Effects of GnRH Administered to Cows at the Onset of Estrus on Timing of Ovulation, Endocrine Responses, and Conception

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ABSTRACT

Two experiments examined effects of GnRH administered within 3 h after onset of estrus (OE) on ovulation and conception in dairy cows. In experiment 1, 46 cows received either saline, 250 μ g of GnRH, or 10 μ g of the GnRH analogue, Buserelin. Cows were observed for estrus, blood samples were collected, and ovulations were monitored by ultrasound. In controls, 76% of cows had intervals from estrus to ovulation of ≤ 30 h and 24% had intervals > 30 h. Treatment with either GnRH or GnRH analogue (data combined) increased magnitude of LH surges and decreased intervals from estrus to LH surge or to ovulation. Treated cows all ovulated ≤ 30 h after OE. Among control cows, plasma estradiol concentrations before estrus correlated positively with amplitudes of LH surges. Higher plasma progesterone was observed in the subsequent estrous cycle in GnRH-treated cows compared to control cows with delayed ovulations. Experiment 2 included 152 primiparous and 211 multiparous cows in summer and winter. Injection of GnRH analogue at OE increased conception rates (CR) from 41.3 to 55.5% across seasons. In summer, GnRH treatment increased CR from 35.1 to 51.6%. Across seasons, GnRH increased CR from 36.0 to 61.5% in cows with lower body condition at insemination and GnRH increased CR (63.2 vs. 42.2%) in primiparous cows compared to controls. Use of GnRH eliminated differences in CR for cows inseminated early or late relative to OE and increased CR in cows having postpartum reproductive disorders. In conclusion, GnRH at onset of estrus increased LH surges, prevented delayed ovulation, and may increase subsequent progesterone concentrations. Treatments with GnRH increased conception in primiparous cows, during summer, and in cows with lower body condition.

(Key words: GnRH, ovulation, conception, cows)

Abbreviation key: OE = onset of estrus, CR = conception rate(s).

INTRODUCTION

In most of the studies on the effect of administration of GnRH to cows during estrus on their conception rate (CR), the hormone was administered at the time of AI without consideration of the time elapsed since the onset of estrus (OE). In some of those studies, but not in others, GnRH or its analogues had a positive effect on fertility. In repeat breeders, the effect of GnRH administration was greater than at the first postpartum insemination (Mee et al., 1990; Stevenson et al., 1990). In a metaanalysis of 40 trials described in 27 papers (Morgan and Lean, 1993), a significant effect of GnRH administration at the time of AI was found: the effect on conception rates in repeat breeders was 22.5 percentage units whereas at the first postpartum AI the effect of GnRH was 5.2 percentage units and that of its analogues was 8.0 percentage units.

Improvement of conception following GnRH treatment during estrus has been attributed to the prevention of ovulation failure or to reduced variation in the interval to ovulation (Coulson et al., 1980; Nakao et al., 1984). However, in these studies, the effect of GnRH on the interval from OE to ovulation was not studied. It has also been suggested that GnRH-induced increase in progesterone concentrations during the subsequent estrous cycle may have an effect on conception; this was found in some studies (Lee et al., 1985; Mee et al., 1993; Ullah et al., 1996), but not in others (Lucy and Stevenson, 1986; Ryan et al., 1994).

In an experiment carried out in winter, it was shown that GnRH or its analogues increased CR when administered at OE but had no effect when administered later in the estrous period (Rosenberg et al., 1991). Also, when GnRH was administered at OE, the height of the spontaneous LH peak more than doubled, whereas

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when it was administered later in the estrous period a second, smaller, LH peak emerged. In a subsequent study, GnRH administered at detection of estrus, following two daily visual observations, increased conception in dairy cows in the summer, when conception rate was very low (Ullah et al., 1996). In contrast, Mee et al. (1990) reported that GnRH administered early in estrus had no effect on the CR of dairy cows. From the conflicting results of the very few published reports on studies involving the administration of GnRH close to OE, it seems that GnRH might have a considerable effect on CR under one set of conditions, but no effect under other conditions.

The objectives of the present study were to examine the effects of GnRH, administered to high-yielding dairy cows at OE, with OE being accurately determined by continuous observations for signs of estrus. Experiment 1 examined the effect of GnRH on preovulatory concentrations of estradiol and LH, the interval from onset of estrus to ovulation, and subsequent plasma progesterone concentrations. Experiment 2 was a large-scale experiment that examined the effects of GnRH on conception rates during summer and winter, in relation to BCS, parity, timing of AI, and reproductive disorders.

