Acta Veterinaria Hungarica 51 (2), pp. 209–218 (2003)

EFFECT OF THE EL NIÑO PHENOMENON ON THE OVARIAN RESPONSIVENESS AND EMBRYO PRODUCTION OF DONOR COWS

B. BÉNYEI[1*], A. GÁSPÁRDY[2] and S. CSEH[3]

[1]Research Institute, Faculty of Veterinary Science, Szent István University, H-2225 Üllő, Dóra major, Hungary; [2]Department of Animal Breeding, Nutrition and Laboratory Animal Science and [3]Department of Animal Reproduction and Obstetrics, Faculty of Veterinary Science, Szent István University, H-1078 Budapest, Hungary

(Received June 25, 2002; accepted December 10, 2002)

The effects of different Temperature Humidity Index (THI) values in cold, hot and El Niño (EN) climates on superovulation and embryo production were analysed on Holstein Friesian donor cows. There were significant differences in the THI among the three climates. The average temperature in the EN period was 6 °C higher than in the summer period of the previous 30 years. The number of corpora lutea (CL) and embryos were log- and back-transformed, Kolmogorov-Smirnoff test was used for normality and Lilliefors test was applied for significance. In the cold season THI was 70.74 ± 1.35 and the average number of CL was 9.84 ± 4.37. In the hot season the THI was 73.99 ± 0.72 and the average number of CL was 9.70 ± 4.49. When the THI, in the EN period, increased up to 79.74 ± 4.01, the superovulation response was significantly ($P < 0.01$) reduced (average number of CL = 5.22 ± 2.53). The embryo production result showed a similar tendency. In the hot period the average number of embryos obtained was 5.87 ± 2.98. However, in the EN period it decreased to 4.21 ± 2.05. Higher temperature reduced embryo quality. The proportion of live embryos (%) was 59.2 ± 37.4 in the cold and 38.2 ± 38.5 in the EN periods of the year ($P < 0.01$). However, ovarian sensitiveness showed adaptation to summer environment while the heat stress, which was more severe in the EN period, negatively affected the superovulation response and embryo production.

Key words: Heat stress, El Niño phenomenon, donor cows, superovulation, embryo production

Cattle, like other mammalian species, are homoiothermic animals that regulate internal temperature within a narrow range (~38.0 to 39.3 °C). An animal maintains homoiothermy by matching the amount of heat produced through metabolism with the heat flow from the animal to the surrounding environment. Heat flow occurs through a process dependent on the surrounding temperature

[*]Corresponding author: Dr. Balázs Bényei, H-1037 Budapest, Erdőalja út 6, Hungary; E-mail: benyeib@axelero.hu; Fax: +36 (1) 478-4124

(conduction, convection and radiation) and humidity (evaporation through sweating and panting). In an environment characterised by high air temperature, flow of heat from the cow can be reduced and, in severe environment, reversed. Intense solar radiation can also provide a source of heat to the animal and exacerbate the problem of homoiothermy. Hyperthermia occurs when heat flow from the animal is less than internal heat production.

Rectal temperature is higher in heat-stressed than in non-heat-stressed environment (Wolff and Monty, 1974; Francos and Mayer, 1983). Intrauterine temperature exceeds even the rectal temperature by 0.1 °C (Wolff and Monty, 1974) or 0.2 °C (Gwazdauskas et al., 1973). Thus, not only the follicular development (Wolfenson et al., 1995) but also the oocyte quality (Rocha et al., 1998) is affected.

The periovulatory period is very critical for embryonic development and survival. Embryonic development and survival was reduced in superovulated cows exposed to heat stress from the onset of oestrus to artificial insemination (AI) performed 15–20 hours later (Putney et al., 1988*b*).

The newly formed conceptus is also very sensitive to hyperthermia. The establishment of pregnancy is very susceptible to disruption by heat stress early in gestation. Hereford heifers exposed to 32.2 °C for 72 hours following AI did not become pregnant. However, 48% of heifers housed at 21.1 °C became pregnant (Dunlap and Vincent, 1971). Similarly, Putney et al. (1988*a*) observed that superovulated heifers kept under hyperthermic conditions for seven days beginning at ~30 hours after the onset of oestrus had a higher incidence of embryos having degenerated blastomeres as well as a higher incidence of embryos classified as abnormal or retarded than heifers housed and superovulated in a thermoneutral environment.

