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**Hormonal and Nutritional Strategies to Optimize
Reproductive Function and Improve Fertility of Dairy
Cattle during Heat Stress in Summer**

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**HORMONAL AND NUTRITIONAL STRATEGIES TO OPTIMIZE REPRODUCTIVE FUNCTION
AND IMPROVE FERTILITY OF DAIRY CATTLE DURING HEAT STRESS IN SUMMER**

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ABSTRACT

The BARD program includes two main parts. In the first, experiments were conducted to complete our understanding of the mechanisms responsible for the impairment of reproductive functions under heat stress. Experiments focused on follicular development and function, since results obtained in our previous BARD project indicate that the preovulatory follicle is susceptible to heat stress. The theca cells, sensitive to thermal stress, produced less androgen during the summer, as well as during the autumn. Similarly, luteinized theca cells obtained from cows in summer produced much less progesterone than in winter. Granulosa cells and luteinized granulosa cells were less susceptible to heat stress. A delayed effect of heat stress on follicular development, on suppression of dominance and on steroid production by theca and granulosa cells was noted. This may be related to the low fertility of cows during the cool months of autumn.

In the second part, experiments were conducted aiming to improve fertility in summer. The timed AI program was developed using two injections of GnRH coupled with PGF_{2α}. It was found effective in improving reproductive performance in lactating cows. Limitations induced by heat stress on estrus detection were eliminated with the timed AI management program. Replacing the second injection of GnRH with hCG instead of GnRH agonist increased plasma progesterone levels post ovulation but did not improve fertility. Use of the timed AI program in summer, shortened days open and increased the net revenue per cow, however, it did not protect the embryo from temperature-induced embryonic mortality. Incorporation of a GnRH-agonist implant into the timed AI program was examined. The implant increased plasma progesterone and LH concentrations and altered follicular dynamics. The use of a GnRH-implant enhanced pregnancy rate in cows with low body conditions. In a timed embryo transfer experiment, the use of fresh or frozen in vitro produced embryos was compared in the summer to improve fertility. The use of fresh embryos (but not frozen ones) improved pregnancy rate, however, substantial embryonic death occurred between 21 and 45 days. The timed AI program, which is now being used commercially, shortened days open, and increased pregnancy rate during summer. Other approaches which were found to improve fertility in small-scale studies, need to be tested again in large-scale field trials.

II

Objectives of the original research proposal

The general objective of this research is to establish a reproductive-nutritional management strategy to eliminate or minimize negative effects of heat stress on the reproductive system, and improve summer fertility in dairy cattle.

The specific aims of the study are:

1) To complete our investigations on the impairment of reproductive functions under heat stress, particularly focusing on the ovulatory follicle as a key factor which interacts with other reproductive events, such as, CL quality, embryonic development, uterine and gonadotrophin secretions, that cause a closed loop of events which subsequently reduce fertility.

2) To examine, via specific fertility experiments, hormonal and nutritional means that, under conditions of optimal heat stress management (sprinkling and forced ventilation), will: 1) induce ovulation of a fresh and healthy ovulatory follicle; 2) increase progesterone support to the utero-conceptus unit in early pregnancy stages; 3) increase the suppressed preovulatory gonadotrophin surge; and 4) suppress the potential elevated luteolytic secretion of uterine $\text{PGF}_{2\alpha}$. The most optimal combination of treatments will finally be tested.

INTRODUCTION TO RESULTS AND DISCUSSION

This report includes 17 different studies, of which 13 studies are published papers, accepted for publication papers or abstracts of studies presented at scientific meetings (see list of publications on pages 222-223).

Part A: Impairment of reproductive functions under heat stress

1. Seasonal effects on steroidogenesis in the dominant follicle (pages 1-11; Reference [Ref.] 1).
2. The effect of the origin of the preovulatory follicle on steroid production by theca and granulosa cells and on the quality of the formed CL (pages 12-42; Ref. 2).
3. Progesterone production by luteinized theca and granulosa cells obtained from dominant follicles in summer and winter (pages 43-46).
4. Delayed effect of heat stress: long term follicular dynamics and biochemical characteristics of dominant follicles (pages 47-48).
5. Delayed effect of heat stress: Ovarian follicular dynamics and steroid production by theca and granulosa cells (pages 49-67; Refs. 3, 4).
6. The effect of the preovulatory LH surge pattern on progesterone secretion from the subsequently formed CL (pages 68-74; Ref. 5).

