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UTERO-OVARIAN-CONCEPTUS RESPONSE TO HEAT STRESS IN THE DAIRY COW AND ITS INVOLVEMENT IN LOW SUMMER FERTILITY

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Title

"UTERO-OVARIAN-CONCEPTUS RESPONSE TO HEAT STRESS IN THE DAIRY COW
AND ITS INVOLVEMENT IN LOW SUMMER FERTILITY"

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ABSTRACT

In a series of experiments in dairy cattle, we examined the multifactorial problem of heat stress on the functions of: ovarian follicles -in particular the dominant, preovulatory follicle-, the corpus luteum, pituitary gonadotrophins, and on uterine prostaglandin F_{20} secretion. Attempts to improve summer fertility in cattle by hormonal therapy also are reported.

Initially, basic information was gained in the absence of stress, with attention focused on control of follicular development and function: LH appeared to maintain dominance of the large follicle; plasma progesterone concentration, via its effect on tonic LH secretion, caused turnover or persistence of that follicle; hormonal secretion by the dominant follicle (estradiol, progesterone, androstenedione, oxytocin, PGF_{20} , IGF-1) characterized its functionality in relation to age and stage of development. Emergence of fresh, healthy preovulatory follicles was induced using a GnRH agonist.

The dominant follicle was highly susceptible to heat stress. Using ultrasonography, we found that follicular dominance was suppressed, and that the preovulatory follicle emerged earlier, resulting in a more aged follicle at ovulation. Theca cells of the dominant follicle secreted much less androstenedione; granulosa cell viability, aromatase activity and inhibin secretion were suppressed drastically. Late summer, and even autumn, were more stressful in terms of estradiol production than early summer, suggesting chronic delayed impairment of follicular function.

Corpora lutea (CL) in summer secreted less progesterone and more PGF_{20} than during the winter. This suggested a temporal impairment in CL function that can be related to the above-described damage to the ovulatory follicle as a possible carry over effect. Low progesterone secretion may affect normal embryonic development in utero. Pulsatile LH, as well as GnRH-induced surges of LH and FSH, were only significantly suppressed during heat stress in cows with low plasma estradiol concentrations. Low plasma concentration of gonadotrophins may alter several ovarian functions. At the uterine level, heat-stressed pregnant heifers secreted more PGF_{20} than cooled heifers. This could be detrimental to the life span of the corpus luteum of pregnancy.

Injection of a GnRH agonist (buserelin) on days 8 or 12 post-insemination in summer-stressed cows did not affect their fertility. Intravaginal supplementation of progesterone from day 8 post-insemination for 12 days yielded equivocal results. Treatment might be beneficial but more needs to be done to substantiate this effect.

The detailed and important information gained in this project could serve to minimize the closed loop of events leading to low summer fertility. Efforts which combine an efficient cooling system and hormonally programmed reproductive management may increase summer conception rates in dairy cattle.

OBJECTIVES OF THE ORIGINAL RESEARCH PROPOSAL

The proposed study aims to shed light on hypophyseal, follicular and conceptus-uterine unit responses under heat stress conditions, and to test potential means to improve low summer fertility. In order to achieve this goal, the following specific aims were undertaken:

1. To investigate heat stress induced effects on follicular growth patterns.
2. To study the effects of exposure of the animal to high body temperature on endocrine function of pre- and/or postovulatory follicles and of the subsequently formed corpus luteum.
3. To examine the pituitary response to heat stress during the follicular phase of the estrous cycle.
4. To study the induction of uterine $\text{PGF}_{2\alpha}$ secretion induced by exogenous oxytocin under conditions of heat stress and possible modification of the response as a result of pregnancy.
5. To examine, under field conditions, feasibility of increasing summer fertility by the use of one or more of the following: 1. exogenous hormones (progesterone or GnRH) at different stages pre- or post AI in order to alter embryo development and or follicular function; 2. embryo transfer technique with frozen embryos collected during cool seasons.

SCIENTIFIC RESULTS

INTRODUCTION

The low summer fertility problem is multifactorial in nature, since hyperthermia of different body organs and tissues results in different functional alterations and impairments. For example, elevated temperature of the ovary alters follicular and luteal endocrine function; the same hyperthermia at the level of the uterus causes direct damage to the developing embryo. Moreover, the level to which tissue temperature is elevated during the thermal stress modulates the responses. Thus, complexity of the problem necessitates an investigation into the effect of heat stress at various levels and on various functions of the reproductive system, in order to identify and locate those parts which are more susceptible to damage by thermal stress. This approach has been taken in the present research, and attempts also were made to determine the time period of each tissue's susceptibility to heat.

Experiments were performed in which functionality of the dominant ovulatory follicle, the corpus luteum (CL), the hypophysis and the utero-embryo unit were studied under conditions of heat stress. Experiments in absence of stress, were performed as needed to establish a basic understanding of the specific organ/tissue being studied, prior to examining its responses under heat stress. This was especially necessary in experiments involving follicular development and function.

All five original objectives of the project were met. Objectives

1 - 4 aimed at investigating impairment of functions by thermal stress; objective 5 was applied in nature and was aimed at using different hormonal therapies or treatments to improve summer fertility.