MATERIALS AND METHODS

Animals

The experiments were carried out in a dairy herd with 305-d average milk yields of 11,400 and 12,100 kg for primiparous and multiparous cows, respectively. Primiparous and multiparous cows were kept under loose housing in separate open sheds. During the summer, a combination of sprinkling and forced ventilation was practiced, as previously described (Flamenbaum et al., 1986). The cows were fed *ad libitum* a total mixed ration containing 1.74 Mcal of NE_L/kg of DM. They were milked three times daily, and milk yield and composition were recorded monthly. The BCS was determined, on a five-point scale (Wildman et al., 1982), by the same individual, once weekly during the 3 wk preceding calving, immediately after calving and at 3-wk intervals thereafter. The BCS recorded closest to insemination was regarded as the BCS at AI.

The herd was under veterinary health care throughout the experiments. All cows were routinely checked and treated for health disorders twice after calving; the first check was carried out approximately 1 wk after calving and the second 6 wk after calving. Cows that were diagnosed as having a retained placenta, or metritis were regarded as having a record of reproductive disorder. Retained placenta was defined as the presence of fetal membranes 24 h after calving. Metritis was diagnosed according to the color, smell, amount and

consistency of the vaginal discharge, and the size, position and tone of the uterus during rectal palpation and vaginal examination.

To maximize milk and calf production, primiparous cows were bred 80 to 100 d after calving and multiparous cows 60 to 80 d after calving. The management of reproduction was based on a scheme of estrous synchronization (Folman et al., 1984). At 3-wk intervals, a cluster of cows was given an estrous synchronization treatment. The experiment was conducted in accordance with the guidelines of the local ethics committee.

Experiment 1

The experiment was carried out during October and November, and involved a total of 46 cows. Estrus was synchronized by inserting a vaginal device containing 1.9 g progesterone (CIDR, Eazi Breed, Hamilton, New Zealand) into the vagina for 9 d, and injecting 500 µg of the PGF_{2α} analogue, cloprostenol (Estrumate, Coopers, Berkhamsted, UK) 2 d before removal of the insert. In order to avoid possible after effects of the synchronization procedure, experimental treatments were applied during the subsequent estrus (73 ± 1.5 d after calving) and not during the synchronized estrous period that followed the insert removal.

Starting 3 wk after the synchronized estrus, continuous visual observation of estrous behavior was carried out for 24 h daily over 5 d, by a team of two people. Immediately following the manifestation of the first signs of the spontaneous standing estrus, 11 cows received an i.m. injection of 250 µg of GnRH (2.5 ml of Fertagyl, Intervet, Holland), 10 cows received an i.m. injection of 10 µg of the GnRH-analogue, Buserelin (2.5 ml of Receptal, Hoechst AG, Germany), and 25 control cows received an i.m. injection of 2.5 ml of saline. The doses of GnRH and GnRH analogue used in this study were the doses recommended by the above-mentioned companies, for improvement of fertility. Blood samples for estradiol and LH determinations were collected every 8 h from 18 to 20 d after the synchronized estrus until the first signs of estrous behavior, and then every 3 h until 24 h after the manifestation of standing estrus. Following ovulation, blood samples for progesterone determination were collected daily during d 1 to 8 and every other day during d 10 to 20 of the cycle. Transrectal ultrasonography of the ovaries, with a 7.5 MHz probe (Aloka 210, Tokyo, Japan), was carried out 18 to 20 d after the synchronized estrus, to detect the preovulatory follicle and to confirm that the ovarian appearance was typical of the follicular phase. Commencing 20 h after the manifestation of standing estrus, ultrasonography of the ovaries was carried out every 4 h until ovulation, or until 50 h after OE in cows that

failed to ovulate by that time. Two hours before the time when ovulation could be discerned was regarded as the time of ovulation. The size of the corpus luteum subsequent to ovulation was determined by a single ultrasonographic screening, carried out 10 to 14 d after estrus.

