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Endocrine and reproductive responses of male and female cattle to agonists of gonadotrophin-releasing hormone

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The pituitary response in cattle to treatment with GnRH agonist has two phases. In the acute phase secretion of LH is increased, while the chronic phase is characterized by a downregulation of GnRH receptors and insensitivity of gonadotrophs to natural sequence GnRH. After long-term treatment with GnRH agonist, cattle do not have pulsatile secretion of LH but maintain basal LH. This is associated with reduced pituitary contents of LH, LH mRNA, FSH and FSH mRNA. Long-term treatment of bulls with GnRH agonist results in an increase in testicular LH receptors and high plasma testosterone. Heifers treated with a GnRH agonist from early in the oestrous cycle develop a larger corpus luteum and secrete more progesterone. Increased steroidogenesis is reflected in increased steroid acute regulatory (StAR) protein and steroidogenic enzymes in the testes and corpus luteum. GnRH agonists have potential as novel strategies for reproductive management in cattle. A GnRH agonist bioimplant was recently used to block the LH surge after FSH stimulation of follicle growth in heifers. Ovulation was induced by injection of LH, and heifers were inseminated relative to the LH injection. This GnRH agonist–LH protocol provides a model for studying the gonadotrophin requirements for follicular growth and oocyte maturation in cattle, and will enable controlled *in vivo* maturation of oocytes before recovery for *in vitro* procedures.

Introduction

Gonadotrophin releasing hormone (GnRH) is a ten amino acid neuropeptide that initiates the cascade of reproductive hormones in mammals. Major reproductive events including the onset of puberty and ovulation rely on increases in GnRH secretion. Increased release of GnRH into hypothalamo–hypophyseal portal vessels results in greater secretion of LH and FSH and enhanced gonadal function. The importance of GnRH for reproductive function has focused attention on this neuropeptide for reproductive therapies in cattle (Thatcher *et al.*, 1993).

Agonists of GnRH were developed initially to treat hypogonadism resulting from insufficient endogenous secretion of GnRH. Typical structural features of GnRH agonists that distinguish them from natural sequence GnRH are substitution of glycine at position 6 of the peptide with a D-amino acid (e.g. D-tryptophan) and removal of glycine from the amino terminus. Substitution with a D-amino acid at position 6 increases the half-life of GnRH agonists in circulation, and removal of the amino terminal glycine increases affinity for the GnRH receptor (Karten and Rivier, 1986).

It was recognized early in the development of GnRH agonists that the reproductive response was dependent on dose of agonist and duration of treatment. The acute response, irrespective of dose, was characterized by increased gonadotrophin secretion (Chenault *et al.*, 1990). Chronic treatment with relatively high doses of GnRH agonist resulted in suppressed gonadotrophin secretion (Lahlou *et al.*, 1987). The latter was due to downregulation of GnRH receptors on

Table 1. Anterior pituitary contents of LH and FSH (ng mg^{-1} anterior pituitary) in intact bulls and castrated bulls (steers) treated with GnRH agonist

	LH		FSH	
	Control	GnRH agonist	Control	GnRH agonist
Bulls[a] ($n = 4$)	≈450	≈25 ($P < 0.01$)	≈950	≈50 ($P < 0.05$)
Bulls[b] ($n = 4–5$)	553	33 ($P < 0.001$)	–	–
Steers[c] ($n = 8–11$)	228	26 ($P < 0.001$)	1515	1390 ($P > 0.05$)

Values are means.
[a]Melson *et al.*, 1986; [b]Aspden *et al.*, 1997a; [c]Aspden *et al.*, 1996.

gonadotrophs (Hazum and Conn, 1988) and an uncoupling (desensitisation) of second messenger systems within these cells (Huckle and Conn, 1988; Hawes *et al.*, 1992).

This review provides an overview of the endocrine and molecular features of the responses of the anterior pituitary and gonads in cattle to treatment with GnRH agonists. Applications for GnRH agonists in the reproductive management of cattle also are considered.

Neuroendocrine Response to GnRH Agonist Treatment

Release of endogenous GnRH into hypothalamo–hypophyseal portal vessels in bull calves occurred in a pulsatile manner (Rodriguez and Wise, 1989). From this observation in male cattle and general patterns of LH release in female cattle, it can be assumed that GnRH release in cattle is pulsatile. In male sheep, treatment with GnRH agonist did not influence GnRH secretory patterns (Caraty *et al.*, 1990). It would appear, therefore, that GnRH agonist treatment does not influence the activity of hypothalamic GnRH secreting neurones. This conclusion is consistent with the major known effects of GnRH agonists at the anterior pituitary gland, which are discussed below.

Anterior Pituitary Response to GnRH Agonist Treatment

The major direct effects of GnRH agonists within the reproductive axis appear to be actions at pituitary gonadotrophs (Hazum and Conn, 1988; Huckle and Conn, 1988). Bulls treated with the GnRH agonist nafarelin had reduced pituitary GnRH receptors after treatment for 15 days (Melson *et al.*, 1986). This was consistent with the classical downregulation of GnRH receptors induced by GnRH agonists (Hazum and Conn, 1988). Studies on second messenger uncoupling (desensitization) (Huckle and Conn, 1988; Hawes *et al.*, 1992) have not been conducted in cattle. However, these studies should be considered, as bulls and heifers treated with GnRH agonists maintain basal secretion of LH (discussed below). This result differs from the significant reduction in circulating LH seen in most species during agonist treatment (D'Occhio and Aspden, 1996).

