Strategies to reduce the impact of heat stress on fertility of cows

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The Problem of Infertility in Dairy Cattle Caused by Heat Stress

Heat stress has two major consequences for the physiology of the cow that reduce its probability of becoming pregnant. First, changes in cow behavior (for example, reduced walking time; López-Gatius et al., 2005) and reduced circulating concentrations of estradiol-17β (Gilad et al., 1993) caused by heat stress reduce ability to detect estrus. On one dairy in Florida, only about 18-24% of estruses in hot months were detected by herdsmen while 45-56% of estrus periods were detected in cool months (Thatcher and Collier, 1986). Secondly, there is a large reduction in fertility. In lactating dairy cows, pregnancy rates per insemination in the summer can be as low as 10-20% (Hansen and Aréchiga, 1999). Fertility is reduced because heat stress can damage both the oocyte and early embryo (Hansen, 2013). The oocyte can be compromised by heat stress as early as 105 days before ovulation (Torres-Júnior et al., 2008) and as late as the peri-ovulatory period (Putney et al., 1989). The early embryo is also initially sensitive to heat stress but quickly becomes resistant so that heat stress on day 1 after estrus reduced embryonic development whereas heat stress at day 3 had no effect (Ealy et al., 1993).

Heat stress is largely a problem of the lactating dairy cow. In Florida, conception rates in Holsteins declined in the summer for lactating cows but not for non-lactating heifers (Badinga et al., 1985). The lactating cow is very susceptible to heat stress because the increased amounts of heat produced as a result of lactation makes it difficult to regulate body temperature during heat stress. Hyperthermia in lactating cows can occur at air temperatures as low as 25-29ºC (Berman et al., 1985; Dikmen and Hansen, 2009). Beef cattle can also be affected by heat stress, particularly in feedlot situations (Mitlöhner et al., 2002), but several factors mitigate against large effects on reproduction in beef cattle. These factors include the existence of beef breeds that are genetically resistant to heat stress (Gaughan et al., 2010) and seasonal breeding patterns that ensure that cows are not bred at the warmest time of year.

The most common approach to reduce effects of heat stress on dairy cattle is to provide housing that minimizes heat stress. Incorporation of features such as shade structures, fans, and sprinklers, misters or foggers can be seen in many dairies in hot regions of the world. While very important, cooling cows does not totally prevent reduced reproductive function during heat stress. In Florida, for example, seasonal variation in pregnancy rate persisted in a herd where cows were cooled with sprinklers and fans (Hansen and Aréchiga, 1999). In Israel, pregnancy rate per insemination in intensively-cooled herds was 19% in summer vs 39% in winter for high-producing herds and 25% in summer vs 40% in winter for low-producing herds (Flamenbaum and Ezra, 2006).

In this paper, some other approaches for reducing effects of heat stress on reproductive function will be discussed. These approaches are based on altering the antioxidant status of the cow, use of hormones to manipulate reproductive processes, and incorporation of embryo transfer to bypass effects of heat stress on the oocyte and embryo.

Administration of Antioxidants to Protect Embryos from Free Radicals Induced by Heat Shock

One of the reasons why heat stress damages the oocyte and embryo may reside in an increase in production of free radical molecules at elevated body temperature. Exposure of maturating oocytes (Nabenishi et al., 2012) and early cleavage-stage embryos to elevated temperature (Sakatani et al., 2004) increases the production of free radicals. In culture, some antioxidants [anthocyananin (Sakatani et al., 2007) and dithiothreitol (de Castro e Paula et al., 2008)] but not all [vitamin E (Paula-Lopes et al., 2003a), glutathione (Ealy et al., 1995), and glutathione ester (Ealy et al., 1995)] reduced the effects of elevated temperature on development of embryos. The possible involvement of free radicals in effects of heat stress on the oocyte and embryo suggests it might be possible to improve fertility by increasing antioxidant concentrations in cells or the blood. There are two papers that suggest that this might be possible.

In the first paper, Aréchiga et al. (1998) found that feeding cows supplemental β-carotene at a rate of 400 mg/d for at least 90 days from about Day 15 after calving increased the proportion of cows that were pregnant at 90 d postpartum during the summer but not during the winter (Table 1). There was no effect of treatment on pregnancy rate at first service so the supplemental β-carotene either improved fertility after first service or estrus detection rate.