In this project, major emphasis was placed on elucidating the involvement of the follicle in low summer fertility. Indirect evidence suggested the preovulatory follicle as an organ most sensitive to thermal stress. However, basic information was lacking regarding growth pattern of follicular waves, and hormonal secretion of the dominant, ovulatory or non-ovulatory follicles and subordinate follicles in the ovaries of nonheat-stressed cows, at different stages of the estrous cycle. Sections 2 of the Scientific Results therefore include detailed studies, in which information on follicular development and function under thermoneutral conditions is presented. Section 3 of the Scientific Results includes studies on the effect of heat stress on follicular development and function.

Section 4 is related to the effect of heat stress on luteal endocrine function. Response of pituitary gonadotrophins -LH and FSH in plasma- in cows exposed to heat stress are presented in Section 5. Section 6 describes the effect of heat stress on uterine $\text{PGF}_{2\alpha}$ secretion in vivo.

In Section 7 of the Scientific Results, the effect of progesterone supplementation after artificial insemination (AI) on

conception rates in normothermic and heat-stressed cows is presented. Studies using a single injection of GnRH analogue (buserelin) at different stages (on day 8 or day 12 post-AI), did not affect summer fertility. Results of these preliminary studies are reported briefly. Part two of original objective 5, which was aimed at transferring frozen embryos collected in winter into cows during the summer, is now being performed in Florida (summer 1993) by M. Drost and W.W. Thatcher. The delay is due to the lower than requested funding allotted to the original project proposal. The relatively high cost of this particular study (\$ 19,000) is being provided by the Florida Dairy Farmers Milk Check-Off program in a grant awarded February, 1993.

In conclusion, efforts to improve summer fertility should combine maximal alleviation of heat stress and efficient hormonal treatments. In the last decade, an efficient cooling system based on cycles of brief periods of water sprinkling and forced ventilation was developed by the two groups of scientists from The Hebrew university in Rehovot, Israel and the University of Florida in Gainesville, U.S.A. This system is now used routinely in both countries. The use of such a cooling system is obligatory if any hormonal treatment is to be used, because hormonal therapy can only increase fertility if strain in the animal is lowered (by cooling) to the point where direct death of the embryo is minimal.

The original objective 5 of the proposal, described in Section 7 of the Scientific Results in this final report, has become the

starting point of a continuing BARD project proposal which is soon to be submitted. In fact, section 7 describes, to the best of our knowledge, the first attempts to improve summer fertility of dairy cattle by hormonal means. We intend, based on the detailed knowledge obtained in the present project, to design and perform additional experiments in which various treatments will be used to improve summer fertility.

The following sections of the Scientific Results are organized and presented, in most cases, in the form of either original papers either published in the literature, or in press. The small number of experiments which have not yet been published, are presented in a scientific paper format.

ALTERATIONS IN FOLLICULAR DEVELOPMENT AND DOMINANCE
DURING THE ESTROUS CYCLE IN HEAT-STRESSED COWS

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SUMMARY

Follicular development was examined by ultrasonography during two consecutive follicular waves (W) in one estrous cycle of heat-stressed (H) or cooled (C) dairy cows. In W1, size of 2nd largest follicle (F) decreased two days (d) later, and number of large Fs (>10 mm) was 30% greater in H cows than in C cows. During W2, H cows had 25% fewer small Fs (3-5 mm) and the dominant (preovulatory) F emerged earlier (d6 < d9), and its size was smaller at estrus (12 < 14 mm), as compared to C cows. Heat stress altered follicular growth and dominance.

INTRODUCTION

Impairment of normal follicular development and function by environmental heat stress may be involved in low summer

fertility. Conception rate in dairy cows has been found to be inversely related to ambient temperature during the follicular phase (1). Heat stress may impair the ovulatory oocyte (2,3), as well as follicular granulosa and theca cell function. Effects of heat stress on follicular development has hitherto not been documented, nor has its possible effect on characteristics of follicular turnover been examined. In each follicular waves, following a selection phase, a dominant F emerges which suppresses growth of subordinate Fs (4). This study examined the effects of thermal stress on follicular growth and pattern of follicular wave dominance in cows.

MATERIALS AND METHODS

Two experiments were conducted during the summer in Florida (Exp. 1) and Israel (Exp. 2), on lactating Holstein cows in which estrous cycles were synchronized. In Exp. 1 (n=12), an intravaginal device that releases progesterone (CIDR, 1.9 g progesterone) was inserted and PGF2 α (25 mg) injected on d12 of the cycle (d0 = estrus), and the CIDR was withdrawn on d17. In Exp. 2 (n=12), PGF2 α was injected on d14. Follicular development was monitored by ultrasonography from d1 until estrus manifestation on d20 (Exp. 1) or d17 (Exp. 2). Diagrams of follicles positions in the ovaries and sizes were drawn at each scanning. Follicles were classified into small (3-5 mm), medium (6-9

mm) and large (>10 mm) size categories. All cows ovulated following estrus. Half of the cows in each experiment were heat-stressed for about 8 hours by daily exposure to direct solar radiation from d1 to subsequent estrus. The other half were managed under shade structures and cooled by a sprinkling and ventilation cooling system. Body temperature was monitored to ensure maintenance of a desired level of hyperthermia in H cows and normothermia in C cows.

