Hormonal treatments to improve fertility in high lactating cows during the summer and autumn- basic and applied studies

Zvi Roth*
*Corresponding author. Department of Animal Science, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University, P.O. Box 12, Rehovot 76100, Israel. Tel.: +972-8-948-9103; Fax: +972-8-9489-552
E-mail: roth@agri.huji.ac.il

Evaporative cooling methods are the most common strategy used to alleviate the effect of heat stress; however, there is a compelling need to find additional ways to improve fertility during the summer and autumn. This review examines the immediate and delayed effects of heat stress on follicular function and describes some potential hormonal strategies for their alleviation. It focuses on how heat stress affects the follicle and its enclosed oocyte, suggesting that perturbations in the follicular microenvironment, to which the oocytes are exposed for long periods of development, reduce their developmental competence. Among the potential alterations are reduction in gonadotropin secretion, alteration in follicular growth, attenuation of dominance, and disruption of steroidogenesis. Potential impairments in the follicle-enclosed oocyte upon heat shock are also described. The current review will focus on hormonal treatment to enhance removal of the impaired follicles by stimulation of follicular growth. A brief treatment with FSH or synchronization of follicular waves with GnRH followed by PGF2α is suggested. A better understanding of the underlying mechanisms by which heat stress impairs fertility may lead to the development of additional approaches to alleviate these effects.
1. Introduction

The most common strategy to alleviate the effect of heat stress in dairy farms is to provide shade and evaporative cooling systems, based on a combination of sprinkling and ventilation, to enable animals to maintain normothermia. This approach can reduce body temperature and increase milk production; however, its effect on summer fertility is limited (Hansen, 1997). Moreover, during the autumn, although the weather is cooler and the cows are no longer subjected to thermal stress, conception rates remain low (Hansen, 1997; Zeron et al., 2001). Thermal-stress-induced alterations in the ovarian pool of follicles and their enclosed oocytes have been suggested as a major cause for reduced dairy cow fertility through the summer and subsequent autumn (Roth, 2008). This review emphasizes the importance of the endocrine milieu and follicular microenvironment to which the ovarian pool of oocytes is exposed and describes potential impairments in the follicle-enclosed oocyte upon heat shock. It describes the immediate and delayed effects of heat stress on follicular function and some potential hormonal treatment to alleviate them.

2. Effects of heat stress on follicular function

The stage of follicular development that is susceptible to thermal stress has not been precisely defined. Study in goats has indicated that follicles exposed to heat stress during recruitment regress and never develop to large sizes or ovulate (Ozawa et al., 2005). Roth et al. (2000) introduced evidence suggesting that early antral follicles of about 0.5 to 1.0 mm in diameter are sensitive to heat stress. In addition, growth of medium-sized follicles was attenuated in cows exposed to direct solar radiation in the previous estrous cycle (Roth et al., 2000). Heat stress impairs the growth of mediumsized follicles (6-9 mm), as indicated by the earlier emergence and delayed decrease of the second follicular wave (Roth et al., 2000). Heat stress reduces the size of the first- and second-wave dominant follicles (Badinga et al., 1993; Wilson et al., 1998a,b); attenuates dominance, as reflected by an increased number of large-sized follicles (Badinga et al., 1993, 1994; Wolfenson et al., 1995; Roth et al., 2000) and delayed regression of the subordinate follicles (Wilson et al., 1998b; Roth et al., 2000). Such alterations
may lead to early emergence of the preovulatory follicle and increased duration of dominance (Wolfenson et al., 1995), both of which are negatively associated with conception rate (Mihm et al., 1994). Similar effects of maternal heat-stress on follicular growth and oocyte developmental competence have also documented in *Bos indicus* cattle (de Torres-Junior, 2008).

The endocrine background governing alterations in follicular dynamics and depression of dominance under heat stress includes alterations in systemic gonadotropin, inhibin and steroid concentrations, as well as in steroidogenesis (for review, see Wolfenson et al., 2000). Heat-induced increases in the number of medium-sized follicles (i.e. immediate effect) is associated with increased concentrations of follicle-stimulating hormone (FSH) and reduced concentrations of inhibin in the plasma (Wolfenson et al., 1995; Roth et al., 2000). Seasonal studies report that lower steroid concentrations in the follicular fluid obtained from large follicles during the hot season are associated with reduced viability of granulose cells and impaired aromatase activity (Badinga et al., 1993; Wolfenson et al., 1995). Thus, heat-induced impairment of granulosa cell function, the main source of plasma estradiol and inhibin, can lead to increased FSH concentrations in the plasma (Roth et al., 2000).

