New clinical uses of GnRH and its analogues in cattle


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**ABSTRACT**

Gonadotropin-releasing hormone (GnRH) and its analogues cause an acute secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) such that concentrations in peripheral blood are elevated for a 3–5 h period. GnRH-induced alterations in the function of the corpus luteum (CL) or follicle appear to be indirect through alterations in LH and FSH secretion. Repeated injections of GnRH during diestrus or single injections late in diestrus will cause acute increases in plasma progesterone and a delay in CL regression. Injections or continual administration of GnRH during early phases of CL development appear to augment CL differentiation and alter subsequent CL function. These effects are attributable to induced increases in LH. Injections of GnRH during the estrous cycle will re-synchronize follicle development owing to ovulation or luteinization of the dominant follicle leading to subsequent recruitment and selection of a new dominant follicle during a 7 day period. Injection of GnRH followed by injection of prostaglandin (PGF$_{2\alpha}$) at 6 or 7 days is a system of estrous synchronization in which follicle development and CL regression are both synchronized and fertility at the induced estrus is good. Injection of GnRH during the luteal phase post-insemination (e.g. Days 12–14) or post-embryo transfer, to alter CL and/or follicular function, has not resulted in a consistent increase in pregnancy rates. An overall assessment of studies that injected GnRH at the time of insemination in first service postpartum cows or in repeat breeders is rather disappointing. Considerable variation existed among studies within both types of cow populations relative to significant differences, directions of pregnancy rate change (+ vs. −), and magnitude of pregnancy rate increases. Recent findings indicate that timing of GnRH injections closer to the onset of estrus may be beneficial in increasing the conception rate. Utilization of GnRH in combination with progesterone and PGF results in an acute treatment sequence to program follicular development, ovulation and a subsequent cycle in cows with follicular cysts. The use of GnRH, with or without PGF, as a reproductive management program in the early postpartum period has not shown a clear improvement in subsequent reproductive efficiency. Development of precise systems to control ovarian function and reproductive efficiency with GnRH and other pharmaceutical agents is possible. However, such advancements must be founded on a clear understanding of GnRH-induced physiological effects and ability to capture any advantage by good management of the farm unit.

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INTRODUCTION

Gonadotropin-releasing hormone (GnRH) is a decapeptide hormone synthesized in cell bodies of neurosecretory neurons located in the mediobasal hypothalamus and secreted into the primary capillary bed of the median eminence. GnRH is responsible for the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the pituitary. With the chemical identification of GnRH and its synthesis, a new and powerful drug became available for reproductive management and production medicine in cattle. Alterations in the chemical structure of the native GnRH molecule have led to the synthesis of potent GnRH agonists. Substitutions usually involve replacement of the glycine molecules at positions 6 and 10 with a D-amino acid at position 6 and/or an N-ethylamide group at position 10. The following GnRH analogues and GnRH agonists are available commercially: Buserelin (D-serine at position 6 and ethylamide at position 10; Receptal, Hoechst AG, Frankfurt, Germany), native GnRH (Gonadorelin diacetate tetrahydrate; Cystorelin, Sanofi Animal Health, Overland Park, KS), native GnRH (Gonadorelin hydrochloride; Factrel, Fort Dodge Laboratories, Fort Dodge, IA), and Fertirelin acetate (ethylamide at position 10; Ovulyse, Takeda Chemical Industries, Ltd., Osaka, Japan). The design of GnRH agonists has been directed toward stabilization of the molecule against enzymatic attack, increasing binding to plasma proteins and membranes, and increasing the affinity of the agonist for the GnRH receptor (Conn and Crowley, 1991).

Owing to alterations in chemical structure, marked differences exist between the various GnRH analogues in relative potencies to release LH and FSH in cattle (Nawito et al., 1977; Chenault et al., 1990). For example, Fertirelin acetate was approximately four to ten times more potent than Gonadorelin as measured by LH and FSH release during the luteal phase of the bovine estrous cycle; Buserelin was 50 times more potent than Gonadorelin (Chenault et al., 1990). Based upon a carryover effect of a moderate dose followed by a low dose of these products, the pituitary's gonadotropes apparently become refractory to GnRH for up to 48 h after injection of doses greater than or equal to 50 μg Fertirelin acetate, 500 μg Gonadorelin and 10 μg Buserelin. A single injection (i.m.) of GnRH and GnRH agonists gives a predictable release of both LH and FSH into the peripheral circulation over a 3 h period.

BIOLOGICAL EFFECTS OF GnRH

Any rational use of GnRH or its agonists, for implementation in various reproductive management systems or their use to increase conception rates, should be based on a thorough understanding of GnRH-induced biological effects on the reproductive-endocrine system. Hypothetically, GnRH-in-
duced effects can be indirect through their induced release of LH and FSH (Chenault et al., 1990) or perhaps direct effects of GnRH on reproductive tissues (Hsueh and Jones, 1981). Two potential gonadotrophin responsive tissues within the ovary are the corpus luteum (CL) and the follicle.

Corpus luteum

The effects of GnRH on CL development and function have been examined in cattle. Milvae et al. (1984) demonstrated that doses of GnRH from 10 to 80 ng failed to alter basal biosynthesis of progesterone by dispersed bovine luteal cells, whereas the high (100 ng) dose of GnRH decreased basal and LH-stimulated production of progesterone. Differences among doses of GnRH were examined by Student’s t-test; a trend for a linear decline due to a dose of GnRH was observed but was not tested statistically by regression analysis. In a subsequent study (Ireland et al., 1990), bovine luteal cells were shown not to have GnRH receptors and progesterone production was not inhibited by GnRH. Collectively, these results indicate that GnRH will not have direct stimulatory or inhibitory effects on the CL to increase progesterone production. Brown and Reeves (1983) demonstrated the absence of specific GnRH receptors in bovine ovarian tissue; thus, potential GnRH effects must be mediated indirectly via alterations in endogenous gonadotropin secretion or directly via a non-receptor mediated mechanism.