Four environmental factors influence effective temperature: (1) air temperature, (2) relative humidity, (3) air movement, and (4) solar radiation (Buffington et al., 1981). When the temperature exceeds 27 °C, even with low humidity the effective temperature is above the comfort zone for high-producing dairy cows. The Temperature Humidity Index (THI) is commonly used to indicate the degree of stress on dairy cattle (Bianca, 1962; Fuquay, 1981). When the THI exceeds 72, high-producing dairy cows are affected adversely, medium stress occurs between 73 and 78, strong stress is marked between 79 and 89 and severe stress is observed between 90 and 98. A THI index value higher than 98 is mortal for the cows (Armstrong, 1994).

The term ‘El Niño’ (EN) is the common term for the localised phenomenon known as ‘El Niño-Southern Oscillations’ or ENSO. EN was first recognized in the late 1800s by fishermen of the coast of Peru as the appearance of abnormally warm water in the Pacific Ocean, which shows up near the end of the year and lasts for several months (Caveman 42’s Page, 2000).

The purpose of this study was to confirm, by retrospective analysis of data collected in an embryo transfer station for 4 years, that (1) ovarian sensitiveness of heat-stressed cows can adapt to elevated temperatures and (2) the effect of EN phenomena altered the superovulation responsiveness and embryo production of donor cows in the semiarid area of Brazil.

Materials and methods

Experimental animals and environment

Superovulation (number of treatments = 368) and embryo production results of adult Holstein-Friesian (HF) cows (n = 100) at a donor centre in Petrolina, PE, Brazil (09º09' S latitude, 40º22' W longitude and 465.5 m altitude) were analysed for meteorological data in this study. The animals were monitored over 4 consecutive years. The cows were kept in pens (capacity 10 animals, 15 m^2/cow) that have high shades with sprinkling cooler system and were milked twice a day in milking parlours. They were fed according to the stage of lactation with freshly chopped whole corn plant and elephant grass, alfalfa hay and concentrate throughout the year. The floor was 5 cm asphalt, embedded with bagasse of sugar cane. Climatological data from Bebedouro, EMBRAPA (40 km from the farm) included daily maximum, minimum and average air temperatures (°C) and relative humidity. During the study, the environmental conditions in this part of semiarid Brazil were consistent on a day-to-day basis, with little fluctuation in either the temperature or the humidity.

The data analysed in this study were obtained in (1) the cold season (between February and August, number of treatments = 138), (2) the hot season (between September and January, number of treatments = 108), and (3) during EN (from December 1997 to June 1998, number of treatments = 122).

Preparation of cows and superovulation treatment

The cows involved in the study were examined by rectal palpation for completing involution, reproductive tract abnormalities and confirming signs of oestrus. Only cows without abnormalities were superovulated. Cows with observed oestrous cycle were synchronised with $PGF_{2\alpha}$, (Veteglan, Serono, Rome, Italy, 2 mL/animal) injected between day 5 and 15 of the natural cycle. For superovulation FSHp (Pluset, Serono, Rome, Italy, 600 IU total amount in progressively decreasing dosages over 4 days) was used, starting on days 8 to 11 of the synchronised cycle (day 0 was the oestrus). On day 3 of the superovulation treatment (day 1 when the superovulation treatment was started) 3 mL $PGF_{2\alpha}$ was administered i. m. (Veteglan, Serono, Rome, Italy, 3 mL dose) and 48 and 60 h later the donors were inseminated artificially (AI) twice (Table 1). Seven days after the first

AI, ovarian structures were evaluated by rectal palpation. The number of CL was counted. Non-surgical embryo collection was performed using DPBS (Dulbecco's phosphate buffer solution, Nutricell, Saõ Paulo, Brazil) supplemented with 25 mg/1000 mL kanamycin sulphate and 0.2% bovine serum albumin.

Day 7 embryos were morphologically evaluated and assigned an embryo quality grade based on a scale from 1 to 4 using criteria of the International Embryo Transfer Society (Stringfellow and Seidel, 1998).