RESULTS

Mean maximal body temperatures ($\pm$ S.E.) of H cows in Exps. 1 and 2 were similar ($40.3 \pm .2^{\circ}\text{C}$); values for C cows were $38.9 \pm .1^{\circ}\text{C}$ and $38.7 \pm .1^{\circ}\text{C}$ in Exps. 1 and 2, respectively. These are typical body temperatures for a state of hyperthermia commonly experienced in summer by cows, and normothermia responses obtained with application of an efficient heat stress relief regimen. Growth rate of dominant and subordinate follicles in 1st and 2nd follicular waves of all (C and H) cows, is presented in Figure 1. Heat stress did not alter the size of W1 dominant F in either experiment. Mean sizes ($\pm$ S.E.) on d10, for C and H cows were: Exp. 1: 13.6 ± 1.0 and $14.3 \pm .9$ mm; Exp. 2: $15.6 \pm .3$ and $14.3 \pm .8$ mm. The number of large Fs during W1 was about 30% greater in H cows than in C cows (Exp. 1: $1.6 \pm .2$ vs. $1.3 \pm .3$, $P < 0.06$; Exp. 2: $1.3 \pm .3$ vs. $1.0 \pm .4$, $P > 0.10$, respectively; Figure 2). On average, the 2nd largest

F in W1 decreased in size 2 days later in H cows than in C cows (Exp. 1.: $d_9 > d_7$, $P > 0.10$; Exp. 2: $d_{11} > d_9$, $P < 0.09$). In both Exps., emergence of the W2 dominant F (preovulatory) occurred earlier in H cows compared to C cows (Exp. 1: $d_5 < d_9$, $P < 0.01$, Figure 1; Exp. 2: $d_7 < d_9$, $P < 0.06$; Figure 3). The preovulatory F was smaller in H cows on estrus day in Exp. 2 ($12 < 14$, $P < 0.08$), but no difference was detected in Exp. 1. This discrepancy between Exps. may be related to the longer period of programmed growth (+ 3 days) of the preovulatory F in Exp. 1, as compared with Exp. 2. During W2, H cows had 25% fewer small Fs than C cows in both Exps ($9.3 < 12.3$ in C cows, $P < 0.10$; Figure 4). Size of the corpus luteum was unaffected by heat stress in Exps. 1 and 2.

DISCUSSION

Despite the fact that the two experiments were conducted in different countries, under widely different management systems, most results are strikingly similar. They suggest that follicular dominance is altered by heat stress, as evidenced in H cows by: (a) a delayed decline in size of 2nd largest F in W1, (b) a greater number of large Fs in W1, and (c) an earlier emergence of the preovulatory W2 F. The latter finding is of particular physiological importance. Estrus cycle length and timing of subsequent ovulation were not affected by heat stress. Therefore, the

earlier growth of the dominant (preovulatory) W2 F, probably caused by depressed dominance of dominant W1 F, may result in a F subjected to a preovulatory surge of gonadotropin, oocyte maturation, luteinization and ovulation while in a more aged or persistent state. Viability of bovine Fs usually lasts only for a few days before onset of atresia (5). Thus, ovulation of aged or persistent follicles may contribute to the low summer fertility syndrome.

REFERENCES

1. Ingraham, R.H., Stanley, R.W. and Wagner, W.C. 1976. J. Dairy Sci., 59: 2086-2090.
2. Baumgartner, A.P. and Chrisman, C.L. 1981. J. Reprod. Fert., 61: 91-96.
3. Putney, D.J., Mullins, S., Thatcher, W.W., Drost, M. and Gross, T.S. 1989. Anim. Reprod. Sci., 19: 37-51.
4. Ireland, J.J. 1987. J. Reprod. Fert. 34 (Suppl.): 39-54.
5. Ireland, J.J. and Roche, J.F. 1987. In: Follicular Growth and Ovulation Rate in Farm Animals. J.F. Roche and D. Callaghan (Eds), Martinus, Dordrecht pp. 1-18.

Figure 1. Growth rate of the dominant and subordinate follicles in 1st and 2nd follicular waves of all (cooled and heat-stressed) cows.

Figure 2. Number of large follicles (≥ 10 mm) during first follicular wave, in cooled and heat-stressed cows.

Figure 3. Growth patterns (mm) of the first and second wave dominant follicles in heat-stressed cows compared to cooled cows during the estrous cycle.

Figure 4. The effect of heat stress on the number of small (3-5 mm) follicles.

Figure 1

Figure 2

Figure 3

Figure 4

THE EFFECTS OF HEAT STRESS AND SEASON ON THE ENDOCRINE FUNCTION OF THE OVARIAN DOMINANT FOLLICLE IN DAIRY COWS

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INTRODUCTION

The low summer fertility of dairy cattle in subtropical regions is a well-documented problem. The aim of this work was to examine the influence of summer heat stress and acute heat stress on hormonal production by follicular cells of the dominant follicle, and its possible involvement in the low fertility phenomenon.

MATERIALS AND METHODS

EXPERIMENT 1

Holstein-Israeli dairy cows were synchronized, and slaughtered on day 6.8 ± 0.5 of the estrous cycle. Granulosa and theca cells of

the dominant follicle were separated by an enzymatic process, and incubated at normal (37.5°C) or high temperature (40.5°C) for 18 h with testosterone (300 ng/ml; granulosa cells) and forskolin (10 μ M; theca cells). The levels of estradiol-17 β (E2), androstenedione and progesterone (P4) in the follicular fluid and medium were determined by RIA, as was that of inhibin in the follicular fluid, in four experimental groups: the summer group, to examine the effect of prolonged heat stress; the temperature-chamber group, involving a short exposure to heat during the final growth period of the dominant follicle (days 3-6) during the winter; the autumn group, to examine the delayed influence of summer heat stress on the follicle of cows in a nonstressful climate; and the winter group (control). Climatic conditions in the three seasons are presented in Table 1.