Subcutaneous injection of a GnRH agonist (Buserelin, 10 µg) four times daily on Days 9–12 of the estrous cycle increased the luteal phase concentrations of plasma progesterone and extended the estrous cycle until 26.2 days (Milvae et al., 1984). Associated with the injections of GnRH agonist were increases in plasma LH that became attenuated with repeated injections; attenuation was likely due to desensitization of gonadotropes. The elevation in plasma progesterone contributed to the well known luteotrophic effect of LH on the bovine CL (Hansel et al., 1973). Even single injections of a potent GnRH agonist (Buserelin, 10 µg) after Day 11 can increase luteal lifespan and cycle length without any associated chronic increase in plasma progesterone concentrations (Macmillan et al., 1985). When a GnRH agonist (Buserelin, 10 µg) was injected once between 12 and 14 days post-insemination in dairy cows, an acute increase in progesterone was detected in both pregnant and non-pregnant cows, and concentrations of progesterone in pregnant cows were subsequently comparable to control cows that were pregnant (Lajili et al., 1991). This single injection of GnRH extended the luteal phase and inter-estrous interval in non-pregnant cows. Short-term increases in concentrations of serum progesterone, 0.5–6.0 h after injection of GnRH, have been reported by numerous workers (Kittok et al., 1973; Zolman et al., 1974; Thatcher and Chenault, 1976; Thompson et al., 1980; Macmillan et al., 1985). Such increases in progesterone concentrations may be attributed to the luteotrophic
response of small luteal cells to LH that is released in response to GnRH (Hansel et al., 1991). Thus, the developed CL does indeed appear to be responsive to treatment with GnRH.

The timing of GnRH treatments may have differential effects on subsequent CL function. For example, injection of GnRH in the periestrous period, either before or concurrently with the preovulatory surge of LH, attenuated subsequent progesterone concentrations in serum during the first 7 days of the estrous cycle (Lucy and Stevenson, 1986). Likewise, exposure of the developing CL (Day 2 of estrous cycle) to GnRH-induced secretion of LH caused a subsequent decrease in serum progesterone concentrations during the estrous cycle (Rodger and Stormshak, 1986). GnRH treatment on Day 2 reduced the concentration of unoccupied LH receptors in luteal tissue collected either on Day 8 or 14 of the estrous cycle. An augmentation in differentiation of the CL was suggested, in which the rate of transformation of small luteal cells to large luteal cells was increased. Indeed, injection of GnRH on both Days 2 and 8 of the estrous cycle increased basal secretion of progesterone by luteal slices, and addition of LH to the media caused no further increase in progesterone secretion (Martin et al., 1991). Such an in vitro luteal

![Graph](image)

Fig. 1. Size of corpus luteum (CL) and plasma progesterone responses to insertion of three implants of a GnRH agonist (Deslorelin) or placebo at the onset of detected estrus and left in place for either 12 (Deslorelin) or 24 days (Deslorelin and placebo) after estrus.
response is characteristic of large luteal cells which exhibit higher basal progesterone production and are less responsive to LH stimulation than small cells (Hansel et al., 1991). Recently, Mee et al. (1993) reported that injection of GnRH at estrus increased the proportion of large luteal cells in the CL on Day 10 of the estrous cycle. It is apparent from these studies that GnRH injections can have variable effects on CL function, depending upon when they are given in the cycle (periestrus or early luteal phases vs. mid- and late-luteal phases of the estrous cycle).

Recent studies in our laboratory (K. Entwistle and W.W. Thatcher, unpublished data, 1993) examined the effect of inserting three implants containing 3.7 mg per implant of a GnRH agonist (Deslorelin, Peptide Technology, Sydney, Australia) at the onset of detected estrus and left in place for either a 12 or a 24 day period. The sustained release of GnRH stimulated CL development based on enhanced CL size (measured by ultrasonography) and higher concentrations of plasma progesterone in the luteal phase of the estrous cycle compared with control cows receiving a placebo implant (Fig. 1). The implants were still active 24 days after insertion since CL regression was completed, but subsequent detected estruses and CL formation had not occurred by 30 days after estrus. The latter inhibition of estrus, ovulation and CL formation may have been due to receptor down-regulation. Early phase modulation of CL function by GnRH manipulation warrants further investigation and potential application to optimize reproductive management.

**Follicle**

Administration of GnRH repeatedly in low doses will stimulate terminal follicle development in cattle. This has been vividly demonstrated within two distinctly physiological periods in cattle. Peters et al. (1985) repeatedly injected small doses of GnRH over a 2 day period in early postpartum dairy cows. Such a treatment stimulated follicle function based on GnRH-induced rises in LH and plasma estradiol concentrations. Thus, restoration of basal LH secretion in the early postpartum period initiates recrudescence of ovarian follicular function.

The high concentration of plasma progesterone during the luteal phase of the estrous cycle inhibits estradiol secretion and causes turnover of anovulatory follicle waves during the estrous cycle (Savio et al., 1993). This is due to the negative feedback effect of luteal phase progesterone concentrations in the circulation that reduces pulsatile secretion of LH. Glencross (1987) demonstrated that pulsatile infusion of GnRH (5 µg given hourly for 5–11 days) during the luteal phase of the estrous cycle increased plasma estradiol to typical preovulatory peak concentrations (7.5 pg ml⁻¹) by 3 days of infusion and was associated with follicular development as assessed by ovarian palpation per rectum. This physiological stimulation of follicle development oc-
curred in the presence of luteal phase progesterone concentrations, and reinforces the importance of LH pulse frequency to control terminal antral follicle development. Thus, selection and sustained dominance of follicles could potentially be regulated pharmacologically by delivery of GnRH through a proper delivery system that induces a proestrus LH pulse frequency of approximately one pulse per hour.

