Oestrous suppression and pregnancy prevention in cattle using GnRH agonists

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Abstract

The aims in this thesis were: (1) to characterise ovarian function in heifers and cows treated long-term with the GnRH agonist deslorelin; and (2) to evaluate the potential of controlled-release deslorelin implants for the prevention of pregnancy in heifers and cows maintained in extensive environments.

Study 1

The first study was conducted on three cattle stations. On Station 1, a total of 100 heifers and cows each received a GnRH agonist implant that contained 12 mg deslorelin. On Stations 2 and 3, a total of 168 heifers and cows each received a GnRH agonist implant that contained 8 mg deslorelin. The heifers and cows used at the three locations were confirmed non-pregnant at the start of the study. Animals were managed under standard conditions as single groups on each station. Bulls (4%) were kept with females for the duration of the study. Control, untreated heifers and cows were included at each site to confirm that bulls were not limiting to fertility in each herd.

Pregnancy and time of conception were estimated based on foetal size as determined by rectal palpation. Pregnancy rates at Station 1 (12 mg implant) were: heifers (n=50), 0% at 6 months and 10% at 11 months; cows (n=50), 0% at 6 months and 6% at 11 months. Pregnancy rates at Station 2 (8 mg implant) were: heifers (n=41), 0% at 6 months and 10% at 12 months; cows (n=48), 4% at 6 months and 12% at 12 months. On Station 3 (8 mg implant) pregnancy rates were: heifers (n=40), 8% at 6 months and 28% at 11 months; cows (n=37), 8% at 6 months and 24% at 11 months. Pregnancy rates for control cattle at Station 1 were: heifers (n=10), 50% at 6

months and 80% (new group introduced at 6 months, n=10) at 11 months; cows (n=10), 100% at 6 months and 80% (new group introduced at 6 months, n=10) at 11 months. Pregnancy rates for control cattle at Station 2 were: heifers (n=8), 12% at 6 months and 62% at 12 months; cows (n=11), 64% at 6 months and 91% at 12 months. On Station 3 pregnancy rates for control cattle were: heifers (n=10), 78% at 6 months and 60% (new group introduced at 9 months, n=10) at 11 months; cows (n=10), 100% at 6 months, and no further control cows were introduced.

At the completion of field observations at 12 months treated animals were slaughtered to determine pregnancy status and characteristics of ovaries and reproductive tracts. Final pregnancy data and estimated average number of days to first conception for deslorelin-treated cattle were: Station 1 (n=99), 9% pregnant and 336 ± 3 days; Station 2 (n=76), 26% pregnant and 231 ± 19 days; Station 3 (n=84), 10% pregnant and 244 ± 13 days. Treatment with a deslorelin implant in heifers and cows restricted ovarian follicular growth to early antral follicles (1-2 mm diameter), as observed by ultrasound scanning.

Study 2

In Study 1, the return to normal fertility in heifers and cows treated with a deslorelin implant appeared to be related to increased pasture availability and accelerated rate of live weight gain, which may put the cattle in a better condition to achieve pregnancy. Study 2 was designed to specifically examine whether treatment with a deslorelin implant suppressed ovarian function for at least 12 months in heifers gaining live weight at a relatively fast rate. GnRH agonist implants that contained three doses of deslorelin were used in Study 2. A group of 198 heifers

showing oestrous cycles at regular intervals were assigned to one of four groups: Control (n=50), not treated; GnRH agonist-low dose (n=50), 3 mg deslorelin; GnRH agonist-medium dose (n=50), 6 mg deslorelin; GnRH agonist-high dose (n=48), 12 mg deslorelin. All heifers and four bulls were maintained on natural pastures. Ultrasonography at monthly intervals was used to monitor ovarian follicular activity, corpus luteum development, conception and maintenance of pregnancy. During the 12-month study, heifers gained 153 ± 3 kg which was approximately 0.4 kg/day. The duration of suppression of ovarian activity was related to the dose of deslorelin in the implants. The 3 mg deslorelin implant suppressed ovarian activity for 3 months with cumulative pregnancies of 4% at 4 months, 57% at 8 months and 75% at 12 months. The 6 mg deslorelin implant suppressed normal ovarian function for 5 months with cumulative pregnancies of 2%, 36% and 54% at 4, 8 and 12 months, respectively. The 12 mg deslorelin implant suppressed ovarian cyclicity for almost 12 months with 6% cumulative pregnancies at 12 months. Pregnancies in control heifers, that were replaced at regular intervals, occurred throughout the study confirming that bulls were not limiting in fertility.

In summary, deslorelin implants were found to suppress ovarian activity in heifers and cows. The duration of suppression of ovarian activity was related to dose of GnRH agonist. Treatment with a GnRH agonist implant that contained 12 mg deslorelin prevented pregnancies for up to 12 months in heifers undergoing continuing live weight gain. The thesis has shown that GnRH agonist implants have the potential for use as a non-surgical technique for the prevention of pregnancies in heifers and cows in extensive beef production systems typical of northern Australia.

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Declaration

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

Signature Redacted

Timothy R. Whyte

Statement of Access

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List of Abbreviations

CL	corpus luteum
FSH	follicle stimulating hormone
Gel PBS	Phosphate Buffered Saline containing Gelatin as blocking agent
GnRH	gonadotrophin releasing hormone
PBS	phosphate buffered saline
$PGF_{2\alpha}$	prostaglandin $F_{2\alpha}$
LH	luteinising hormone

Acknowledgments

I have received tremendous support in undertaking this study. I would like to acknowledge this support and thank the personnel listed below. I thank my supervisors, Dr Michael Coates and Dr William Aspden. I need to acknowledge the initial support and supervision in the early stages of this thesis of Professor Michael D'Occhio.

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For advice, guidance and technical assistance during the field phases of this study I thank Geoffry Fordyce, Neil Cooper and Felicity Hill from the Queensland Department of Primary Industries (QDPI). Also from the Queensland Department of Primary Industries, and in acknowledgemen for use of the facilities of Brigalow Research Station, I thank Tony Barnes, Doug Rynne and Tony Hawke.

For support and provision of the deslorelin implants my thanks to Dr Tim Trigg and John Walsh (Peptech Animal Health Pty Limited).

Dedication

This thesis is dedicated to the ethical management of scientific Research and the honest and just treatment of researchers undertaking scientific research.

Vale, Reproduction and Endocrinology. August 1997

Chapter 1: Introduction and literature review

1.1 Introduction

The viability of the beef industry, particularly in northern Australia, is influenced by many factors including environment and practices associated with management of reproduction (D'Occhio, 1998). Of increasing importance is the value of heifers and cows surplus to breeding requirements. A significant target market for heifers is the live export trade, which requires non-pregnant animals. Standard industry practice for the prevention of pregnancy in female cattle is to surgically remove the ovaries (paralumbar and trans-vaginal spaying) or to sever the ovaries from the reproductive tract (Willis Dropped-Ovary Technique) (Meat and Livestock Australia, [MLA], 2002). These invasive procedures cause production loses due to mortality and morbidity and animals require a recovery period. Intrauterine contraceptive devices would appear unsuitable for use in extensive environments typical of northern Australia (D'Occhio, 1998). Therefore, an effective and efficient technique for management of fertility in cattle, such as a contraceptive implant that did not involve surgical intervention, would be of major benefit in extensive beef herds in northern Australia.

1.2 Literature review

This review will firstly introduce the female reproductive system, with particular reference to cattle, and focus on: (i) the hypothalamic-pituitary-gonadal axis; (ii) the structure, synthesis, secretion and actions of gonadotrophin releasing hormone (GnRH) also known as luteinising hormone releasing hormone (LHRH); (iii) the

concentration and patterns of secretion of the anterior pituitary gonadotrophic hormones luteinising hormone (LH) and follicle stimulating hormone (FSH); (iv) the effects of LH and FSH on ovarian activity, and in particular steroidogenesis and oogenesis; and (v) the structure and actions of GnRH agonists and their use in female cattle.

1.3 Anatomy, physiology, and endocrinology of female reproductive system

1.3.1 General overview

Many of the processes of reproduction in mammals are regulated by the central nervous system and cyclical patterns of activity in females are also controlled by the hypothalamic-pituitary-ovarian axis (Arthur *et al.*, 1983). External stimuli (for example, visual or olfactory, Ellendorff, 1978) act on the central nervous system and affect the hypothalamus. This in turn releases GnRH which acts at the anterior pituitary gland, resulting in the synthesis and release of gonadotrophic hormones LH and FSH. These gonadotrophic hormones then regulate ovarian function. The ovaries, by responding with the biosynthesis and release of steroid hormones and other factors are able, in turn, to affect feedback regulation at the pituitary in some species, and at the brain (Hansel and Convey, 1983; Figure 1.1). Steroid feedback has been demonstrated at the brain and directly at the anterior pituitary gland in livestock (Tilbrook and Clarke, 1995; McWilliams *et al.*, 1998).

1.3.2 The hypothalamic - pituitary - ovarian axis

Normal reproductive function in female cattle is dependent on endocrine signaling between the brain, anterior pituitary gland and ovaries (Johnson and Everitt, 2000) (Figure 1.1). The hypothalamus forms the base of the brain and surrounds the

third ventricle (Karsch, 1984). Gonadotrophin releasing hormone is synthesised at various nuclei within the hypothalamus and GnRH secreting neurons track to and terminate within the basal hypothalamus-median eminence (Johnson and Everitt, 2000). The median eminence is vascularised by the hypothalamo-hypophyseal portal blood system and GnRH neuron endings are found within this capillary network (Karsch, 1984). GnRH is released in a pulsatile manner in many species and enters fenestrated capillaries of the hypothalamo-hypophyseal system, which coalesce and drain to the anterior pituitary where they form a secondary capillary plexus (Karsch, 1984). The secondary capillary plexus allows GnRH to diffuse into the anterior pituitary (Karsch, 1984). GnRH has been shown to be a principal regulator of hormones within the reproductive axis in female cattle (Thatcher *et al.*, 1989; Drost and Thatcher, 1992).