EXPERIMENT 2

This experiment was conducted in summer, on lactating Holstein cows in which estrous cycles had been synchronized. Half of the cows were heat-stressed for about 8 h by daily exposure to direct solar radiation from days 1 to 11 of the estrous cycle. The other half were managed under shade structures and cooled by a sprinkling and ventilation cooling system. The concentrations of E2, P4 and inhibin in peripheral blood were determined by RIA's.

RESULTS AND DISCUSSION

Granulosa cell viability in the summer and autumn groups was at

least 40% lower as compared to the winter group (Table 2). Brief exposure to heat (temp. chamber group) or the incubation of cells at 40.5°C did not decrease granulosa cell viability. Neither season nor acute heat stress influenced the percentage of live theca cells (over 95%). High incubation temperature did not affect viability of either granulosa or theca cells (Table 4).

The concentration of E2 in the follicular fluid of autumn cows was lower than that in winter cows (Table 3). A decrease in E2 concentration was also found in the summer and temperature chamber groups (NS). The concentration of androstenedione in the follicular fluid was higher in the summer group than in the autumn or winter groups. The concentration of inhibin, produced by granulosa cells, in the follicular fluid of the winter follicles was higher than in the summer and temperature-chamber follicles.

In the presence of testosterone, granulosa cells from the autumn group produced more than double the amount of E2 produced by summer and temperature-chamber groups (Table 7). In contrast, the theca cells of the autumn group produced minute amounts of androstenedione, whereas those of the winter group produced the largest amount (Table 5). Incubation at 40.5°C lowered E2 production by granulosa cells but did not affect androstenedione production by theca cells (Tables 6,8).

Low androstenedione levels in the follicular fluid in autumn

represent the low capacity of the theca cells to produce this hormone. Low E2 concentrations are due to shortage of substrate for aromatase activity, and/or to the low viability of the granulosa cells. In the summer group, a high concentration of androstenedione in the follicular fluid resulted from low aromatase activity in the granulosa cells. A low level of androstenedione in the follicular fluid in the winter group indicates its efficient use by the granulosa cells for E2 production.

Increased P4 production is a clear sign of follicular atresia. Consequently a high level of P4 production was evident in situations in which the ability of theca and granulosa cells to produce androstenedione and E2 was impaired (Tables 9-10).

In experiment 2, mean maximal air temperature was 30.9°C and relative humidity at peak temperature was 52%. Body temperatures of cooled cows were below 39.0°C all day, whereas in heat-stressed cows it reached 40.0°C at noon and 40.5°C at the afternoon hours. Plasma E2 levels were lower in heat-stressed cows than in the control group, from days 5-8 of the estrous cycle, suggesting earlier atresia of the follicle (Figure 1). Accordingly, there was a decrease in inhibin concentration in the peripheral blood of hyperthermic cows (Figure 2). This decrease may affect FSH secretion and change the dynamics of follicular development during heat stress. Concentration of P4 in plasma of cooled and heat-stressed cows is presented in Figure 3.

In conclusion, exposure of dairy cows to prolonged heat stress caused decreased viability of granulosa cells in the dominant follicle. Prolonged heat stress markedly decreased theca cell production of androstenedione, and aromatase activity and inhibin production in granulosa cells. Exposure to a short period of heat stress during the final period of follicular growth caused similar, but less pronounced decreases. The results suggest that impairment of function of the dominant-ovulatory follicle during heat stress may be involved in the low summer fertility syndrome.

Table 1. Average climatic conditions during Summer (August-September), Autumn (October-November) and Winter (December-February).

	Air Temperature		Relative Humidity		THI	
	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
*August	39.0 ± 8.0	25.4 ± 6.0	48.0 ± 2.0	15.0 ± 2.0	81.5	72.1
*September	36.4 ± 7.0	23.7 ± 6.0	54.0 ± 2.0	19.0 ± 2.0	79.9	70.5
+October	28.8 ± 9.0	14.8 ± 12.0	91.0 ± 2.0	47.0 ± 2.0	76.3	58.6
+November	24.2 ± 13.0	11.2 ± 12.0	90.0 ± 4.0	46.0 ± 4.0	70.3	52.5
+December	19.4 ± 10.0	8.2 ± 12.0	91.0 ± 2.0	51.0 ± 3.0	64.5	47.3
+January	17.9 ± 13.0	6.6 ± 8.0	91.0 ± 4.0	51.0 ± 5.0	62.5	44.6
+February	19.2 ± 14.0	6.8 ± 7.0	92.0 ± 3.0	49.0 ± 4.0	64.1	44.8

THI- Temperature-Humidity Index.

Values are given as mean ± SEM.

* Data from the Southern Arava.

+ Data from Bet Dagan.

Table 2. Characteristics of the dominant follicle on day 7 of the estrous cycle during different seasons.