Table 1. Effect of supplemental feeding of β-carotene in the postpartum period on reproductive function of lactating Holstein cows in Florida.[a]

Experiment	Time of year	Treatment	No. of cows	Pregnancy rate at first insemination	Pregnancy rate, 90-d postpartum
1	Hot	Control	128	9.4%	21.1%
		β-carotene	48	12.9%	35.4%*
2	Cool	Control	55	17.6%	33.3%
		β-carotene	52	12.9%	26.2%

[a] From Aréchiga et al. (1998)
* P<0.05.

More recently, Garcia-Ispierto et al. (2013) tested whether administration of 18 mg melatonin implants (9-12 implants per cow) beginning at 220 d of gestation to cows during the summer would affect postpartum reproduction. Melatonin has antioxidant properties in the follicle (Tamura et al., 2013). Cows treated with melatonin had reduced interval to conception and decreased incidence of cows experiencing > 3 breedings per conception (Table 2). It is not clear whether the effect of melatonin is due to blocking effects of heat stress on the oocyte and embryo or whether it acts through some effect independent of heat stress. Results are promising but experiments should be repeated in both hot and cool seasons.

Table 2. Effect of administration of melatonin implants at 220 d of gestation on postpartum reproduction in the summer in Spain.[a]

Treatment	No. of cows	Cows with > 3 AI/conception	Interval from calving to conception, days
Control	67	28.4%	123
Melatonin	72	12.5%**	103*

[a] From Garcia-Ispierto et al., 2013
* P<0.05
** P< 0.01

Use of Hormones to Bypass Effects of Heat Stress on Reproduction

Protocols for timed artificial insemination (AI) can completely bypass problems associated with detecting estrus during heat stress because timing of ovulation is synchronized and insemination can be implemented at a fixed time without the need for estrus detection. What timed AI does not do is reverse damage to the oocyte or embryo caused by heat stress. Implementation of timed AI programs during heat stress can increase the rate at which at which cows get pregnant after calving because it increases the number of eligible cows that are inseminated. This is despite the fact that actual conception rates are not improved.

The effectiveness of timed AI during heat stress can be examined by looking at results from two experiments conducted in the summer (Table 3). The first experiment, that of Aréchiga et al. (1998), was conducted in south Florida, where the magnitude of heat stress is very high. Cows were assigned to either be bred at first detected estrus after the voluntary waiting period of 70 days or were subjected to the Ovsynch timed AI procedure to be inseminated at 70 d after calving. Implementation of timed AI reduced the interval from calving for first service by 10 d. There was no difference in the proportion of cows that became pregnant after first insemination between treatments. Moreover, few animals became pregnant, undoubtedly because of the high degree of heat stress. Nonetheless, more cows were pregnant by 90 d postpartum in the timed AI group, presumably because more cows had been inseminated.

Similar results were obtained in Kansas by Cartmill et al. (2001). In this experiment, one group of cows were subjected to an estrous synchronization regimen (Select Synch) whereas another group was inseminated using the Ovsynch protocol. The overall level of fertility was higher than for the Florida study. Like for the Florida experiment, timed AI did not increase the percent of cows pregnant at first insemination after calving. However, more cows were inseminated within 7 d after the TAI treatment than after the estrous synchronization treatment so the percent of cows pregnant at 90 d postpartum was almost twice as high for the timed AI group (Table 3).

Table 3. Effectiveness of timed insemination protocols for increasing pregnancy rates of lactating Holsteins when implemented during periods of heat stress in Florida (Experiment 1) and Kansas (Experiment 2).

Experiment[a]	Treatment[b]	No. of cows	Interval, calving to first service, days	Cows bred within 7 d after PGF	Pregnancy rate at first service	Pregnancy rate at 90-d postpartum
1	Estrus	184	82.4	--	12.5%	9.8%
	TAI	169	72.4***	--	13.6%	16.6%*
2	SS	128	--	57%	32.0%	17.9%
	TAI	207	---	100%	33.3%	33.3%**

[a] Experiment 1: Aréchiga et al. (1998); Experiment 2, Cartmill et al. (2001).
[b] Estrus = breeding at each observed estrus beginning at Day 70 postpartum; TAI = timed artificial insemination followed by breeding at all observed estrous periods thereafter; SS=Select Synch; GnRH followed by prostaglandin F-2α (PGF) 7 d later and breeding at detected estrus for the next 21 d .
* $P<0.05$ ** $P<0.01$ *** $P<0.001$

