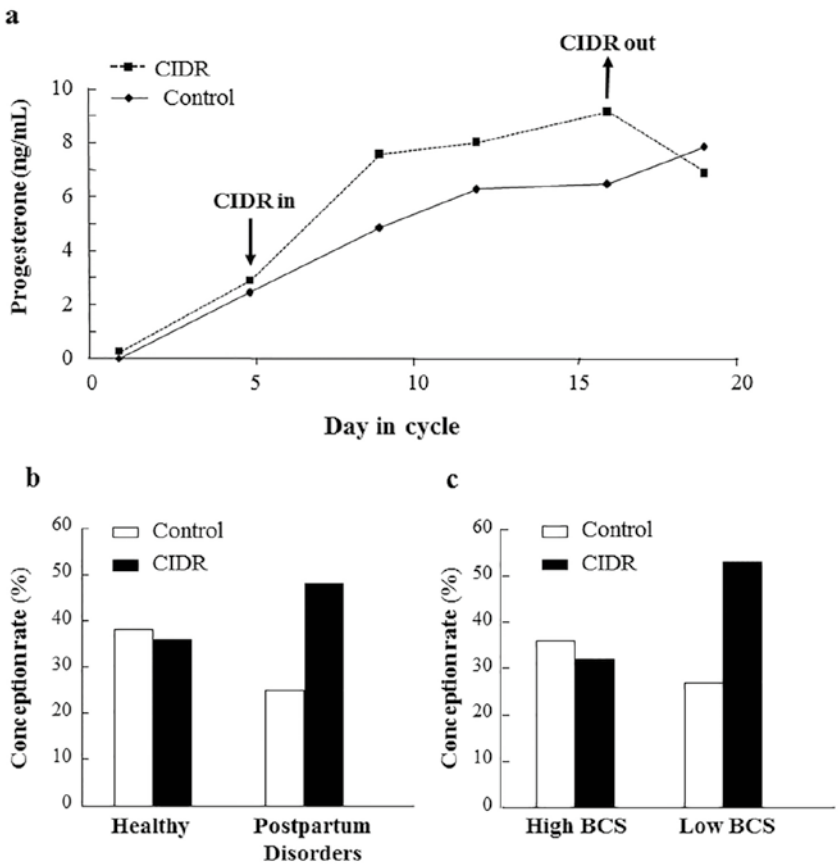

Fig. 4. CIDR inserted post-AI to improve conception rate during the summer and fall. Cows were treated with insert containing 1.9 g of P_4 on day 5 $\pm$ 1 post-AI for 13 days. Analysis revealed (a) a day-by-treatment interaction ($P < 0.06$), with approximately 1.5 ng/mL greater progesterone concentration in plasma from day 9 to 16 of the cycle. (b) Interaction ($P = 0.035$) between treatment and low BCS at peak lactation, reflected by a 26-percentage-unit increase in conception rate. (c) A 23-percentage-unit increase in conception rate in cows treated with CIDR and diagnosed with postpartum reproductive disease. Data are presented as means.

its enclosed oocytes. Hormonal treatments with GnRH/$PG_{2\alpha}$ to induce follicular turnover were found successful for a subpopulation of cows (primiparous, high BCS, low SCC), but had only a limited impact on the herd as a whole. The deleterious effect of heat stress on CL function can be mitigated by insertion of a CIDR device 5 days post-AI to increase P_4 levels in order to support the developing embryo, and this has been found to be beneficial for a subgroup of cows with postpartum uterine diseases. The effect of heat stress on the oocyte and early-cleaved embryos can be bypassed by thermoresistant embryo transfer. Nevertheless, to date, none of these suggested approaches have been widely implemented on farms. It is proposed that combining two or more of these potential approaches into one program might be more efficient for improving reproductive responses under heat stress. For example, combining hormonal treatments before (GnRH/$PG_{2\alpha}$) and after (CIDR) insemination would compensate for