Pituitary contents of LH (Table 1) and LH mRNA (Table 2) were reduced in intact bulls treated with GnRH agonist. This was associated with a lack of pulsatile secretion of LH (Melson *et al.*, 1986) but maintenance of basal LH secretion (D'Occhio and Aspden, 1996; Aspden *et al.*, 1997a,b; Aspden *et al.*, 1998). In an intensive study of LH secretory patterns in bulls treated with the GnRH agonist nafarelin, it was confirmed that basal LH secretion in bulls is slightly but significantly increased during agonist treatment (Jimenez-Severiano *et* al., 1998). Similar increases in basal plasma LH were observed in heifers treated with leuprolide (Evans and Rawlings, 1994) and buserelin (Gong *et al.*, 1995).

An effect of GnRH agonist on pituitary LH in cattle was demonstrated clearly using castrated bulls (Aspden *et al.*, 1996), which have an increased secretion of LH and provide a useful experimental model to demonstrate any decreases in circulating LH (Fig. 1), and pituitary contents of LH and LH mRNA, which might occur during treatment with deslorelin. Although plasma LH

Table 2. Anterior pituitary contents of LH mRNA and FSH mRNA (mean arbitrary relative units) in intact bulls and castrated bulls (steers) treated with the GnRH agonist deslorelin

	LH β-subunit mRNA		FSH β-subunit mRNA	
	Control	GnRH agonist	Control	GnRH agonist
Bulls[a] (n = 4–5)	0.65	0.22 (P = 0.003)	–	–
Steers[b] (n = 8–11)	1.56	0.08 (P < 0.001)	1.01	0.34 (P < 0.001)

Values are means.
[a]Aspden *et al.*, 1997a; [b]Aspden *et al.*, 1996.

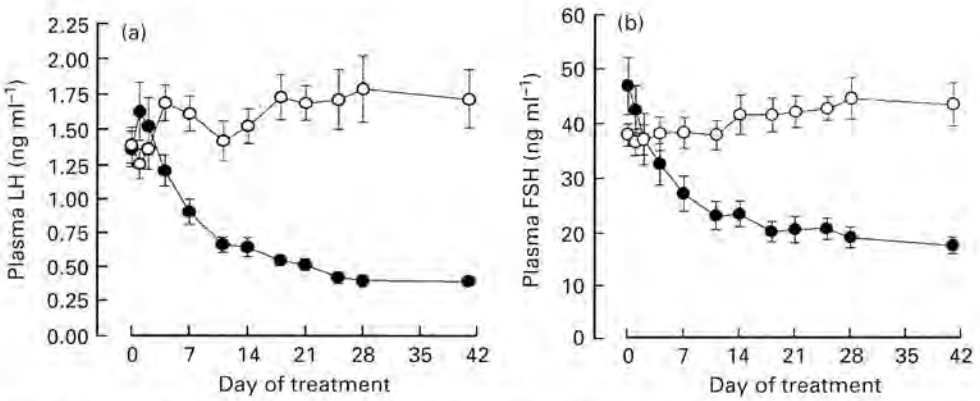

Fig. 1. Longitudinal profiles of plasma (a) LH and (b) FSH in control (open circles) castrated bulls (steers) and steers treated with the GnRH agonist deslorelin (solid circles) commencing on day 0. Results are means ± SEM (n = 8) (Aspden *et al.*, 1996).

was reduced in castrated bulls treated with agonist, basal secretion was maintained, similar to the finding in intact bulls (Fig. 1). The understanding that has emerged from GnRH agonist studies in cattle, therefore, is that pulsatile secretion of LH is blocked, which is consistent with GnRH receptor downregulation, but basal LH secretion is tonically increased (Evans and Rawlings, 1994; Jimenez-Severiano *et al.*, 1998).

The mechanism(s) that promotes increased basal secretion of LH in cattle treated with GnRH agonist has not been defined. The basal secretion could be constitutive and does not require typical second messenger pathways (Huckle and Conn, 1988). Alternatively, GnRH agonists may stimulate second messenger pathways in cattle to maintain increased basal LH secretion. The latter suggestion might be considered inconsistent with the downregulation of GnRH receptors in cattle during agonist treatment. Endogenous GnRH is not required for continued secretion of LH in bulls treated with GnRH agonist, as bulls treated with agonist, and simultaneously actively immunized against GnRH, maintained basal secretion of LH (Aspden *et al.*, 1997b).

Treatment of intact bulls with the GnRH agonist nafarelin caused a decrease in pituitary FSH content (Table 1). In castrated bulls, treatment with deslorelin was associated with a decrease in FSH mRNA (Table 2) but there was no apparent change in pituitary FSH content (Table 1). The latter observation was inconsistent with the reduction in plasma FSH in castrated bulls during treatment with deslorelin (Fig. 1).