Table 1

Superovulation hormonal treatment protocol applied

Day	Treatment	
0	Oestrus	
9–11	Beginning of FSH injections (Day 1)	
Day 1	a.m. 125 IU	p.m. 125 IU
Day 2	a.m. 100 IU	p.m. 100 IU
Day 3	a.m. 50 IU	p.m. 50 IU + PGF2α (3 mL)
Day 4	a.m. 25 IU	p.m. 25 IU
Day 5		p.m. AI
Day 6		p.m. AI
Day 12	a.m. Retrieval of embryos	

Statistical analyses

As the number of CL did not follow the normal distribution, it was transformed by using logarithmic transformation (LogCL) to fit to the Gaussian curve. Kolmogorov-Smirnoff test (K-S) was used for normality and Lilliefors test was applied for significance (for CL K-S $d = 0.14$, $P < 0.01$ and Lilliefors $P < 0.01$; for LogCL K-S $d = 0.05$, $P > 0.20$ and Lilliefors $P < 0.05$). Because the number of LogCL rose by the number of treatment, therefore it was corrected for the first treatment {C1LogCL = LogCL – [0.0659 × (number of treatment – 1)]; $b = 0.17$, $P < 0.01$}.

The corrected LogCL figures gave the basic data for the further calculation of the logarithmically transformed number of washed, live and degenerated embryos (LogEMB, LogLIVE and LogDEG, respectively) using the original frequencies.

The parameters examined in different environments were processed by one-way analysis of variance, in which the independent effect was the climatic environment.

THI was calculated from Dry Bulb Temperature (DB in °Fahrenheit) and Relative Humidity (RH, %) by the following formula (Kelly and Bond, 1971):

$$THI = DB - [0.55 - (0.55 \times RH)] \times (DB - 58).$$

The difference of the actual temperature from the 30-year mean ($DIFF_{30}$ in °F) was also evaluated.

Finally, back-transformation was used to evaluate the ovarian response by the number of CL (BackCL = $e^{C1LogCL}$) and the same process was applied for embryos washed and evaluated as living or degenerated (BackEMB, BackLIVE and BackDEG, respectively) in the three environmental groups. The SD in groups was determined using the following form: ± SD = $e^{C1Log\ (CL,\ EMB,\ LIVE\ or\ DEG) \pm SD}$.

Results

Meteorological measurements

Significant differences were found in the THI values between the cold, hot and EN climatic groups (70.74 ± 1.35; 73.99 ± 0.72; 79.74 ± 4.01, $P < 0.01$, respectively) underlining the rightfulness of setting up and application of our climatic groups.

In the daily deviation of temperature on treatment days from the 30-year mean ($DIFF_{30}$) there were no remarkable differences between the non-EN climatic groups (cold vs. hot). However, there were significant differences between the EN group and the non-EN groups (Table 2). The daily temperature was nearly 11 °F higher (this corresponds to a 6 °C temperature elevation) during the EN period that appeared from December 1997 to June 1998 than the 30-year mean of the same period of the year.

Table 2

Mean ± SD for THI and for $DIFF_{30}$ values through the experiment

Parameters	Climatic groups			F and P values	$LSD_{1\%}$ and $LSD_{5\%}$
	Cold	Hot	El Niño		
THI ± SD	70.74 ± 1.35	73.99 ± 0.72	79.74 ± 4.01	371.739; < 0.01	0.67; 0.88
$DIFF_{30}$ ± SD	–0.80 ± 1.48	0.14 ± 0.15	10.82 ± 5.50	408.708; < 0.01	0.89; 1.17

THI: Temperature Humidity Index; $DIFF_{30}$: temperature difference of the actual one from the 30-year mean (in °F)

Superovulation and embryo production results

There are significant differences in the logarithmically transformed number of CL (2.27 ± 0.62 vs. 1.65 ± 0.66), collected embryos (1.77 ± 0.71 vs. 1.42 ± 0.68) and live embryos (1.00 ± 0.95 vs. 0.59 ± 1.06) between the hot season of non-EN and the EN periods. However, in the cold season during non-EN years the corresponding figures of embryo production tend to be higher than in the hot season; the differences are not significant (Table 3).

Table 3

Means ± SD for log- and back-transformed numbers of CL and embryo production results in the non-EN and EN periods