At the anterior pituitary gland, GnRH acts through gonadotroph cell GnRH receptors. The bovine GnRH receptor is a member of the 7-transmembrane, G protein-coupled receptor family (Kakar *et al.*, 1993). Binding of GnRH to its receptor activates the G-protein through signal transduction and subsequently sets in motion associated second messenger pathways. Binding of GnRH to its receptor also results in dimerisation of receptors and internalisation within the gonadotroph cell, (Kaiser *et al.*, 1997). Therefore, even under normal circumstances gonadotroph cells are transiently insensitive to stimulation from GnRH. Receptivity is typically restored over 1-2 hours and involves synthesis of new GnRH receptors and their return to the surface of gonadotroph cells (Kaiser *et al.*, 1997). During chronic treatment with a GnRH agonist, GnRH receptors are not replenished at the surface of gonadotroph cells and the cells remain insensitive to endogenous GnRH. Second messenger systems of the GnRH receptor, such as calcium mobilisation and its interaction with calmodulin

and activation of protein kinase C, that stimulate the synthesis and release of the gonadotrophic hormones, LH and FSH, may become uncoupled (Hazum and Conn, 1988).

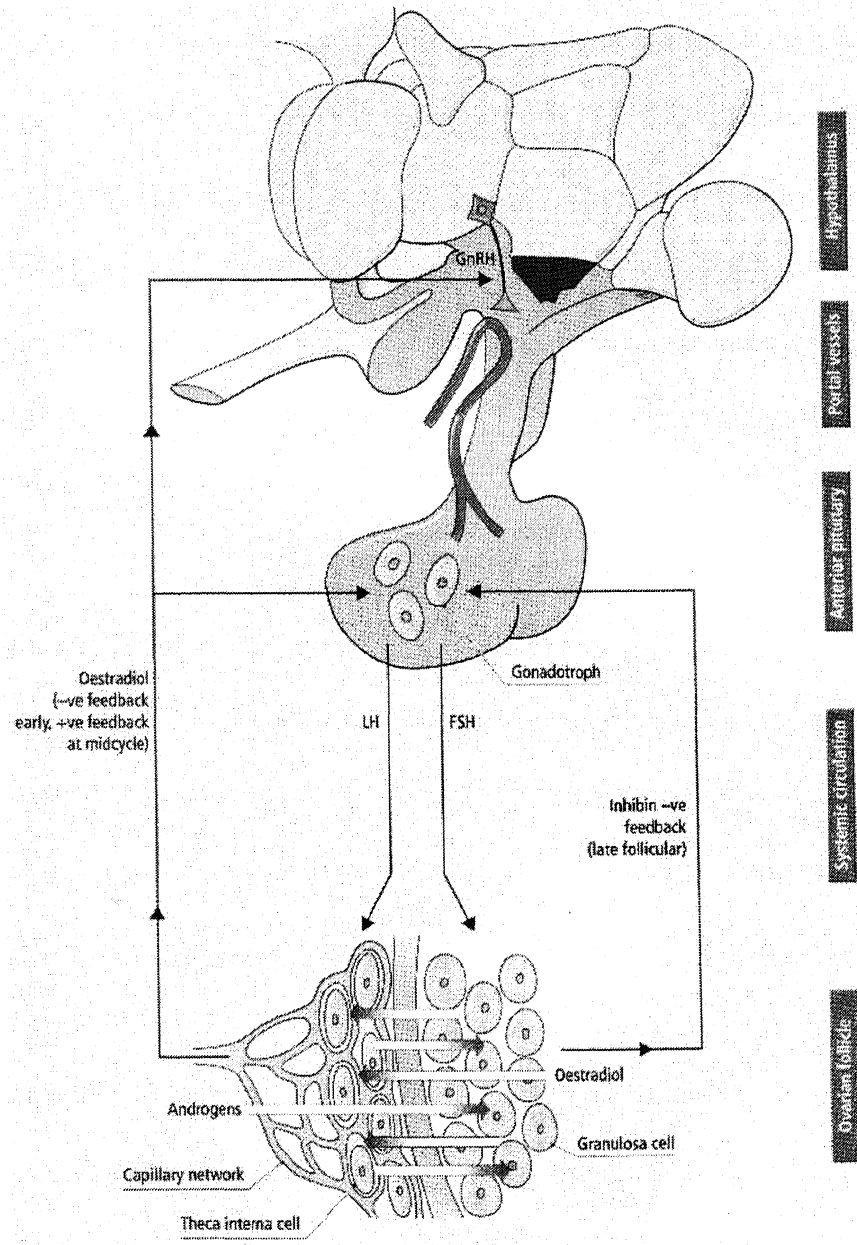

Figure 1.1. Summary of reproductive endocrine axis in the female. GnRH released from the hypotahlamus acts at the anterior pituitary to stimulate the release of LH and FSH, which act at the gonads to induce steroid biosynthesis which regulates ovarian function, and provides feedback to the brain. Inhibin provides negative feedback to the hypothalamus to reduce secretion of FSH. (Reproduced from Johnson and Everitt, 2000, p115).

1.3.3 GnRH structure

Mammalian GnRH is a decapeptide neurohormone and has the linear sequence pyro-Glu^{1}-His^{2}-Trp^{3}-Ser^{4}-Tyr^{5}-Gly^{6}-Leu^{7}-Arg^{8}-Pro^{9}-Gly^{10}-NH_2 (Matsuo *et al.*, 1971; Burgus *et al.*, 1972). GnRH is conserved across mammalian species with respect to peptide length, amino acid sequence, and the NH_2-terminal (residues pGlu-His-Trp-Ser) and the COOH-terminal (residues Pro-Gly-NH_2). These terminal sequences are involved in GnRH receptor binding at the surface of anterior pituitary gonadotroph cells. The NH_2- terminal is also essential for receptor activation (Sealfon *et al.*, 1997). Endogenous GnRH from the hypothalamus, and natural sequence GnRH administered exogenously, are rapidly metabolised, with a circulating half-life of 2-5 minutes in humans (Candas *et al.*, 1990).

1.3.4 GnRH secretion

Procedures developed for the collection of serial samples of blood from the hypothalamo-hypophyseal portal system in sheep have permitted the measurement of the secretory pattern of GnRH (Clarke and Cummins, 1982). Rodriguez and Wise (1989) observed pulsatile secretion of GnRH in the hypothalamo-hypophyseal system in bull calves and similar pulsatile secretion of GnRH was recently demonstrated in female cattle (Yoshioka *et al.*, 2001). The average pulse frequencies of both GnRH and LH were greater during proestrous and the early luteal phase than during the luteal phase in female cattle (Yoshioka *et al.*, 2001). However, mean concentration and pulse amplitude of GnRH and LH did not differ between these phases (Yoshioka *et al.*, 2001). During the proestrous and early luteal phases approximately 80% of GnRH pulses were accompanied by a pulse of LH. In the mid-luteal phase, the proportion of GnRH pulses that resulted in an LH pulse decreased to around 60%. Pulsatile

secretion of GnRH followed by corresponding pulses of LH are illustrated schematically in Figure 1.2. A general relationship between a pulse of LH coinciding with the secretion of a pulse of GnRH has been elucidated; however, it has also been shown in other species generally that GnRH pulses may not always produce corresponding pulses of LH (Karsch *et al.,* 1987; Clarke and Cummins, 1982). The patterns and concentration of LH and FSH secreted by the anterior pituitary are mediated primarily by the concentration, frequency and amplitude of pulses of GnRH (Conn, 1986). Other factors such as inhibin, activin and follistatin are additional regulators of FSH release (Ying, 1988).

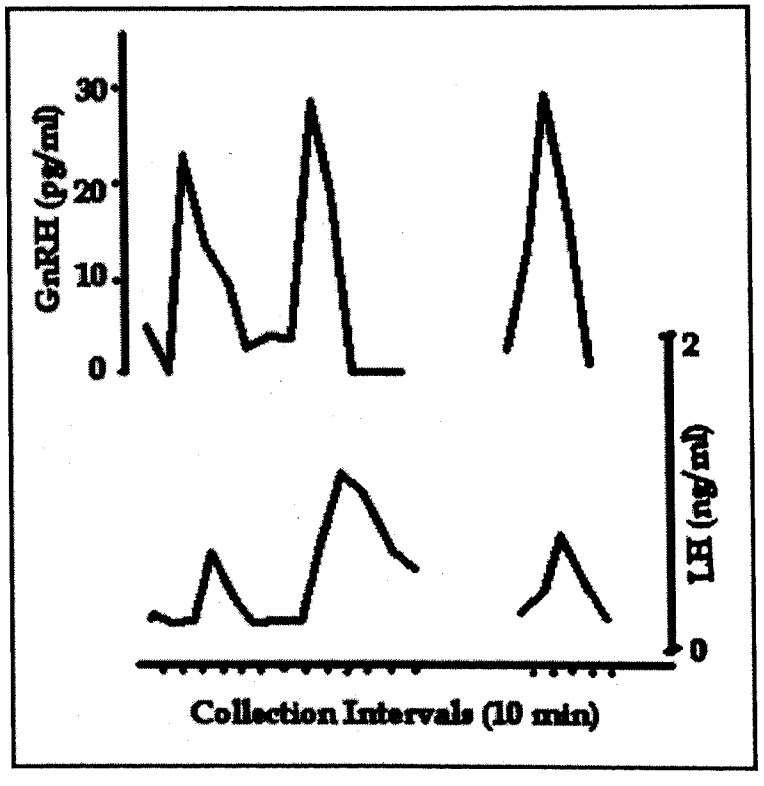

Figure 1.2. Schematic representing concentrations of GnRH in the hypophyseal portal system and cavernous sinus, and corresponding pulses of LH from the anterior pituitary entering the general circulation (Redrawn from Rodriguez and Wise, 1989).

