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Reproductive responses of cattle to GnRH agonists

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Abstract

The response in cattle to treatment with gonadotrophin releasing hormone (GnRH) agonist includes downregulation of GnRH receptors on gonadotrophe cells, desensitisation of the anterior pituitary gland to endogenous GnRH, and the abolition of pulsatile release of LH. In bulls, a tonic pattern of LH release is associated with increased secretion of testosterone, which persists for the duration of treatment with GnRH agonist. The mechanism for this response in bulls has not been elucidated, but clearly pulsatile release of LH is not required to stimulate the synthesis of steroidogenic enzymes that sustain elevated secretion of testosterone. In heifers, desensitisation to endogenous GnRH prevents the occurrence of the pre-ovulatory surge release of LH, thus blocking ovulation. The latter provided the opportunity to evaluate the potential of a GnRH agonist bioimplant to control fertility in heifers under extensive management. Bioimplants that contained graded amounts of GnRH agonist prevented pregnancies in heifers for periods of 3 to 12 months. Zebu crossbred heifers treated with GnRH agonist from 14 to 23 months of age failed to conceive, but showed normal conception patterns when introduced into mating herds at around 26 months of age. After treatment with GnRH agonist for 4 to 6 weeks, ovarian follicular growth in heifers is restricted to relatively small (2–4 mm) antral follicles. Suppressed follicular growth in heifers treated long-term with GnRH agonist is due to a lack of gonadotrophin support, rather than a direct action of agonist at the ovaries. This was demonstrated by the ability to induce apparently normal follicular growth and ovulation by acute treatment with FSH for 4 days, followed by an injection of LH, in heifers that had been exposed to GnRH agonist for around 6 months, and which had only small (2–4 mm) antral follicles at the start of FSH treatment. GnRH agonist

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bioimplants have been incorporated into new multiple ovulation and embryo transfer protocols that allow control of the time of ovulation subsequent to superstimulation of ovarian follicular growth with FSH. In these protocols, the endogenous surge release of LH is blocked by treatment with agonist and ovulation is timed by injection of exogenous LH, allowing fixed-time AI. It can be concluded from recent studies that GnRH agonist bioimplants have considerable potential for both pro-fertility and anti-fertility applications in cattle. It is likely that commercial bioimplants will be available within the next 3 to 5 years.

Keywords: GnRH agonist; Testes; Ovaries; Fertility control; Cattle

1. Introduction

Gonadotrophin releasing hormone (GnRH) occupies a central role in the reproductive function in mammals. GnRH is a neuropeptide that is released in a synchronised, pulsatile manner from neurons that terminate at the medial basal hypothalamus–median eminence, an area bathed by the hypothalamo-hypophyseal portal vessels. GnRH moves into the portal vessels and is delivered to gonadotrophe cells in the anterior pituitary gland. At gonadotrophe cells, GnRH binds to specific cell-surface receptors, and this triggers a sequence of events that include, GnRH receptor micro-aggregation and internalisation, activation of second messenger signal transduction pathways, release of LH and FSH, and de novo synthesis of LH and FSH. The internalisation of GnRH receptors after binding by GnRH induces a transient state of insensitivity in gonadotrophe cells to GnRH. Under normal circumstances, new GnRH receptors are synthesised and returned to the surface of gonadotrophe cells, thereby re-instating responsiveness to subsequent stimulation from GnRH.

Two distinguishing features of GnRH agonists, compared with natural sequence GnRH, are that agonists have a higher affinity for GnRH receptors and a longer half-life in circulation (Karten and Rivier, 1986). These properties of GnRH agonists allow them to be used at substantially lower doses than natural sequence GnRH. The response to chronic treatment with GnRH agonist has two components. The acute phase of treatment, which can last for several days, is characterised by an immediate large increase in plasma LH and FSH, followed by a return to basal concentrations (Melson et al., 1986; Gong et al., 1995, 1996). Continued exposure to GnRH agonist leads to a chronic phase during which pulsatile secretion of LH is blocked (D'Occhio and Aspden, 1996). The latter occurs as a consequence of downregulation of GnRH receptors on gonadotrophe cells (Melson et al., 1986; Hazum and Conn, 1988) and an uncoupling of second messenger pathways within gonadotrophe cells (Huckle and Conn, 1988). A lack of pulsatile secretion of LH, and likely FSH, is maintained for as long as GnRH agonist is present in circulation at a threshold concentration (D'Occhio and Aspden, 1996).