Thecal cells incubated at high temperatures or collected from heat-stressed cows produce less androstenedione when stimulated by LH, but not by forskolin, implying a disruption in LH receptor function upon heat stress (Wolfenson et al., 1995; Roth et al., 2001b). Follicle pieces obtained from heat-stressed cows secrete lower levels of androstenedione and estradiol upon gonadotropin stimulation (Bridges et al. 2005). Moreover, the alterations of steroidogenic capacity induced by summer heat stress carry over to the final stage of follicle development, as evidenced by reduced androstenedione production by thecal cells and low estradiol concentrations in follicular fluid collected from dominant follicles in the autumn (Wolfenson et al., 1997). Decreased estradiol and androstenedione production was recorded in granulosa and thecal cells obtained from follicles three to four weeks after acute heat stress (Roth et al., 2000). Similarly, estradiol content in the follicular fluid aspirated from cows was relatively low in late summer and increased throughout the autumn (Roth et al., 2004). Thus, the extent of the heat stress effects on follicular function is transient as reflected by the spontaneous improvement of fertility throughout autumn and early winter (Zeron et al., 2001).
3. Effect of heat stress on oocyte developmental competence

*In-vivo* and *in-vitro* studies support the view that bovine oocytes are susceptible to thermal stress at various stages of follicular development. Perturbation in the physiology of the follicle-enclosed oocyte during the lengthy period of follicular development could potentially lead to an oocyte with reduced competence for fertilization and subsequent development. Intensive cooling from one day before estrus to eight days post-Al did not improve the conception rate of cows in the summer (Her et al., 1988). Similarly, using a fan-and-fogger cooling system 42 days before oocyte collection did not increase the proportion of oocytes that developed to the blastocyst stage (Al-Katanani et al., 2002). Thus, either the oocytes were already compromised prior to the period of heat-stress relief or the cooling was not efficient enough to decrease heat stress. A study performed from late summer to early winter indicated that a period of two to three estrous cycles is required for recovery from heat damage and appearance of competent oocytes (Roth et al., 2001a). Respectively, study from Brazil reported that the low fertility of repeat-breeder cows during summer is related to a low oocyte developmental competence (Ferreira et al., 2011). It appears that not only the individual ovulated oocyte, but also the ovarian pool of oocytes can be damaged during heat exposure. However, the exact follicular stage at which the enclosed oocyte is susceptible to thermal stress has not been defined.

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. Exposure of GV-stage bovine oocytes to 41°C did not impair GV breakdown but reduced the proportion of oocytes that progressed to metaphase II (MII) and was associated with further impairment of blastocyst development (Payton et al. 2004). Exposing animals to heat stress between the onset of estrus and insemination (i.e. the time of in vivo maturation) disrupts subsequent embryonic development, with an increased proportion of abnormal and retarded embryos (Putney et al., 1989; Ealy et al., 1993). Similarly, *in-vitro* exposure of COCs to elevated temperatures during the first 12 h of maturation decreases their cleavage rate (Roth et al., 2004; Roth and Hansen, 2005) and the proportion of oocytes that develop into blastocysts (Edwards and Hansen, 1997; Ju et al., 2005; Roth and Hansen, 2004 a,b;
Edwards et al., 2009). Culturing oocytes at 41ºC does not compromise their nuclear and cytoplasmic maturation but accelerates the process, thus a fertilization performed 5 h earlier is suggested to attenuate the deleterious effect on oocyte development (Edwards et al. 2005). Using a time-laps system we have recently provided evidences that the adverse effect of summer thermal stress on oocyte developmental competence is associated with delayed cleavage of the two first embryonic deviations (Gendelman et al., 2010). The timing of first cleavage (early vs. delayed) is considered to have major long-lasting effects on subsequent embryonic developmental potential (Fenwick et al., 2002), which may explain the inferior developmental competence of oocytes collected during the summer.