Parameters (± SD)	Climatic groups			F and P values	$LSD_{1\%}$ and $LSD_{5\%}$
	Cold	Hot	El Niño		
C1LogCL	$2.29^{a} \pm 0.59$	$2.27^{a} \pm 0.62$	$1.65^{b} \pm 0.66$	25.524; <0.01	0.20; 0.26
BackCL	9.84 ± 4.37	9.70 ± 4.49	5.22 ± 2.53		
LogEMB	$1.83^{a} \pm 0.64$	$1.77^{a} \pm 0.71$	$1.42^{b} \pm 0.68$	22.464; <0.01	0.25; 0.33
BackEMB	6.25 ± 2.95	5.87 ± 2.98	4.21 ± 2.05		
LogLIVE	$1.28^{a} \pm 0.77$	$1.00^{a} \pm 0.95$	$0.59^{b} \pm 1.06$	11.663; <0.01	0.28; 0.37
BackLIVE	3.61 ± 1.94	2.73 ± 1.67	1.80 ± 1.18		
Proportion of live embryos (%)*	$59.2^{a} \pm 37.4$	$41.3^{b} \pm 37.0$	$38.2^{b} \pm 38.5$	8.199; < 0.01	7.8; 10.3
LogDEG	$0.96^{ab} \pm 0.81$	$1.14^{a} \pm 0.96$	$0.79^{b} \pm 0.84$	3.067; < 0.05	0.27; 0.35
BackDEG	2.62 ± 1.46	3.14 ± 1.94	2.21 ± 1.25		

*According to the original occurrences; [ab]Means within rows with differing superscripts differ significantly; C1LogCL: logarithmically transformed number of CL corrected for the first treatment; BackCL: back-transformed number of CL (from C1LogCL); LogEMB: logarithmically transformed number of washed embryos; BackEMB: back-transformed number of washed embryos (from LogEMB); LogLIVE: logarithmically transformed number of live embryos; BackLIVE: back-transformed number of live embryos (from LogLIVE); Proportion of live embryos (%): proportion of live embryos within all washed embryos; LogDEG: logarithmically transformed number of degenerated embryos; BackDEG: back-transformed number of degenerated embryos (from LogDEG)

The proportions of live embryos were significantly different (59.2 ± 37.4 vs. 38.2 ± 38.5, $P < 0.01$) between the cold non-EN and EN periods. No significant differences were found between the hot non-EN and EN climates. However, the tendency of embryo mortality is clearly visible.

There was a significant negative correlation between the number of CL and the THI values ($r = 0.21$, $P < 0.01$). Based on our findings, the following equation can estimate the number of CL from the THI values: Number of estimated CL = 36.535 – (0.3342 × THI actual).

Discussion

El Niño is the name used for warmer than normal sea surface temperatures in the Pacific Ocean of the West Coast of South America. It occurs when the easterly winds die down, in turn allowing for warmer waters normally kept in the

western Pacific to drift eastward towards the Americas. The periodical warming of South America is due to this event. The cause of this phenomenon has not been discovered yet but there are four theories trying to explain the occurrence: (1) underwater earthquake, (2) subsidence of prevailing easterly trade winds, (3) time travel and width of the Pacific Ocean and (4) global warming (Caveman 42's Page, 2000).

We investigated three climatic periods in our study. The analysis of THI values shows differences between the cold (70.74 ± 1.35; weak heat stress), hot (73.99 ± 0.72 which refer to an adverse stress) and EN periods (79.74 ± 4.01 which refers to a strong heat stress) proving the right group distribution (Armstrong, 1994). The EN occurred from December to June while in the non-EN years the temperature starts to be colder from February. During this meteorological phenomenon this did not happen, even the heat stress turned more intensive. During our study, the temperature of investigated cold and hot seasons corresponded to the 30-year mean with differences of –0.80 and 0.14 °Fahrenheit. However, in the EN period the temperature was 11 °Fahrenheit (6 °C) higher. This change of the ambient temperature was enough to reduce the ovarian sensitiveness of donor cows and the proportion of collected live/degenerated embryos.

Follicular development is closely correlated with the ovarian response in the superovulated cows. The effect of high ambient temperature on follicular development was investigated by Badinga et al. (1993). They found that as a result of high temperature, on day 8 the dominant follicles in the first wave were smaller and contained less fluid than in shaded animals. They concluded that elevated body temperature might have a detrimental effect on the number, differentiation and/or function on granulosa and theca cells, thereby weakening the negative feedback of the dominant follicle on the hypothalamic-ovarian axis. Ultrasonographic records during the first 7 days of the cycle showed that follicular dominance was altered, as indicated by the lack of decrease in the number of medium-sized follicles and the size of the second large, subordinate follicle. Wolfenson et al. (1995) reported another experiment at a similarly hot ambient temperature. Cows were monitored throughout the cycle and it was found that follicular dynamics were altered and follicular dominance was depressed by heat stress. This was supported by significant alterations in the number of large follicles and an associated decrease in the number of medium-sized follicles, the earlier emergence of the preovulatory follicle and a lack of decline in the number of medium-sized follicles during the follicular phase. These findings explain the alteration in the superovulation sensitiveness.