Experiment 2

The experiment was carried out during the summer (July to October) and winter seasons (November to May). During the summer, mean daily maximum and minimum air temperatures and relative humidity were 29.7 and 18.9°C, and 71.6 and 51.7%, respectively; in the winter, the respective values were 22.0 and 11.1°C, and 76.8 and 54.4%, respectively. A total of 152 primiparous and 211 multiparous cows, all healthy, were included. Before the first postpartum AI, the cows were grouped into clusters that were synchronized at 3-wk intervals with two 500- μ g cloprostenol injections given 14 d apart. Estrous behavior of each cluster of cows was monitored for 6 d, starting 36 h after the second injection of PGF_{2 α} . Constant visual observation of estrous behavior was carried out for 19 h daily (between 0500 and 2400 h) by a team of two people. Only cows that were observed to be in estrus were included in the experiment. Cows of each cluster were randomly allotted to control or GnRH treatment groups, according to parity and BCS. Within 3 h following the detection of estrus, GnRH-treated cows received an i.m. injection of 10 μ g of the GnRH analogue Buserelin (2.5 ml of Receptal, Hoechst AG, Germany) and control cows received an i.m. injection of 2.5 ml of saline. Cows were inseminated once daily at a fixed time by a single inseminator. Cows that did not conceive as a result of the first postpartum AI, and returned to estrus, were assigned to the opposite treatment and re-inseminated. Approximately 45 d after AI, pregnancy diagnosis by rectal palpation was performed by the herd veterinarian. The experiment was carried out only with respect to the first two postpartum inseminations. The conception rate was defined as the number of cows diagnosed pregnant, expressed as the percentage of total number of inseminations performed within group.

Hormone Analyses

Plasma samples were extracted for estradiol determination with diethyl ether as described by Badinga et al. (1992). Extracted plasma samples were analyzed for estradiol-17 β concentrations by means of RIA, according to Badinga et al. (1992), and as validated in our laboratory (Shaham-Albalancy et al., 1997). The antibody, purchased from Diagnostic Products (Los

Angeles, CA) did not cross-react with testosterone or progesterone, and had low cross-reactivity with estriol (0.2%) and estradiol-17 α (0.01%). Assay sensitivity was 0.5 pg/ml, and the intra- and inter-assay coefficients of variation were 3 and 5%, respectively. Plasma progesterone concentrations of unextracted samples were analyzed with a solid-phase RIA kit (Diagnostic Product Corp., Los Angeles, CA), against a standard curve prepared in our laboratory by dissolving progesterone in plasma from an ovariectomized cow, as described previously (Shaham-Albalancy et al., 2000). The assay sensitivity was 0.2 ng/ml and the intra- and interassay coefficients of variations were 3.9 and 8.6%, respectively. Plasma LH concentrations were measured by enzyme immunoassay (EIA) with a biotin-streptavidin amplification system as described and validated for bovine plasma by Mutayoba et al. (1990). A highly purified bovine LH, USDA-bLH-B-6, was labeled with the Biotin Labeling Kit (Boehringer Mannheim, GmbH, Germany) according to the manufacturer's instructions. The assay was performed according to Mutayoba et al. (1990). A highly specific bovine LH antibody (USDA-309-684p) was used in a final dilution of 1:150,000, and a standard USDA-bLH-B-6 was used to prepare the standard curve (in hormone-stripped, charcoal-dextran-treated plasma) ranging from 0.39 ng/ml to 25 ng/ml. Twenty μ l of standard or unknown plasma were used in the assay. Absorbance was measured at 450 nm in a microtitration plate photometer. The assay was performed in duplicate; the intra- and inter-assay coefficients of variation were 8.5 and 10.6%, respectively, and the assay sensitivity was 7.8 pg/well. Values are expressed in ng of bovine LH per ml.

Statistical Analyses

In experiment 1, the elapsed times from OE to the peak of the LH surge and to ovulation, and the concentrations of the hormones were analyzed by means of the General Linear Model procedure of the Statistical Analysis System (SAS User's Guide, 1987, Cary, NC, USA). Differences between the two experimental groups, in those elapsed times, were analyzed by one-way ANOVA. The statistical model for hormonal concentrations (repeated measures design) included the effects of treatment (control vs. GnRH groups), cows (within treatment) which served as an error term for treatment, day of estrous cycle, and treatment-by-day interaction. In Experiment 2, multiple logistic regression was used to model the success of AI for winter and summer separately, as a function of treatment, parity, BCS, milk yield and reproductive disorders. The model also included interactions between treatment and parity, and the other variables. When interactions were

Table 1. Time intervals (h) between the onset of estrus, peak of the preovulatory LH surge and ovulation.