1.3.5 Ovarian function in female cattle

The length of the oestrous cycle in female cattle is around 21 days (Figure1.3) (Knopf *et al.*, 1989). The luteal phase occurs from approximately Day 4 to Day 17, and the follicular phase and luteolysis occur from Day 17 to Day 21, depending upon recruitment of a dominant follicle from the second or third follicular wave. Follicular development during the oestrous cycle in the bovine ovary occurs in a series of follicular waves (Knopf *et al.*, 1989). In each wave, a cohort of follicles from the pool of early antral follicles begins to grow in response to stimulation by a transient increase in FSH (Adams *et al.*, 1992; Ireland *et al.*, 2000) (Figure 1.3). In female cattle, the first follicular wave regresses, with the dominant follicle undergoing atresia, which is associated with a reduction in plasma concentrations of FSH (Adams, 1999). Atresia of the first-wave dominant follicle leads to a decline in oestradiol and inhibin, which reduces feedback at the pituitary on FSH and results in a transient increase in FSH which recruits gonadotrophin-sensitive early antral follicles to form the second wave of follicular development (Savio *et al.*, 1993; D'Occhio *et al.*, 1999). The same result occurs if the second wave dominant follicle also becomes atretic and results in the third wave of follicular development of the oestrous cycle. A small proportion of oestrous cycles can have 4-waves of follicular development (Sirois *et al.*, 1988; D'Occhio *et al.*, 1999).

A decrease in episodic secretion of LH has been associated with the loss of dominance and the end of a non-ovulatory follicular wave (Ireland *et al.*, 2000). A number of follicular growth factors, including inhibins, activins, and insulin-like growth factors and related binding proteins, have been found in follicular fluid of bovine follicles. *In vitro* studies have shown that these factors may modify

gonadotrophin-stimulated follicular growth and differentiation (Ireland *et al.*, 2000). However, the precise role of intra-follicular growth factors in turnover of dominant follicles has not been determined. The dominant follicle of the second, third, or occasionally fourth wave of follicular development is recruited for maturation towards ovulation. Ovulation occurs in response to a surge release of LH from the anterior pituitary gland (Adams, 1999).

The first dominant follicle of oestrous cycles in heifers was detected on about Day 4 of the oestrous cycle, attained a maximum size on Day 6, remained stable from Days 6-10 and decreased in size until being undetectable by Day 15 (Savio *et al.*, 1988). The second dominant follicle of an oestrous cycle was detectable at Day 12, attained maximum size on average by Day 16, when non-ovulatory, and was undetectable by Day 19. When the dominant follicle from the second wave of the oestrous cycle was ovulatory, it reached maximum size around Day 19. Where the dominant follicle from the second wave of follicular development regressed, the third ovulatory follicle of the third wave was detectable by Day 16 and maximum size was achieved on Day 21 (Savio *et al.*, 1988). During three waves of follicular development in heifers, each dominant follicle was shown to be larger than the preceding ones (Savio et al., 1988). Ovulation then occurs when stimulated by the pre-ovulatory surge release of LH and the tissues of the dominant follicle develop into a corpus luteum.

It is believed that the dominant follicle causes regression of other follicles in a follicular wave (Ko *et al.*, 1991; Garcia *et al.*, 1999). Also, the dominant follicle during its growing phase acts as a suppressive factor against the next follicular wave. The dominant follicle secretes oestradiol and inhibin which feed back on FSH at the hypothalamus and/or pituitary and prevent the transient increase in FSH required for recruitment of follicles into a follicular wave (Ko *et al.*, 1991; *Garcia et al.*, 1999).

The mechanisms that promote the selection of the dominant follicle are not fully understood.

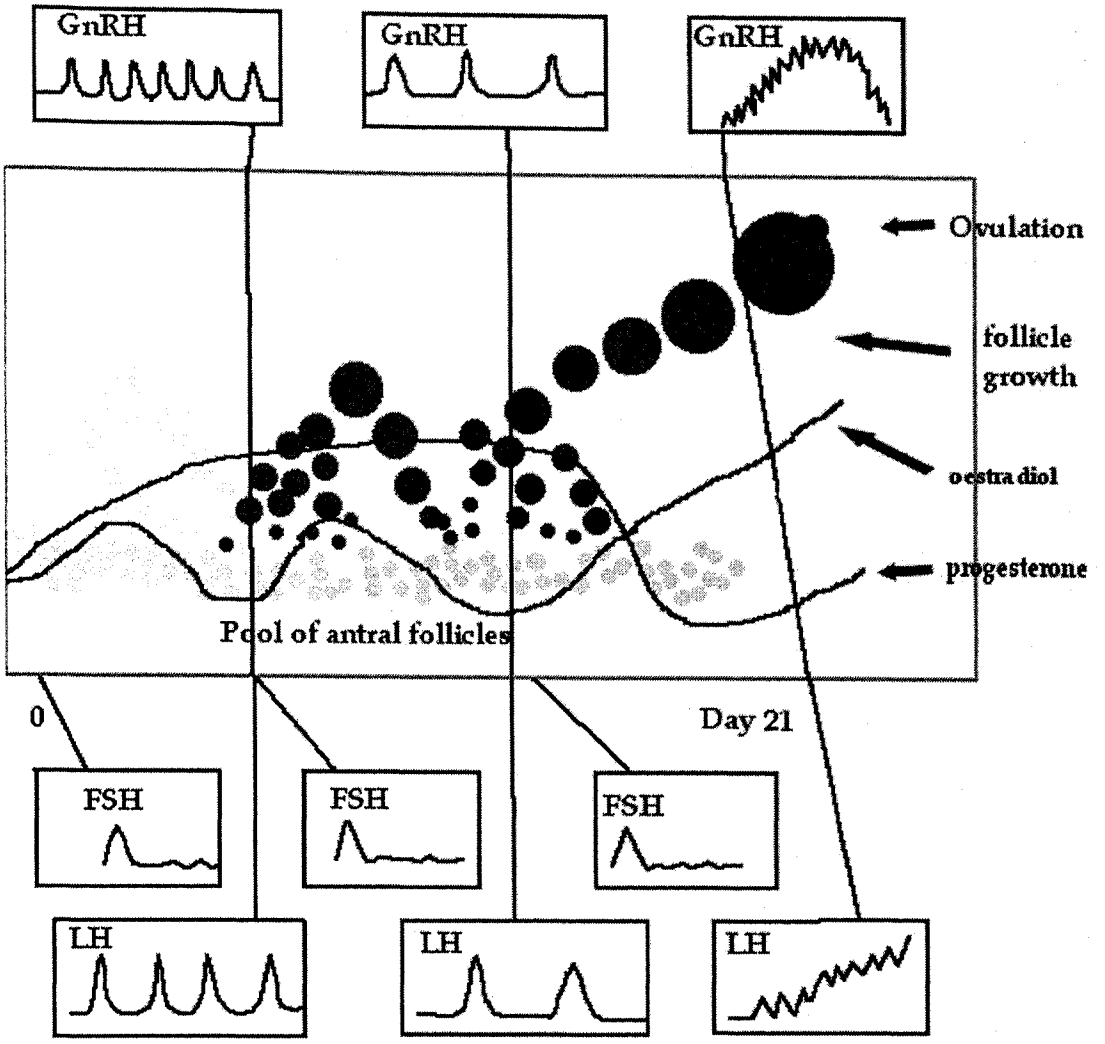

Figure 1.3. Schematic view of follicular recruitment and development during the normal bovine oestrous cycle. Three ovarian follicular waves are shown in this schematic, each stimulated by a transient increase in FSH. The dominant follicles of the first two follicular waves undergo regression as no 'significant' LH surge occurs as a corpus luteum is present and progesterone feedback on pulsatile secretion of GnRH precludes a preovulatory surge release of LH, even though the follicles may produce sufficient oestradiol for a positive feedback effect. Ovulation occurs from the dominant follicle of the third follicular wave in response to the LH surge. The LH surge occurs in response to an increase in oestradiol released by the follicle stimulating a single pulse or series of pulses of increasing frequency that become superimposed and lead to the preovulatory surge of GnRH that produces the preovulatory surge of LH. The LH surge is similarly comprised of superimposed pulses of LH. (Redrawn/adapted from Pitcher, 1999).

1.3.6 Gonadotrophin releasing hormone agonists

GnRH agonists were first developed to manage conditions caused by insufficient endogenous secretion of GnRH, such as hypothalamic hypogonadism (Crowley *et al.*, 1980). It was soon found, however, that chronic administration of GnRH agonist downregulated anterior pituitary gland release of LH and suppressed the reproductive endocrine axis (de Koning *et al.*, 1978). Differences between the GnRH agonist deslorelin, used exclusively in these previous trials, and natural sequence GnRH include the substitution of L-glycine with D-tryptophan at position 6 of the decapeptide (Figure 1.4). This altered structure of the peptide increases the circulating half-life of the agonist, by reducing enzymatic cleavage in circulation. A second structural difference, which increases the affinity of the agonist for the GnRH receptor, is the removal of the amino-terminal glycine (Karten and Rivier, 1986).