The two phases of the response to GnRH agonists has led to studies on potential pro-fertility and anti-fertility applications of agonists in cattle. The present article reviews studies in bulls and heifers, with a particular focus on recent studies on the use of GnRH agonists to control ovarian follicular growth and ovulation in heifers. The development of new GnRH agonist bioimplants has allowed long-term studies in which a suppression of ovarian follicular growth was achieved in heifers for up to 12 months.

This finding confirmed that GnRH agonists have a potential as the next generation technology for fertility control in heifers, and it is anticipated that commercial long-acting GnRH agonist bioimplants will be available in 3 to 5 years.

2. Effects of GnRH agonists on the anterior pituitary

2.1. Gonadotrophe cells

Studies on the effects of GnRH agonists on the anterior pituitary gland in cattle have been conducted primarily in bulls. Similar to findings in other species, bulls treated with the GnRH agonist nafarelin underwent a decrease in gonadotrophe cell GnRH receptors (Melson et al., 1986). Pituitary contents of LH and LH mRNA were also decreased in bulls treated with the GnRH agonists nafarelin (Melson et al., 1986) and deslorelin (Aspden et al., 1997). A corresponding decrease in pituitary FSH content was observed in the entire group of bulls (Melson et al., 1986) but not in one study in castrated bulls (Aspden et al., 1996); however, in the latter study, pituitary FSH mRNA was decreased by treatment with deslorelin. It can be concluded that cattle have a downregulated anterior pituitary gland during treatment with GnRH agonist.

2.2. Secretion of LH and FSH

Gonadotrophin secretion during continuous treatment with GnRH agonist can be characterised by two phases. During the acute phase, which can last for several days, LH secretion is increased in both bulls (Melson et al., 1986) and heifers (D'Occhio et al., 1996). A similar acute increase in FSH was reported for heifers (Gong et al., 1995, 1996). The acute increase in LH is followed by a chronic phase during which pulsatile release of LH is blocked but basal secretion is maintained (Melson et al., 1986; D'Occhio et al., 1996). In several studies, both bulls (D'occhio and Aspden, 1996; Jimenez-Severiano et al., 1999) and heifers (Evans and Rawlings, 1994; Gong et al., 1995) receiving GnRH agonist had elevated basal concentrations of LH compared with control animals. It would appear that elevated basal LH during treatment with GnRH agonist is maintained long-term in bulls, as in one study, bulls treated with bioimplants containing deslorelin had increased plasma concentrations of testosterone for over 100 days (D'Occhio and Aspden, 1996). Patterns of LH and FSH secretion in heifers during long-term treatment with GnRH agonist were characterised by Gong et al. (1995, 1996). In one study, treatment with buserelin for 3 weeks suppressed pulsatile secretion of LH but resulted in elevated basal concentrations of both LH and FSH (Gong et al., 1995). When buserelin was administered for a longer period, basal plasma concentrations of FSH were suppressed after around 30 days of treatment (Gong et al., 1996). The latter observation was consistent with an absence of significant follicular growth in heifers after approximately 6 weeks of treatment with a deslorelin bioimplant (D'Occhio and Whyte, unpublished results). Further studies are required to characterise gonadotrophin secretion in heifers treated with GnRH agonist for periods of up to 12 months, and to relate changes in gonadotrophin secretion to ovarian follicular activity.

3. Testicular function in bulls treated with GnRH agonist

As noted above, bulls treated with GnRH agonist show an increase in plasma concentration of testosterone, which is maintained for the duration of treatment with agonist (D'Occhio and Aspden, 1996). A similar increase in testosterone secretion was reported for red deer stags (Lincoln, 1987). These testosterone responses in bulls and red deer differ, however, to the classical decrease in testosterone secretion reported for males of others species during treatment with GnRH agonist (Aspden et al., 1997). The basis for increased testosterone secretion in bulls during treatment with GnRH agonist has not been fully elucidated, but is likely related, in part, to the elevated basal plasma concentrations of LH discussed above. During treatment with agonist, bulls have increased testicular levels of steroid acute regulatory (StAR) protein and steroidogenic enzymes involved in testosterone biosynthesis (Aspden et al., 1998). In one study, young Zebu bulls treated with deslorelin bioimplants showed an increased rate of testicular growth (D'Occhio and Aspden, 1996). In other short-term studies, no effect (Melson et al., 1986; Ronayne et al., 1993) and a retardation (Chandolia et al., 1997) of testicular growth were reported in young bulls treated with GnRH agonist. The potential to influence testicular growth in young bulls by treatment with GnRH agonist bioimplants is the focus of ongoing research.