	Control n = 22	GnRH n = 21
Interval from OE ¹ to peak of LH surge.	3.1 ± 0.5	1.7 ± 0.4*
Interval from peak of LH surge to ovulation.	25.0 ± 0.4	23.9 ± 0.4
Interval from OE to ovulation of cows that ovulated within 50 h from OE.	28.2 ± 0.6	25.6 ± 0.6**
Interval from OE to ovulation of all cows ² .	30.8 ± 1.5	25.6 ± 0.4**

¹OE = onset of estrus.²Control group (n = 25) included three cows that ovulated later than 50 h after OE, and for which the interval to ovulation was designated as 50 h.**P* < 0.05.***P* < 0.01.

significant at *P* < 0.05 or treatment effects seemed to differ among subgroups, CR in the GnRH and control groups were compared by means of the chi-square test. Other specific comparisons between the GnRH and control groups were also made with the chi-square test.

RESULTS

Experiment 1: Effects of GnRH Injection at Onset of Estrus on Timing of Ovulation and Endocrine Responses

In the control group (n = 25), 19 cows ovulated within 30 h of OE, three cows ovulated later than 30 h, and three cows did not ovulate within the 50 h of ultrasonographic observation. The ultrasonographic screening that was performed 10 to 14 d after estrus revealed that the three control cows that did not ovulate within 50 h, had a corpus luteum indicative of eventual ovulation. Therefore, for those last three cows, a minimum value of 50-h was designated to the interval from OE to ovulation. Of the six cows (24% of control cows) that ovulated later than 30 h after OE, one was primiparous and the other five were multiparous animals. In the GnRH group (n = 21), all cows ovulated within 30 h of OE. As there were only small and nonsignificant differences, between cows that were treated with Fertagyl and cows that were treated with Buserelin, the data of the two subgroups were combined and are presented as those of the GnRH group. The GnRH treatment decreased the time interval from OE to the LH surge peak (3.1 ± 0.5 vs. 1.7 ± 0.4 h; *P* < 0.05), but did not affect the interval from the peak of LH surge to ovulation (Table 1). Thus, the significant difference between the control and the GnRH groups (that ovulated within 50 h of OE), in the interval from OE to ovulation, was the result of the differing intervals from OE to the peak of the LH surge (Table 1).

The peak of the preovulatory LH surge of control cows (excluding the three cows that ovulated later than 50

h after OE) was 9.7 ± 1.1 ng/ml, compared with 15.5 ± 1.1 ng/ml in the GnRH group (*P* < 0.01; Figure 1). The area under the curve of the LH surge was calculated as the integral of the LH concentrations over a period extending from 6 h before to 6 h after the peak of the LH surge. Analysis indicated that the area under the curve was increased by the GnRH treatment: 46.1 ± 3.6 and 61.2 ± 3.7 ng/ml in the control and treatment groups, respectively (*P* < 0.05).

Plasma estradiol concentrations at OE did not differ between the treatments (Figure 2). In the control group, the coefficients of correlation between plasma estradiol concentrations 8 h before OE and at OE, and the height of the LH peak were 0.40 (*P* < 0.05) and 0.56 (*P* < 0.01), respectively. In the GnRH-treated group, the coefficients of correlation between the same variables were small and not statistically significant. Plasma progesterone concentrations before estrus did not differ among

Figure 1. Peripheral plasma LH concentrations before and after the preovulatory LH surge of control cows (□) and GnRH-treated cows (■). The height of the surge and the area under the curve of control cows are less than those of treated cows (*P* < 0.05).

Figure 2. Peripheral plasma concentrations of estradiol before and after the onset of standing estrus in control (□) and GnRH-treated cows (■).

the experimental groups (data not shown). There was also no significant difference in progesterone concentrations between the GnRH and control groups during the subsequent estrous cycle (Figure 3). Similarly, the mean diameter of the corpus luteum, as determined between 10 and 14 d after estrus, did not differ between the control and the GnRH-treated groups (25.4 ± 0.6 and 25.9 ± 0.6 mm, respectively). On d 11 to 16 of the estrous cycle, the mean progesterone concentration of the GnRH group was 6.5 ± 0.41 , compared with 5.6 ± 0.33 ng/ml in the control group; this difference only tended to be significant ($P < 0.12$). However the proges-

Figure 3. Peripheral plasma progesterone concentration during the estrous cycle following the GnRH administration in control (□) and GnRH-treated cows (■).

terone concentration of the GnRH group was higher than that of the six control cows that did not ovulate within 30 h of OE (6.5 ± 0.4 vs. 4.5 ± 0.2 ng/ml; $P < 0.01$).