Part B: Improvement of Summer Fertility

The studies numbered 7 to 13 deal with implementation of the timed artificial insemination program in the summer management system of fertility.

7. Evaluation of timed insemination using gonadotropin releasing hormone agonist (pages 75-83; Ref. 6).
8. Evaluation of timed insemination during summer heat stress (pages 84-93; Ref. 7).
9. Veterinary aspects of Timed AI in dairy cattle (pages 94-101; Ref. 8).
10. Economics of the OVSYNCH – Timed AI protocol in dairy cattle (pages 102-118).
11. Differential response of the luteal phase and fertility, in the winter and summer,

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following ovulation of the first-wave follicle with hCG or GnRH agonist (pages 119-128; Ref. 9).

12. Use of a GnRH agonist or hCG for timed artificial insemination protocol in cattle (pages 129-136; Ref. 10).
13. Effects of timed insemination and supplemental β -carotane on reproduction and milk yield of dairy cows under heat stress (pages 137-149; Ref. 11).
14. Evaluation of the effect of a subcutaneous GnRH implant on CL function, and follicle dynamics (pages 150-164; Ref. 12)
15. Evaluation of the effect of a subcutaneous GnRH implant incorporation into a timed AI management program (pages 165-178; Ref. 13).
16. Enhancing pregnancy rate during heat stress by timed embryo transfer at a stage when embryos are less susceptible to heat stress (pages 179-208).
17. Improving fertility by hormonal therapy that is aimed at enhancing the hormonal support of embryonic development and induction of fresh follicle ovulation (pages 209-212)

FERTILITY STUDIES - ISRAEL

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A series of fertility studies were conducted, the rationale of which was (a) to increase circulating plasma progesterone to support embryonic development under heat stress conditions; (b) to induce ovulation of a fresh follicle; (c) to optimize the preovulatory surge of LH which is lowered under heat stress. Experiments included: (1) injection of PGF₂ α on day 7 or day 14 to induce ovulation and insemination (AI) of fresh first- or second-wave dominant follicles; (2) insertion of an intravaginal device (CIDR) containing progesterone on day 7 post-AI for 11 days to improve progesterone support to the developing embryo; (3) injection of a GnRH analogue on day 12 post-AI; (4) injection of a GnRH analogue at the start of estrus prior to AI. Experiments were conducted in winter and in summer. All experiments were conducted on high milk-yielding cows in commercial farms. Hormonal treatments were applied, on average, between 50 and 70 days postpartum to cows that showed estrous activity prior to treatment. Pregnancy was confirmed by rectal palpation 40-50 days post-AI. Conception rate was calculated as the number of pregnancies per number of cows inseminated.

Induction of fresh follicle ovulation by PGF₂ α injection

Two experiments were conducted. In the winter experiment, Givaat-Chayeem herd, cows were injected with PGF₂ α on day 7 (PG7) or on day 14 (PG14) of the cycle and inseminated at estrus within 96 h of injection. Conception rate of PG7, PG14 and intact controls were 56% (n=17), 53% (16) and 38% (17), respectively. The second experiment (Eilot herd) was started in autumn (November) and continued throughout the winter. Conception rates of cows of

PG7, PG14 and intact controls were 45% (29), 40% (27) and 21% (39), respectively. The low conception rate of control cows reflected the low fertility of cows in the autumn.

Progesterone supplementation post-insemination

Two experiments were conducted during the summer months. A CIDR device was inserted on day 7 post-AI for 11 days. In the first experiment total conception rates were 26% (n=215) for treated cows and 22% (223) for control cows. In the second experiment (Ayelet-Illashachar herd), conception rate of progesterone-treated cows was 44% (23) and of control group 41% (27). In both studies, a nonsignificant 4% increase in conception rate with the use of the CIDR was recorded.