The underlying mechanism by which heat stress disrupts oocyte developmental competence is not entirely clear; it may directly affect the oocyte (i.e. cytoplasm and nuclear maturation) or mediate negative effects through an impaired follicular environment (i.e., follicular fluid steroid content) or impaired function of surrounding cumulus cells. Gendelman and Roth (2011) have recently suggested that heat-stress-induced alterations in GV-stage oocytes are further expressed in the transcriptional levels of genes involved in oocyte maturation and early embryonic development. In the later study, bovine oocytes were collected during cold (December-April) and hot (May-November) seasons. Total RNA and poly(A) mRNA of oocytes and developing embryos were isolated and subjected to semi-quantitative and real-time PCR for C-MOS, GDF9 and POU5F1, genes. In GV-stage oocytes, the mRNA levels did not differ between the seasons. However, following maturation (MII-stage oocytes) and through the subsequent developmental stages (2-, 4-, and 8- cell stage embryos and blastocysts), the mRNA levels prominently differed between seasons. It appears that exposing the ovarian pool of oocytes to environmental stress impairs maternal mRNA storage and/or the mechanism of transcription renewal, which in turn affects embryo gene expression before and after embryonic genome activation. Such impairment might partially explain the carry-over effect of summer heat stress on dairy cow conception rates.

Heat shock impairs both nuclear and cytoplasmic maturation events, such as translocation of cortical granules to the oolemma (Payton et al., 2004), cytoskeleton rearrangement (Roth and Hansen, 2005), and spindle formation (Ju et al., 2005; Roth and
Hansen, 2005). Similarly, heat-shock-induced perturbations of the spindle apparatus have been reported in mature porcine oocytes (Ju and Tseng, 2004) and in parthenogenetically activated bovine oocytes (Tseng et al., 2004). Such alterations may potentially lead to incomplete nuclear maturation (Payton et al., 2004; Roth and Hansen, 2005), fertilization failure, and/or abnormal zygote formation. Most heatshocked oocytes fail to undergo maturation and fertilization and are arrested at stages MI through MII (Roth and Hansen 2005).

Heat-shock-induced apoptosis has been documented for bovine oocytes exposed to elevated temperatures during maturation. Heat stress increases the proportion of oocytes that express high caspase activity (specifically, group II caspases, i.e. caspases 2, 3 and 7) and nuclear fragmentation, as determined by terminal deoxynucleotidyl transferase (TdT)-mediated d-UTP nick end-labeling (TUNEL) (Roth and Hansen 2004a, b). Soto and Smith (2009) reported that heat shock during maturation (41°C, 22 h) increases the proportion of oocytes with TUNEL-positive chromatin and loss of mitochondrial membrane potential (Δψm). Recently we provided evidences that heat-shock-induced apoptosis in bovine oocytes involves membrane alterations such phosphatidylinerine externalization and ceramide activity (Kalo and Roth, 2010); both considered early apoptotic events, upstream of DNA fragmentation. The findings support the view that heat-shock-induced apoptosis via the sphingomyelin pathway (i.e. ceramide formation) is functionally related to reduced developmental competence of the oocyte.

The sphingomyelin pathway is a signal-transduction system initiated by the hydrolysis of sphingomyelin, a membrane phospholipid, to generate the second messenger ceramide (Peña et al. 1997). Whereas ceramide is associated with growth arrest and apoptosis sphingosine 1-phosphate (S1P) is associated with proliferation and cell survival (Pyne and Pyne 2000). Roth and Hansen (2004b) provided evidence that the anti-apoptotic molecule S1P blocks the effect of heat shock on bovine oocytes. On the other hand maturation of bovine oocytes with C2-ceramide increases the proportion of annexin-V-positive oocytes (i.e. indicating early apoptosis stages) and impairs oocyte developmental competence in a dose-dependent manner (Kalo and Roth, 2011). It was also shown that specific inhibitors of ceramide formation alleviated, to some extent, the effect of heat shock on oocyte developmental competence. Since the balance between the amounts of ceramide and S1P is an important determinant of cell fate,
administration to reduce the amounts of intracellular ceramide upon heat stress might alleviate its effect on oocytes. This point warrants further investigation.

4. Strategies to improve ovarian function

Our previous findings indicate that heat stress affects the ovarian pool of small antral follicles, approximately 0.5-1.0 mm in diameter therefore, enhanced removal of summer impaired follicles might be a potential means of improving fertility in dairy cows. Based on this assumption, mechanical and hormonal strategies to enhance the removal of impaired follicles and the reappearance of healthy follicles and competent oocytes have been suggested (for review see Roth, 2008). Frequent follicular aspiration of small and medium-sized follicles (3 to 7 mm), in four consecutive estrous cycles during the fall and early winter, enhanced follicular removal from the ovary and led to earlier emergence of healthy follicles (Roth et al., 2001a). The aforementioned concept of removing impaired follicles also seems to be relevant to improving oocyte quality, since frequent follicle aspirations by an ovum pick-up procedure improved the morphology and developmental competence of oocytes aspirated in the fall following the hot summer. These findings led to the development of hormonal-treatment strategies to enhance the emergence of follicular waves.