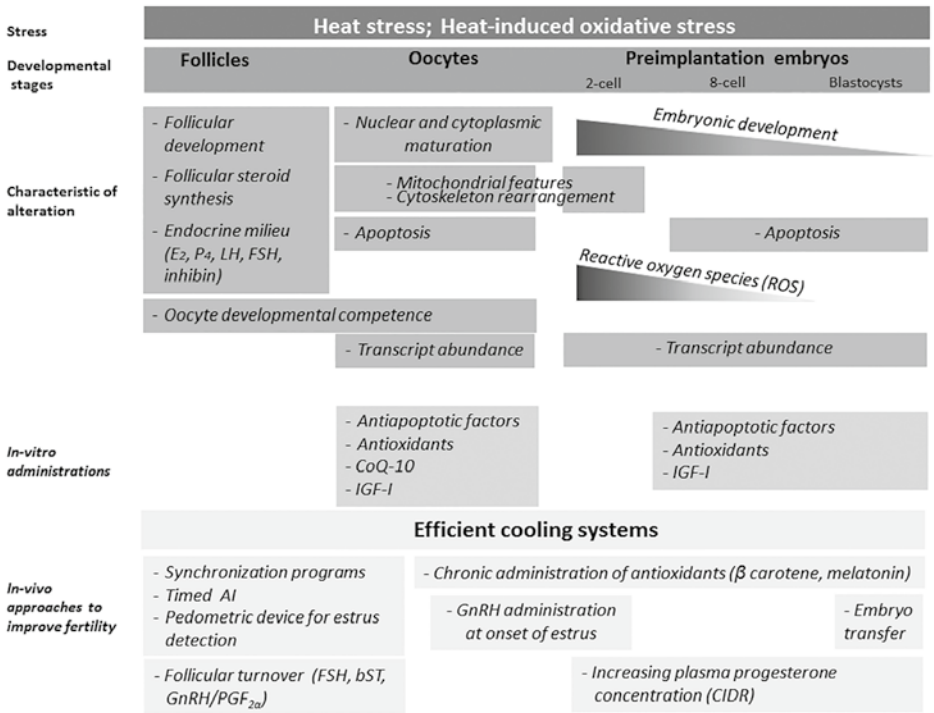

Fig. 5. Diagram illustrating the effects of heat stress and heat-induced oxidative stress on oocyte and embryo competence at various developmental stages. Also presented are various compounds that can be administered to counteract the effects of heat shock in vitro, and some potential approaches to improving fertility. Heat stress can impair follicular development and steroid synthesis, which in turn can disrupt the endocrine milieu. Heat stress also affects oocyte maturation and developmental competence, as reflected by impaired nuclear, cytoplasmic maturation and cytoskeletal rearrangement during maturation, as well as altered mitochondrial features and transcript abundance. Heat-induced apoptosis has been documented in matured oocytes and preimplantation embryos, but not in the 2-cell-stage embryo. The embryo is more susceptible to thermal stress and reactive oxygen species (ROS) in the early stages of development and becomes more thermotolerant coincident with activation of the embryonic genome. In-vitro administration of antiapoptotic, antioxidant and growth factors can counteract the effects of heat shock on the oocyte and embryo. However, these have not yet seen practical use. Various approaches have been examined to improve the fertility of lactating cows under heat stress. While an efficient cooling system is required to maintain cows in normothermia, it is not sufficient to improve fertility. Other suggested approaches, to be combined with cooling, are: (1) hormonal administration to induce follicular turnover; (2) transfer of in-vitro-derived embryos treated with IGF-I. In both approaches, increasing plasma progesterone concentration to support the developing embryo might help increase conception rate during the summer.

the deleterious effects of summer heat stress on two different reproductive tissues, the ovarian pool of follicles and their enclosed oocytes and the CL, respectively. This program has been found to extend the population of cows that benefit by 16 percentage units relative to each treatment alone (Z Roth *et al.*, unpublished observations). It is suggested that a comprehensive strategy, rather than correcting the function of a single part of the reproduction tract, is required to cope with the whole heat-stress problem.

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