While timed AI can increase the number of cows inseminated, it cannot increase fertility during the summer. Unfortunately, no pharmacological treatment has been identified that can consistently increase pregnancy rate per AI during heat stress (see Hansen, 2011 for review). Among the treatments that have been examined are treatment with human chorionic gonadotropin at Day 5 of the estrous cycle to increase circulating progesterone concentrations, treatment with bovine somatotropin to increase secretion of the embryoprotective molecule insulin-like growth factor-1 and treatment with gonadotropin releasing hormone to extend lifespan of the corpus luteum. The ineffectiveness of hormonal treatments is probably related to the broad period of time in which the oocyte and early embryo are susceptible to disruption by heat shock. Treatments that might reverse effects of heat stress at one physiological window cannot reverse effects at others. Consider, for example, use of chorionic gonadotropin to increase output of progesterone by the corpus luteum. Such a treatment might be effective at reversing effect of heat stress on circulating progesterone concentrations after ovulation (Wolfenson et al., 2000) but this effect would not improve fertility in a cow whose oocyte was already damaged by heat stress occurring at some time before ovulation.

Embryo transfer to bypass death of oocytes and embryos caused by heat stress

Embryo transfer was developed as a tool to increase the number of offspring from genetically-superior females. This technology can also be used for improving fertility during heat stress. In fact, it remains the best method available for increasing pregnancy rate in lactating cows exposed to heat stress. Indeed, it has been shown repeatedly that embryo transfer can alleviate the reduction in fertility caused by heat stress (Figure 1).