4.1. FSH administration to remove damaged follicles

Short-term treatment with FSH (2 X 200 mg Folltropin-V at a 12-h interval) increased the number of follicles emerging within follicular waves and resulted in earlier ovarian recovery following summer thermal stress. This was also reflected by increased proportion of oocytes with grade-1 morphology and cleaved to the 2- and 4- cell-stage embryo in the autumn (Roth et al., 2002). It should be noted, however, that FSH administration did not improve the proportion of embryos that developed to the blastocyst stage. The high doses of FSH generally used for super-ovulatory protocols limit this treatment’s prospects in terms of wide use on commercial farms due to economic circumstances but also because of its potentially deleterious effect on
oocyte developmental competence (Greve et al., 1995). On the other hand, administration with low doses of FSH (4.4 mg, Ovagen), precisely timed to synchronize with the emergence of follicular waves, efficiently induces follicular turnover with a modest effect on the steroidogenic capacity of the preovulatory follicle in the treated cycle but not in the subsequent cycle (Friedman et al., 2011). While not entirely clear, the beneficial effects of the up maintained FSH administrations could be related to enhanced removal of follicles that has been damaged in the previous summer and/or to direct or indirect effects on the follicular microenvironment.

4.2. Induction of follicular waves with GnRH followed by PGF2α

An alternative approach based on synchronization protocols is suggested. Induction of several consecutive follicular waves by GnRH and PGF2α resulted in the development of healthy preovulatory follicles in heat-stressed cows relative to normothermal cows (Guzeloglu et al., 2001). Similarly, induction of 6 successive 9- day follicular waves with GnRH and PGF2α through the autumn has a beneficial effect on developing follicles since it increased follicular volume and estradiol concentration in preovulatory follicles aspirated from previously heat-stressed cows (Roth et al., 2004). As the above approach appears to improve ovarian function, its possible beneficial effect on fertility of dairy cows during the summer and the subsequent autumn was recently examined (Friedman et al., 2011). The experiment was conducted from July to December in 2 commercial herds in Israel and included 382 healthy Holstein cows. Cows (50 to 60 days in milk [DIM]) were hormonally treated to induce 3 consecutive 9-day follicular waves. Both control (n = 187) and treated (n = 195) cows were inseminated following estrus and pregnancy was determined by palpation 45 days postinsemination. Induction of follicular waves during the summer and fall significantly improved conception rate in primiparous cows by 16% and pregnancy rate at 120 DIM by 14%. Interestingly, interaction between treatment and high body condition score was reflected by a 14% increase in pregnancy rate at 90 DIM. The findings indicate that administration of hormonal treatment (i.e. GnRH followed by PGF2α ) to induce successive follicular waves improved fertility of primiparous cows and cows with high BCS at the
initiation of treatment. Implementation of such hormonal treatment in combination with an efficient cooling system may improve reproductive performance of dairy cows during the summer and subsequent autumn. Although not examined at the hot season, double Ovsynch (Souza et al., 2008) and Doublesynch (Ozturk et al., 2010) protocols, which also based on GnRH + PGF2α administration, had a beneficial effect on the fertility of primiparous but not on multiparous cows. Thus, reproductive management that is based on hormonal treatment in subpopulations of cows may improve efficiency of treatment and reduce expenses.

5. Summary

Disruption of the follicle and its enclosed oocyte seems to be a pivotal factor in the complex mechanism via which heat stress impairs fertility. This includes alterations in the endocrine milieu and follicular microenvironment to which the ovarian pool of oocytes is exposed, leading to their decreased developmental competence. Hyperthermia can directly disrupt follicular function, but a carry-over effect on the follicle and its enclosed oocyte is also evident. Evaporative cooling systems used to maintain normothermia in high-lactating cows are likely to remain obligatory in dairy management during the hot season. However, since they only partially restore normal fertility, there is a compelling need for additional approaches. Beneficial strategies might involve hormonal treatment to enhance the removal of impaired follicles. The current review focused on follicular-wave synchronization with the use of GnRH and PGF2α, or stimulation of follicular growth with brief treatments of low FSH doses. More precise treatments designed to mitigate the deleterious effect of heat stress on the follicle and its enclosed oocyte should be further examined.
References