The response of the pituitary to natural sequence GnRH is reinstated over several weeks after treatment with GnRH agonist in bulls (Bergfeld *et al.*, 1996a) and heifers (Bergfeld *et al.*, 1996b). It is not known whether the gradual recovery of pituitary responsiveness to GnRH is related to a gradual replenishment of GnRH receptors on gonadotroph cells, or to a gradual re-establishment of second

Table 3. Volume fractions and absolute volume (ml) per testis of seminiferous epithelium, lumen and interstitium for control bulls and bulls treated with deslorelin.

	Volume fraction			Absolute volume per testis		
	Seminiferous epithelium	Lumen	Interstitium	Seminiferous epithelium	Lumen	Interstitium
Control	0.693 ± 0.024^a	0.116 ± 0.018^a	0.192 ± 0.015^a	119.5 ± 5.9^a	19.8 ± 7.0^a	33.3 ± 3.6^a
Deslorelin	0.707 ± 0.014^a	0.110 ± 0.013^a	0.183 ± 0.007^a	168.0 ± 12.5^b	27.1 ± 4.1^a	43.4 ± 3.4^a

Results are means ± SEM ($n = 6$).
^{a,b}Means within columns without a common superscript are significantly different ($P < 0.01$).
^{a}Means within columns with a common superscript are not significantly different ($P > 0.05$).

messenger pathways within gonadotroph cells (Gorospe and Conn, 1988). Consistent with a gradual return to normal pituitary function after GnRH agonist treatment, post-pubertal heifers treated with a deslorelin bioimplant for 10, 28 or 56 days ovulated approximately 20 days after treatment (D'Occhio and Kinder, 1995; D'Occhio *et al.*, 1996). Heifers infused with buserelin for 48 days displayed oestrus 8–11 days after treatment, but a preovulatory surge release of LH had not occurred 22 days after treatment (Gong *et al.*, 1996). In young bulls, treatment with leuprolide from 6 to 20 weeks of age delayed the occurrence of a prepubertal rise in plasma LH and testosterone by 4 weeks, from 20 weeks to 24 weeks (Chandolia *et al.*, 1997).

Gonadal Responses to GnRH Agonist Treatment

Male cattle

Testosterone secretion, LH receptors and testis growth. The outstanding feature of the response in bulls to treatment with GnRH agonist is an increase in the secretion of testosterone which is maintained for the duration of treatment, irrespective of the dose of agonist (D'Occhio and Aspden, 1996). Increased testosterone secretion in bulls during agonist treatment was consistently demonstrated for deslorelin (Bergfeld *et al.*, 1996a; D'Occhio and Aspden, 1996; Aspden *et al.*, 1997a,b; Aspden *et al.*, 1998), nafarelin (Melson *et al.*, 1986), buserelin (Rechenberg *et al.*, 1986) and leuprolide (Ronayne *et al.*, 1993). However, relatively young bulls treated with leuprolide had reduced plasma concentrations of testosterone (Chandolia *et al.*, 1997).

In a long-term study, 20-month-old Brahman and Brahman × Hereford Shorthorn bulls treated with deslorelin maintained high plasma testosterone for more than 120 days (D'Occhio and Aspden, 1996). Over this period, bulls receiving GnRH agonist showed a faster rate of testis growth and at the end of treatment had larger testes (Aspden *et al.*, 1998). Increased testis size was associated with greater absolute volume per testis of seminiferous epithelium (Table 3). The numerical density of round spermatids in the testis was not increased in bulls treated with GnRH agonist, but the absolute number of round spermatids per testis was greater in bulls treated with agonist ($23.5 \pm 2.5 \times 10^9$) than in control bulls ($16.2 \pm 1.4 \times 10^9$). Increases in rate of testis growth in bulls during GnRH agonist treatment were not observed consistently in shorter-term studies (Melson *et al.*, 1986; Ronayne *et al.*, 1993). Notwithstanding the latter studies, chronic treatment with GnRH agonist may provide practical applications to influence testis growth and function in bulls, as discussed below.

Bulls treated with GnRH agonist had increased numbers of testicular LH receptors which might explain, in part, increased testosterone secretion (Melson *et al.*, 1986). However, in *in vitro* studies using rat Leydig cells, only approximately 1% LH receptor occupancy was required for a maximal testosterone response to gonadotrophin stimulation (Mendelson *et al.*, 1975). It is possible, therefore, that increased testosterone secretion in bulls treated with GnRH agonist is due to a combination of