An alternative to repeated physiological injections are pharmacological injections of GnRH; the acutely induced change in LH (Chenault et al., 1990) should act like a normal preovulatory surge of LH and FSH that induces a decline in plasma estradiol (associated with luteinization), ovulation of the dominant periestrus follicle (Thatcher and Chenault, 1976), and subsequent recruitment and selection of an anovulatory dominant follicle over a 7 day period (Sirois and Fortune, 1988; Savio et al., 1988; Ginther et al., 1989). Whether this complete sequence of events is initiated at all stages of the estrous cycle when GnRH is given will depend on ovarian follicular status at the time of injection.

Macmillan and Thatcher (1991) evaluated the effects of a single injection of a GnRH agonist (Buserelin, 10 µg, i.m.) on the characteristics of ovarian follicles in cows and heifers when given between Days 11 and 13 of the cycle. The total number of follicles observed on the ovaries by ultrasonography was not altered by Buserelin treatment. However, Buserelin increased the number of cloudy follicles in small (3–5 mm), medium (6–9 mm) and large (over 9 mm) follicular classes and reduced the number of clear follicles compared with the control group. The classification of cloudy applied to follicles that contained flocculent material or that had an indistinct basement membrane; these changes gave the follicular fluid a cloudy appearance in contrast to a uniform black one. The induction of a cloudy appearance in follicles is probably associated with induced luteinization. The Buserelin-induced changes were detected by Day 13 for medium and large follicular classes but were greatest on Days 14 and 16. The average number of medium and large follicles which were clear or cloudy were similar on Days 18 and 20, indicating that treatment effects on follicular status lasted for 4–6 days. By Day 20, a large clear follicle had emerged that became the ovulatory follicle in each animal injected with Buserelin. The presence of a single clear ovulatory follicle by Day 20 represented a newly selected follicle compared with the control group. In essence, treatment with the GnRH agonist synchronized follicular development. Some of the large follicles present at the time of injection ovulated. Comparable changes in ovarian follicular dynamics, induced by Buserelin, have been reported by Guilbault et al. (1990).

The process of premature luteinization could alter the functional integrity of the theca and granulosa layers of a developing follicle, including the potential capacity to produce estradiol. Buserelin injection reduced the variance in plasma estradiol concentrations on a within-cow basis (Macmillan and
Thatcher, 1991). Rettmer et al. (1992b) demonstrated that injection of a less potent GnRH agonist (Fertirelin acetate, 200 μg) between Days 11 and 13 of the estrous cycle caused a biphasic response in serum estradiol: an acute increase in estradiol occurred between 4 and 6 h, followed by estradiol concentrations that were chronically lower during the subsequent 7 day period. Collectively, these results clearly indicate that follicular development and function can be regulated by administration of GnRH analogues during the luteal phase of the estrous cycle.

The most dramatic increase in LH secretion during the estrous cycle is the endogenous preovulatory surge of LH occurring at the onset of estrus. In a comparison between beef cattle that conceived or did not conceive to an artificial insemination, there was no difference in the timing of the preovulatory surge of LH that occurred within 6 h after onset of estrus in all cows (Grieger et al., 1991). Thus, a delayed preovulatory surge of LH does not appear to be a factor contributing to lack of conception. LH of the preovulatory peak induces meiotic maturation of oocytes in vivo. Perhaps injection of a GnRH agonist at the time of the endogenous preovulatory LH surge amplifies plasma LH and affects the process of oocyte maturation. Indeed, if GnRH or GnRH agonists are given just prior to, or at the time of the LH surge, the spontaneous/induced preovulatory surge of LH is amplified (Lucy and Stevenson, 1986; Laurincik et al., 1991; Rosenberg et al., 1991). Injection of a GnRH agonist in superovulated (20 mg of FSH given in declining doses over 4 days beginning on Day 13 of the cycle with Cloprostenol injected 48 h after the first FSH dose) heifers 40 h after Cloprostenol injection increased the rate of oocyte maturation by 65 h after Cloprostenol injection (Laurincik et al., 1991). The distinguishing difference in LH profiles was that the timing of maximal LH concentrations associated with the preovulatory surge were the same, but maximal release of LH (either maximal LH concentration or area under the curve) was markedly greater in GnRH agonist treated heifers. Consequently, the timing of GnRH or GnRH agonist injections to complement the endogenous preovulatory LH surge may be critical to increasing the efficiency of subsequent events associated with oocyte maturation and ovulation. This point needs to be considered when developing or interpreting strategies to improve reproductive efficiency with the administration of GnRH or its agonists.