Experiment 2: Effects of GnRH Injection at Onset of Estrus on Conception Rate, in Relation to Season, Parity, Body Condition and Reproductive Disorders

Logistic regressions to model CR as a function of treatment, BCS at AI and parity, together with interactions of treatment by BCS and treatment by parity, showed that in summer BCS had a significantly positive effect on CR ($P < 0.03$), and the tendency to significance of treatment by parity interaction ($P < 0.08$) suggested a stronger effect of treatment on primiparous cows. The same model for the winter data showed a positive effect for treatment ($P < 0.05$) and borderline significance ($P < 0.06$) for treatment by BCS interaction, showing a weaker treatment effect for cows with high BCS. For data of both seasons together, the season effect was added to the above model. The treatment, BCS and winter main effects were significantly positive ($P < 0.02$, $P < 0.01$ and $P < 0.002$, respectively), and the treatment by BCS and treatment by parity interactions were also significant ($P < 0.03$ and $P < 0.05$, respectively). Further chi-square tests showed the following specific results. Administration of GnRH at OE increased the overall CR by 14.2 percentage units ($P < 0.01$). This increase was due mainly to the significant effect of GnRH in summer, when it increased the CR from 35.1 to 51.6% ($P < 0.01$; Table 2). The effect of GnRH treatment on primiparous cows was statistically significant ($P < 0.01$), whereas the effect on multiparous cows was not significant (Table 2). The increase was again mainly due to a significant effect of treatment during the summer, when GnRH almost doubled the CR of primiparous cows (from 33.3 to 60.3%; $P < 0.01$). It is noteworthy that GnRH-treated cows had a similar CR in summer to that of control cows in winter.

Cows were divided into low- and high-BCS subgroups. For each experimental group, within parity and season, the value of the median BCS at AI was calculated. Cows with BCS lower or higher than the respective median values were designated to low- or high-BCS groups. Mean values of BCS of the low- and high-BCS subgroups were, respectively, 3.0 ± 0.02 and 3.3 ± 0.03 ($P < 0.01$) at calving, and 2.2 ± 0.02 and 2.8 ± 0.02 ($P < 0.01$) at AI, and the decreases in BCS between calving and AI were 0.80 ± 0.02 and 0.55 ± 0.02 ($P < 0.01$), respectively. In both seasons, GnRH increased the CR of cows with a low BCS at AI from 36.0 to 61.5% ($P < 0.01$), but did not affect the CR of cows with a

Table 2. The effect of GnRH administered to cows at onset of estrus on conception rates¹ (%) in relation to BCS at AI² and parity following the first and second postpartum AI, during summer and winter.

	Summer		Winter		Summer and Winter	
	Control	GnRH	Control	GnRH	Control	GnRH
BCS at AI						
Low	30.7 (75) ³	53.8** (78)	47.2 (36)	76.9*** (39)	36.0 (111)	61.5** (117)
High	39.0 (82)	49.4 (79)	61.5 (39)	50.0 ^b (42)	46.3 (121)	49.6 (121)
Parity						
Primiparous	33.3 (57)	60.3** (63)	57.6 (33)	68.8 (32)	42.2 (90)	63.2*** (95)
Multiparous	36.0 (100)	45.7 (94)	52.4 (42)	59.2 (49)	40.8 (142)	50.3 ^b (143)
Overall	35.1 (157)	51.6** (157)	54.7 (75)	63.0 (81)	41.3 (232)	55.5** (238)

¹Conception rate = Number of cows diagnosed pregnant, expressed as the percentage of total number of inseminations performed within group.

²BCS = body condition score. Cows of each experimental group, within parity and season, were divided into low and high BCS by their respective medians. Mean values of low- and high-BCS groups at AI are: 2.2 and 2.8, respectively.

³No. of AI in parentheses.

** $P < 0.01$, significantly different from the control group.

^{a,b}Conception rates within the same column with no common superscript letters differ significantly ($P < 0.05$).

high BCS at AI (Table 2). Interestingly, among GnRH-treated animals in the winter, cows with a low BCS at AI had a higher CR than cows with a high BCS at AI (76.9 vs. 50.0%; $P < 0.05$).