It was recognised during early studies with GnRH agonists that treatment with agonist produced a reproductive response that was dependent upon dose and duration of treatment. Regardless of dose, the acute response to GnRH agonist treatment was increased gonadotrophin secretion (Chenault *et al.*, 1990). Prolonged treatment caused anterior pituitary gonadotrophin secretion to decrease as a result of down regulation of GnRH receptors on gonadotroph cells, as shown in male cattle (Melson *et al.*, 1986).

GnRH

PyroGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2

1 2 3 4 5 6 7 8 9 10

Deslorelin

PyroGlu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-NH-CH2-CH3

1 2 3 4 5 6 7 8 9 (ethylamide group)

Figure 1.4. Structure of deslorelin compared with natural sequence GnRH.

1.3.7 GnRH agonist treatment - anterior pituitary response

The pituitary gonadotroph cells are the target sites for direct effects of GnRH agonists (Melson *et al.*, 1986; Conn *et al.*, 1987; Hazum and Conn, 1988; Huckle and Conn, 1988; Conn *et al.*, 1995; Kaiser *et al.*, 1997). Melson *et al.* (1986) showed reduced numbers of pituitary GnRH receptors in bulls treated with the GnRH agonist nafarelin. This supports the downregulation of receptors affected by GnRH agonists (Hazum and Conn, 1988). Downregulation occurs when GnRH agonists cause the micro-aggregation and internalisation of GnRH receptors rendering the gonadotroph cell insensitive to further GnRH stimulation (Conn and Hazum, 1981). With continued exposure to GnRH agonist, receptors at the surface of gonadotroph cells are not replaced (Huckle *et al.*, 1988; Hawes *et al.*, 1992).

Bulls and heifers treated with GnRH agonists showed an abolition of pulsatile secretion of LH and FSH but appeared to maintain a basal LH and FSH secretion (Melson *et al.,* 1986; Evans and Rawlings, 1994; Gong *et al.*, 1995, 1996; D'Occhio and Aspden, 1996; Aspden *et al.*, 1997; Jimenez-Severiano *et al.*, 1998). An example of the abolition of pulsatile LH and reduction in transient increases of FSH in female cattle treated with a GnRH agonist is depicted in Figure 1.5.

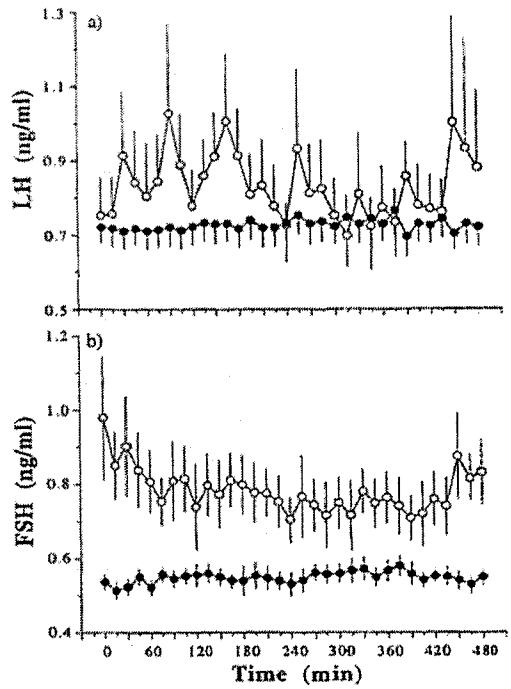

Figure 1.5. Mean (± SEM) circulating plasma concentrations of LH (a) and FSH (b) in control heifers (open circles) and GnRH agonist-treated heifers (closed circles) on Day 38 of treatment. Heifers treated with GnRH agonist did not have pulsatile secretion of LH and FSH (Reproduced from Gong *et al.*, 1996).

1.3.8 Gonadal response in female cattle to GnRH agonist treatment

1.3.8.1 Ovarian follicular response

Treatment with GnRH agonist induced an acute plasma LH increase in heifers, which mimicked the pre-ovulatory LH surge observed in normal heifers (Chenault *et al.,* 1990). Macmillan and Thatcher (1991) showed that this LH surge induced ovulation from follicles that were at least 6 mm in diameter in *Bos taurus* cattle. Luteinisation, without ovulation, can also be induced by treatment with GnRH agonists (Macmillan and Thatcher, 1991; Rettmer *et al., 1992).* Treatment with GnRH agonists also blocked the endogenous preovulatory surge release of LH, thus blocking ovulation (D'Occhio *et al.,* 1997).

Following the acute response, follicle stimulating hormone secretion is suppressed in GnRH agonist-treated heifers, restricting follicle recruitment (Gong *et al.*, 1996). With suppression of pulses of FSH, follicles were not recruited from the gonadotrophin-sensitive pool, hence follicular development was restricted to early antral follicles. Follicular growth to early antral follicles is generally regarded as gonadotrophin independent (Bao *et al.*, 1997).

1.3.8.2 Corpus luteum response

Prolonged treatment of heifers with GnRH agonists starting on Day 3 of the oestrous cycle resulted in a larger corpus luteum (CL) and increased secretion of progesterone during the luteal phase of the cycle compared with that of untreated heifers (Pitcher *et al.*, 1997; Table 1.1).

Table 1.1. 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. Results are means ± SEM [t].

	N	Corpus luteum (grams)	Plasma progesterone (ng/ml)
Control	16	3.1 ± 0.2^{a}	9.1 ± 1.3^{a}
Deslorelin	16	4.2 ± 0.4^{b}	18.9 ± 3.5^{b}

[t] Pitcher et al., 1997
[a, b] Means within columns without a common superscript differ ($P<0.05$; independent t- test).

Deslorelin treatment, commenced on Day 3 of the oestrous cycle was associated with greater basal concentrations of LH in blood plasma, which most likely contributed to increased size and function of the corpus luteum (Pitcher *et al.*, 1997). Heifers treated during the oestrous cycle with the GnRH agonist buserelin had a

greater number of large luteal cells in the corpus luteum; large luteal cells have been shown to produce more progesterone as compared with small luteal cells (Twagiramungu *et al.*, 1995).

Increased gonadal steroidogenesis in cattle treated with GnRH agonist (Bergfeld *et al.*, 1996; D'Occhio *et al.*, 1996; Pitcher *et al.*, 1997) could be related to the absence of pulsatile secretion of LH, but the maintenance of (Evan and Rawlings, 1994) or increase in (Jimenez-Severiano *et al.*, 1998) basal LH secretion. In male cattle treated chronically with GnRH agonists, there was an increase in numbers of LH receptors (Melson *et al.*, 1986) and content of steroidogenic enzymes (Aspden *et al.*, 1998) in the testes. Therefore, it cannot be excluded that there are changes in numbers of LH receptors in the corpus luteum of female cattle during treatment with a GnRH agonist. It was suggested that pulses of LH may serve to maintain LH receptor numbers in cattle gonads within normal ranges and with the abolition of pulses of LH in GnRH agonist-treated cattle, numbers of LH receptors may increase (Melson *et al.*, 1986).

1.4 Aims in the present thesis

Chronic treatment with GnRH agonists in female cattle blocked the transient increase in FSH necessary for recruitment of a wave of follicular development and development of a dominant follicle, resulting in follicles restricted in growth to the early antral stage. Chronic treatment with GnRH agonists in cattle also blocked the pre-ovulatory surge release of LH. Previous studies with GnRH agonists in cattle were conducted only over a relatively short time period of one to two months.

The two studies reported in this thesis examined whether implants containing the GnRH agonist deslorelin had the potential for long-term suppression of cyclic

ovarian activity, and, therefore, prevention of pregnancies in cattle. The first study utilised heifers and mature cows and experiments were undertaken on three different stations. In these three experiments, GnRH agonist-treated animals were assessed for return of cyclic ovarian activity and time to pregnancy, for up to a 12 month period. The second study examined heifers treated with implants containing different doses of deslorelin. These heifers were managed under improved pasture conditions to observe the effectiveness of the GnRH agonist implant technology during ongoing weight gain. These heifers were observed for return to ovarian cyclic function and time to pregnancy.

The aims of this study were:

1. To determine if controlled release implants containing the GnRH agonist deslorelin suppressed the oestrous cycle and prevented pregnancies in female cattle treated for up to 12 months.
2. To determine the relationships between content of deslorelin in GnRH agonist implants and period of suppression of fertility.
3. To determine if fertility suppression with deslorelin implants was maintained in female cattle undergoing a continuing and relatively rapid rate of gain in weight.

Chapter 2 - General Materials and Methods

2.1 Animal Ethics

Studies in this thesis were conducted following the guidelines of the Australian Code of Practice for the Use of Animals for Scientific Purposes. The two studies reported in this thesis received ethical approval and an Ethical Clearance Certificate No. TBC 87 was issued by the Tropical Beef Centre Animal Experimentation Ethics Committee, Rendel Laboratory, Rockhampton, Queensland.

2.2 GnRH agonist implant

The GnRH agonist implant (deslorelin, D-Trp^{6}-Pro^{9}-des-Gly^{10}-GnRH ethylamide) (Karten and Rivier, 1986) is being developed by Peptech Animal Health Pty Ltd, North Ryde, Sydney (Figure 2.1). The implant consists of the GnRH agonist deslorelin contained in a bees wax slow-release matrix. When incubated *in vitro* each implant released approximately 10 to 20 μg deslorelin/24 hours depending upon formulation (J. Walsh, unpublished data, Peptech Animal Health Pty Limited, Sydney). The implants are cylindrical with a diameter of about 2 mm. The deslorelin implant is inserted subcutaneously in the dorsal surface of the ear using the commercial implanting device supplied and aseptic technique.