Combined treatment: induction of ovulation of first-wave dominant follicle by PGF_{2α} injection on day 7 and GnRH injection on day 12 post-AI

The experiment was performed in the winter months (Ashdot-Yaakov herd). Each of the treated cows were injected with PGF_{2α} on day 7 and inseminated at estrus. A GnRH analogue was injected (10 µg Receptal) on day 12 post-AI. Conception rates of treated and control cows were 43% (n=48) and 48% (45), respectively (NS).

Combined treatment: Induction of ovulation of fresh first- or second-wave dominant follicle by PGF_{2α} injection on day 7, progesterone supplementation by CIDR insertion on day 7 post-AI for 11 days, and GnRH injection on day 12 post-AI.

Winter experiment Givaat Brener herd – first year. Cows were randomly divided into three groups - control cows, and cows injected with PGF_{2α} on day 7 (PG7) or on day 14 (PG14) of the cycle. Cows were inseminated at estrus, a CIDR was inserted on day 7 post-AI for 11 days, and Receptal was injected on

day 12 post-AI. Conception rates of control, PG7 and PG14 groups were: 40% (n=65), 65% (43) and 44% (34), respectively.

Based on the results obtained in the first year, in the second year of the study, the PG14 treatment was eliminated and the experiment was repeated with PG7 and control groups, in the same herd as well as in the Saad herd. Total conception rates for the three experiments were: for control cows 45% (206) and for PG7 cows 56% (153). This difference was statistically significant ($P < 0.03$).

Summer experiment The experiment was conducted during July-September in a commercial herd (Yavne Herd) equipped with a sprinkling and ventilation cooling system. Cows were randomly assigned to control or treated cows. The latter group was injected with $\text{PGF}_{2\alpha}$ on day 7 and inseminated at estrus, then a CIDR was inserted on day 7 post AI and Receptal injected on day 12. Conception rates of control and treated mature cows (second and more lactations) were 35% (n=23) and 47% (15), respectively. For first-calf heifers, conception rate of control animals was, for unknown reasons, extremely high - 69% (26), above the routine figures of summer fertility in that particular herd or the averages for the whole country, as compared to a conception rate of 36% (28) in treated cows. The total conception rate was 53% (49) and 40% (43) for control and treated cows, respectively (NS).

Injection of GnRH analogue at the start of estrus before insemination

During the summer, the LH surge is lowered. Injection of GnRH during early stages of estrus coincides with the endogenous LH surge. It may induce a higher LH surge and it may also better synchronize the time intervals between estrus, LH surge, ovulation and insemination.

Lactating cows were synchronized with two injections of $\text{PGF}_{2\alpha}$ 14 days apart. For 4 days, starting 48 h after the second injection, cows were watched continuously for estrous behavior 18 h a day. At first signs of standing estrous, cows were injected with 10 μg Receptal.

Winter experiment: Conception rates of control and GnRH-treated cows were similar: 49.4% (n=89) and 57.3% (89), respectively (NS).

Summer experiment : The study was conducted during June-November. Conception rate of first-calf heifers rose significantly ($P<0.01$) to 60.3% (n=68) from 37.5% (64) in controls. In contrast, conception rate of mature cows (second or more lactations) did not differ between groups, though it was higher in GnRH cows 43.5% (115) than in controls - 33% (109). The reason that GnRH was only effective in first-calf heifers and not in mature cows is unclear, but could be related to lower milk yield by the former, which may influence their ability to maintain normothermia under heat stress conditions.

In summary, Progesterone supplementation post AI was not effective in summer. The combined treatment that significantly increased conception rate in winter was also not effective in summer. Injection of GnRH at estrus which was not effective during the winter increased summer fertility in first-calf heifers but not in mature cows. A main limiting factor in improving summer fertility by hormonal means, in our opinion, is that hyperthermia which was not prevented in many of the cows, although they were cooled during summer. Random measurements of body temperature (data not shown) recorded during summer indicated that it rose during the hot hours of the day to levels which are considered detrimental to embryonic development (39.5-40.5°C). In such a situation no hormonal therapy is likely to efficiently improve summer fertility. Cooling practices need to be improved in commercial herds to lower hyperthermia before more research efforts are made.