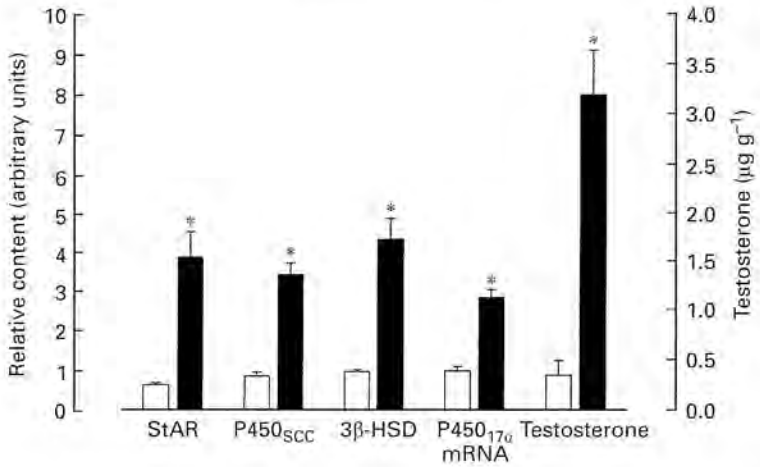

Fig. 2. Relative contents of testicular steroid acute regulatory (StAR) protein, P450scc, 3β-HSD, $P450_{17\alpha}$ mRNA (arbitrary units) and testosterone (μg g^{-1} tissue) in control bulls (open squares) and bulls treated with the GnRH agonist deslorelin (solid squares) for 10 days. Results are means ± SEM ($n = 4$ to 7); *$P < 0.001$ for all data (Aspden *et al.*, 1998).

increased LH receptors and tonically increased basal concentrations of LH. The increase in the number of LH receptors in bulls treated with agonist could be interpreted to mean that pulses of LH typically observed in bulls downregulate LH receptors in Leydig cells. The requirement of LH for increased testosterone secretion during GnRH agonist treatment was confirmed by the observation that plasma testosterone declined in bulls treated with agonist and simultaneously actively immunized against LH (Aspden *et al.*, 1997b).

StAR protein and steroidogenic enzymes in bulls. Steroid acute regulatory (StAR) protein facilitates the transport of cholesterol from the cytoplasm to the inner mitochondrial membrane, which is the rate-limiting step in steroid biosynthesis (Stocco, 1997). StAR protein mRNA was demonstrated in bovine testis tissue (Pilon *et al.*, 1997). Testicular content of StAR protein was increased in bulls treated with GnRH agonist, and which had increased testicular testosterone content and enhanced testosterone secretion (Aspden *et al.*, 1998). Major steroidogenic enzymes were similarly increased in bulls treated with GnRH agonist (Fig. 2). It would appear, therefore, that increased testosterone synthesis and secretion in bulls treated with GnRH agonist results from the stimulation of normal steroidogenic mechanisms in Leydig cells.

Bulls that had an increased plasma concentration of testosterone in response to chronic treatment with deslorelin showed a further acute increase in testosterone secretion subsequent to injection of human chorionic gonadotrophin (Table 4). This finding was interpreted as indicating that StAR protein and presumably steroidogenic enzymes are not maximally stimulated during GnRH agonist treatment in bulls.

Female cattle

Follicles. The acute increase in plasma LH that occurs at initiation of GnRH agonist treatment (Chenault *et al.*, 1990) can induce ovulation of growing preovulatory follicles (Macmillan and Thatcher, 1991). Luteinization, without ovulation, also can be induced by treatment with GnRH agonist (Macmillan and Thatcher, 1991; Rettmer *et al.*, 1992a). In Brahman heifers, ovulation was

Table 4. Plasma concentrations of testosterone at commencement of treatment with deslorelin (day 0) and on day 56 of treatment, and changes in plasma concentrations of testosterone after intramuscular injection of hCG (2 000 iu; 0 min) on day 56 for control bulls and bulls treated with deslorelin

		Testosterone (ng ml^{-1})			
			Day 56		
	n	Day 0	0 min	120 min	Δ Testosterone*
Control	6	$1.6 \pm 0.5^{a,x}$	$1.6 \pm 0.4^{a,x}$	$3.7 \pm 0.6^{a,y}$	2.1 ± 0.7^{a}
Deslorelin	8	$1.9 \pm 1.0^{a,x}$	$5.3 \pm 1.4^{b,y}$	$11.1 \pm 1.0^{b,z}$	5.8 ± 1.4^{b}

Results are means ± SEM (M. J. D'Occhio, unpublished).
*Increase in plasma concentration of testosterone after injection of hCG.
^{a,b}Means within columns without a common superscript are significantly different ($P < 0.05$).
^{x,y,z}Means within rows without a common superscript are significantly different ($P < 0.05$).

induced by GnRH agonist treatment on day 4 and day 6 of the oestrous cycle, but not day 2 or day 8 (Table 5). These findings were consistent with previous observations in which ovulation was consistently induced by treatment with GnRH agonist at about day 5 to day 6 of the oestrous cycle (Schmitt *et al.*, 1996b). Practical applications of the ovulation-inducing response to acute GnRH agonist treatment are discussed below.

In post-pubertal heifers, the absence of pulsatile secretion of LH associated with continued treatment with GnRH agonist prevented the development of follicles (Gong *et al.*, 1996) beyond the stage at which follicles acquire significant LH receptors and become LH-dependent (7–9 mm) (Bao *et al.*, 1997). Subsequently, plasma FSH was suppressed and follicle growth was restricted to early stages of development (≤ 4 mm) (Gong *et al.*, 1996). The GnRH agonist-treated heifer therefore provides an experimental model to study gonadotrophin requirements for growth, development and maturation of ovarian follicles.