The timing of AI within the estrous period affected the CR of control cows in summer but not in winter (Table 3): the CR of cows inseminated between 4 and 20 h after the detection of estrus was almost twice as high as those of cows receiving AI before 3 h or after 20 h ($P < 0.06$). In summer, the greatest increases in CR, in GnRH-treated compared with control cows, were associated with the early AI (+20 percentage units; NS) and late AI (+47%; $P < 0.01$). No such tendency could be detected during the summer in the GnRH-treated group, in which a nonsignificant increase in CR with increasing time between OE and AI was noted. It should be noted that the data on early and late AI should be treated with caution, because of the small numbers of such inseminations.

The effect of GnRH on CR was not affected by milk yield. When the data from cows of both treatments were combined, cows with a high milk yield (46.2 ± 0.7 FCM, kg/d; average of the higher median) during the second and third months after calving, had a higher CR (53.7 vs. 43.5%; $P < 0.05$) than those with a lower milk yield (38.8 ± 0.6 FCM, kg/d; average of the lower median).

In summer, control cows with a low BCS at AI, and those that had postpartum reproductive disorders, had a lower CR than healthy cows (11.8 vs. 38.7%, $P < 0.05$; Table 4), whereas similar cows that received GnRH treatment had a similar CR to that of healthy cows. Overall, GnRH treatment more than doubled the CR of cows with reproductive disorders ($P < 0.01$), whereas its effect in healthy cows was nonsignificant.

DISCUSSION

It was previously suggested that administering GnRH at estrus might prevent ovulation failure or re-

Table 3. The effect of GnRH on conception rates¹ (%) of cows in relation to timing of AI during the estrous period in summer and winter.

Time interval from OE ³ to AI, h	Summer		Winter	
	Control	GnRH	Control	GnRH
0–3	22.7 (22) ²	42.9 (21)	55.6 (9)	55.6 (9)
4–20	38.7 (124)	51.3* (119)	51.9 (54)	62.5 (64)
21–24	18.2 ⁴ (11)	64.7* (17)	66.7 (12)	75.0 (8)

¹Conception rate = Number of cows diagnosed pregnant, expressed as the percentage of total number of inseminations performed within group.

²No. of AI in parentheses.

³OE = onset of estrus.

⁴0 to 3 h and 21 to 24 h AI in summer control cows gave lower conception rates than in control cows AI to 20 h after OE ($P < 0.06$).

* $P < 0.05$ significant from the control group in the summer.

Table 4. The effect of GnRH on conception rates¹ (%) in the summer in cows with postpartum reproductive disorders.

BCS at AI ²	Healthy cows		Cows with disorders	
	Control	GnRH	Control	GnRH
Low	38.7 (75) ³	50.8 (65)	11.8* (17)	51.9** (27)
High	38.8 (49)	50.0 (46)	31.3 (16)	57.9 (19)
Overall	38.7 (124)	50.0 (111)	21.2 (33)	54.4** (46)

¹Conception rate = Number of cows diagnosed pregnant, expressed as the percentage of total number of inseminations performed within group.

²BCS = body condition score. Cows of each group and parity were divided into low and high BCS by their respective medians.

³No. of AI in parentheses.

* $P < 0.05$, significant from the respective healthy control group.

** $P < 0.01$, significant from respective control cows with disorders.

duce the variation in the time interval to ovulation (Coulson et al., 1980; Nakao et al., 1984). In the present study, all GnRH-treated cows ovulated within 30 h after OE, whereas 24% of the control cows ovulated later. This delay in ovulation relative to the timing of estrus and AI might reduce fertilization (Saacke et al., 2000) and pregnancy rates. An extreme delay in ovulation, such as those recorded in the three control cows that ovulated later than 50 h after OE, in the present study, could affect fertilization and also delay the establishment of luteal function (Larson et al., 1997) and consequently reduce fertility. Thus, if GnRH treatment were given at OE, failure or delay of ovulation might be prevented and CR might increase. On the other hand, if GnRH is administered at the time of AI, especially when detection of estrus is not carried out frequently, the administration is likely, in most cases, to be rather late in estrus, with unknown effects on the timing of ovulation, and a prospective decline in plasma progesterone levels in the subsequent luteal phase (Ryan et al., 1994).