EVALUATION OF THE RESEARCH ACHIEVEMENTS

Part A: Impairment of reproductive functions under heat stress

Seven studies were performed. Five of them focused on the functional impairment of the preovulatory follicle under heat stress. The preovulatory follicle is a key factor interacting with other reproductive functions to cause a closed loop of events which subsequently reduces fertility during the summer. One study examined the seasonal effects on steroidogenesis in the dominant follicle; one dealt with the effect of the origin of the preovulatory follicle (first or second wave) on steroid production by theca and granulosa cells and on the quality of the subsequently formed CL; one examined the effect of follicle origin on luteinized theca and granulosa cells obtained from cows in summer and winter. Three studies evaluated the delayed effect of heat stress on follicular function. Finally, one study examined the effect of the preovulatory LH surge pattern on progesterone secretion from the subsequently formed CL.

1. The theca cells were found to be susceptible to heat stress, as evidenced by the reduction in androstenedione production. Chronic heat stress (exposure to summer heat), short-term heat stress (exposure of cows to heat in an environmental chamber in the winter), and a delayed heat stress effect (evident during the autumn) effectively decreased androgen production. Estradiol production by granulosa cells was less susceptible to heat stress: production dropped in summer but not in autumn; the summer drop only tended to be lower than winter production.

2. We compared the endocrine function of the first- and second-follicular wave dominant follicles (DF1 and DF2) and the subsequently formed CLs. Production of estradiol by granulosa cells was similar in DF1 and DF2, but androstenedione production was significantly higher in DF1 than in DF2. Similarly, during luteinization in vitro, progesterone production was similar by luteinized granulosa cells derived from DF1 or DF2, but luteinized theca cells derived from DF1 produced two to three times more progesterone than their DF2 counterparts. In agreement with the in vitro study, in two out

of three in vivo studies, plasma progesterone concentration was higher during the luteal phase following ovulation of DF1 than DF2. The higher steroidogenic capacity of DF1 relative to DF2 may be related to the hormonal milieu in which the follicles developed.

3. Progesterone production during luteinization in vitro of theca and granulosa cells obtained from cows in summer and in winter was compared. Theca-derived luteal cells were found to be much more susceptible to heat stress than granulosa derived luteal cells. Progesterone production dropped from 500 ng/100,000 cells in winter to 100 ng in summer.

4. Three experiments were conducted to evaluate the delayed effect of heat stress on follicular function. In the first experiment, the follicular fluid of the dominant follicle was aspirated from cows following heat exposure for 7 days in an environmental chamber. Aspiration was performed 10 times every 8 days in each of 10 follicular cycles programmed by GnRH injection, CIDR insertion and PGF_{2α} injection. The number of small (class 1) and large (class 3) follicles increased during heat stress, but follicular fluid concentrations of estradiol, heat-shock protein 90, and of IGFBP-2 and 3 were not altered by heat stress. Repeat of GnRH injections to induce the follicular cycle may have dissipated the adverse effects of heat stress on follicular function. In the second experiment, cows were heat-stressed during the entire estrous cycle. In the subsequent (cooled) cycle, less medium-size (class 2) follicles developed, indicating a delayed effect of heat stress on follicular development. The slower rate of decline of the medium follicles in previously heat-stressed cows indicates depression of dominance in the large follicle. In a third experiment, cows were heat-stressed in a hot chamber for 5 days. Three weeks later, in the subsequent cycle, steroid production by theca and granulosa cells from medium follicles was significantly lower and more atretic follicles were recorded on the ovaries of previously heat-stressed cows.

5. A study conducted in our previous BARD project indicated that the LH surge is lowered under conditions of thermal stress. We therefore examined the effect of LH surge pattern on progesterone secretion. In vivo, a high LH surge induced, after ovulation a

higher plasma progesterone concentration curve, relative to that induced by low LH surge. In vitro, luteinized granulosa cells that were exposed to high LH "surge" at the beginning of 8-day culture produced more progesterone at the end of luteinization. The study indicated a clear relationship between the LH surge pattern and the function of the developing CL.

Part B: Improvement of summer fertility

Progress in this area is summarized as four main sections in which a series of basic and fertility studies were conducted aiming to improve summer fertility.