	Summer	Autumn	Winter	Winter-HS
No. of cows	5	5	9	5
Follicle diameter (mm)	13.8 ± 1.2	12.0 ± 1.2	13.3 ± 0.9	12.4 ± 1.2
Fluid volume (cc)	1.2 ± 0.2 ^{ab}	1.3 ± 0.2 ^{ab}	1.5 ± 0.2 ^a	1.0 ± 0.2 ^b
Granulosa cell (no. * 10 ⁶)	7.6 ± 1.2	6.6 ± 1.1	7.0 ± 0.8	6.4 ± 1.1
Cell viability (%)	23.2 ± 7.7 ^b	21.0 ± 6.9 ^b	38.3 ± 4.9 ^a	32.3 ± 6.9 ^{ab}

HS - Heat stress.

Values are given as mean ± SEM.

Different letters within a row denote statistically significant differences (P < 0.05).

Table 3. Steroid and inhibin concentrations in the follicular fluid of dominant follicles on day 7 of the estrous cycle during different seasons.

	Summer	Autumn	Winter	Winter-HS
Estradiol 17β (ng/ml)	72.4 ± 18.7 ^{a*}	41.4 ± 18.7 ^b	110.8 ± 15.8 ^a	78.2 ± 19.1 ^{ab}
Androstenedione (ng/ml)	2.2 ± 0.7 ^a	0.5 ± 0.6 ^b	0.8 ± 0.6 ^{b†}	1.5 ± 0.6 ^{ab}
Inhibin (μg/ml)	17.1 ± 3.9 ^b	25.0 ± 3.9 ^{ab}	28.0 ± 3.1 ^a	16.3 ± 3.9 ^b
Progesterone (ng/ml)	13.1 ± 1.9	10.4 ± 1.7	10.9 ± 1.1	8.9 ± 1.4

HS- Heat stress

Values are given as mean ± SEM.

Different letters within a row denote statistically significant differences (P < 0.05 ; * P < 0.06 ;

† P < 0.08).

Table 4. Number of granulosa and theca cells (per well) incubated at different temperatures during 18 hours.

	37.5°C	40.5°C
Granulosa cell	37450 ± 2146	38954 ± 2146
Theca cell	38974 ± 1671	39280 ± 1671

Values are given as mean ± SEM.

Table 5. Basal and forskolin-stimulated androstenedione production by theca cells (ng/10⁵ cells)¹.

	Summer	Autumn	Winter	Winter-HS
Basal	0.23 ± 0.12 ^b	0.05 ± 0.03 ^b	1.17 ± 0.14 ^a	0.21 ± 0.06 ^b
Stimulated	1.05 ± 0.72 ^b	0.58 ± 0.72 ^b	4.07 ± 0.54 ^a	1.86 ± 0.72 ^{b*}

¹After 18 hours of incubation.

HS- Heat-stress.

Values are given as mean ± SEM.

Different letters within a row denote statistically significant differences (P < 0.01;

* P < 0.03)

Table 6. Basal and forskolin-stimulated androstenedione production by theca cells incubated at different temperatures (ng / 10⁵ cells)¹.

	37.5°C	40.5°C
Basal	0.47 ± 0.58	0.65 ± 0.14
Stimulated	2.02 ± 0.14	1.76 ± 0.14

¹After 18 hours of incubation.

HS- Heat-stress.

Values are given as mean ± SEM.

Table 7. Estradiol production by granulosa cells during different seasons (ng/10⁵ cell

	Summer	Autumn	Winter	Winter-HS
Estradiol 17 β	6.6 $\pm$ 2.9 ^b	18.3 $\pm$ 2.9 ^a	12.6 $\pm$ 2.2 ^{ab}	8.4 $\pm$ 2.9 ^b

¹ After 18 hours incubation.

HS- Heat-stress.

Values are given as mean $\pm$ SEM.

Different letters denote statistically significant differences (P < 0.01).

Table 8. Estradiol production by granulosa cells incubated at different temperatures (ng / 10⁵ cells)¹.

	37.5°C	40.5°C
Estradiol 17 β	13.3 $\pm$ 0.6 ^a	10.1 $\pm$ 0.6 ^b

¹ After 18 hours of incubation.

Values are given as mean $\pm$ SEM.

Different letters denote statistically significant differences (P < 0.01).

Table 9 . Basal progesterone production by granulosa cells during different seasons (ng / 10⁵ cells)¹.

	Summer	Autumn	Winter	Winter-HS
Progesterone	3.52 ± 0.49 ^a	2.06 ± 0.49 ^b	1.47 ± 0.36 ^b	2.04 ± 0.49 ^b

¹ After 18 hours of incubation.

HS- Heat-stress.

Values are given as mean ± SEM.

Different letters denote statistically significant differences (P < 0.05).

Table 10 Basal progesterone production by theca cells during different seasons (ng / 10⁵ cells)¹.

	Summer	Autumn	Winter	Winter-HS
Progesterone	0.69 ± 0.14 ^a	0.68 ± 0.14 ^a	0.36 ± 0.10 ^{b*}	0.25 ± 0.14 ^b

¹ After 18 hours of incubation.

HS- Heat-stress.

Values are given as mean ± SEM.

Different letters denote statistically significant differences (P < 0.05 ; * P < 0.08)

Figure 1. Concentration of estradiol in plasma of cooled (n=6) and heat-stressed (n=6) cows during days 1 to 10 of the estrous cycle.

Figure 2. Concentration of inhibin in plasma of cooled (n=6) and heat-stressed (n=6) cows during days 1 to 10 of the estrous cycle.

Figure 3. Concentration of progesterone in plasma of cooled (n=6) and heat-stressed (n=6) cows during days 1 to 10 of the estrous cycle.