APPLICATIONS OF GnRH FOR REPRODUCTIVE MANAGEMENT

Estrous synchronization

Programming follicular development by injection of a GnRH agonist (Macmillan and Thatcher, 1991), provided a basis for development of an estrous synchronization system in which both follicular development and CL
regression are controlled (Thatcher et al., 1989). Treatment of heifers with Buserelin (6 \mu g) followed 7 days later by injection with prostaglandin F_{2\alpha} (PGF_{2\alpha}) increased the number of animals synchronized within a 5 day period and also enhanced the precision of synchrony 2 and 3 days after PGF_{2\alpha} injection compared with cows treated with PGF_{2\alpha} only. Additional studies have been conducted in which estrus was synchronized with the injection of Buserelin (8 \mu g; experimental Day 0) followed 8 (Experiment 1) or 7 (Experiment 2) days later by an injection of PGF_{2\alpha} (25 mg; Lutalyse, Upjohn, Kalamazoo, MI). Following the synchronized estrus, heifers were assigned randomly to receive a Buserelin injection (8 \mu g) at 12 days post-insemination or no treatment. Approximately 89% of the heifers were detected in estrus for Experiments 1 and 2 (Table 1); however, a 7 day treatment interval (Experiment 2) gave a better synchrony of estrus than an 8 day interval (Experiment 1). With the 7 day interval between GnRH agonist and PGF_{2\alpha} injection, 96% of the detected estruses (n = 157) occurred on experimental Days 7–11 (distribution of estruses by experimental days (D): D4, 2.6% (n = 4); D5, 1.3% (n = 2); D6, 0%; D7, 7.6% (n = 12); D8, 6.4% (n = 10); D9, 52.2% (n = 82); D10, 19.1% (n = 30); D11, 10.8% (n = 17)). In contrast, the 8 day treatment interval gave a less precise synchrony in which the distribution of detected estruses (n = 168) by experimental days was: D5, 3.0% (n = 5); D6, 1.8% (n = 3); D7, 6.6% (n = 11); D8, 6.0% (n = 10); D9, 3.0% (n = 5); D10, 14.9% (n = 25); D11, 39.9% (n = 67); D12, 7.7% (n = 13); D13, 12.5% (n = 21); D14, 4.8% (n = 8). For the 6 day period following the initial Buserelin injection, virtually all estrous activity was blocked owing to either luteinization or atresia of existing follicles or acute induction of ovulation. By 7 days after GnRH injection, newly recruited follicles and CL sensitive to

| TABLE 1 |
| Effect of Buserelin (8 \mu g) on conception rate when given at +12 days post-estrus in heifers synchronized with Buserelin (8 \mu g) followed by PGF_{2\alpha} (25 mg) at 8 (Experiment 1) or 7 (Experiment 2) day intervals |

<table>
<thead>
<tr>
<th>Responses</th>
<th>Synchronization: Buserelin + PGF_{2\alpha}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8 day interval</td>
</tr>
<tr>
<td>Heifers treated</td>
<td>189</td>
</tr>
<tr>
<td>Heifers in estrus</td>
<td>168 (88.9%)</td>
</tr>
<tr>
<td>Conception rate to synchronized estrus</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>61.9 (n = 71)</td>
</tr>
<tr>
<td>Buserelin + at 12 days</td>
<td>60.9 (n = 69)</td>
</tr>
<tr>
<td>Inter-estrous interval (days)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>21.0 (n = 18)*</td>
</tr>
<tr>
<td>Buserelin at +12 days</td>
<td>23.2 (n = 19)</td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01.
PGF$_{2\alpha}$ were available to respond to treatment with PGF$_{2\alpha}$. Of the heifers that were artificially inseminated, conception rates were 61.9% and 56.5% for control animals, and 60.9% and 58.7% for heifers treated on Day 12 post-insemination with Buserelin of Experiments 1 and 2, respectively (Table 1).

Several reports have indicated that a 6 or 7 day interval between Buserelin and PGF$_{2\alpha}$ is a satisfactory system for synchronization of estrus with good fertility (Coleman et al., 1991; Guilbault et al., 1991) comparable to that achieved following inseminations to synchronized estruses after two injections of PGF$_{2\alpha}$ given 11 days apart (Coleman et al., 1991). Narasimha Rao and Venkatramiah (1991) were able to both induce and synchronize estrus with an acceptable level of fertility in anoestrous buffaloes receiving a GnRH agonist followed by Cloprostenol (7 day interval) compared with untreated controls. Thus, GnRH agonist and Cloprostenol treatment appeared to induce follicle development leading to ovulation in anoestrous buffaloes; such a program also warrants testing in anoestrous Bos taurus animals. Guilbault et al. (1991) reported that a 6 day Buserelin–PGF$_{2\alpha}$ program was also efficient for estrous detection and did not adversely affect fertility.

Synchronization of estrus with a GnRH agonist followed by PGF$_{2\alpha}$ at a 6 or 7 day interval is an additional option for controlled breeding. Owing to follicle management, such a program reduces the probability that the induced estrus is a consequence of an estrogenic follicle that has been retained on the ovary for a considerable period of time and which would lead to a reduction in fertility (Patterson et al., 1989). The 7 day treatment interval is convenient for weekly farm visits in a herd reproductive-health management program.

**Post-insemination treatments to improve fertility**

Follicular waves occurring during diestrus lead to atresia of a large dominant anovulatory follicle by mid-cycle. Development of an estrogenic follicle during the second half of the luteal phase initiates the luteolytic process via secretion of estradiol (Fogwell et al., 1985). Follicle-induced luteolysis occurs because estrogenic effects are imposed upon a progesterone-primed endometrium capable of synthesizing and secreting PGF$_{2\alpha}$. Given at 3 day interval from 12 to 48 days post-estrus, Buserelin extended CL lifespan owing to the repeated luteinization of follicles that would normally cause luteolysis and/or induce an accessory CL (Thatcher et al., 1989). Following the last injection of Buserelin on Day 48, an interval of approximately 6–8 days was required before a healthy follicle was recruited, selected, and induced luteolysis with a subsequent estrus.