1. Implementation of the timed AI program in the summer management system of fertility.

An ovarian control system has been developed that permits the programmed recruitment of an ovulatory follicle after the injection of a commercially available Gonadotrophin Releasing Hormone (GnRH). On day 7 after injection of GnRH, cows are injected with Prostaglandin ($\text{PGF}_{2\alpha}$; Lutalyse) to regress the corpus luteum that permits final maturation of the ovulatory follicle. On day 9 cows are injected again with GnRH that causes the ovulatory follicle to ovulate 30 hours later. All cows are artificially inseminated at 15 h after the injection of GnRH which is also 15 hours before the time of ovulation. This permits the sperm to develop the capacity to fertilize the egg. This system eliminates the need for heat detection since insemination is implemented at a fixed time. The timing of injections is very conducive to farm management allowing the system to be implemented in groups of animals on a weekly basis. Research trials completed on a commercial dairy farm indicate that pregnancy rates are comparable to what is achieved in control cows (receiving GnRH and $\text{PGF}_{2\alpha}$ 7 days apart, and inseminated at detected heat). The timed AI program gave consistent pregnancy rates from month to month (January to May) and higher levels during the warmer months of April and May compared to the control group. Undoubtedly, these systems offer a distinct advantage over a non-programmed system that would normally be practiced on the farm. These reproductive management systems have been implemented with nutritional targeted

programs of fat feeding to stimulate ovarian follicular activity.

Due to reductions in days to first insemination and days to conception, there are potential savings of implementing a Timed Insemination Program. Potential savings as net revenue per cow in the herd varies depending upon the management scenarios followed. The following table provides different examples. Programmed insemination of cows will also permit a greater number of cows to be inseminated prior to the more difficult heat stress season when embryonic deaths are high. The programmed breeding system also permits a greater opportunity to predict herd production and performance to optimize overall profit of the farm in the future. Since a large amount of the reproductive decisions and tasks are managed at specific times during the week, it should permit a reduction in labor costs. Implementation of the timed insemination program increases net revenue per cow from \$15 (use in cool season for first service) to \$71 (use in April before hot season on all cows that are open) and \$25 when used for first service in the hot summer months only.

Application of timed insemination during the hot summer season of low fertility improved reproductive management. Timed insemination (TI) was compared with a control insemination (CI) at observed estrus on a commercial dairy in Florida during the hot season of May to September 1995. Lactating dairy cows were injected with $\text{PGF}_{2\alpha}$ (25 mg) at 30 ± 3 d postpartum (PP). For TI cows ($n = 148$), injections of GnRH agonist (GnRHa; Buserelin, 8 μg) were administered at 50 ± 3 d PP, followed in 7 d with $\text{PGF}_{2\alpha}$ and GnRHa 48 h later. Cows were inseminated 16 h after the second injection of GnRHa. The CI cows ($n = 156$) were inseminated at detected estrus following injection of $\text{PGF}_{2\alpha}$ only at 57 d PP. Estrus detection or insemination rate for CI cows was $18.1 \pm 2.5\%$ versus 100% for TI cows ($P < 0.01$). Mean interval from $\text{PGF}_{2\alpha}$ to insemination was shorter for TI cows (3 ± 2.1 d $< 35.5 \pm 1.9$ d; $P < 0.01$). Pregnancy rate was greater for TI cows ($13.9 \pm 2.6 > 4.8 \pm 2.5\%$; $P < 0.01$) as was overall pregnancy rate by 120 d PP ($27.0 \pm 3.6 > 16.5 \pm 3.5\%$; $P < 0.05$). Number of days open for cows conceiving by 120 d PP was less for TI cows ($77.6 \pm 3.8 < 90.0 \pm 4.2$ d; $P < 0.05$), as was interval to first service ($58.7 \pm 2.1 < 91.0 \pm 1.9$ d; $P < 0.01$). Services per conception were greater for TI

cows ($1.63 \pm 0.10 > 1.27 \pm 0.11$; $P < 0.05$). The TI program did improve group reproductive performance. However, the TI program will not protect the embryo from temperature induced embryonic mortality, but management limitations induced by heat stress on estrus detection are eliminated.

2. Evaluation of the effect of a subcutaneous GnRH implant on CL function, and follicle dynamics, and its incorporation into a timed AI management program.