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Studies in this thesis were conducted following the guidelines of the Australian Code of Practice for the Use of Animals for Scientific Purposes. The two studies reported in this thesis received ethical approval and an Ethical Clearance Certificate No. TBC 87 was issued by the Tropical Beef Centre Animal Experimentation Ethics Committee, Rendel Laboratory, Rockhampton, Queensland.

2.2 GnRH agonist implant

The GnRH agonist implant (deslorelin, D-Trp^{6}-Pro^{9}-des-Gly^{10}-GnRH ethylamide) (Karten and Rivier, 1986) is being developed by Peptech Animal Health Pty Ltd, North Ryde, Sydney (Figure 2.1). The implant consists of the GnRH agonist deslorelin contained in a bees wax slow-release matrix. When incubated *in vitro* each implant released approximately 10 to 20 μg deslorelin/24 hours depending upon formulation (J. Walsh, unpublished data, Peptech Animal Health Pty Limited, Sydney). The implants are cylindrical with a diameter of about 2 mm. The deslorelin implant is inserted subcutaneously in the dorsal surface of the ear using the commercial implanting device supplied and aseptic technique.

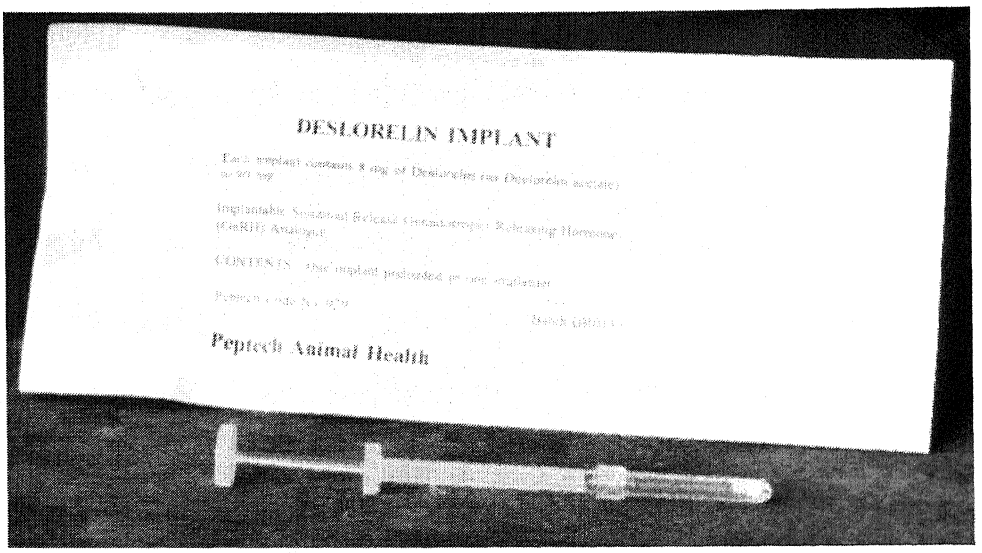


Figure 2.1. Deslorelin implanting device.

2.3 Blood sampling

Blood samples for progesterone analysis were collected by venepuncture using Vacutainer™ tubes (Becton-Dickinson, Rutherford, New Jersey, USA). The collection tubes contained lithium heparin as anticoagulant. Samples were kept on ice until centrifuged (800 g/30 min) and the plasma stored at –20°C until assayed.

2.4 Progesterone assay protocol

Concentrations of plasma progesterone for the studies in Chapter 3 were determined using an extraction, single-antibody radioimmunoassay (D'Occhio *et al.*, 1988).

For preparation of a standard curve, progesterone (4-pregnene, 3,20-dione; Sigma Chemical, St Louis, Missouri, USA) in ethanol (AR grade) was used. A

standard curve was prepared by serial dilution of the stock progesterone in the range of 0.07 to 10 ng/ml. Standards (100 µl aliquots) were pipetted in triplicate into labeled 10x75 mm borosilicate glass tubes. Quality controls of known concentration (100 µl) and unknown samples (100 µl) were pipetted into duplicate glass tubes. One milliliter of heptane (scintillation grade) was added to samples, quality controls and standards; these were then extracted for 10 minutes by shaking on a vortex orbital shaker. Following extraction, tubes were allowed to stand for 10 minutes to allow for separation of aqueous and solvent phases. Racks of tubes were then placed in a 70% ethanol bath, frozen with liquid nitrogen to solidify the aqueous layer. The supernatant was poured off into appropriately labeled tubes. Contents of these tubes were then evaporated to dryness in a water bath at 45°C under a stream of filtered air.

After drying, 200 µl of tritiated progesterone (10,000 cpm/100 µl in 0.01 M, pH 7.5, PBS), containing 0.2% gelatin (Gel PBS); 1,2,6.7-^{3}H-progesterone; 80-100 Ci/mmol TRK413; Amersham Australia Pty Ltd, North Ryde, NSW, Australia) was added to each tube. Two hundred microlitres of progesterone antiserum (sheep-anti-progesterone-11α-hemisuccinate-BSA conjugate, Dr RI Cox, Hormone Assay Development Group, Blacktown, NSW, Australia) in Gel PBS was also added using Eppendorf Pipettemaster™ multi-aliquot pipettors. The antiserum was used at a 1:15,000 dilution to ensure a 45% reference binding. After shaking to ensure mixing of tracer and antisera the assay was incubated overnight (at least 16 hr) in a cool room at 4°C. Removal of unbound isotope was accomplished by the addition of 200 µl of dextran-coated charcoal (62.5 mg Dextran T70 and 625 mg charcoal Norit A in 100 ml PBS). A further incubation of 10 min at 4°C followed, then tubes were centrifuged 1000 x g for 20 minutes at 4°C using a Beckman J-6M Induction Drive centrifuge.

The resultant supernatant was immediately decanted into appropriately labeled tubes. Four milliliters of scintillation fluid (Optiphase HiSafe 2) was added to each tube. These tubes were counted on a β-counter for 2 minutes (Wallac 1410 Liquid Scintillation Counter, Pharmacia, Wallac Oy, Finland).

Progesterone concentrations were calculated using the Prince Henry's Hospital Institute Of Medical Research, Radioimmunoassay Program (Monash Medical Centre, Melbourne, Australia).

Sensitivity of the assays was 0.2 ng/ml progesterone, and inter- and intra-assay coefficients of variation were less than 10% based on duplicate samples.

2.5 Statistical analyses

Data were analysed by the independent t-test procedure of SAS/STAT, where two independent groups were being compared and by analysis of variance procedures using the General Linear Models (GLM) procedure of SAS/STAT, where more than two groups were compared (SAS, 1992). Comparisons within the same group over time were undertaken using the paired sample t-test of SAS/STAT for two times and repeated measures for more than two times (SAS, 1992). SAS/STAT corrected probabilities for non-homogeneity of variance were used when appropriate. Number of pregnancies, occurrences of progesterone ≥ 2.0 ng/ml, and number of follicles in controls as compared with deslorelin-treated animals were analysed by SAS/STAT Chi Square (SAS, 1992). Significance was pre-set at $P<0.05$. Results are reported as untransformed means ± SEM, unless otherwise stated.

Chapter 3: Ovarian activity in heifers and cows treated long-term with a GnRH agonist implant

3.1 Introduction

Ovulation did not occur in female cattle treated with a GnRH agonist for 4 to 6 weeks (D'Occhio *et al.*, 1996, 1999, 2000; Gong *et al.*, 1995, 1996). This disruption of ovarian function in cattle treated with a GnRH agonist is believed to be due to the downregulation of the anterior pituitary gonadotroph cell GnRH receptors (Melson *et al.*, 1986), and associated abolition of pulsatile secretion of LH and FSH (D'Occhio *et al.*, 1996; Gong *et al.*, 1995, 1996). Reduced plasma concentrations of FSH and disruption of normal release patterns of FSH resulted in restriction of ovarian follicular growth to the early antral stage (Gong *et al.*, 1995, 1996). Treatment with GnRH agonist also prevented occurrence of a pre-ovulatory surge release of LH (D'Occhio *et al.*, 1997), a further barrier to ovulation. Therefore, GnRH agonists in the form of slow-release implants offer the potential to provide a non-surgical method for contraception in female cattle. GnRH agonist implant technology offers many advantages compared with conventional surgical sterilisation including animal welfare, increased efficiency of livestock production and ease of application. The latter is of particular importance in extensive beef production systems typical of northern Australia. In previous studies, treatment with GnRH agonist has been of relatively short duration (D'Occhio *et al.*, 1996; Gong *et al.*, 1995, 1996).

The aim in the present study described in this Chapter was to ascertain the efficacy of a GnRH agonist implant to induce infertility in heifers and cows. The hypothesis was that a controlled-release GnRH agonist implant would suppress pregnancies for a period of 12 months in heifers and cows.

3.2 Materials and methods

3.2.1 General materials and methods

Details on the GnRH agonist (deslorelin) implant (Section 2.2), progesterone assay (Section 2.4) and statistical analyses (Section 2.5) are provided in Chapter 2.