Figure 1

Estradiol in plasma

Figure 2

Inhibin in plasma

Figure 3

Progesterone in plasma

FIELD STUDIES IN SUMMER: PRELIMINARY RESULTS ON THE EFFECTS OF
EXOGENOUS SUPPLEMENTATION OF PROGESTERONE AND GnRH POST-
INSEMINATION

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GENERAL INTRODUCTION

Two hormones, progesterone and synthetic GnRH or its analogues, have been widely used to improve fertility in cattle in different studies, with very limited success. However, the majority, if not all of the studies, examine the effect of the hormones not under summer conditions. Such stress could be harmful, as described in detail in previous sections, to the growing embryo, to the ovulatory follicle, and to the subsequently developed corpus luteum (CL). The rationale behind adding progesterone post-AI is based on reports that: (a) the embryo in heat-stressed cows is less developed and its growth is retarded. (b) the CL produces less progesterone in summer. (c) as a result, uterine $\text{PGF}_{2\alpha}$ secretion during thermal stress is increased and the chances for early luteal regression rise. GnRH is used post-AI because of reports stating that: (a) a single injection of a GnRH analogue on day 12 post-AI increases fertility in some experiments. (b) injection of GnRH can

luteinize the large follicles in the ovary. Since destruction of large follicles increases the CL's life span in cows, GnRH may prevent early luteolysis and may also increase progesterone secretion via its induction of LH secretion.

The fertility studies performed within the framework of this project provided preliminary results. As a consequence, we describe them only briefly in the following paragraphs.

PROGESTERONE SUPPLEMENTATION

METHODS

Progesterone was supplied as described in the above paper by Van Cleef et al. CIDR's were inserted intravaginally in all experiments on days 7-8 post AI for 12 days. The day of CIDR insertion was chosen: (a) in light of published and nonpublished reports that CIDR's inserted early post-AI (days 1-4) may cause a reduction in fertility; (b) CIDR's had a positive effect on fertility in New-Zealand cows when inserted on day 7; (c) exogenous supply of progesterone between days 7 and 19 coincides with the sensitive period of pregnancy recognition, when a well-developed embryo is important to the process.

RESULTS

Three experiment were conducted in the summers of 1991 and 1992. In experiment 1 (1991 and 1992 combined; July-October, Naan, IS), overall conception rates of control and CIDR-treated lactating

cows were 25% (n=91) and 39% (n=59), respectively. In experiment 2 (July-September 1992, Samar, IS), conception rates of 1st to 3rd AI's of control and CIDR-treated lactating cows were 11% (n=96) and 15% (n=59), respectively. In experiment 3 (June-October 1992, Yotveta, IS), conception rates in 1st and 2nd AI's in control and CIDR-treated cows with no postpartum reproductive disorders were: 25% (n=32) and 23% (n=31), respectively. Results are equivocal, and more detailed experiments are needed to obtain more convincing ones. It could be, however, that local climate and farm management conditions (e.g. cooling system operation) interact with the treatment.

GNRH INJECTION

METHODS

Two experiments were performed to determine if GnRH injected on either day 8 or day 12 post-AI would increase conception rates during the summer heat stress period. Administrations of the hormone on these days aimed at luteinizing the first-wave or second-wave dominant follicles, respectively. In Experiment 1 (Larson Dairy, Okeechobee, FL), 8 µg buserelin (Receptal, Hoechst-Roussel) was injected im. to lactating cows on day 11±1 post-AI (May-August). In Experiment 2 (Netzer-Sireni, IS), 250 µg GnRH (Fertagyl, Intervet) was injected im. into lactating cows on day 8±1 post-AI (July-September)

RESULTS

In Experiment 1, Conception rates of control and GnRH-treated cows were: 37% (n=191) and 33% (n=196), respectively. In Experiment 2, Conception rates of control and GnRH-treated cows were: 15% (n=39) and 18% (n=33), respectively. Results from these experiments show no significant benefit to using GnRH post AI, during summer months, given as a single injection on days 8 or 12. Time, doses and sequence of GnRH administration need to be reevaluated before further studies can be conducted.

CONCLUSIONS

Hormonal therapy to summer-stressed cows with progesterone and GnRH agonists post-insemination was tested. We found that adding progesterone might be beneficial but more studies need to be conducted to substantiate this. We did not find any effect of GnRH injected on days 8 or 12 post-insemination. More studies will be conducted in the future, taking into consideration other aspects, such as quality of the follicles, corpus luteum, embryo, gonadotrophin support, etc. All of the above must be combined with efficient cooling systems that are able to alleviate, at least partially, the heat stress, to prevent direct embryonic death in utero.

COOPERATION OF RESEARCH ACTIVITIES

Cooperation between the groups in Florida (University of Florida) and Israel (Hebrew University/Agric.) can be found throughout most of the research conducted. Productivity was indeed synergistic in the opinion of the investigators. The following is a short description of that cooperation.

1. One of the Israeli scientists learned and practiced the ultrasound technique in Florida, with the guidance of an experienced scientist there. Two experiments performed in Florida characterized follicular development in the thermoneutral state. Later two complementary, key studies on follicular development under heat stress conditions were performed both in Florida and in Israel.