Follicular development is attenuated on the CL-bearing ovary ipsilateral to the pregnant uterine horn (Guilbault et al., 1986; Pierson and Ginther, 1987; Driancourt et al., 1991; Thatcher et al., 1991). This localized attenuation of
ovarian follicular development and potential estradiol secretion would complement anti-luteolytic effects of the conceptus to attenuate endometrial secretion of PGF$_{2\alpha}$. If reduced secretion of the conceptus-produced anti-luteolytic protein (Bovine Trophoblast Protein-1 complex (bTP-1)) is a cause of embryonic mortality, embryo survival may be improved by enhancing the CL lifespan and allowing the conceptus more time to undergo development and secrete bTP-1 for induction of an anti-luteolytic effect. Macmillan et al. (1986) reported that cows injected with 10 $\mu$g Buserelin on either Days 11, 12, or 13 after insemination had higher pregnancy rates than control cows (72.4% vs. 60.9%). Among cows that did not conceive, Buserelin reduced the proportion of animals having return-to-service intervals of less than 22 days by approximately the same percentage as the increase in fertility rate. This suggested that the GnRH agonist was acting to delay luteolysis, via follicular alterations, in cows that would otherwise have lost their embryos because of an earlier luteolysis. If a viable conceptus is present within the uterine lumen, follicular development is not essential for maintenance of pregnancy. Pregnancy can be established following embryo transfer in ovariectomized cattle by simple progestin replacement following estrogen priming (Inskeep and Baker, 1985).

At this time, numerous additional studies have been completed to test whether GnRH agonists given in the luteal phase after insemination improve conception rates. Buserelin (8 $\mu$g) given 12 days after insemination in both experiments involving synchronization with Buserelin and PGF$_{2\alpha}$ failed to enhance conception rates in Florida experiments (Table 1). However, Buserelin treatment did extend mean inter-estrous intervals in both experiments by 2–5 days. In evaluating fertility responses, it may be critical to consider the reproductive management of animals leading to the experimental estrus for insemination. For example, the above studies utilized a synchronization system that would have recruited a fresh follicle for ovulation. An additional study was conducted with lactating dairy cows during the hot summer months in south Florida to evaluate the effects of an injection of Buserelin (8 $\mu$g) 12 days after insemination. The experiment was conducted in two separate barns of the same commercial dairy. All cows were inseminated artificially to a spontaneous estrus. Buserelin failed to increase conception rates (Buserelin, 33.3% vs. control, 37.2%; Table 2). The fertility rate differed appreciably between barns; however, an advantage of Buserelin was not detected in either population of cows. Perhaps the devastating effects of heat stress on early embryo death (Putney et al., 1988) precludes the ability of Buserelin to improve embryo survival.

Jubb et al. (1990) were unable to show an improvement in conception rates when Buserelin (10 $\mu$g) was injected between 11 and 13 days after insemination. The experiment was conducted across 19 farms and involved 2050 dairy cows artificially inseminated to spontaneously detected estruses. Lack
TABLE 2

Effect of Buserelin (GnRH agonist, 8 μg) on conception rates of lactating dairy cows during summer heat-stress periods; Buserelin given 12 days post-insemination

<table>
<thead>
<tr>
<th>Response</th>
<th>Number</th>
<th>Percentage conception</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buserelin</td>
<td>195</td>
<td>33.3</td>
</tr>
<tr>
<td>Control</td>
<td>191</td>
<td>37.2</td>
</tr>
<tr>
<td><strong>Barn</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>176</td>
<td>27.8**</td>
</tr>
<tr>
<td>2</td>
<td>210</td>
<td>41.4</td>
</tr>
<tr>
<td><strong>Treatment by barn</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barn 1: Buserelin</td>
<td>93</td>
<td>25.8</td>
</tr>
<tr>
<td>Barn 1: Control</td>
<td>83</td>
<td>30.1</td>
</tr>
<tr>
<td>Barn 2: Buserelin</td>
<td>102</td>
<td>40.2</td>
</tr>
<tr>
<td>Barn 2: Control</td>
<td>108</td>
<td>42.5</td>
</tr>
</tbody>
</table>

**P<0.01.

of a Buserelin effect was consistent among herds of low, average and high fertility. Biologically, Buserelin was active in that the frequency of shortened inter-estrous intervals was reduced. Furthermore, no Buserelin effect was detected on fertility to the insemination following treatment. Coleman et al. (1991) also failed to detect an effect of Buserelin (8 μg) given 14 days post-insemination in beef cattle inseminated to estruses synchronized by either a 7 day Buserelin–PGF2α program or two injections of PGF2α given 11 days apart. Lajili and coworkers (1991) reported a beneficial effect of Buserelin (10 μg) on conception rates when given to dairy cows 12–14 days following inseminations to a PGF2α-synchronized estrus but not following spontaneous estruses. Based upon the management of the herd, cows synchronized with PGF2α were considered to be cows not previously detected in heat at the expected time. Authors suggested that PGF2α-synchronized animals would have had a reduced luteal phase or exposure to progesterone which may reduce fertility that was corrected by subsequent treatment with Buserelin. Whether this difference is real needs additional verification. The percentage difference in fertility was the same between both groups; however, the number of animals not treated with PGF2α was disproportionally lower. Rettmer et al. (1992a) examined the effects of Buserelin given between 11 and 14 days after insemination in synchronized heifers (synchronized by a sequence of melengestrol acetate (MGA) feeding followed by PGF2α injection) and cycling suckled cows given one injection of PGF2α. In most locations, inseminated cows and heifers were exposed to fertile bulls after insemination. When fertility was evaluated by rectal palpation between 40 and 60 days after insemination or by calving dates, there was no statistically significant difference in fertility at a significance level of P<0.05. Utilizing a more liberal interpretati-
tion of the data, involving rectal palpations up to 85 days after insemination (there was difficulty in differentiating between ages of fetuses 15–20 days apart), pregnancy rates in heifers were increased. In a recent report with dairy cows from the UK (Drew and Peters, 1991), injection of Buserelin (10 μg) 12 days post-insemination increased conception rates by 12% when evaluated across ten herds and a total of 643 cows (approximately 64 cows per herd or 32 cows per treatment group within each herd). Collectively, across experiments, there is not a clear indication that treatment with a GnRH agonist between 12 and 14 days after insemination will consistently increase pregnancy rates.