It has been shown previously, in first postpartum AI cows, that when GnRH is administered at OE, the GnRH-induced LH peak coincides with the spontaneous LH peak, and the resulting merged peak is higher than either the spontaneous LH peak in the control group or the peak induced late in estrus by GnRH (Rosenberg et al., 1991). In the present study similar results were obtained: GnRH at OE increased both the LH peak height and the area under the curve, whereas, in contrast, in most other studies GnRH administered at AI induced a second LH peak and did not increase the spontaneous one (Lee et al., 1985; Lucy and Stevenson, 1986; Ryan et al., 1994). These findings could be related to the fact that repeat breeder heifers have a smaller preovulatory LH surge than virgin heifers (Gustafsson et al., 1986) and, therefore, an increase in the spontaneous surge that results from the administration of GnRH at OE affects the CR favorably.

The fact that six control cows ovulated more than 30 h after OE—three of them more than 50 h after OE—could be related to the low preovulatory LH surge in the control group. Recently, we characterized a subgroup of high-yielding Holstein cows with very long intervals between estrus and ovulation, and between LH surge and ovulation; they were associated with a markedly low preovulatory LH surge and lower than usual concentrations of estradiol in the follicular phase before estrus (Bloch et al., 2001). These findings are in agreement with those of the present study, in which we found in the control group a significant correlation between estradiol concentrations at and before estrus, and the height of the LH peak. Collectively, these results suggest that the phenomenon of an unusually extended interval between estrus and ovulation may result from impairment of the preovulatory follicle function. The results indicate that the GnRH treatment may have enabled cows with low estradiol concentrations before estrus to achieve a larger LH peak, which, in turn, enabled all the cows to ovulate within 30 h of OE. This interpretation is also supported by the lack of significant correlation, in the treatment group, between estradiol levels during proestrus and the height of the peak LH surge. It should be mentioned, however, that, to the best of our knowledge, there is no direct evidence for a relationship between a low LH surge and a long interval to estrus.

In various studies, progesterone concentration has either increased, decreased or remained unchanged during the luteal phase, following GnRH administration (Lee et al., 1985; Lucy and Stevenson, 1986; Mee et al., 1993; Ryan et al., 1994; Ullah et al., 1996). In these experiments GnRH or its analogues were administered in different seasons, at first postpartum AI or to repeat breeders, either at OE or later in estrus, following estrous behavior or at a fixed time after PGF_{2α} treatment. The many variations in the conditions under which GnRH was administered may have led to dif-

fering effects on the formation of the corpus luteum and, hence, on the plasma progesterone levels during the subsequent luteal phase. In the present study, no significant difference in plasma progesterone concentrations between overall control and GnRH groups could be discerned. However, control cows with a long OE-to-ovulation interval had lower progesterone concentrations than GnRH-treated cows, during days 11 to 16 of the subsequent estrous cycle. Furthermore, we have previously shown that untreated cows with a very long interval between estrus and ovulation had significantly lower plasma progesterone concentrations in the mid-luteal phase of the subsequent estrous cycle than cows with a normal interval (Bloch et al., 2001). These results indicate that at least some cows have a low preovulatory LH surge, ovulate late after estrus, and may be deficient in progesterone in the subsequent luteal phase. In light of reports that repeat breeder cows and heifers had lower plasma progesterone levels than control animals in the estrous cycle subsequent to AI (Gustafsson et al., 1986; Kimura et al., 1987; Maurer and Echternkamp, 1985), the GnRH-induced increase in blood progesterone levels in repeat breeder cows (Mee et al., 1993) might be one of the causes of the increased conception rates in these cows, as well as of those found in other experiments in which cows exhibited low progesterone concentrations (Ullah et al., 1996).

Administering GnRH or its analogues to cows at the time of AI increased CR in some experiments, but not in others (Mee et al., 1990; Stevenson et al., 1990). With late AI, cows treated early in estrus had a higher CR than those treated late in estrus (Rosenberg et al., 1991). The latter study, conducted in the winter, and that by Ullah et al. (1996) under heat-stress conditions, both indicated that GnRH treatment at OE significantly improved CR, particularly when the CR of untreated cows was low. In keeping with these results, the present study found that CR was improved by GnRH in summer, when the CR of control cows was low, and not in the winter when it was markedly higher. It is interesting that within the control group, in summer but not in winter, early and late AI resulted in lower CR than AI administered between 4 and 20 h after OE. These findings are similar to those reported by Nebel et al. (2000) based on a large data set collected from 17 herds and 2661 inseminations, in which OE was detected by the radiotelemetric HeatWatch system. In contrast, the CR in GnRH-treated cows increased gradually with increasing time between OE and AI, and the difference in CR between those receiving AI early and those inseminated late reached a maximum of 47%. These findings are in agreement with those of Saacke et al. (2000) and Dalton et al. (2001), who reported that

early AI reduced the fertilization rate, and that late AI reduced the embryo quality.

