HEAT STRESS RESEARCH ON EMBRYO SURVIVAL: CONCEPTS RELATED TO SUPEROVULATION, EMBRYO TRANSFER AND HEAT STRESS

by

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Introduction

A major constraint to reproductive efficiency in subtropical, tropical and arid environments is seasonal periods of heat stress. During the last four years, we have implemented the techniques of superovulation and embryo transfer as tools to study at what stage heat stress causes embryo death and whether embryo transfer of normal embryos can bypass the early detrimental effects of heat stress. Prior research in our laboratory has examined the effects of heat stress on the endocrinology of the estrous cycle, and development of management systems to reduce heat stress on the cow so that fertility can be maintained during the summer months. Heat stress under conditions of our Florida environment does not prevent animals from having normal estrous cycles. Temperature measurements of the uterus indicate that as uterine temperature approaches 40°C pregnancy does not occur and embryos are apparently loss. To further develop systems to improve fertility, we need to know specifically how elevated body temperature interferes with embryo development, what stages of embryo development are most sensitive to heat stress, and whether this new knowledge can be applied to improve fertility during the summer months. The purpose of this presentation is to update our dairy producers on our research with the early embryo that was supported by a USDA-CSRS Competitive Research Grant for a 4-year period (1984-1988). The grant contributed to the support of a graduate student, Dr. D. James Putney, and some of the research also was conducted on Florida dairy farms. The authors are deeply appreciative of the cooperation provided by the dairy industry to support our various on-farm research efforts.

Early Embryo Development

Reproductive efficiency is depressed when lactating dairy cows are maintained under environmental conditions of high ambient temperature and relative humidity, resulting in conception rates as low as 10 to 15 percent during stressful months of the year. Heifers do not have these clear summer time drops in fertility because they are not lactating and

can tolerate the summer temperature and humidity without a major increase in body temperature. For our experimental purposes, we can use heifers if we expose them to sufficiently high temperatures within an environmental chamber that elevates their body temperature to a level that you find during the summer months in a lactating dairy cow. Holstein heifers (n=16) were used to determine whether heat stress prior to ovulation increases the incidence of embryonic abnormalities in dairy cattle [2]. Heifers were superovulated with (FSH-P; 32 mg total), beginning on Days 10 or 11 of the estrous cycle. Prostaglandin $F_2\alpha$ (Lutalyse; 60 mg total) was administered on Day 3 of FSH-P treatment. Heifers were maintained at either thermoneutrality (24°C) or under hyperthermic conditions (exposure to 42°C for 10 h) beginning at the onset of estrus. After the short 10-hour heat stress, the treated heifers were cooled off so that their body temperatures were normal and then inseminated artificially at 15 and 20 h after the onset of estrus. Heifers were continuously maintained under environmental conditions of thermoneutrality for 7 days as provided by environmental shade structures. On Day 7 post estrus, embryos were recovered nonsurgically and evaluated morphologically for stage of development and quality. The distribution of embryos classified as normal, retarded and/or abnormal, or as unfertilized ova differed ($P<0.001$) between heat stress and thermoneutral treatments (Table 1). Only 12.0% of 25 embryos recovered from stressed heifers were normal compared to 68.4% of 19 embryos from thermoneutral heifers. Stressed heifers had a higher ($P<0.001$) incidence of retarded and/or abnormal embryos with degenerate blastomeres. We were able to use a fluorescent stain (4'-6'-Diamidino-2-Phenylindol) that permits us to identify dead cells. Cells that fluoresce are dead cells and the viability of embryos can be classified as: healthy with negative or no staining; partial staining in which some of the cells in the embryo are dead and the embryo is probably in the process of undergoing degeneration and death; positive staining in which all cells of the embryo are dead and the embryo is considered dead. An appreciably higher level of embryo cell death occurred in the heat-stressed group (Table 2). Responses indicated that thermal stress during the preovulatory period increases the incidence of retarded and/or abnormal embryos in superovulated heifers. This is a very important observation in that it means the ovum within the follicle of the ovary on the day of estrus is extremely sensitive to heat stress. This is the time that the oocyte undergoes final maturation following the LH surge at the onset of estrus and is being prepared for subsequent fertilization following ovulation. **THE IMPORTANT POINT FOR THE DAIRY PRODUCER IS THAT THE DAY OF HEAT IS EXTREMELY SIGNIFICANT AND COWS NEED TO BE KEPT COOL. OTHERWISE, SUBSEQUENT EMBRYO DEVELOPMENT FOLLOWING FERTILIZATION WILL BE ALMOST COMPLETELY BLOCKED AND EMBRYOS WILL DIE.**