The influence of Buserelin injection and Deslorelin (a GnRH analogue) implants administered on day 5 of the estrous cycle, on plasma concentrations of LH and progesterone (P_4), accessory CL formation and follicle and CL dynamics was examined in non lactating Holstein cows. On day 5 (day 1=ovulation) following a synchronized estrus, 24 cows were assigned randomly ($n=4$ per group) to receive 2 ml saline im (Control), 8 μ g Buserelin, im, or Deslorelin implant sc, in concentrations of 75 μ g (DES-75 μ g), 150 μ g (DES-150 μ g), 700 μ g (DES-700 μ g) or 2100 μ g (DES-2100 μ g). Ovulation of the first wave dominant follicle occurred in all cows following GnRH treatment, the mean interval from treatment to ovulation being 28.0 ± 1.2 h. Both induced and spontaneously formed CL regressed at the same time in all groups of cows. Days to CL regression did not differ between treatments, but return to estrus was significantly delayed (44.2 vs 27.2 days; $P < 0.01$) in cows of DES-2100 μ g group. In conclusion, Buserelin (8 μ g) or low doses (75, 150 μ g) of Deslorelin did not alter the endocrine profiles or follicle dynamics of treated cows. Deslorelin implants of 700 μ g increased plasma P_4 and LH concentrations and slightly delayed the emergence of the third dominant follicle. On the contrary, Deslorelin implants of 2100 μ g drastically altered the endocrine profiles and follicle dynamics in non lactating cows.

Based upon the dose titration of the Deslorelin implant described above, our objectives were to investigate the effect of a synthetic GnRH-agonist (Deslorelin) implant on CL function and follicle dynamics when administered 48 h after $PGF_{2\alpha}$, in a timed-insemination protocol, and to determine if the incorporation of a Deslorelin implant into a timed-insemination protocol to synchronize ovulation would be beneficial

to the establishment of pregnancy. In Experiment I, 15 non lactating cyclic Holstein cows received Buserelin (8 μ g, im) on Day -9, Lutalyse (25 mg, im) on Day -2, and then on Day 0 received either a Deslorelin implant (700 μ g, sc; n=5), Buserelin (8 μ g, im; n=5), or no treatment (control; n=5). From Days 0 to 15, the rate of increase of plasma progesterone (P_4) was greater ($P<0.01$) for Deslorelin than for control and Buserelin. Establishment of the first-wave dominant follicle (FWDF) as a Class 3 (>9 mm) follicle was delayed ($P<0.01$) with Deslorelin (14.2 ± 1.3 d) compared with the control (4.6 ± 1.3 d) and Buserelin (5.0 ± 1.5 d) treatments. The FWDF resumed growth after Day 13 in all 5 Deslorelin-treated. In Experiment II, 16 lactating potentially subfertile (body condition score 2.25) cows received Cystorelin (100 μ g, im; Day -9), Lutalyse (25 mg, im; Day -2), and either a Cystorelin injection (100 μ g, im; n=8) or Deslorelin implant (700 μ g, sc; n=8) on Day 0 and inseminated 16 h later. Deslorelin-treated cows had a higher plasma P_4 concentration between Days 0 and 16 ($P<0.05$) than the 2 other groups, and 5 of the 8 cows in this group were pregnant (Day 45, palpation) compared with 1 of 8 cows in the Cystorelin group ($P<0.05$). Incorporation of a Deslorelin implant into a timed-insemination protocol enhanced the pregnancy rate in cows of poor body condition. The results support the hypothesis that enhanced CL function and delayed establishment of the first-wave dominant follicle may enhance embryo survival.

3. Enhancing pregnancy rate during heat stress by timed embryo transfer at a stage when embryos are less susceptible to heat stress.