4. Ovarian follicular function in heifers treated with GnRH agonist

4.1. Pro-fertility applications of GnRH agonists

The pro-fertility applications of GnRH agonists in heifers have utilised the acute increase in LH secretion that occurs in response to treatment with agonist. An early exploitation of the acute increase in LH was in the management of cystic follicles in dairy heifers and cows by injection of GnRH agonist (Thatcher et al., 1993), which followed similar studies with natural sequence GnRH (Kesler and Garverick, 1982). Recently, GnRH agonists have been used to induce ovulation in oestrus synchronisation protocols (Schmitt et al., 1996; see also Pursley et al., 1998).

Another recent pro-fertility application of GnRH agonists was the development of the GnRH agonist–LH protocol for ovarian follicle superstimulation (D'Occhio et al., 1997). In this protocol, heifers and cows to be superstimulated are implanted with a deslorelin bioimplant, which prevents the occurrence of the endogenous pre-ovulatory surge release of LH subsequent to stimulation with FSH. The time of ovulation is controlled by injection of exogenous LH, which allows for fixed-time insemination (D'Occhio et al., 1998). The GnRH agonist–LH protocol provides the opportunity to study the effects of follicle 'coasting' (delaying ovulation), after superstimulation with FSH, on ovulation rate, fertilising capacity of oocytes, and embryo developmental competency. These studies will define the timing of LH injection, which optimises ovulation rate and the recovery of high quality embryos in multiple ovulation and embryo transfer programs. The protocol also provides an experimental model for basic studies on the development and maturation of follicles and oocytes, and the associated

Table 1
Reproductive status of heifers 4 months after treatment with graded doses of the GnRH agonist deslorelin using subcutaneous bioimplants (D'Occhio and Whyte, unpublished results). Results are respective proportions of heifers

Reproductive parameter	GnRH agonist dose			Control heifers
	Low	Medium	High	
Follicle size				
Small (≤ 5 mm)	33/50 (66%)	43/49 (88%)	43/48 (89%)	–
Medium (6–9 mm)	2/50 (4%)	2/49 (4%)	2/48 (4%)	–
Large (≥ 10 mm)	8/50 (16%)	3/49 (6%)	3/48 (6%)	–
Corpus luteum	5/50 (10%)	0/49 (0%)	0/48 (0%)	–
Pregnant	2/50 (4%)	1/49 (2%)	0/48 (0%)	12/19 (63%)

interrelationships between follicles and oocytes. With regard to follicles, apparently normal follicular growth and maturation occur in the absence of pulsatile secretion of LH in the GnRH agonist–LH protocol. Follicles ovulate in response to exogenous LH, release fertilisable oocytes, and result in embryos that establish normal pregnancies after transfer to recipients (Nogueira et al., 2000).

4.2. Fertility control with GnRH agonists

The use of GnRH agonists for fertility control in heifers relies on the absence both of pulsatile secretion of LH and the pre-ovulatory surge release of LH during the chronic phase of treatment with agonist. The recent development of long-acting GnRH agonist bioimplants (Walsh et al., unpublished results; Peptech Animal Health Pty Limited) provided the opportunity to examine whether treatment with agonist could be used for fertility control in heifers and cows maintained in extensive beef production enterprises. In a dose–response study, bioimplants containing graded amounts of deslorelin suppressed ovarian follicular growth, ovulation and conception in a dose-dependent manner

Table 2
Reproductive status of heifers 8 months after treatment with graded doses of the GnRH agonist deslorelin using subcutaneous bioimplants (D'Occhio and Whyte, unpublished results). Results are respective proportions of heifers

Reproductive parameter	GnRH agonist dose			Control heifers
	Low	Medium	High	
Follicle size				
Small (≤ 5 mm)	2/49 (4%)	13/49 (26%)	41/48 (85%)	–
Medium (6–9 mm)	0/49 (0%)	5/49 (10%)	1/48 (2%)	–
Large (≥ 10 mm)	10/49 (20%)	15/49 (30%)	5/48 (10%)	–
Corpus luteum	9/49 (18%)	1/49 (2%)	1/48 (2%)	–
Pregnant	28/49 (57%)	15/49 (30%)	0/48 (0%)	27/34 (79%)