A second experiment with Holstein heifers (n=14) was conducted to determine whether thermal stress increases the incidence of embryonic abnormalities when heat stress was imposed from the time of ovulation (day after insemination) until day 7 of embryo development [1]. Heifers were acclimated to environmental chambers at 20°C for 9 days and superovulated with Follicle Stimulating Hormone (FSH-P; 40 mg total), beginning on Days 9 to 11 of the estrus cycle. Prostaglandin $F_2\alpha$ (Lutalyse: 60 mg total) was administered on Day 3 of FSH-P treatment. At estrus, heifers were inseminated artificially and then maintained either at thermal neutrality (20°C) or under hyperthermic conditions (16 h at 30°C and 8 h at 42°C per

Table 1. Distribution* of normal and retarded-abnormal embryos and unfertilized ova recovered from thermoneutral and heat-stressed heifers on Day 0.

Treatment		Embryo		
		Normal	Retarded abnormal	Unfertilized ova
Thermoneutral:	n	13	4	2
	%	68.4	21.1	10.5
Heat stress:	n	3	17	5
	%	12.0	68.0	20.0

* X^2=15.05; P<0.001 distribution of embryos different between treatments.

Table 2. Distribution of 4'-6'-diamidino-2-phenylindol (DAPI)[a] fluorescence reaction of embryos classified by light microscopy into developmental and quality groups and as effected by environmental temperature on Day 0.

Group	N	DAPI Reaction %**		
		Negative % (n)	Partial % (n)	Positive % (n)
Normal	16	100.0 (16)	---	---
Retarded-abnormal	21	---	47.6 (10)	52.4 (11)
Unfertilized ova	7	---	---	100.0 (7)
Good to excellent	10	100.0 (10)	---	---
Poor to fair	27	22.2 (6)	37.0 (10)	40.7 (11)
Thermoneutral	19	68.4 (13)	21.1 (4)	10.5 (2)
Heat stress	25	12.0 (3)	24.0 (6)	64.0 (16)

[a] The DAPI staining is characterized by a brilliant yellow-colored fluorescence of single nuclei (partial fluorescence) or of all nuclei (positive fluorescence) within an embryo.

** X^2 = 15.05; P<0.001 distribution of fluorescence reaction different among developmental groups.

** X^2 = 31.53; P<0.001 distribution of fluorescence reaction of embryos different between quality groups.

** X^2 = 17.04; P<0.01 distribution of fluorescence reaction of embryos different between treatments.

day) for an additional 7-day period beginning at 30 h after onset of estrus. Respiratory rates and rectal temperatures were monitored throughout the treatment period. On Day 7 post estrus, embryos were recovered nonsurgically and evaluated by light microscopy as to their morphological stage of development and quality. Heifers maintained under hyperthermic conditions had higher ($P<.01$) rectal temperatures and respiration rates compared to heifers at thermoneutrality. The distribution of embryos classified as normal, abnormal, retarded and unfertilized ova differed ($P<.001$) between heat stress and control treatments (Table 3). Stressed heifers had a higher incidence of abnormal and retarded embryos with degenerate nonviable blastomeres (vital fluorescent staining: 4'-6'-Diamidino-2-Phenylindol; Table 4). Responses indicated that thermal stress (from 30 h post onset of estrus) increased the incidence of abnormal or retarded embryos in superovulated heifers.

Table 3. Distribution[a] of normal, abnormal or retarded embryos and unfertilized ova collected from thermoneutral and heat-stressed heifers (Days 1 to 7).

	Embryo category			
	Normal	Abnormal	Retarded	Unfertilized
Thermoneutral:				
n = 68	35	9	11	13
%	51.47	13.24	16.18	19.12
Heat stress:				
n = 82	17	22	28	15
%	20.73	26.83	34.15	18.29

[a] $X^2=18.09$; $P<0.001$ distribution of embryos different between treatments.

It is obvious that embryos during this period were sensitive to heat stress but the magnitude of the effect was the same as the single 10-hour stress on the day of estrus before ovulation. These results suggest that the embryo becomes somewhat more resistant to heat stress as it ages or becomes more developmentally mature. **NEVERLESS, THE 7-DAY PERIOD FOLLOWING FERTILIZATION IS A TIME WHEN CATTLE NEED TO BE PROTECTED FROM HEAT STRESS TO MAINTAIN FERTILITY.**

Since embryos that are less than 7 days of age are sensitive to controlled heat stresses imposed on the mother in vivo, we examined whether environmental temperatures were associated with both quality of embryos from donors and pregnancy rates of recipients in cattle managed under conditions associated with a **COMMERCIAL EMBRYO TRANSFER UNIT IN TEXAS** [3]. Transfer records on embryo donor (n=3,908; beef 99%, dairy 1%) and recipient (n=19,936; beef 92%, dairy 8%) cattle, collected for 4

Table 4. Distribution of 4'-6'-diamidino-2-phenylindol (DAPA)[a] fluorescence reaction of embryos classified by light microscopy into four developmental groups as influenced by environmental temperature (Days 1 to 7).