In prepubertal heifers, treatment with deslorelin for 28 days was associated with increased plasma concentrations of oestradiol (Bergfeld *et al.*, 1996b). This finding was analogous to increased testosterone secretion in bulls treated with GnRH agonist. There may be a common response mechanism in cattle that leads to increased gonadal steroidogenesis during GnRH agonist treatment. This suggestion is supported by increased progesterone secretion by the corpus luteum in heifers treated with GnRH agonist commencing early in the oestrous cycle (Thatcher *et al.*, 1993; D'Occhio *et al.*, 1996; Pitcher *et al.*, 1997). Increased gonadal steroidogenesis in cattle receiving GnRH agonist could be related to the absence of pulsatile secretion of LH, but maintenance of tonically increased basal LH secretion (Evans and Rawlings, 1994; Jimenez-Severiano *et al.*, 1998).

Since bulls had increased testicular LH receptors during GnRH agonist treatment (Melson *et al.*, 1986), increased secretion of oestradiol in prepubertal heifers receiving agonist (Bergfeld *et al.*, 1996b) may be due to increased stimulation of thecal cells by LH and a greater supply of androgen for follicular aromatization to oestradiol (Berndtson *et al.*, 1995). However, this contention is not supported by the finding that post-pubertal heifers implanted with deslorelin, and undergoing superstimulation of ovarian follicle growth with FSH tended to have lower plasma concentrations of oestradiol (Fig. 3; heifers nos. 23, 50, 146 and 904). In the latter study, reduced secretion of oestradiol may have been due to reduced androgen precursor synthesis by thecal cells, due to a lack of pulsatile secretion of LH during stimulation of follicular growth with FSH.

It was suggested that the acute increase in LH that occurs when GnRH agonist treatment is initiated at the mid-luteal phase of the oestrous cycle (day 12–13) causes luteinization of follicles, which accounts for reduced secretion of oestradiol (Thatcher *et al.*, 1993; Rettmer *et al.*, 1992a). The steroidogenic response of follicles to GnRH agonist would appear to be influenced by age, ovarian status and other endocrine factors.

Heifers treated chronically with GnRH agonist cannot initiate an endogenous preovulatory surge release of LH and ovulation does not occur (D'Occhio *et al.*, 1997). Chronic GnRH agonist

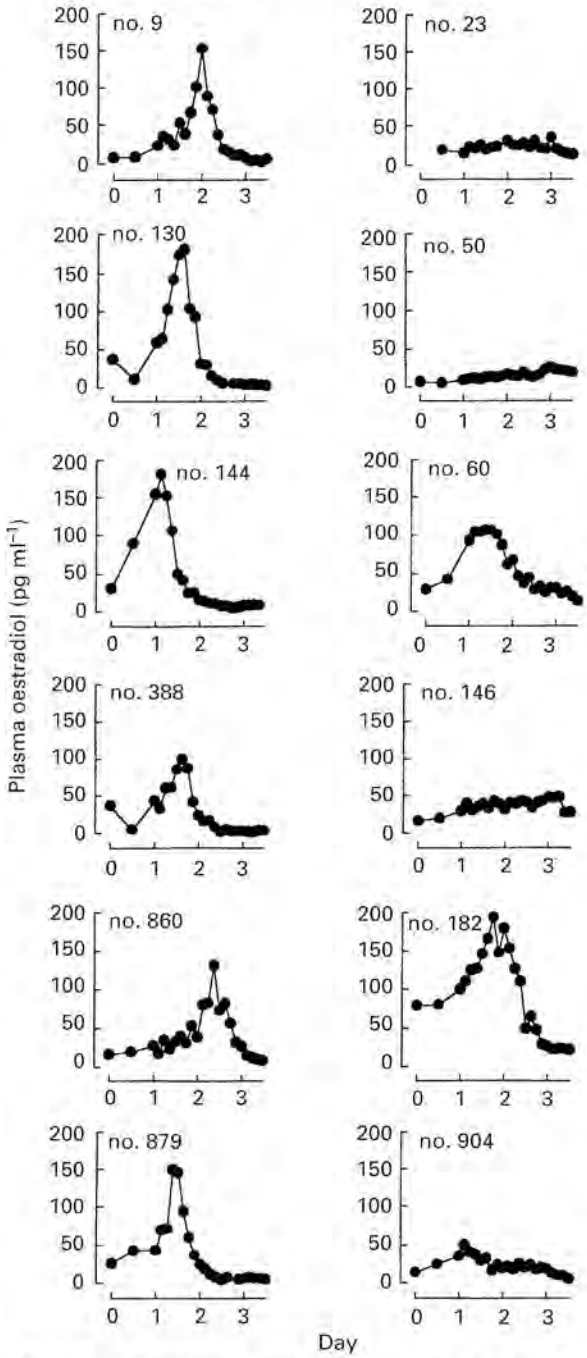

Fig. 3. Plasma concentrations of oestradiol for individual control heifers (left panel) and heifers treated with the GnRH agonist deslorelin (right panel). The latter heifers were implanted with a deslorelin bioimplant one week before treatment with FSH. FSH was used to stimulate ovarian follicle growth in both groups of heifers over 4 days, with FSH treatment ending on day 2 of the profiles (M. J. D'Occhio and J. E. Kinder, unpublished).