Table 3
Reproductive status of heifers 12 months after treatment with graded doses of the GnRH agonist deslorelin using subcutaneous bioimplants (D'Occhio and Whyte, unpublished results). Results are respective proportions of heifers

Reproductive parameter	GnRH agonist dose			Control heifers
	Low	Medium	High	
Follicle size				
Small ($\leq$ 5 mm)	1/48 (2%)	10/48 (20%)	37/45 (77%)	–
Medium (6–9 mm)	1/48 (2%)	0/48 (0%)	1/45 (2%)	–
Large ($\geq$ 10 mm)	6/48 (12%)	10/48 (20%)	3/45 (6%)	–
Corpus luteum	4/48 (8%)	2/48 (4%)	1/45 (2%)	–
Pregnant	36/48 (75%)	25/48 (52%)	3/45 (6%)	44/47 (93%)

for up to 12 months in Zebu crossbred heifers (Tables 1–3). Heifers were approximately 26-months-old and showing regular oestrous cycles at the time of implantation with deslorelin. After 2 months of treatment, follicular growth in the majority of heifers was restricted to $\leq$ 5 mm (Low Dose, 45/50 heifers; Medium Dose, 43/50 heifers; High Dose 39/48 heifers). Follicular growth progressively returned to normal in heifers treated with the Low Dose and Medium Dose and this was associated with conceptions (Tables 1–3). In the majority of heifers treated with the High Dose, follicular growth remained restricted to $\leq$ 5 mm, after 12 months of treatment (Table 3). The High Dose GnRH agonist treatment therefore has considerable potential as a non-surgical approach for the management of fertility in heifers and older cows maintained in extensive environments, where the control of bulls is often difficult. In a preliminary study to evaluate the efficacy of GnRH agonist bioimplants under industry conditions, heifers and cows on three extensive sub-tropical beef cattle stations were treated with the Medium Dose or High Dose GnRH agonist bioimplant and maintained with bulls for approximately 12 months.

Conception rates at the end of the industry trail are shown in Table 4. The Medium Dose bioimplant prevented conception for around 230 days, while the High Dose bioimplant was effective for approximately 330 days. These findings provided further support for the potential of GnRH agonist bioimplants for fertility control in heifers and cows maintained under extensive management.

Table 4
Pregnancy data at slaughter for heifers and cows treated with a GnRH agonist bioimplant. Results are for heifers and cows combined (D'Occhio et al., unpublished results)

	GnRH agonist treatment	Number of animals	Duration (days)	Pregnant (%)	Day at first conception
Station A	Medium Dose	76	387	20 (26%)	231 $\pm$ 19
Station B	Medium Dose	84	376	8 (10%)	244 $\pm$ 13
Station C	High Dose	99	394	9 (9%)	336 $\pm$ 3

Table 5
Pregnancies for control heifers and heifers treated with a Low Dose or Medium Dose GnRH agonist bioimplant. Results are proportion of heifers pregnant at the end of treatment with GnRH agonist and cumulative pregnancies after introduction into mating herds (D'Occhio and Fordyce, unpublished results)

	GnRH agonist	Days after the start of mating		
		30	60	90
Control	20/50 (40%)	38/50 (76%)	48/50 (96%)	50/50 (100%)
Low Dose	5/50 (10%)	34/50 (68%)	47/50 (94%)	49/50 (98%)
Medium Dose	2/50 (4%)	31/48 (65%)	48/48 (100%)	48/48 (100%)

4.3. Temporary suppression of fertility with GnRH agonists

To examine whether GnRH agonist bioimplants might be used for temporary suppression of fertility in heifers destined for future breeding, Zebu crossbred heifers were treated with either a Low Dose or Medium Dose deslorelin bioimplant from approximately 14 to 23 months of age. Heifers were grazed in an extensive beef system together with non-treated contemporary heifers and herd bulls. All pregnancies were terminated between 50 and 100 days, and these heifers were then segregated and maintained as a separate group in the absence of bulls until the second phase of the study. Bioimplants were removed from all heifers at about 23 months and, at approximately 26 months of age, all heifers were placed into mating herds. Results for pregnancies at the end of treatment with deslorelin, and during subsequent herd mating, are shown in Table 5. The pregnancy rate for untreated heifers from 14 to 23 months of age (20/50) was typical for Zebu crossbred heifers in a dry tropical environment. Treatment with both Low Dose and High Dose deslorelin significantly reduced the proportion of heifers which conceived from 14 to 23 months (5/50 and 2/50, respectively). After introduction into mating herds, most heifers were pregnant within 60 days, irrespective of whether they had previously been treated with a deslorelin bioimplant (Table 5). These findings indicated that GnRH agonist bioimplants have potential application in the control of the time of first conception in young heifers maintained in extensive environments. There are likely to be broader applications for GnRH agonist bioimplants in other cattle production systems where a temporary suppression of fertility in heifers and cows is desired.