**Embryo transfer**

The potential for a GnRH agonist (Buserelin) to enhance embryo survival in embryo transfer recipients was tested by two commercial embryo transfer units (Ellington et al., 1991). Buserelin (8 μg) was given either at the time of embryo transfer (Days 7 or 8; Group 1, n=254; Day 0 = day of estrus) or 4–7 days after transfer (Days 11–15; Group 2, n=252), and pregnancy rates compared with a control group receiving no Buserelin (Group 3, n=258). Buserelin given at the time of transfer or 4–7 days later, did not affect pregnancy rates (72%, 66% and 68% for Groups 1, 2 and 3, respectively). Furthermore, there were no interactions between treatment and embryo score (excellent, good and fair), or treatment and plasma progesterone determined at the time of embryo transfer. Thus, Buserelin did not have a beneficial effect in maintaining pregnancy for embryos of poorer quality or in recipients with lower progesterone concentrations.

We had hypothesized that follicular development in recipients, after the time of normal embryo transfer (Day 7) contributed to the need of close synchrony between donor and recipient (Thatcher et al., 1989). By controlling follicular dynamics of the recipient and maintaining CL function, the need for a close synchrony may be reduced. A preliminary experiment tested whether repeated Buserelin injections (8 μg given at 3 day intervals beginning on Day 12 after estrus and continued until 12 days after embryo transfer) would permit asynchronous embryo transfer of Day 7 embryos into recipients that ranged between 9.5 and 14.5 days after estrus. Results were encouraging in that 38.9% (7/18) of the asynchronous embryo transfers resulted in pregnancies. An additional experiment has been conducted in which pregnancy rates of asynchronous transfers were compared between recipients receiving repeated Buserelin injections and untreated contemporary controls. A total of 30 recipient heifers received on Day 12 after estrus gave either excellent or good quality Day 7 embryos (two blastocysts, 24 expanded blastocysts and four hatched blastocysts) from superovulated donor cows. Treated recipients (n=19) received 8 μg injections of Buserelin on Days 12, 15, 18, 21, and 24
after estrus, whereas the non-injected control group \((n = 11)\) received no Buserelin injections. Pregnancy was determined by ultrasonography at Day 30 and rectal palpation at Day 40 after estrus. Pregnancy rates for asynchronous transfers in the Buserelin group were \(4/19\) (21%) at Day 30 and \(2/19\) (11%) at Day 40; responses for the control group were \(3/11\) (27%) at Day 30 and \(2/11\) (18%) at Day 40 after estrus. There were no differences among the groups, indicating that repeated Buserelin injections did not increase pregnancy rate to asynchronous embryo transfer. Return-to-service intervals in non-pregnant heifers differed between Buserelin \((38.6 \pm 1.6\) days\) and control \((25.8 \pm 2.6\) days\) groups. Thus, Buserelin-induced alterations in follicular development and maintenance of CL or a progesterone environment did not promote continued development of embryos that were placed in an advanced uterus. Based on extended inter-estrous intervals for the control group, embryos appeared to initiate the process of maternal recognition of pregnancy but were not visible by Day 30.

**Repeat breeders and normal inseminations**

A considerable number of studies examined whether GnRH treatments given at the time of insemination would improve conception rates in cattle at first service (see summary by Mee et al., 1990) and in repeat breeders (cows not conceiving by at least two previous services; see summary by Stevenson et al., 1990). Considerable variation existed among the studies within both types of cow populations relative to significant differences, direction of pregnancy rate change \((+ \text{ vs. } -)\), and magnitudes of pregnancy rate increases. In general, GnRH-induced increases in pregnancy rates were of a greater magnitude in cows identified as repeat breeders. It is important to understand the differences among locations, herds, management, and physiological states that contribute to the differential fertility responses to GnRH treatments. In an economic assessment, Weaver et al. (1988) concluded that herds with conception rates of 45% or less benefitted from GnRH treatment at first or second inseminations; herds with conception rates of 60% or over benefitted from GnRH treatment only at second or later services. The enhancement of fertility necessary to achieve the break-even point with GnRH treatment at third service was 2% for low- and 5% for high-conception-rate herds. With conception rates varying among studies in first service dairy cows from \(-7\) to \(+17\%\) (Mee et al., 1990), it is difficult to accept that GnRH is eliciting a predictable increase in fertility that can be reliably applied from herd to herd. Recent findings (Rosenberg et al., 1991; Mee et al., 1990) indicate that timing GnRH injections close to detection of estrus contributes to the success of a GnRH treatment at first postpartum inseminations in cows with low conception rates. In cows with two to four inseminations, injections of GnRH near detection of estrus needed to be coupled with inseminations 4–30 h after
detection of estrus to obtain an increase in fertility as opposed to early inseminations (less than 3 h; Rosenberg et al., 1991). The role of GnRH to indirectly alter the rate or efficiency of oocyte maturation in low fertility cows warrants study.

The concept of treating repeat breeder cows has become clouded relative to defining a true class of infertile cows and whether they specifically respond to GnRH. A component of the traditional definition of a repeat breeder, as defined by Casida (1961), is that semen quality and insemination circumstances should be optimal in identifying this class of cows that have greater rates of defective ova, ova loss, fertilization failure and embryonic loss. If cows with at least three services are defined as repeat breeders, it is critical that management factors such as accurate estrous detection, timing of insemination, handling of semen and inseminator skills are optimal, and that cows with palpable abnormalities of the reproductive tract are excluded. This array of management factors undoubtedly contributes to the herd-to-herd variability in the success of GnRH treatments. In addition to the periestrous treatment of repeat breeders with GnRH, Thibier et al. (1985) reported that a mid-luteal phase injection of a GnRH agonist prior to insemination in repeat breeders increased the rate of recovery and quality of embryos. Perhaps the GnRH agonist led to recruitment, selection and ovulation of a better quality follicle and oocyte.