Table 5. Proportion of Brahman heifers that ovulated in response to treatment with a GnRH agonist bioimplant commencing on different days of the oestrous cycle

Day of oestrous cycle	Diameter of the largest follicle (mm)	Proportion of heifers that ovulated
2	4.7 ± 0.3	0/4
4	6.5 ± 0.7	2/4
6	10.0 ± 0.0	4/4
8	8.6 ± 0.9	0/4

Values are mean ± SEM (M. J. D'Occhio and F. Cremonesi, unpublished).

Table 6. Size of the corpus luteum and plasma concentrations of progesterone on day 13 of the oestrous cycle in control heifers and heifers treated with deslorelin from day 3 of the cycle[†]

	n	Corpus luteum (g)	Plasma progesterone (ng ml^{-1})
Control	16	3.1 ± 0.2^{a}	9.1 ± 1.3^{a}
Deslorelin	16	4.2 ± 0.4^{b}	18.9 ± 3.5^{b}

Results are means ± SEM.
[†]Pitcher *et al.* (1997).
[a,b]Means within columns without a common superscript are significantly different ($P < 0.05$).

treatment therefore has potential as a contraceptive approach in heifers and this is discussed below. The absence of an endogenous LH surge in heifers treated with agonist also provides the opportunity to control the time of ovulation with exogenous gonadotrophin. The value of this approach is discussed below in relation to multiple ovulation and embryo transfer.

Structure and function of the corpus luteum. Heifers treated chronically with GnRH agonist commencing early in the oestrous cycle (day 3) had a larger corpus luteum and secreted more progesterone than did untreated heifers (Table 6). Treatment with deslorelin was associated with higher basal plasma concentrations of LH (Pitcher *et al.*, 1997) which could have contributed, in part, to increased size and function of the corpus luteum. The corpus luteum in heifers treated with deslorelin had a greater content of StAR protein and the steroidogenic enzyme, cytochrome P450 side-chain cleavage enzyme (P450scc; Pitcher *et al.*, 1997). Treatment of heifers with buserelin early in the oestrous cycle caused an increase in the relative numbers of large luteal cells in the corpus luteum (Twagiramungu *et al.*, 1995).

As noted above, heifers treated with GnRH agonist from about day 4 to day 6 of the oestrous cycle can ovulate and develop an accessory corpus luteum. In this situation, increased progesterone secretion during the oestrous cycle is contributed by the existing spontaneous corpus luteum and the accessory corpus luteum. The potential application of GnRH agonist treatment early in the oestrous cycle to increase plasma progesterone and enhance the likelihood of pregnancy recognition and conception is discussed below.

Application of GnRH Agonists in Cattle

Male cattle

Puberty and sperm production. Pubertal (20-month-old) Brahman (*Bos indicus*) bulls treated chronically with deslorelin for 120 days had a faster rate of testis growth and at the end of treatment

had a greater sperm production capacity than untreated bulls (Aspden *et al.*, 1998). These findings indicate that reproductive development during the prepubertal period in bulls might be accelerated by chronic treatment with GnRH agonist. However, 5-month-old Freisian (*Bos taurus*) bulls treated with leuprolide for 56 days did not show enhanced testis growth (Ronayne *et al.*, 1993). In another study, Holstein bulls (*Bos taurus*) treated with natural sequence GnRH from 1.5 to 3.0 months of age showed a delay in puberty (Miller and Amann, 1986). In addition, Hereford (*Bos taurus*) bulls treated with a slow-release formulation of leuprolide at approximately 1.5, 2.5 and 3.5 months of age had smaller testes and fewer spermatids at about 12 months of age (Chandolia *et al.*, 1997). The present information therefore indicates that treatment with natural sequence GnRH or GnRH agonist occurring relatively early (1–3 months of age) may be detrimental to pubertal development, but treatment during the peripubertal period or after puberty may enhance testicular function.

Semen quality. Bulls treated with GnRH agonist had increased intratesticular testosterone and high plasma concentrations of testosterone (Aspden *et al.*, 1998). It is possible that GnRH agonist treatment may be beneficial in situations in which testosterone production is reduced and is limiting to sperm production, semen quality or both factors. Increased testosterone synthesis in response to GnRH agonist treatment may enhance semen quality by effects within the testes, epididymides or accessory sex glands.

Female cattle

Treatment of cystic follicles. The acute increase in plasma LH that occurs at initiation of GnRH agonist treatment in female cattle (Chenault *et al.*, 1990) can induce ovulation or luteinization of a cystic follicle (Thatcher *et al.*, 1993). GnRH agonists are used for the treatment of cystic follicles, particularly in post-partum dairy cows.