5. Potential pituitary and ovarian sites of action of GnRH agonists

Studies, particularly in rodent models, have provided evidence for actions of GnRH agonists at the gonads (Sharpe et al., 1981). In support of the latter studies, both GnRH-like peptides (Ireland et al., 1988) and GnRH receptors (Whitelaw et al., 1995) have been identified in gonadal tissues. It was possible, therefore, that suppressed ovarian function in heifers treated long-term with GnRH agonist may have been due, in

Table 6
Ovarian follicular distribution subsequent to superstimulation with FSH in control heifers ($n = 5$) and heifers implanted with GnRH agonist ($n = 5$) for around 6 months. Results indicate the number of follicles in each category, with number of heifers in parentheses; there were no significant differences between control and implanted heifers for any parameters (D'Occhio and Cremonesi, unpublished results)

Follicle size (mm)	Days relative to FSH stimulation			
	Control		GnRH agonist	
	4	6	4	6
2	0.6 ± 0.6 (1)	0.0 ± 0.0 (0)	1.0 ± 0.6 (2)	0.0 ± 0.0 (0)
4	8.8 ± 2.2 (5)	1.6 ± 1.0 (2)	5.2 ± 1.5 (4)	0.8 ± 0.8 (1)
6	10.6 ± 2.6 (5)	14.6 ± 2.2 (5)	9.0 ± 3.4 (5)	9.2 ± 2.0 (5)
8	1.8 ± 0.6 (4)	6.2 ± 2.2 (5)	1.6 ± 1.2 (2)	5.8 ± 1.2 (5)
≥ 10	0.8 ± 0.2 (4)	4.0 ± 1.1 (5)	0.2 ± 0.2 (1)	4.6 ± 2.8 (3)

part, to a direct action of deslorelin at the ovaries. To test this possibility, heifers that had been exposed to deslorelin for around 6 months were treated with a conventional 4-day FSH superstimulation protocol (D'Occhio et al., 1997) and follicular growth was monitored. After treatment with FSH, heifers received an injection of exogenous LH (D'Occhio et al., 1997) to determine whether ovulation could be induced in any follicles that had been stimulated to grow in response to FSH. At the commencement of treatment with FSH, heifers receiving deslorelin had follicles with a mean diameter of 2.7 ± 0.7 mm compared with 6.0 ± 0.6 mm for controls that were showing regular oestrous cycles. After 4 days of FSH treatment, heifers implanted with deslorelin had developed follicles ranging in size from 4 to 10 mm, similar to control heifers. Follicles continued to grow to Day 6 (Table 6).

Injection of LH induced ovulation in heifers implanted with deslorelin and, 7 days after ovulation, the number of corpora lutea were similar ($P = 0.327$) for implanted heifers (11.0 ± 4.7 corpora lutea/heifer) and control heifers (16.0 ± 0.9). It was concluded that suppressed ovarian follicular activity in heifers treated long-term with GnRH agonist is due to gonadotrophin insufficiency, rather than a direct action of agonist at the ovaries. Another interesting observation in the latter study was the range of follicle diameters after 4 days of treatment with FSH in heifers implanted with GnRH agonist (Table 6). At the start of treatment with FSH, implanted heifers had predominantly 2-mm follicles that grew at different rates during stimulation. It could be suggested from this observation that populations of early antral follicles in heifers already differ in their potential to respond to FSH stimulation.

6. Conclusions

Recent studies have confirmed potential opportunities for the use of GnRH agonists in a range of pro-fertility and anti-fertility applications in cattle. The likely realisation of these opportunities has been enhanced by the development of GnRH agonist bioimplants, which deliver agonist for periods greater than 12 months. The potential to influence pubertal development in bulls with long acting GnRH agonist bioimplants is

presently under investigation. A particular opportunity in heifers is the development of a commercial GnRH agonist bioimplant to replace surgical sterilisation for the management of fertility in extensive environments. GnRH agonist bioimplants also have application in the development of experimental models to address basic questions relating to follicular and oocyte development and maturation.

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