Objective of this program area was to determine whether pregnancy rates in heat stressed dairy cattle could be enhanced by timed embryo transfer relative to timed insemination. Ovulation in Holstein cows was synchronized by a GnRH injection followed 7 d later by PGF_{2 α} and a second treatment with GnRH 48 h later. Cows were assigned to either timed insemination (TI) or timed embryo transfer (TET). Control cows (TI, n=129) were inseminated 16 h (Day 0) after the second GnRH injection. On Day 7, a fresh (TET-Fresh, n=133) or frozen-thawed (TET-Frozen, n=142) embryo was transferred to all TET cows after categorizing the corpus luteum (CL) via palpation per rectum as 3 (excellent) 2 (good, fair) 1 (poor) and 0 (non palpable). Of the 404 cows,

76.2% responded to the synchronization protocol as determined by plasma progesterone concentration of 1.5 ng/ml on Day 0 and 2.0 ng/ml on Day 7. At rectal palpation (45 d), TET-Fresh cows had a greater pregnancy rate than TET-Frozen and TI ($14.3 > 4.8, 4.9 \pm 2.3\%$; $P < 0.01$). Body condition of cows influenced the pregnancy. In summary, timed embryo transfer using fresh in vitro produced embryos in heat stressed dairy cattle improved pregnancy rate relative to timed insemination.

4. Improving fertility by hormonal therapy that is aimed at enhancing the hormonal support of embryonic development and induction of fresh follicle ovulation.

The main finding of a series of fertility studies conducted in Israel are:

1. Supplementation of progesterone post-AI by insertion of CIDR on day 7 for 11 days did not improve conception in summer.
2. A combined treatment which included ovulation of a fresh, first-wave dominant follicle, CIDR insertion on day 7 post-AI for 11 days and GnRH analogue injection on day 12 post AI - found to be significantly effective in improving fertility in the winter - was not effective in summer. The relatively small scale summer study needs to be repeated, however, because of the large difference in the responses of mature cows (positive effect) and first calf heifers (negative effect).
3. The opposite situation was noted regarding GnRH injection at the start of estrus before AI: this treatment which was not effective during the winter was found to be effective in summer. Conception rate rose in first-calf heifers but not in mature cows. We can not yet explain the discrepancy of responses in the two latter experiments between first-calf heifers and mature cows.
4. A main limitation to improving summer fertility by hormonal means is hyperthermia which was not prevented in a large proportion cows, although they were cooled during the summer. Random measurements of body temperature (data not shown) taken during summer experiments indicated that it rose during the hot hours of the day to levels which are considered detrimental to embryonic development ($39.5\text{--}40.5^\circ\text{C}$). In such a situation no hormonal therapy is likely to efficiently improve summer fertility, and cooling practices need to be improved in commercial herds before more research efforts are made.

COOPERATION

The two research teams, Florida and Israel, have a long and close collaboration which is reflected in two previous fruitful BARD grants. The problem of summer fertility is multifactorial and, therefore requires the use of different methods and experimental approaches. The two groups complement each other in terms of their specializations. For example, the delayed effect of heat stress was characterized in both Florida and Israel. The ovum pick up technique available in Florida was used to collect follicular fluid during eight follicular cycles from the same cows following heat exposure in environmental chambers. A complementary study in which steroid production by theca and granulosa cells was determined following heat exposure was conducted in Israel. Another example: the timed AI procedure was developed in Florida and it was therefore quite natural to perform the fertility studies there using this procedure during summer. In Israel, two other hormonal approaches, not related to the timed AI program, were examined – induction of ovulation of first-wave dominant follicles and injection of GnRH at start of estrus.

CONCLUSIONS

1. Heat stress is detrimental to steroid production in the preovulatory follicle, particularly androgen production by the theca cells.
2. The delayed effect of heat stress on the depression of follicular dominance and function could be related to the low fertility of dairy cattle in the cool autumn season.
3. The first-wave dominant follicle and the subsequently formed CL are more steroidogenic than the second-wave dominant follicle and CL.
4. A lower preovulatory LH surge (typical for summer) induces lower production of progesterone by the luteal cells in vivo and in vitro.
5. Use of the timed AI program reduces days open and increases pregnancy rate and net revenue per cow during the summer.
6. Incorporation of a GnRH-agonist implant into the timed AI program may improve follicular function, CL quality and pregnancy rate.
7. Implementation of embryo transfer in the Timed AI protocol may improve pregnancy rate in summer.
8. Replacement of the second injection of GnRH in the timed AI program with hCG does not improve fertility although luteal plasma progesterone concentration is higher.
9. Supplemental β -carotene may increase pregnancy rates for cows in the summer managed with the timed AI program.

LIST OF PUBLICATIONS

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