Group	No.	DAPI Reaction % (No.)		
		Negative	Partial	Positive
Normal[b]	19	68.4 (13)	31.6 (6)	--------
Abnormal	12	16.7 (2)	83.3 (10)	--------
Retarded	8	---------	100.0 (8)	--------
Unfertilized	12	---------	----------	100.0 (12)
Thermoneutral[c]	24	45.8 (11)	33.3 (8)	20.8 (5)
Heat stress	27	14.8 (4)	59.3 (16)	25.9 (7)

[a]DAPI stains dead nuclei of the embryo with a yellow fluorescence.

[b]χ^2 = 70.110; P<0.001 distribution of fluroescence reaction different among embryo groups.

[c]χ^2 = 6.11; P<0.005 distribution of fluorescence reaction different between treatments.

years, were analyzed for environmental effects. Embryos (n=42,428) were recovered on Days 5 to 8 post estrus from superovulated donors (FSH-P). Numbers of ova, fertilized embryos and embryos of transferable quality were recorded. Transferable embryos were classified as to stage of development and morphological quality. Embryos (n=19,936) were transferred nonsurgically. These responses were analyzed statistically to determine if environmental temperatures were associated with either quality of the embryos following superovulation of donor cattle and conception rates of recipient animals that received these embryos. Fluctuations in mean daily maximum temperature (1 to 43°C), for Days 0 to 7 of embryo development, had no effect on distribution of embryos classified as good (48%), fair (40%) and poor (12%). Temperature did not affect percentage of donors flushed with recoverable ova (89%), mean number of ova (12.2 $\pm$ 0.3), fertilization rate (76%) or percent transferable embryos (57%). Recipient pregnancy rate (56%) was unaffected by mean daily maximum temperature for days 0 to 10 post transfer. Interactions between temperature and breed type (dairy vs beef), parity (cow vs heifer), or lactational status (lactating vs dry) on pregnancy rate were not detected. Although heat stress of donors induces embryonic abnormalities (i.e., Experiments one and two described above) present data indicate that elevated environmental temperature does not adversely affect reproductive responses of donors in a **COMMERCIAL TRANSFER UNIT.** Furthermore, pregnancy rates of recipients were unaffected by temperature.

Of course, a commercial embryo transfer unit is in the business to generate pregnancies following embryo transfer and the management systems are likely to partially protect the donor and recipient cattle from the stresses of the environment. However, embryo transfer may provide an alternative to artificial insemination to circumvent heat stress-induced infertility in cattle. The potential for on-farm embryo collection, screening and transfer of only good quality embryos is a modern day reality. An evaluation of pregnancy rates to embryo transfer, as compared to artificial insemination during summer months, warrants investigation.

Several physiological strategies should be combined with environmental modification. In dairy systems, utilization of frozen semen from proven bulls is essential for improving genetic merit. Furthermore, heat stress effects on the bull are avoided by use of artificial insemination in which semen can be collected and frozen during cooler times of the year. Prostaglandin $F_2\alpha$ works effectively to regress the CL in heat stressed cattle [4], and effective synchronization system should be implemented with environmental management systems. Parts of the herd could be synchronized and bred to avoid the heat stress season. Alternatively, groups of cows could be synchronized during hot weather to intensify accuracy of heat detection, and these synchronized groups managed under shade management systems for a limited time (e.g., 20 to 40 days) to maximize conception rates. It is anticipated that, with continued technological advancements, frozen embryos will be utilized successfully (with a normal rate of fertility) for embryo transfer in cattle. Furthermore, on farm non-surgical embryo transfers may develop into a routine reproductive manipulation as artificial insemination is today. Hypothetically, these techniques may offer a means of bypassing the early pre- and post-ovulatory periods of thermal sensitivity. For example, excellent quality embryos that are frozen could be transferred into recipients at Day 7 when the embryo appears to be less sensitive to heat stress. Alternatively, embryos could be collected from heat stressed donors, screened for quality and development, and only good quality embryos transferred to recipients that are not as sensitive to heat stress (e.g., non-lactating recipients).