2. Follicular endocrine function was characterized in both laboratories, via different approaches which provided insight on the subject from different biological perspectives. Thus, a better understanding of follicular function during thermal stress was achieved. For example: in Florida, the whole follicle was dissected from the ovary and incubated in vitro, in conditions closely approximating tissue arrangement in vivo. In Israel, the two layers of the follicular wall were separated, and dispersed cells (theca and granulosa cells) were incubated separately, enabling an evaluation of hormone synthesis by the two layers that form a functional unit. Each approach complemented the other.

3. Each group's specialization in a specific area also was reflected in the study on uterine response to heat stress performed in Florida, and on responses of the CL to thermal stress performed in Israel. In fertility studies, as well as in ultrasound experiments, climate and management in Israel and Florida were slightly different, as were the experimental approaches taken in each country. As a result, any effect or lack of one found in both places proved the findings to be of a more general nature and applicable to regions experiencing seasonal periods of heat stress.

RESEARCH ACHIEVEMENTS AND BENEFIT TO AGRICULTURE

The project was very fruitful, and most of the original aims of the research proposal were fulfilled. The main achievements are listed below.

1. This project yielded valuable information about the dominant follicle, regarding regulation of its turnover, and the levels of various steroids, prostaglandins, peptide hormones and growth factors secreted by the large dominant follicle (Section 2 of the Scientific Results). Increased LH support appeared to maintain a dominant follicle, whereas high progesterone concentrations decreased LH pulse frequency, leading to turnover of the dominant follicle (Savio et al., 1993a). Low levels of progesterone caused persistence of the dominant follicle, resulting in an aged ovulatory follicle. Subsequently, insemination of heifers that ovulated persistent follicles resulted in poor fertility (Savio et

al., 1993b). Another study demonstrated the value of using follicular oxytocin and estradiol concentrations to physiologically classify the functional state of large bovine follicles (Meidan et al., 1993). Follicles could be classified as being harvested before or after the preovulatory gonadotrophin surge, or atretic.

In a particularly detailed study, evolution of the first-wave dominant follicle and its subordinate follicle was characterized (Badinga et al., 1992). The dominant follicle and the CL were found to have important regulatory effects on LH and FSH secretion, which regulate ovarian folliculogenesis and steroidogenesis. As a result of this and related experiments, the concept of manipulating follicular development to bring about ovulation of a fresh young follicle emerged. Such follicles could increase conception rates in cattle. The use of a GnRH agonist (buserelin) in synchronization programs, combined with intra-vaginal progesterone and im. PGF_{2α} injection, gave a more precise synchronization of estrus. One experiment which was part of the project (Wolfenson et al., 1990; manuscript submitted for publication is presented in Section 2) demonstrated that a single injection of GnRH in mid-luteal phase followed by an injection of PGF_{2α} (7 days later) induces the rise of a "new" wave of follicles from which the ovulatory follicles were fresh and healthy, and more dominant in nature than control follicles. Such a system leads to a synchronization system that programs synchronization of both follicle development and CL regression.

2. The most important finding of this project is probably the fact that the dominant-ovulatory follicle is highly susceptible to heat stress (Section 3 of the Scientific Results). This was reflected in various findings under heat stress: dominance of the large follicle was suppressed (Wolfenson et al., 1992); theca cells produced less androstenedione and granulosa cell aromatase activity was lower, resulting in low estradiol production; granulosa cells produced less inhibin possibly affecting follicular development; heat stress drastically reduced the viability of granulosa cells (Lew et al., 1993; manuscript in preparation is presented in Section 3) early summer was less stressful than late summer months in terms of estradiol secretion, suggesting chronic delayed impairment of follicular function (Badinga et al., 1993); a significant delayed effect of summer heat stress was detected during the cooler autumn months (Lew et al., 1993).

3. Three more key components of the reproductive system - The corpus luteum, the pituitary, and the uterus (Sections 4, 5 and 6 of the Scientific Results, respectively) were impaired during heat stress.

Corpora lutea during summer were shown to secrete less progesterone than during the winter (Wolfenson et al., 1993b). This could be a direct result of the above-described damage caused by heat stress to the ovulatory follicle. In addition, CLs studied during summer periods of heat stress produced more PGF_{20} than those

characterized in winter which may act synergistically with elevated secretion of $\text{PGF}_{2\alpha}$ from the uterus (see below) to induce luteolysis.

Pulsatile secretion of LH as well as the preovulatory surges of LH and FSH, were suppressed significantly under conditions of summer heat stress (Gilad et al., 1993). The latter could be demonstrated only in animals with low plasma estradiol concentration. Low levels of LH and FSH, in turn, may alter normal functioning of the ovarian components, e.g. follicular development, oocyte maturation, ovulation, luteinization and subsequent differentiation of the CL.

At the uterine level, another study demonstrated in vivo that heat-stressed pregnant heifers secreted more $\text{PGF}_{2\alpha}$ than controls in response to oxytocin (Wolfenson et al., 1993a). This important in vivo finding confirmed previous studies performed in vitro. In a situation where the CL itself produces more $\text{PGF}_{2\alpha}$, this could be detrimental to CL maintenance during periods of heat stress.

4. Field Studies (Section 7 of the Scientific Results): Injection of a GnRH agonist (buserelin) on days 8 or 12 post-insemination in summer-stressed cows did not affect their fertility (Thatcher and Wolfenson, Section 7). Intravaginal supplementation of progesterone from day 8 post-insemination for 12 days yielded equivocal results (Van Cleef et al., 1991; Thatcher and Wolfenson, Section 7). Treatment might be beneficial but more needs to be done to substantiate this effect.