Gordon et al. (1987) analysed the effect of season on superovulatory response. They found significantly higher ovulation number and higher proportion of live embryos in the cold than in the hot season (12.2 and 73% vs. 7.1 and 46%) in Saudi Arabia. Putney et al. (1988*a*, 1988*b* and 1989) reported data of 3908 embryo collection cases and concluded that heat-stressed cows produced fewer ova and yielded a lower percentage of fertilised ova and an increased

number of retarded and/or abnormal embryos. Alfuraiji et al. (1993) and Boland et al. (1988) reported similar observations. In contrast, Critser et al. (1980) as well as Massey and Oden (1984) disagree with these observations, because the number of CL and live embryos throughout the year did not differ.

In our study, we did not find differences in superovulation responsiveness between the cold and hot period in non-EN years. The back-transformed number of CL shows nearly the same superovulation results (9.84 ± 4.37 vs. 9.70 ± 4.49). However, we found figures significantly different from these in the EN period (5.22 ± 2.53). This might be explained by the fact that in the tropical regions where HF cows are kept and continuously heat stressed, they may adapt to the elevated temperature. However, in the EN period the heat stress was much higher (THI was 79.4 referring to a long-lasting strong heat stress period) and follicular development was disturbed which was manifested in lower hormonal responsiveness. Analysing the reproductive performance of HF cattle in tropical regions, Ingraham et al. (1972 and 1975) came to a similar conclusion. They observed that conception rates improved slightly as the cows had an opportunity to adapt to higher THI values. Conforming to the superovulation results, the total number of embryos and live embryos obtained were significantly different between the hot non-EN and EN seasons (5.87 ± 2.98 vs. 4.21 ± 2.05).

These observations support the findings of two Californian studies where embryo quality of heat-stressed superovulated cows was analysed between the onset of oestrus and AI (Putney et al., 1989) and day 1 to 7 post insemination (Putney et al., 1988*a*). In both cases it was found that heat-stressed heifers had a higher presence of unfertilised ova and retarded/degenerated embryos. Gordon et al. (1987) and Sartori et al. (2000) also reported that the proportion of eggs/degenerated embryos was higher in hot than in winter periods.

Similarly, in our study high THI values affected the embryo quality. As the THI rose (from 70.74 ± 1.35 in the cold season to 73.99 ± 0.72 in summer), the number of degenerated embryos and the proportion of live/degenerated embryos significantly changed. We observed that this change was more expressed between the cold and hot season of the year and the EN periods.

The final conclusion is that HF cows can adapt to a higher temperature and the superovulation results and the total number of yielded embryos did not undergo significant changes. However, embryo production is reduced in an extreme hot period when the donor cows are strongly heat stressed. As it was previously demonstrated in earlier studies on IVF and *in vivo* embryo production, elevated ambient temperature affects the embryo development. Our results support these observations: in the summer season the proportion of live/degenerated embryos and the number of degenerated embryos changed. We found evidence that during the El Niño phenomena embryo production was more affected by the long-lasting hot environment than in the summer period in semiarid Brazil.

References

Alfuraiji, M. M., Alibrahim, R. M. and Elnouty, F. D. (1993): Seasonal variations in superovulatory responses of Holstein cows treated with pregnant mare serum gonadotrophin in Saudi Arabia. J. Reprod. Fertil. Abs. Ser. **11,** 75. (Abstr. 140).

Armstrong, D. V. (1994): Heat stress interaction with shade and cooling. J. Dairy Sci. **77,** 2044–2050.

Badinga, L., Thatcher, W.W., Diaz, T., Drost, M. and Wolfenson, D. (1993): Effect of environmental heat stress on follicular development and steroidogenesis in lactating Holstein cows. Theriogenology **39,** 797–810.

Bianca, W. (1962): Relative importance of dry and wetbulb temperatures in causing heat stress in cattle. Nature (Lond.) **195,** 251.

Boland, M. P., Gordon, I., McGovern, H. and Lynn, G. (1988): Superovulatory responses of cows and heifers in summer in Saudi Arabia. Theriogenology **29,** 227. 9 (Abstr.).