3.2.2 Experimental design

This study was conducted on three cattle stations. Cattle for the experiments were selected randomly from animals available on the respective stations. At Station 1, 51 heifers and 48 cows received an implant that contained 12 mg deslorelin. On Station 2, 41 heifers and 48 cows received an implant that contained 8 mg deslorelin. On Station 3, 39 heifers and 37 cows received the same 8 mg deslorelin implant as at Station 2. Ten heifers and 10 cows representing control animals (cycling and untreated) were included at the start of the experiment at Stations 1 and 3, and 8 control heifers and 11 control cows included at Station 2. Heifers and mature cows not treated with deslorelin (control animals) were maintained within the experimental herds on all three stations. These control animals were used to evaluate the fertility of the bulls and ensure that the inability of cattle treated with deslorelin to conceive was due to the deslorelin implant and not to other factors such as bull fertility. As significant numbers of control cattle became pregnant, replacement groups of control cattle, where available, were introduced to monitor pregnancy in untreated control heifers during the latter part of the study. At Station 1, replacement groups of control cows and heifers (n=10 each) were introduced at 6 months of the study. At Station 2, no new untreated control cattle were introduced during the study. At Station 3, untreated control heifers (n=10) were introduced at 9 months but no cows were

introduced at this time. Approximately 4% bulls were included as part of the herd with the female cattle. Before being assigned to the study, heifers and cows were tested for pregnancy by rectal palpation and animals detected as pregnant were excluded. Heifers and cows not detected as pregnant by rectal palpation were given a luteolytic dose of prostaglandin ($PGF_{2\alpha}$) (5ml Lutylase, Intervet (Aust) Pty Ltd, Castle Hill, NSW) to ensure that any animals at a stage of early pregnancy, that could not be detected by ultrasonography, would abort the pregnancy. Hence, all heifers and cows were non-pregnant at the start of the study. At 2 monthly intervals, heifers and cows were weighed, scored for body condition and checked for pregnancy status by rectal palpation or ultrasonography. A blood sample was taken for subsequent measurement of plasma progesterone which was used as an indicator of the presence of a CL.

At approximately 12 months of the study GnRH agonist-treated treated cattle were slaughtered and reproductive tracts observed for pregnancy status and ovarian activity. Time of conception was determined from estimates of foetal age.

3.2.3 Experimental sites

(a) Station 1. This experimental site was Canobie Station (latitude 19°45'S; longitude 140°30'E) is owned and operated by the Australian Agricultural Company and is located 250 km north of Julia Creek in the dry tropics of north Queensland.

(b) Station 2. This experimental site was Flora Valley Station (latitude 18°30'S; longitude 128°20'E) is owned and operated by the Heytesbury Beef and is located 300 km south of Kunanurra in the dry tropics of northern West Australia.

(c) Station 3. This experimental site was Havilah Station (latitude 20°50'S; longitude 147°50'E) owned and operated by Stanbroke Pastoral Company and located 60 km west of Collinsville in the dry tropics of north Queensland. The respective stations owned the animals used in the three experiments. Animals were 12- to 18-month-old Brahman or Brahman crossbred heifers (Station 1, 303±2 kg; Station 2, 307±5 kg; Station 3, 307±4 kg) and mature cows (Station 1; 445±8 kg. Station 2; 317±5 kg. Station 3; 401±8 kg; 6-10 years old).

3.2.4 Detection of pregnancy

Rectal palpation was the method used for the detection of pregnancies on the three stations. The operator was a veterinary surgeon with much experience in pregnancy diagnosis by rectal palpation. All animals used in this study were examined every 2 months for pregnancy.

3.2.5 Progesterone index of ovarian activity

Plasma concentrations of progesterone in heifers and cows were used as an index to evaluate ovarian activity. A plasma concentration of progesterone ≥ 2 ng/ml was considered indicative of the presence of an active CL in a heifer or cow (Kinder *et al.*, 1997).

3.2.6 Body condition score

Body condition scoring in cattle was conducted every 2 months for the duration of the study. The person who recorded body condition score was a veterinary surgeon with extensive experience in evaluation of this variable.

Body condition was scored as follows:

1 Moribund; 2 Very poor; 3 Poor; 4 Backward store; 5 Store; 6 Forward store; 7 Prime; 8 Fat; 9 Over-fat.

3.3 Results

3.3.1 Body weights and body condition score

Heifers and cows at Station 1 showed seasonal fluctuations in weight which were typical of cattle in tropical and subtropical environments. During the dry winter weight was stable in heifers (Figure 3.1; Table 3.1) and declined in cows (Figure 3.2; Table 3.2), followed by an increase in weight in both heifers and cows during the typically wet summer. Body condition score in heifers declined during winter and increased during summer, following a similar pattern to weight (Figure 3.3; Table 3.3). Cows demonstrated similar but less marked season-related changes in body condition score (Figure 3.4; Table 3.4). A new group of control cows were introduced at 6 months which made comparisons of body condition score with treated cows inappropriate.

At Station 2, seasonal changes in weight in heifers (Figure 3.5; Table 3.5) and cows (Figure 3.6; Table 3.6) followed similar trends as described above for cattle at Station 1. Heifers and cows at Station 2 showed a reduction in body weight and condition score from winter to late spring. This was followed by a rapid increase in weight and improvement in body condition score in summer and early autumn (Figures 3.7 and 3.8; Tables 3.7 and 3.8).

Heifers and cows at Station 3 did not show a reduction in body weight or condition score during winter. Instead, heifers and cows at Station 3 showed a progressive, steady increase in weight (Figures 3.9 and 3.10; Tables 3.9 and 3.10) and body condition score (Figures 3.11 and 3.12; Tables 3.11 and 3.12) during the study.

Data for relative changes in weight during the experiment are shown in Table 3.13. At all three stations heifers and cows gained weight during the study. There were no real differences in weight gain or condition score between control animals and deslorelin-treated animals on any of the three stations.

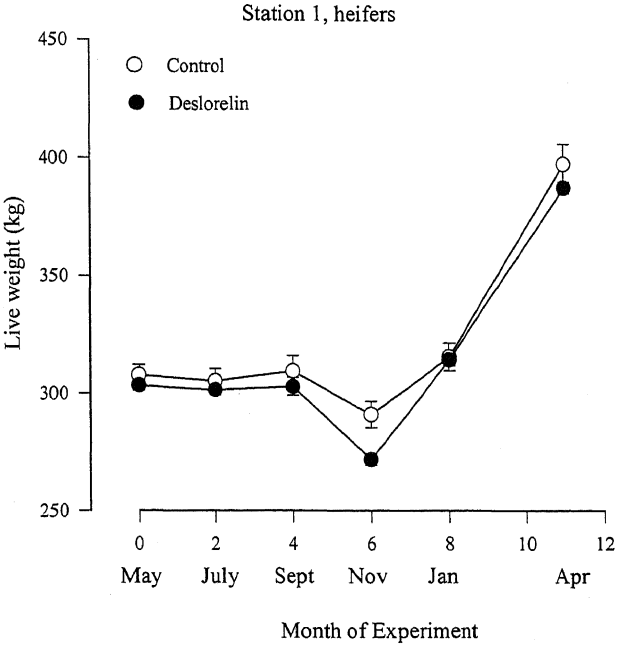


Figure 3.1. Seasonal changes in body weight of heifers on Station 1 (means ± SEM).

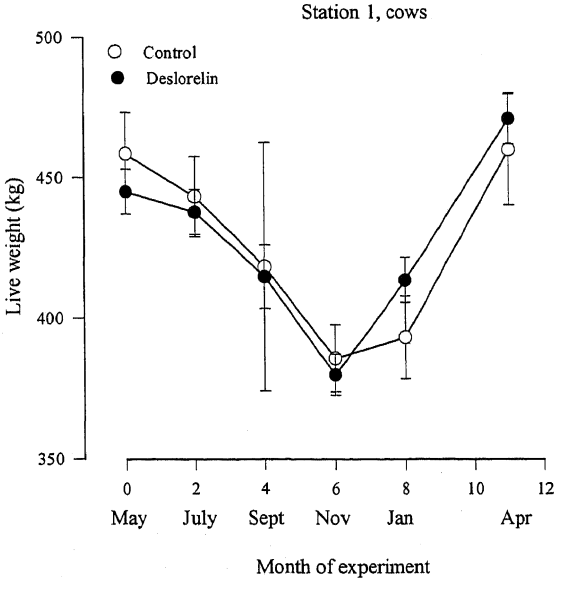


Figure 3.2. Seasonal changes in body weight of cows on Station 1 (means ± SEM).

Table 3.1. Seasonal changes in body weight of heifers on Station 1 (kg, means ± SEM).

	Months			
Treatment	0	4	8	11
Control	307 ± 4 (10)[a]	309 ± 7 (9)[a]	316 ± 6 (10)[a]	398 ± 8 (10)*, [a]
Deslorelin	303 ± 2 (51)[a]	303 ± 4 (27)[a]	314 ± 2 (51)[a]	338 ± 3 (51) [b]

* numbers in parentheses indicate number of heifers
[a,b] Means in columns without a common superscript differ ($P<0.05$; independent t-test). Difference shown at 11 months due to introduction of new control heifers.

Table 3.2. Seasonal changes in body weight of cows on Station 1 (kg, means ± SEM).

	Months			
Treatment	0	4	8	11
Control	459 ± 15 (10)	418 ± 44 (3)	393 ± 15 (10)	460 ± 20 (10)*
Deslorelin	445 ± 8 (48)	415 ± 11 (23)	414 ± 8 (48)	471 ± 9 (46)

* numbers in parentheses indicate number of cows
Means within columns do not differ ($P>0.05$; independent t-test)

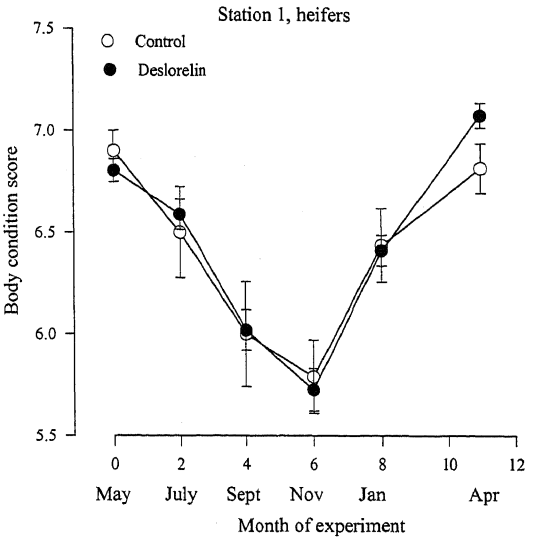


Figure 3.3. Seasonal changes in body condition score of heifers on Station 1 (means ± SEM).