Cystic ovaries

Kittok et al. (1973) reported the first success in treating follicular cysts in cows with GnRH, with a recovery rate ranging from 70 to 95% and a mean recovery period from 18 to 25 days. Based upon the ability of GnRH agonists to alter follicular dynamics (Macmillan and Thatcher, 1991), part of the GnRH and/or agonist treatment is to alter follicular development in cystic cows. This is vividly demonstrated in Figs. 2 and 3. A cow containing two follicular cysts, that had a combined diameter of 48 mm, and were maintained for at least 10 days in the absence of a CL, was subjected to a treatment sequence involving a single injection of Buserelin (8 μg), concurrent intravaginal insertion of a controlled internal drug releasing device (CIDR; containing 1.9 g progesterone) for 9 days, and injection of PGF2α on Day 7 or 2 days before removal of the CIDR (Fig. 2(A)). Immediately following the injection of Buserelin the high concentrations of plasma estradiol declined (Fig. 3(A)) and ovulation was induced in a non-cystic follicle that was spontaneously recruited during the pretreatment cystic period (Fig. 2(B)). Follicular turnover may or may not occur during periods of cystic development (Savio et al., 1990). During the CIDR insertion period, progesterone concentrations rose due to its release from the CIDR device and induction of a new CL (Fig. 3(B)). Following PGF2α injection and CIDR removal, an initial
Fig. 2. Ovarian follicular responses of cystic cow A to injection of Buserelin (8 μg) and concurrent intravaginal insertion of a controlled internal drug releasing device (CIDR; containing 1.9 g of progesterone) for 9 days, and injection of PGF2α 2 days before removal of the CIDR device. (A) Size (mm) of two cystic follicles during the 10 day control period (CP) and CIDR period (CIDR). I, decrease in size of cysts. (B) Size (mm) of subordinate follicles (SF) and new dominant follicle during treatment period (DFTP). II, selection of new dominant follicle.

Buserelin-recruited follicle underwent terminal development (Fig. 2(B)) leading to a proestrous rise in estradiol (Fig. 3(A)), induction of estrus, ovulation and formation of a robust CL that secreted substantial luteal phase concentrations of progesterone in plasma (Fig. 3(B)). During the normal luteal phase, the cow underwent three follicular waves leading to a normal 22 day estrous cycle (Fig. 3(A)). It is clear from the induced hormonal changes that the population of cystic follicles gradually declined during periods of progesterone exposure (CIDR insertion; early luteal phase of normal estrous cycle). Treatment with a GnRH agonist alters follicle dynamics and when combined with other treatments as described leads to a short and predictable recovery interval.
Postpartum reproductive management

The administration of GnRH in the postpartum period has been utilized for various experimental and clinical objectives. Administration of GnRH at various times during the early postpartum period, between 3 and 40 days postpartum, indicated that GnRH-induced secretion of LH was essentially restored by 10 days postpartum in lactating dairy cows (Kesler et al., 1977; Fernandes et al., 1978). Removal of the inhibitory effects of pregnancy and
the recrudescence of pituitary responsiveness to the GnRH-induced secretion of LH at this time is consistent with the early occurrence of postpartum ovarian follicular activity. As early as 14 days postpartum, administration of GnRH induced ovulation and cyclic activity (Britt et al., 1974). Associated with the induction of an ovulation was an acute increase in the rate of uterine involution during the week following administration of GnRH. Perhaps this decrease in uterine size is due to elimination or luteinization of an estrogen-active follicle by injection of GnRH as depicted for the cystic cow of Fig. 2. The CL formed by treatment with GnRH at 10–14 days postpartum will undergo luteolysis in response to an injection of prostaglandin between 20 and 24 days postpartum with a luteolytic rate of approximately 47% (Benmrad and Stevenson, 1986). This rate was no different from control cows receiving prostaglandin that were not pretreated with GnRH (33%).

The fertility of lactating dairy cows at the time of normal breeding (beginning at 60 days postpartum) was related positively to the number of previously detected estruses during the postpartum period (Thatcher and Wilcox, 1973). Cows expressing one or more estruses during the first 30 days postpartum required fewer services than cows with no estruses. However, the incidence of estruses between 31 and 60 days postpartum was not related to subsequent reproductive performance. This type of observation indicates that physiological and hormonal events associated with estrus (follicle turnover, uterine contractions, exposure to estradiol, and dilation of the cervix) restore uterine and ovarian function to a state more conducive to the subsequent establishment of pregnancy. As described above, GnRH is a potential drug that may regulate ovarian and uterine function in a manner that improves postpartum reproductive efficiency.

Numerous studies have been conducted to improve reproductive efficiency by the treatment of postpartum cows with GnRH or GnRH followed later by injection of PGF2α. The varied effects and efficiency of such trials are summarized in the reports by Leslie et al. (1984) and Stevenson and Call (1988a). Administration of GnRH has reduced the frequency of ovarian cysts and decreased culling due to infertility (Britt et al., 1977). GnRH has improved reproductive efficiency by reducing the interval from calving to conception and decreasing the number of services per conception. Furthermore, the effects of GnRH were more obvious in cows experiencing periparturient disorders. However, other studies have demonstrated no beneficial effects of GnRH administration in the early postpartum period. Once again, variability among studies relative to the efficacy of GnRH effects is related to unknown factors unique to the experimental units and the management systems of the farms. A review of several recent studies reinforces this point.