Buffington, D. E., Colazo-Arocho, A., Canton, G. H., Pitt, D., Thatcher, W. W. and Wilcox, R. J. (1981): Black-globe-humidity index (BGHI as comfort equation for dairy cows). Trans. Am. Soc. Agric. Eng. **26,** 1798.

CAVEMAN 42'S PAGE (2000): http://www.geocities.com/SouthBeach/Surf/9379/scipro.html.

Critser, J. K., Rowe, R. F., Del Campo, M. R. and Ginther, O. J. (1980): Embryo transfer in cattle: factors affecting superovulatory response, number of transferable embryos, and length of post-treatment estrous cycles. Theriogenology **13,** 397–406.

Dunlap, S. E. and Vincent, C. K. (1971): Influence of postbreeding thermal stress on conception rate in dairy cattle. J. Anim. Sci. **32,** 1216–1218.

Francos, G. and Mayer, E. (1983): Observations on some environmental factors connected with fertility in heat-stressed cows. Theriogenology **19,** 625–634.

Fuquay, J. W. (1981): Heat stress as it affects animal production. J. Anim. Sci. **52,** 164.

Gordon, I., Boland, M. P., McGovern, H. and Lynn, G. (1987): Effect of season on superovulatory responses and embryo quality in Holstein cattle in Saudi Arabia. Theriogenology **27,** 231. (Abstr.)

Gwazdauskas, F. C., Thatcher, W. W. and Wilcox, C. J. (1973): Physiological, environmental, and hormonal factors at insemination which may affect conception. J. Dairy Sci. **56,** 873–877.

Ingraham, R. H., Gillette, D. D. and Wagner, W. D. (1972): Relationship of temperature and humidity to conception rate of Holstein cows in subtropical climate. J. Dairy Sci. **57,** 476–481.

Ingraham, R. H., Stanley, R. W. and Wagner, W. C. (1975): Relationship of temperature and humidity to conception rate of Holstein cows in Hawaii. J. Dairy Sci. **59,** 2086–2090.

Kelly, C. F. and Bond, T. E. (1971): Bioclimatic factors and their measurement. In: A guide to environmental research on animals. National Academy of Sciences, Washington, D. C. p. 7.

Massey, J. M. and Oden, A. J. (1984): No seasonal effect on embryo donor performance in the southwest region of the USA. Theriogenology **21,** 196–217.

Putney, D. S., Drost, M. and Thatcher, W. W. (1988*a*): Embryonic development in superovulated dairy cattle exposed to elevated ambient temperatures between days 1 to 7 post insemination. Theriogenology **30,** 195–209.

Putney, D. S., Mullins, S., Thatcher, W. W., Drost, M. and Gross, T. S (1989): Embryonic development in superovulated dairy cattle exposed to elevated ambient temperatures between the onset of estrus and insemination. Anim. Reprod. Sci. **19,** 37–51.

Putney, D. J., Thatcher, W. W., Drost, M., Wright, J. M. and DeLorenzo, M. A. (1988*b*): Influence of environmental temperature on reproductive performance of embryo donors and recipients in the southwest region of United States. Theriogenology **30,** 905–922.

Rocha, A., Randel, R. D., Broussard, J. R., Lim, J. R., Blair, R. M., Roussel, J. D., Godke, R. A. and Hansen, W. (1998): High environmental temperature and humidity decrease oocyte quality in *Bos taurus* but in *Bos indicus* cows. Theriogenology **49,** 657–665.

Sartori, R., Sartor-Bergfelt, R., Mertens, S. A., Guenther, J. N., Parrish, J. J. and Wiltbank, M. C. (2000): Early embryonic development during summer in lactating dairy cows and nulliparous heifers. Biol. Reprod. **62,** (Suppl. 1) 155. (Abstr.)

Stringfellow, D. A. and Seidel, S. M. eds. (1998): Manual of the International Embryo Transfer Society. 3rd ed., IETS, Savoy, IL. pp. 167–170.

Wolfenson, D., Thatcher, W. W., Badinga, L., Savio, J. D., Meidan, R., Lew, B. J., Braw-Tal, R. and Berman, A. (1995): Effect of heat stress on follicular development during the estrous cycle in lactating dairy cattle. Biol. Reprod. **52,** 1106–1113.

Wolff, L. K. and Monty, D. E. (1974): Physiologic response to intensive summer heat and its effect on the estrous cycle of nonlactating and lactating Holstein-Friesian cows in Arizona. Am. J. Vet. Res. **35,** 187–192.