Synchronization of oestrus. The capacity of acute treatment with GnRH and GnRH agonists to induce ovulation and formation of a corpus luteum has been used to develop oestrous synchronization protocols. A particularly successful protocol involves injections of GnRH or GnRH agonist, in combination with prostaglandin, to induce the emergence of a new dominant preovulatory follicle, and to control the time of ovulation for fixed-time insemination (Twagiramungu *et al.*, 1995; Burke *et al.*, 1996; Schmitt *et al.*, 1996a; Pursley *et al.*, 1997).

Induction of accessory corpus luteum. The relationship of conception rates in heifers to absolute concentrations of progesterone in circulation remains equivocal (Rettmer *et al.*, 1992b; Macmillan and Peterson, 1993). If it is demonstrated that increased plasma concentrations of progesterone are associated with increased pregnancy rates, treatment with a GnRH agonist bioimplant commencing at about day 6 of the oestrous cycle may prove practical for inducing an accessory corpus luteum and increasing plasma progesterone, in combination with artificial insemination, embryo transfer, or both procedures. Treatment with GnRH agonist at about day 12 of the oestrous cycle after mating may extend the luteal phase and increase the opportunity for maternal recognition of pregnancy (Thatcher *et al.*, 1993).

Post-partum anoestrus. The use of GnRH agonist treatment to induce ovulation during the post-partum period was examined extensively in beef and dairy cattle (D'Occhio *et al.*, 1989; Roberge *et al.*, 1992; Twagiramungu *et al.*, 1995). Chronic treatment with agonist induced ovulation in post-partum anoestrous cows, but the life-span of the resulting corpus luteum was reduced. A corpus luteum of normal life-span could be induced if GnRH agonist treatment was preceded by progesterone priming (D'Occhio *et al.*, 1989). Most previously anoestrous animals induced to ovulate with GnRH or GnRH agonist treatment did not initiate regular oestrous cycles. The application of

GnRH agonist treatment to induce ovulation in post-partum anoestrous cows would therefore appear to be restricted to situations in which it can be combined with progestagen priming and artificial insemination.

Superovulation

Sexually mature heifers and cows. A GnRH agonist bioimplant was recently used to develop a new protocol for multiple ovulation and embryo transfer (MOET) in cattle (D'Occhio *et al.*, 1997). In the new protocol, donor heifers or cows are implanted with GnRH agonist approximately one week before commencement of FSH treatment. The preovulatory surge release of LH that typically occurs subsequent to FSH treatment is blocked due to the desensitizing actions of GnRH agonist described above and, accordingly, ovulation does not occur. However, ovulation can be induced by injection of exogenous LH (D'Occhio *et al.*, 1997).

An outstanding feature of the GnRH agonist–LH protocol for MOET is that the time of ovulation is determined by programming the injection of exogenous LH (D'Occhio *et al.*, 1998b). In a time-course study, it was found that optimal fertilization and recovery of embryos occurred when injection of exogenous LH was delayed by about 12 h, relative to normal occurrence of a preovulatory LH surge after stimulation with FSH (D'Occhio *et al.*, 1997). It was considered that a delay in the occurrence of an LH surge allowed additional follicles to develop and acquire sufficient LH receptors for an ovulatory response.

The observation of oestrus subsequent to FSH stimulation is not required in the GnRH agonist–LH protocol, and donors are inseminated relative to the injection of exogenous LH (D'Occhio *et al.*, 1997). In a recent study, typical rates of fertilization and embryo recovery were obtained after donor heifers received one insemination, 12 h after injection of exogenous LH (D'Occhio *et al.*, 1998a).

Heifer calves

The ability to recover viable oocytes from heifer calves provides opportunities for genetic improvement in cattle (Davis *et al.*, 1997). However, current production of viable embryos *in vitro* from oocytes of heifer calves remains relatively low and variable. Ovarian follicular waves are initiated early in the life span of heifer calves (Evans *et al.*, 1994) and there is evidence to indicate that follicular dominance is also established early. These factors may contribute to variability in the follicular response of young heifers to stimulation with gonadotrophins. In addition, heifer calves can have a surge release of LH during stimulation of follicle growth, which may further contribute to the recovery of a heterogeneous population of oocytes (Maclellan *et al.*, 1997).

A GnRH agonist bioimplant was recently used to block pulsatile secretion of LH and prevent the occurrence of a surge release of LH during stimulation of follicle growth with FSH in heifer calves (Maclellan *et al.*, 1997, 1998). Calves treated with deslorelin had more follicles after stimulation with FSH, and this translated into a greater number of oocytes and embryos (Maclellan *et al.*, 1997). This result was not observed in a subsequent study (Maclellan *et al.*, 1998) and additional studies are required in larger numbers of heifer calves to determine whether treatment with a GnRH agonist confers an advantage in follicle growth, oocyte recovery and oocyte developmental competency.