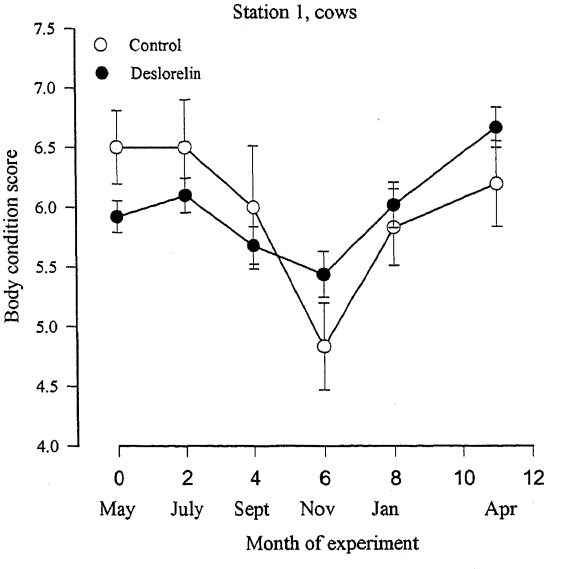


Figure 3.4. Seasonal changes in body condition score for cows on Station 1 (means ± SEM).

Table 3.3. Seasonal changes in body condition score of heifers on Station 1 (means ± SEM).

	Months			
Treatment	0	4	8	11
Control	6.9 ± 0.1 (10)	6.0 ± 0.3 (10)	6.4 ± 0.2 (10)	6.8 ± 0.1 (10)*
Deslorelin	6.8 ± 0.1 (51)	6.0 ± 0.1 (51)	6.4 ± 0.1 (51)	7.1 ± 0.1 (51)

* numbers in parentheses indicate number of heifers
Means in columns do not differ (P>0.05; independent t-test)

Table 3.4. Seasonal changes in body condition score of cows on Station 1 (means ± SEM).

	Months			
Treatment	0	4	8	11
Control	6.5 ± 0.3 (10)	6.0 ± 0.5 (6)	5.8 ± 0.3 (10)	6.2 ± 0.4 (10)*
Deslorelin	5.9 ± 0.1 (48)	5.7 ± 0.2 (48)	6.0 ± 0.2 (48)	6.7 ± 0.2 (46)

* numbers in parentheses indicate number of cows
Means in columns do not differ (P>0.05; independent t-test)

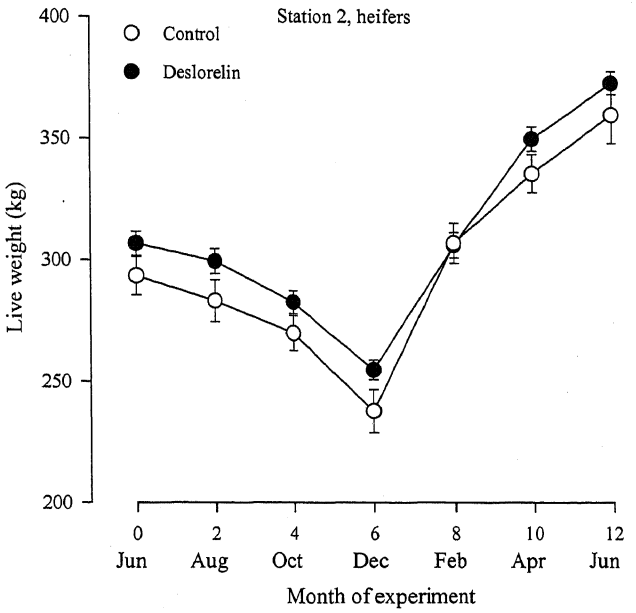


Figure 3.5. Seasonal changes in body weight for heifers on Station 2 (kg, means ± SEM).

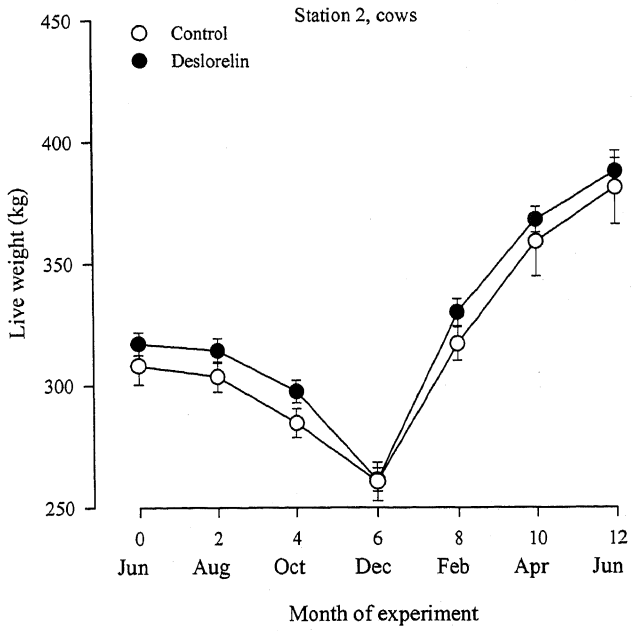


Figure 3.6. Seasonal changes in body weight for cows on Station 2 (kg, means ± SEM).

Table 3.5. Seasonal changes in body weight of heifers on Station 2 (kg, means ± SEM).

	Months			
Treatment	0	4	8	12
Control	294 ± 8 (8)	270 ± 7 (8)	307 ± 8 (7)	360 ± 12 (8)*
Deslorelin	307 ± 5 (41)	283 ± 5 (39)	306 ± 5 (41)	373 ± 5 (40)

Means within columns do not differ (P>0.05; independent t-test)
* numbers in parentheses indicate number of heifers

Table 3.6. Seasonal changes in body weight of cows on Station 2 (kg, means ± SEM).

	Months			
Treatment	0	4	8	12
Control	308 ± 8 (11)	285 ± 6 (11)	317 ± 7 (9)	382 ± 15 (10)*
Deslorelin	317 ± 5 (48)	298 ± 5 (47)	330 ± 6 (47)	388 ± 5 (48)

Means within columns do not differ (P>0.05; independent t-test)
* numbers in parentheses indicate number of cows

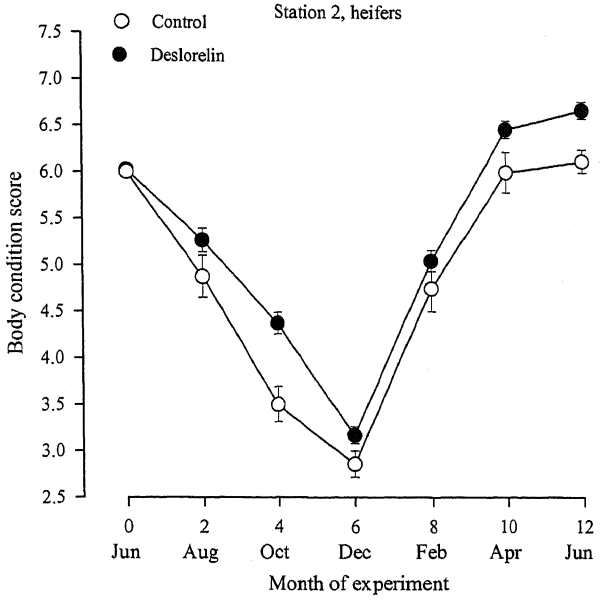


Figure 3.7. Seasonal changes in body condition score for heifers on Station 2 (means ± SEM).

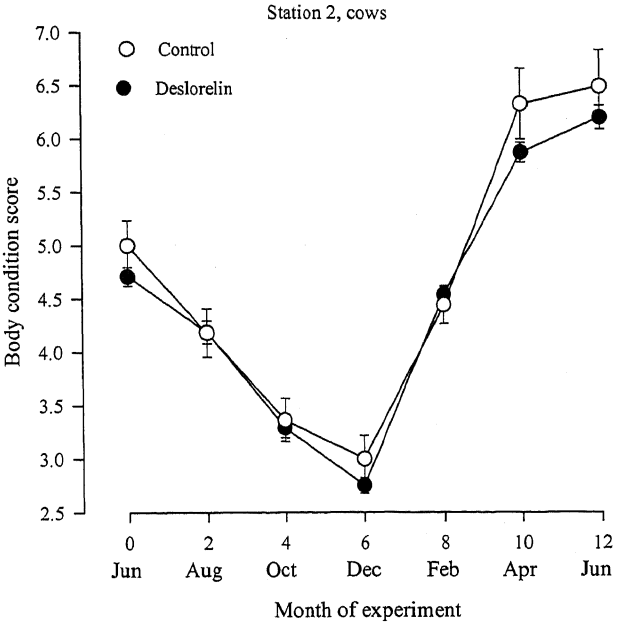


Figure 3.8. Seasonal changes in body condition score for cows on Station 2 (means ± SEM).

Table 3.7. Seasonal changes in body condition score of heifers on Station 2 (means ± SEM).

	Months			
Treatment	0	4	8	12
Control	6.0 ± 0.0 (8)	3.5 ± 0.2[a] (8)	4.8 ± 0.2 (8)	6.1 ± 0.1[a] (8)*
Deslorelin	6.0 ± 0.1 (41)	4.4 ± 0.1[b] (40)	5.1 ± 0.1 (41)	6.7 ± 0.1[b] (40)

[a,b] Means in columns (months 4 and 12) without a common superscript differ ($P<0.05$; independent t-test)
* numbers in parentheses indicate number of heifers

Table 3.8. Seasonal changes in body condition score of cows on Station 2 (means ± SEM).