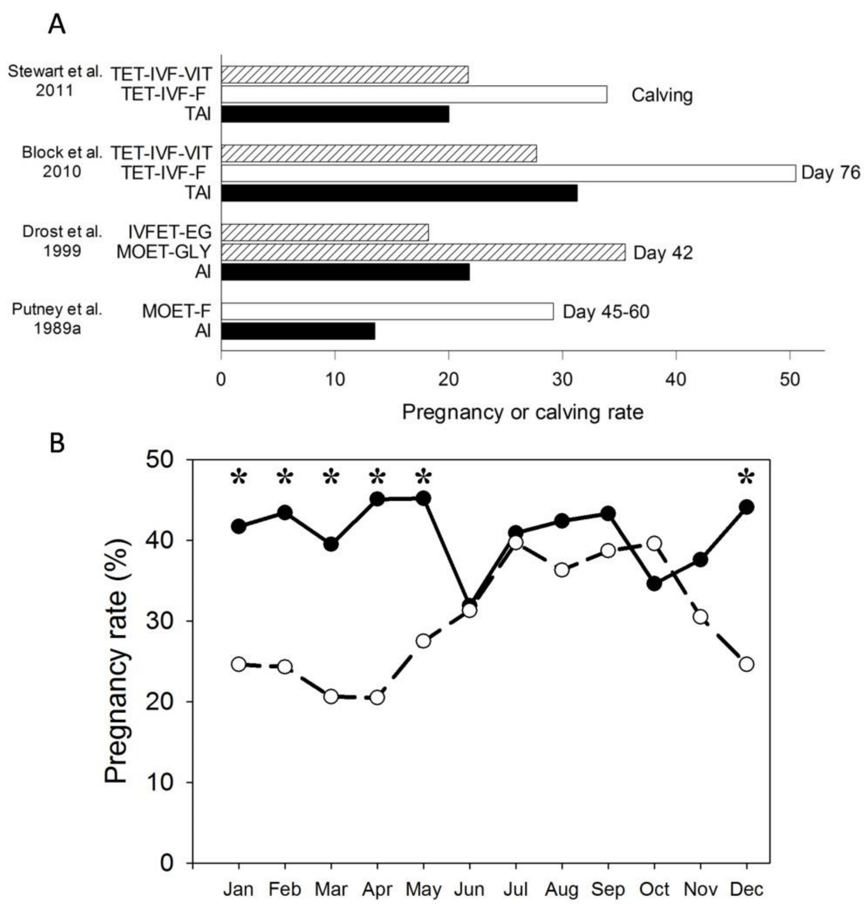

Figure 1. Enhancement of pregnancy rates during heat stress using embryo transfer. Data in Panel A represent results from various experiments in the summer in Florida. Abbreviations are as follows: AI: artificial insemination; EG, frozen in ethylene glycol; F, fresh; Gly, frozen in glycerol; IVFET, embryo transfer with an in vitro produced embryo; MOET, multiple ovulation embryo transfer; TAI, timed artificial insemination; TET-IVF, timed embryo transfer with an in vitro produced embryo; VIT, vitrified. The numbers in the graph represent the day of gestation at which pregnancy diagnosis was carried out. Panel B represents data from a commercial dairy in Brazil in which cows were either inseminated or received an embryo produced by superovulation (Rodriques et al., 2004). Asterisks represent months in which pregnancy rate was different between AI and ET. The figure is reproduced from Hansen (2013).

Fertility is low in the summer largely because of damage to the growing follicle, oocyte and embryo caused by exposure to maternal hyperthermia (elevated body temperature). The oocyte can be damaged by heat stress as early as 105 days before ovulation (Torres-Júnior et al., 2008) and remains sensitive to heat stress on the day of ovulation (Putney et al., 1989b). The early embryo, too, can be damaged by heat stress but soon acquires biochemical mechanisms that protect it elevated temperature (Hansen, 2013). Thus, heat stress at Day 1 after estrus reduced embryonic development but heat stress at Days 3, 5, and 7 had no effect (Ealy et al., 1993).

In embryo transfer protocols, the only embryos typically transferred are those that have developed develop. Thus, the embryo that is transferred into a heat-stressed recipient has, for one reason or another, escaped effects of heat stress. In addition, embryos have become resistant to heat stress by the time they reach the stage of development where they are ready to be transferred into a recipient (the morula or blastocyst stage). Thus, it is unlikely that maternal hyperthermia will kill an embryo after Day 7 of pregnancy than an embryo in the first day or two of life.

Embryos can be produced using either superovulation or in vitro fertilization. For superovulation, cows are injected with follicle stimulating hormone to cause the growth and ovulation of multiple follicles. For in vitro fertilization, oocytes are harvested either from growing follicles using transvaginal, ultrasound guided aspiration (called oocyte pickup or OPU) or from ovaries recovered at slaughter or ovariectomy. Oocytes are then fertilized with sperm and the resultant embryos allowed to develop in the laboratory until transferred into recipients. Embryos produced by superovulation are superior to those produced in vitro in terms of ability to establish pregnancy after transfer and survive cryopreservation for long-term storage. As shown in Figure 1, transfer of superovoulated embryos improves fertility during heat stress regardless of whether embryos are cryopreserved or transferred fresh. However, the poor survival of in vitro produced embryos to cryopreservation means that transfer of in vitro produced embryos improved fertility only when embryos were transferred without cryopreservation.

Despite the problems caused by poor embryo freezability, in vitro production systems are superior to superovulation in terms of the maximum number of embryos that can be produced from a cow per year. In addition, sexed semen can be used very efficiently for in vitro fertilization since one straw can be used to inseminate dozens of oocytes. Production of in vitro produced embryos using oocytes collected at a slaughterhouse is much less expensive that production of embryos by superovulation or by in vitro fertilization of oocytes harvested using OPU.

The decision as to whether to use embryo transfer during the summer depends on the magnitude of the reduction in fertility caused by heat stress, the degree of improvement in fertility caused by embryo transfer and the cost of the embryo available for transfer. Ribeiro et al. (2012) estimated the cost to produce a female pregnancy in lactating cows as a function of the pregnancy rate per AI or embryo transfer. As an example, consider the case where pregnancy rate in the summer is 15% to AI using conventional semen and 25% using embryo transfer with sexed semen. In this scenario, embryo transfer would be profitable. It would cost $1157 to produce a female pregnancy using timed AI, $1042 to produce an embryo using an oocyte harvested by ultrasound, and $820 to produce an embryo using an oocyte recovered from a slaughterhouse ovary.

Embryo transfer will become more profitable during heat stress as improvements in the process increase the competence of the embryo to establish pregnancy and survive cryopreservation. In addition, cost advantages of using embryos derived from oocytes recovered from the slaughterhouse should not be overlooked. Embryos of high genetic merit can be produced by using elite bulls because one straw of semen can produce dozens of embryos. Improvements in cryopreservation will also make embryo biopsy for genotyping more practical.

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