In vivo *oocyte maturation*

The GnRH agonist–LH protocol for MOET described above (D'Occhio *et al.*, 1997) provided an experimental model for examining whether it would be possible to achieve *in vivo* maturation of oocytes before recovery for *in vitro* procedures. Post-pubertal heifers were implanted with GnRH agonist, stimulated with FSH, and injected with LH 24 h after the final injection of FSH (Lindsey *et al.*, 1998). Oocytes were recovered 12 h after injection of LH (i.e. before ovulation) and immediately exposed to spermatozoa for *in vitro* fertilization. The oocytes showed a fertilization potential and

Table 7. Fertilization rate (cleavage) and embryo development (blastocyst) for oocytes recovered after superstimulation of follicle growth with FSH in heifers treated with deslorelin, and heifers treated with deslorelin and treated with exogenous LH

	n	Total number of oocytes	Cleavage	Blastocyst
Deslorelin	9	163	61 (42.3%)[a]	9 (5.5%)[a]
Deslorelin + LH	9	112	61 (54.5%)[a]	25 (22.3%)[b]

Oocytes were recovered 12 h after injection of exogenous LH (Lindsey *et al.*, 1998).
[a, b]Percentages within columns without a common superscript are significantly different ($P < 0.05$).

embryo developmental competency that were typical of oocytes exposed to a conventional 24 h *in vitro* maturation before fertilization (Table 7). Oocytes obtained from heifers treated with GnRH agonist and FSH, but not injected with LH, had normal rates of fertilization but embryo developmental competency was compromised (Table 7). On the basis of these preliminary findings, the capacity for fertilization and embryo developmental competency would appear to have a differential requirement for exposure to pulsatile LH, a surge release of LH, or both. It is possible that oocyte cytoplasmic maturation may be more dependent on exposure to an LH surge than is nuclear maturation.

It would be of interest to examine whether *in vivo* maturation of oocytes using the GnRH agonist–LH protocol could be applied to heifer calves to increase embryo development rates. The GnRH agonist–LH protocol could be further applied to examine fundamental questions relating to the gonadotrophin requirements for normal follicle and oocyte growth and maturation in cattle.

Contraception

The absence of surge releases of LH in heifers and cows treated with GnRH agonist led to studies on the potential of a long-acting GnRH agonist bioimplant as a new contraceptive approach in female cattle (D'Occhio *et al.*, 1996). In a recent study, a substantial contraceptive response was achieved over a period of approximately 12 months with a prototype GnRH agonist bioimplant (Table 8). The use of GnRH agonists provides opportunities for achieving a controlled, reversible suppression of fertility in female cattle.

Conclusion

The response of the anterior pituitary in cattle to treatment with GnRH agonist involves an acute phase (0 to 24 h) during which LH secretion is increased and a chronic phase during which pulsatile secretion of LH is suppressed, but basal release of LH is slightly but significantly increased. The mechanism(s) for continued release of LH during agonist treatment has not been elucidated, but may reflect non-stimulated constitutive release, or an action of the GnRH agonist on gonadotrophs to maintain functional second messenger pathways. An outstanding feature of the gonadal response in cattle treated with GnRH agonists is the increase in steroidogenic activity. This was demonstrated for increased testosterone secretion in bulls, increased oestradiol secretion in pre-pubertal heifers, and enhanced progesterone secretion in post-pubertal heifers treated with agonist commencing early in the oestrous cycle. The basis for the increase in steroidogenesis during agonist treatment is not understood but is likely to be related to tonically elevated basal secretion of LH which appears to be associated with an increase in gonadal LH receptors.

Both the acute and chronic phases of the LH response in cattle to treatment with GnRH agonist provide opportunities for practical applications of agonists. The acute increase in plasma LH that

Table 8. Ovarian activity in female cattle treated with a long-acting GnRH agonist bioimplant

	n	Duration of trial (days)	Number showing ovarian activity	Approximate duration of anoestrus in heifers and cows that showed a return of ovarian function (days)
Trial 1	76	387	20 (26%)	231 ± 19
Trial 2	84	376	8 (10%)	244 ± 13
Trial 3	99	394	9 (9%)	336 ± 3

Heifers and cows were grazed in the presence of bulls, and a return to cyclic ovarian activity was extrapolated from the time of conception (M. J. D'Occhio, G. Fordyce, T. Jubb and T. Whyte, unpublished). Contemporary untreated heifers and cows were introduced at regular intervals and conceived progressively during the trials (data not shown).

occurs at the beginning of agonist treatment has been used in the treatment of cystic follicles, in oestrous synchronisation protocols, and in new superovulation programmes. The lack of preovulatory surge releases of LH in heifers treated chronically with GnRH agonist will lead to the development of a long-acting contraceptive GnRH agonist bioimplant for female cattle. This will have application in the management of fertility in extensively managed beef herds and in the prevention of conception in pre-feedlot heifers. Other possible applications of GnRH agonists could be to increase progesterone secretion in combination with artificial insemination and embryo transfer to enhance conception rates, and to increase testosterone secretion chronically in bulls that have poor semen quality due to reduced testosterone synthesis.

The GnRH agonist-treated heifer will continue to provide an experimental model for studying gonadotrophin requirements for normal follicle and oocyte growth and development in cattle. These studies should lead to GnRH agonist-based protocols for *in vivo* maturation of oocytes before collection for *in vitro* procedures.

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