We recently examined whether embryo transfer could be used under routine conditions to bypass early heat stress effects on the embryo. Lactating Holstein dairy cows were managed on two commercial dairies **(NORTH FLORIDA HOLSTEINS, BELL, FLORIDA; RICHARDSON'S DAIRY, SANDERSON, FLORIDA)** during summer heat stress periods to determine if pregnancy rate to embryo transfer (n=113) was higher compared to contemporary control cows (n=524) that were artificially inseminated (AI) [5]. Holstein heifers (n=55) were superovulated with FSH-P (32 mg total), beginning on Day 10 of the estrous cycle, and administered prostaglandin $F_2\alpha$ (Lutalyse; 50 mg total) on Day 3 of FSH-P treatment. Heifers were inseminated artificially during estrus and subsequently managed under shade systems available at the farms to minimize summer heat stress. On Day 7 post-estrus, embryos were recovered, and good quality embryos transferred nonsurgically to estrus-synchronized lactating Holstein cows (n=113). Lactating control cows were managed under routine conditions of exposure to summer heat stress ambient temperatures and relative humidity. Pregnancy was determined by milk progesterone concentrations at Day 21 and per rectum at 45 to 60 days post-estrus (Table 5). Pregnancy rates of

cows presented for AI (Day 21, 18.0%; Days 45 to 60, 13.5%) were typical for lactating cows inseminated during periods of summer heat stress in Florida. Pregnancy rate of embryo recipient cows was higher ($P<0.001$) than that of cows presented for AI (Day 21, 47.6%; Days 45 to 60, 29.2%). Summer heat stress had no adverse effect on heifer superovulatory response but increased ($P<0.05$) the incidence of retarded embryos (less than 16 cells) and embryos graded as fair to poor quality. Increased pregnancy rate of recipient lactating cows indicates that the early bovine embryo (Days 1 to 7 post estrus) is extremely sensitive to maternal heat stress. Embryo transfer may bypass this period of extreme embryonic sensitivity and provide an alternative to AI to partially circumvent heat stress-induced infertility in cattle. It is also clear that even though pregnancy rates are higher for the embryo transfer group, there are still additional embryo losses between Day 21 and Day 40. This is an additional area of current research investigation, and the probability of increasing embryo survival during this latter period is higher. The embryo is more developmentally mature and controlling growth rates of the embryo is more likely since we can treat the cow to delay regression or maintain the corpus luteum which will allow embryos more time to grow and reduce embryo mortality.

Table 5. Pregnancy rates of embryo recipients and artificially inseminated lactating cows at North Florida Holsteins and Richardson's dairies.

Group	(N)	Pregnancy rate**	
		Day 21[a]	Day 40[b]
Embryo transfer	(113)	47.6	29.2
Artificial insemination	(524)	18.0	13.5

[a] By milk progesterone
[b] By rectal palpation
** $P < .01$.

The application of embryo transfer and superovulation has been essential to our understanding of heat stress effects in dairy cattle. It is dramatically clear from the present results that management of dairy cattle on the day of estrus (even prior to insemination and fertilization) is important to minimize heat stress on fertility. Furthermore, as the embryo develops from fertilization to Day 7, it also is sensitive to daily re-occurring heat stress. Awareness of this sensitivity will permit producers to better manage their cattle to improve fertility. Embryo transfer of Day 7 embryos will reduce losses to heat stress but not totally alleviate the problem. This suggests that conceptus development after Day 7 is also sensitive to heat stress but not as devastating as the earlier periods.

References

[1] Putney, D.J., Drost, M., and Thatcher, W.W. Embryonic development in superovulated dairy cattle exposed to elevated ambient temperatures between days 1 to 7 post insemination. Theriogenology 30: (1988) 195.

[2] Putney, D.J., Mullins, S., Thatcher, W.W., Drost, M., and Gross, T.S. Embryonic development in superovulated dairy cattle exposed to elevated ambient temperatures between the onset of estrus and insemination. Anim. Reprod. Science: (1988; In press).

[3] Putney, D.J., Thatcher, W.W., Drost, M., Wright, J.W., and DeLorenzo, M.A. Influence of environmental temperature on reproductive performance of bovine embryo donors and recipients in the southwest region of the United States. Theriogenology 30: (1988) 905.

[4] Thatcher, W.W., and Chenault, J.R. Reproductive physiological responses of cattle to exogenous prostaglandin $F_2\alpha$. J. Dairy Sci. 59 (1976) 1366.

[5] Putney, D.J., Drost, M., and Thatcher, W.W. Influence of summer heat stress on pregnancy rates of lactating dairy cattle following embryo transfer or artificial insemination. Theriogenology 31: (1989) 765.