Benefit to agriculture

The above experiments demonstrate the complexity of the summer heat stress syndrome. They suggest that intact, fresh ovulatory follicles are essential for successful conception. They show that impairment of follicular function, e.g. lowered estradiol secretion, can lead to lower preovulatory surges of LH/FSH which in turn affects follicular development, as well as the luteinization process. The latter may impair subsequent luteal function, lower progesterone secretion and alter normal embryo development in utero. An embryo retarded in development under conditions of thermal stress, with low progesterone support, is unable to inhibit PGF_{2α} output, resulting in early luteolysis of the CL and termination of pregnancy.

Ways to intervene in the above-described closed loop of events occurring under heat stress are to use on-farm cooling systems as efficiently as possible and to complement this system with the use of hormonal therapy at the right time. Supplementation of exogenous progesterone could be one way, and manipulation of follicular development to produce a fresh, healthy ovulatory follicle, even under conditions of stress is another alternative. Hormonal manipulations to ensure proper gonadotrophin secretion and the production of a high quality ovulatory follicle and subsequent CL are other approaches to be tested. The uterine environment needs to be better controlled under conditions of heat stress, and could be regulated by nutritional means that antagonize PGF_{2α} secretion.

The ultimate goal of research in this area is to test and develop means of improving low summer fertility. First steps in this direction are demonstrated in the field studies (Section 7 of Scientific Results). This provides the main objective of a continuing BARD project, to be submitted in the future. Our findings could be of great value to agriculture if conception rates during the summer months could be raised in dairy cattle from 10-20%, to the winter values of 40-60%. Furthermore, we may reduce the detrimental effects of heat stress that carryover into the cooler season of fall.

LIST OF PUBLICATIONS

1. Badinga, L., Driancourt, M.A., Savio, J.D., Wolfenson, D., Drost, M., de la Sota, R.L. and Thatcher, W.W. Endocrine and ovarian responses associated with the first-wave dominant follicle in cattle. *Biol. Reprod.*, 47:871-883, 1992.
2. Badinga, L., Thatcher, W.W., Diaz, T., Drost, M. and Wolfenson, D. Effect of environmental heat stress on follicular development and steroidogenesis in lactating Holstein cows. *Theriogenology*, 39:797-810, 1993.
3. Gilad, E., Meidan, R., Berman, A., Graber, Y. and Wolfenson, D. Alterations in plasma concentrations of gonadotrophins, by acute or seasonal heat stress and oestradiol in cyclic cows. *J. Reprod. Fertil.*, in press, 1993.
4. Lew, B.J., Wolfenson, D. and Meidan, R. Heat stress affects steroid content of follicular fluid and steroid production by granulosa and theca cells in the bovine dominant follicle. *Israel Endocrine Soc., Ann. Meeting (Abstract 21)*, Tel- Aviv, January 1993.
5. Meidan, R., Wolfenson, D., Thatcher, W.W., Gilad, E., Aflalo, L., Graber, Y., Shoshani, E. and Girsh, E. Oxytocin and estradiol concentrations in follicular fluid as a means for the classification of large bovine follicles. *Theriogenology*, 38:421-432, 1993.
6. Savio, J.D., Thatcher, W.W., Badinga, L., de la Sota, R.L. and Wolfenson, D. Regulation of dominant follicle turnover during the oestrous cycle in cows. *J. Reprod. Fertil.*, 97:197-203, 1993a.
7. Savio, J.D., Thatcher, W.W., Morris, G.R., Entwistle, K., Drost, M. and Mattiacci, M.R. Induction of low plasma progesterone concentrations with a progesterone releasing intravaginal device affects follicular turnover and fertility in cattle. *J. Reprod. Fertil.* 98: 77-84, 1993b.
8. Van Cleef, J., Drost, M. and Thatcher, W.W. Effects of postinsemination progesterone supplementation on fertility and subsequent estrous responses of dairy cows. *Theriogenology*, 36:795-807, 1991.
9. Wolfenson, D., Bartol, F.F., Badinga, L., Barros, C.M., Marple, D.N., Cummins, K., Wolfe, D., Lucy, M.C., Spenser, T.E. and Thatcher, W.W. Secretion of $\text{PGF}_{2\alpha}$ and oxytocin during hyperthermia in cyclic and pregnant heifers. *Theriogenology*, 39:1129-1141, 1993a.

10. Wolfenson, D., Luft, O., Berman, A. and Meidan, R. Effects of season, incubation temperature and cell age on progesterone and prostaglandin $F_{2\alpha}$ production in bovine luteal cells. Anim. Reprod. Sci., 32:27-40, 1993b.

11. Wolfenson, D., Thatcher, W.W., Savio, J.D. and Lucy, M.C. The effect of a GnRH analogue and $PGF_{2\alpha}$ on follicular development and dominance in the cyclic lactating dairy cow. Amer. Dairy Sci. Assoc. Ann. Meeting (Abstract P176), J. Dairy Sci. Suppl. 1, Raleigh, June 1990.

12. Wolfenson, D., Thatcher, W.W., Badinga, L., Savio, J.D., Philosof, Y., Maman, M., Meidan, R. and Berman, A. Alterations in follicular development and dominance during the estrous cycle in heat-stressed cows. Proc. 12th Int. Cong. Anim. Reprod. Vol. 4, Paper 623, pp. 2117-2119, The Hague, August 1992.