The findings of the present study indicate that GnRH treatment increased the CR mainly in cows with a low BCS at AI. The greater postpartum body condition deterioration in cows with a low BCS at AI, observed in the present study, suggests that these cows had a greater negative energy balance after calving than those with a high BCS at AI (Butler and Smith, 1989; Ferguson and Otto, 1989). It seems reasonable to assume that the main reason for the positive effect of GnRH on the CR of low BCS cows is that these cows also had lower CR than the high-BCS ones within each season, so that there was a greater potential for fertility improvement. A greater effect of GnRH, administered at OE, on cows exhibiting low fertility was also reported by Rosenberg et al. (1991). Recently, Butler (2000) suggested that the low fertility of the dairy cow under negative energy balance is associated with low estrogen and LH secretion, which could be due to low glucose, insulin and IGF-I secretion, and that part of the low-fertility syndrome could be related to low secretion of progesterone as well. Interestingly, in contrast to the present study, in timed AI protocols (Ovsynch), in which GnRH was injected at a fixed time after PGF_{2α} injection and cows were inseminated at a further fixed time later (and not at OE, as in the present study), CR was higher in cows with high BCS than in cows with low BCS (Moreira et al., 2000). The higher response to GnRH in cows with high BCS, found in the latter study, was attributed to an earlier postpartum resumption of cyclicity.

The reason for the greater beneficial effect of GnRH on primiparous cows than on multiparous cows is unclear. Primiparous cows usually have a higher CR than multiparous cows; a 7- to 8-percentage unit higher conception rate for primiparous cows was recorded in 2000 in the Israeli Holstein Herd book. It may be speculated that GnRH treatment removes various limiting factors that decrease CR, and thus allows better realization of the fertility potential, which may be higher in primiparous cows. Similarly, the reason for the greater beneficial effect of GnRH on cows with postpartum reproductive disorders is also unclear. It has been shown that postpartum reproductive disorders are associated with increased risk for abnormal cyclicity (Opsomer et al., 2000). It may be speculated that abnormal cyclicity is associated with low preovulatory LH secretion, delayed ovulation and low progesterone secretion after estrus. It is hypothesized that GnRH at OE improved endocrine responses that had been impaired by the previous uterine disorders.

GnRH treatment almost annulled the negative effect of summer heat stress on fertility; however, the effect was pronounced and significant in primiparous cows

but not in multiparous cows. A possible explanation for the greater effect of GnRH in summer could be related to an attenuated preovulatory LH surge and its possible association with low postovulation progesterone concentrations. Lactating dairy cows under chronic summer heat stress or under acute heat exposure exhibited a reduced preovulatory LH surge (Gilad et al., 1993). Recently, possible relationships between low LH surge and low in vitro secretion of progesterone by luteinized granulosa cells, and between a low GnRH-induced LH surge and low plasma progesterone concentrations at mid-luteal phase were reported (Less et al., 1998; Biger et al., 2000). A similar relationship between low LH surge and low postovulation progesterone concentration was found in primates (Zelinski-Wooten et al., 1997). In agreement with these findings, hCG administered to cows on day 5 of the cycle induced a greater increase in progesterone concentrations than the GnRH analogue Buserelin, that is known to induce a narrow LH surge (Schmitt et al., 1996), and the potent GnRH implant Deslorelin induced a higher surge of LH and higher postovulation progesterone concentrations than Buserelin (Ambrose et al., 1998; Rajamahendran et al., 1998). Collectively, the above observations could be associated with the findings that plasma progesterone concentrations are lower in cows under chronic summer heat stress than in their counterparts under winter conditions (Wolfenson et al., 2002). Under the former conditions, GnRH treatment at OE has a greater potential to improve hormonal secretion and hence the fertility of lactating cows in the summer.

CONCLUSIONS

Use of GnRH at the onset of estrus increases the spontaneous LH peak, prevents delays in ovulation, and induces uniformly high postovulation progesterone concentrations. Under these conditions, GnRH has the potential to increase conception rates considerably in high-yielding dairy cows under low-fertility conditions. Treatment with GnRH may yield better fertility results in cows with a low BCS at AI, and in primiparous cows than in multiparous ones. The effect of GnRH on increasing conception rate is greater in summer, under heat-stress conditions, than in winter.

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