In a large field trial, the effects of administering GnRH (250 µg) on Day 15 and Cloprostenol (Clop; 500 µg) on Day 24 postpartum were evaluated in a 2 × 2 factorial experiment (GnRH–saline; GnRH–Clop.; saline–Clop.; sa-
line–saline) involving 305 cows (Etherington et al., 1984). No significant differences were detected among the groups in services per conception, number of estruses before first service or culling rates for infertility. Cows treated only with GnRH had increased intervals of calving to first estrus and to first insemination, and an increase in the incidence of pyometra (17.8% vs. 6.3%). In contrast, the administration of Cloprostenol on Day 24 decreased intervals from calving to first estrus and to conception, and decreased the incidence of pyometra, regardless of GnRH treatment. Benmrad and Stevenson (1986) conducted a similar factorial experiment with 234 cows and detected a different type of GnRH response. GnRH alone (200 μg) or Lutalyse alone (PGF$_{2\alpha}$, 25 μg) reduced the interval from calving to conception, but this effect was most evident in cows experiencing an abnormal puerperium (e.g. dystocia, retained fetal membranes, uterine infection, purulent discharge, milk fever, ketosis, or abnormal enlargement of uterine horns or cervix). Cows receiving GnRH–saline and saline–PGF$_{2\alpha}$ had a reduction in services per conception regardless of whether the puerperium was classified as normal or abnormal. There was no evidence that the incidence of persistent CL maintenance, perhaps associated with pyometra, was statistically higher in cows receiving only GnRH. Treatment with GnRH increased the proportion of cows with an ovulation between 3 and 5 days after injection (GnRH, 75% vs. saline, 28%). Perhaps the high incidence of retained fetal membranes (16.7%) and metritis (25.9%) of the trial reported by Etherington et al. (1984) contributed to the differential responses to GnRH between the two trials. Stevenson and Call (1988a) conducted an additional field trial (843 cows) that focused on a single injection of GnRH (100 μg, between Days 11 and 25) or Lutalyse (PGF$_{2\alpha}$, 25 mg) given between Days 11 and 25 or between Days 25 and 40 postpartum. Control cows received no treatment. No beneficial effect of treatments were detected on any measurement of fertility regardless of puerperium classification (normal vs. abnormal). Thus, the inability of GnRH or PGF$_{2\alpha}$ to elicit a repeatable improvement in efficiency of reproductive management points to the variety of unknown environmental factors that influence the responses. Stevenson and Call (1988b) pointed out that their trial was conducted with cows milked three times per day compared with the earlier study that involved twice daily milking (Benmrad and Stevenson, 1986).

Reproductive disorders (dystocia, twinning, still birth, retained placenta, cystic ovaries, anovulation, pyometra, metritis, and abnormal health status) in the postpartum period do indeed reduce subsequent reproductive efficiency (Stevenson and Call, 1988b). The potential of GnRH treatment to correct the cystic ovarian condition was described earlier. Leslie et al. (1984) examined the reproductive performance of dairy cows that had a retained placenta following treatment with GnRH (200 μg, n = 187) or saline (n = 191) between 8 and 14 days postpartum. GnRH failed to alter intervals from parturition to either first observed estrus, first insemination, or conception. The
total number of services per cow regardless of conception, number of services per conception and incidence of culling for infertility were not altered by GnRH. When data analyses were restricted to herds that began inseminations at a mean of 80 days or less from parturition, GnRH treatment reduced the intervals from parturition to first service and conception and decreased services per cow regardless of conception. Perhaps any reproductive advantage of GnRH treatment of cows with retained placenta in the early postpartum period is lost with a farm management system of delayed insemination.

Collectively, the array of field trials with GnRH in the postpartum period points out that the potential physiological and endocrine effects induced by GnRH treatment may not evolve into a clear advantage in reproductive efficiency with the wide variety of environmental factors that influence the experimental responses.

CONCLUSIONS AND IMPLICATIONS

GnRH and analogues of GnRH elicit LH release in a very predictable manner that is characteristic of the specific molecule. Consequently, as pharmaceutical agents, these compounds are very reliable in altering the endogenous secretion of both LH and FSH. The variability of LH response to GnRH among animals is due to the physiological state of the animal at the time of injection (e.g. stages of the estrous cycle, pregnancy or the postpartum period). The GnRH-induced secretion of LH has numerous physiological and endocrine effects associated with altered CL function, luteinization or ovulation of ovarian follicles, and recruitment of new ovarian follicles. These GnRH-induced effects, via altered gonadotrophin secretion, have the potential to be combined with the use of other pharmaceutical agents (e.g. PGF sub 2 alpha ) to reproductively manage individual animals. Consequently, GnRH and analogues of GnRH have the potential to be utilized in systems to synchronize estrus, stimulate CL development, alter rate of uterine involution, induce postpartum ovulations, delay CL regression and treat animals with cystic ovaries. Additional uses may be possible with altered systems of GnRH delivery (e.g. sustained release of low quantities of GnRH to induce ovarian activity in anestrous or prepubertal animals, or sustained release of large amounts of GnRH to terminate reproductive activity for desired periods of time). Presently, considerable variability exists in the responsiveness of animal groups to GnRH or analogues of GnRH when evaluations of the response are overall measurements of reproductive efficiency of the group. Unfortunately, the economic responses (e.g. interval from parturition to conception) include a host of other variables that impact on the response dependent and independent of GnRH. This undoubtedly has contributed to the marked variation in reported efficiencies of GnRH use from one field trial to the next. Reproduction, production and health management systems vary markedly
among production units. Use of pharmaceutical agents to alter reproductive function is not a substitute for poor management. As management systems are controlled more rigorously, the probability of utilizing GnRH effectively should be increased. However, this may not be the case if the improved management alters animal productivity (e.g. milk production) to a level that compromises the intended use of the GnRH treatment. These types of management interactions need to be characterized in future studies for the rational and realistic utilization of GnRH or GnRH analogues.

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REFERENCES