	Months			
Treatment	0	4	8	12
Control	5.0 ± 0.2 (11)	3.4 ± 0.2 (11)	4.4 ± 0.2 (9)	6.5 ± 0.3 (10)*
Deslorelin	4.7 ± 0.1 (48)	3.3 ± 0.1 (48)	4.5 ± 0.1 (48)	6.2 ± 0.1 (48)

Means within columns do not differ ($P>0.05$; independent t-test)
* numbers in parentheses indicate number of cows

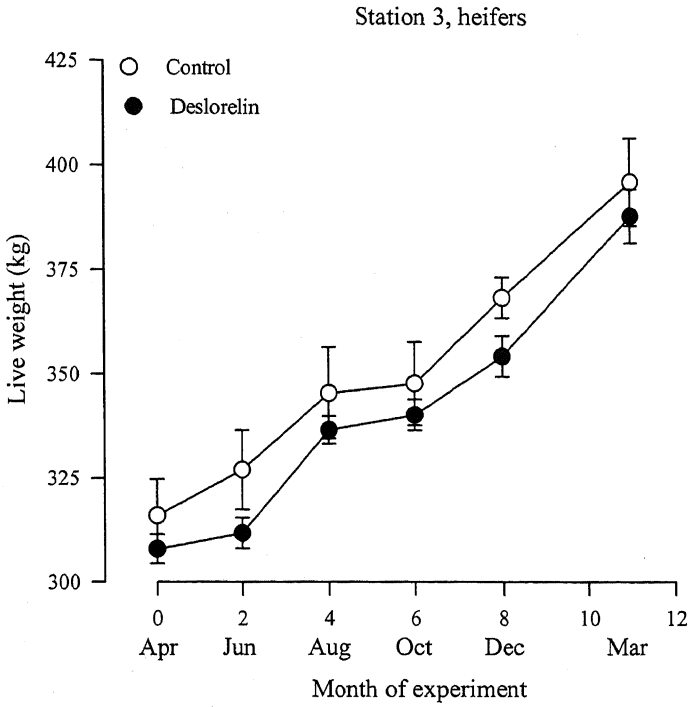


Figure 3.9. Seasonal changes in body weight of heifers on Station 3 (kg, means ± SEM).

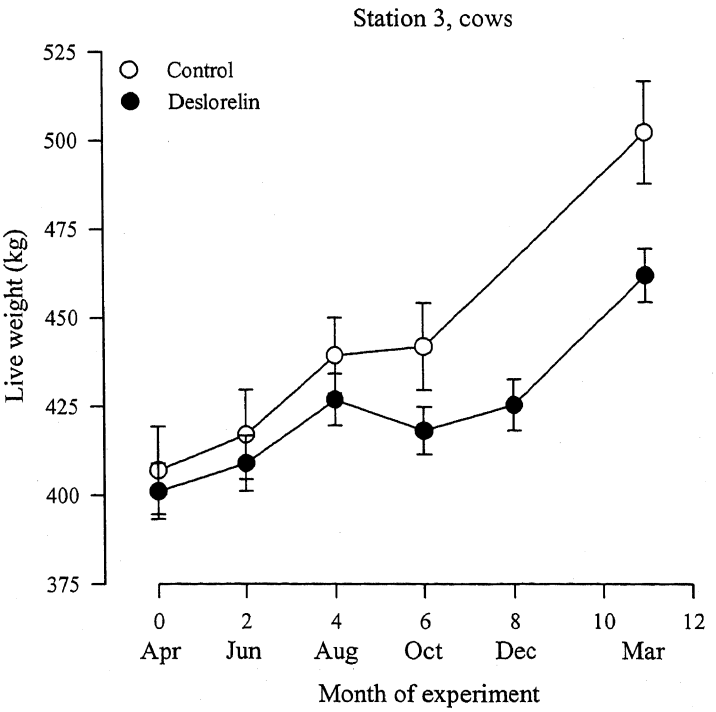


Figure 3.10. Seasonal changes in body weight of cows on Station 3 (kg, means ± SEM).

Table 3.9. Seasonal changes in body weight of heifers on Station 3 (kg, means ± SEM).

	Months			
Treatment	0	4	8	11
Control	316 ± 9 (10)	345 ± 11 (10)	368 ± 5 (3)	396 ± 11 (10)*
Deslorelin	307 ± 4 (39)	337 ± 3 (39)	354 ± 5 (35)	387 ± 6 (39)

Means within columns do not differ (P>0.05; independent t-test)
* numbers in parentheses indicate number of heifers

Table 3.10. Seasonal changes in body weight of cows on Station 3 (kg, means ± SEM).

	Months			
Treatment	0	4	8	11
Control	407 ± 12 (10)[a]	439 ± 11 (10)[a]	416 ± 6 (2)[a]	502 ± 15 (6)*, [a]
Deslorelin	401 ± 8 (37)[a]	427 ± 7 (37)[a]	425 ± 7 (37)[a]	462 ± 8 (37) [b]

a,b Means in columns without a common superscript differ (P<0.05; independent t-test; difference at 11 months may be due to pregnancies, or un-mustered animals)
* numbers in parentheses indicate number of cows

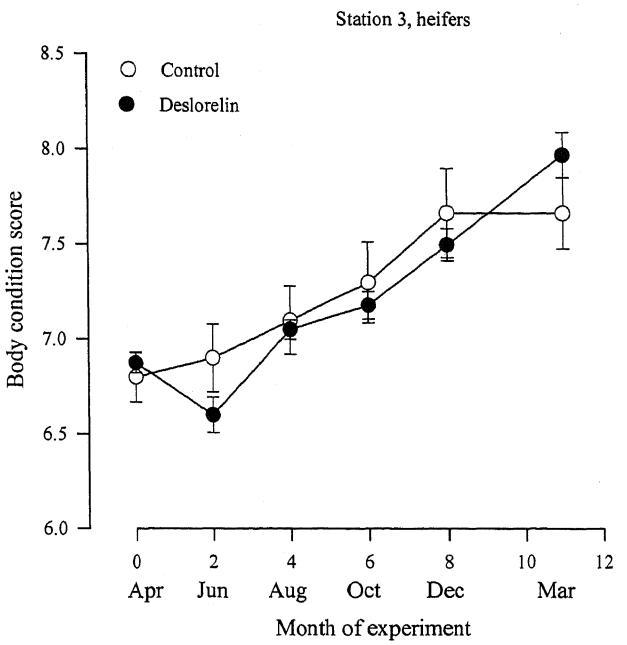


Figure 3.11. Seasonal changes in body condition score of heifers on Station 3 (means ± SEM).

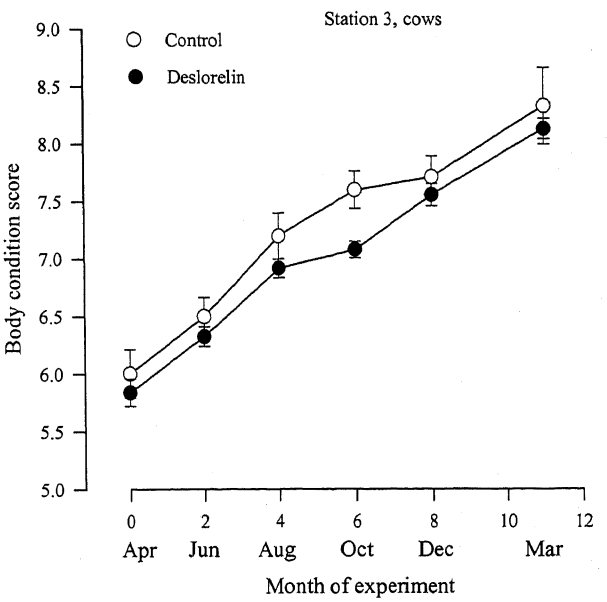


Figure 3.12. Seasonal changes in body condition score of cows on Station 3 (means ± SEM).

Table 3.11. Seasonal changes in body condition score of heifers on Station 3 (means ± SEM).

	Months			
Treatment	0	4	8	11
Control	6.8 ± 0.1 (10)	7.1 ± 0.2 (10)	7.7 ± 0.2 (9)	7.7 ± 0.2 (10)*
Deslorelin	6.9 ± 0.1 (39)	7.1 ± 0.1 (39)	7.5 ± 0.1 (36)	8.0 ± 0.1 (39)

Means within columns do not differ (P>0.05; independent t-test)
* numbers in parentheses indicate number of heifers

Table 3.12. Seasonal changes in body condition score of cows on Station 3 (means ± SEM).

	Months			
Treatment	0	4	8	11
Control	6.0 ± 0.2 (10)	7.2 ± 0.2 (10)	7.7 ± 0.2 (7)	8.3 ± 0.3 (6)*
Deslorelin	5.8 ± 0.1 (37)	6.9 ± 0.1 (37)	7.6 ± 0.1 (34)	8.1 ± 0.1 (37)

Means within columns do not differ (P>0.05; independent t-test)
* numbers in parentheses indicate number of cows

Table 3.13. Summaries of changes in body weight during the experiments for heifers and cows treated with deslorelin.

Months (days)	Number of animals	Heifers		Cows	
		Body weight gain (kg)	Daily body weight gain (kg/day)	Body weight gain (kg)	Daily body weight gain (kg/day)
			STATION 1		
0 to 6 (189)	51 heifers 48 cows	-31 ± 1[a]	-0.17 ± 0.01[a]	-66 ± 4[a]	-0.35 ± 0.02[a]
7 to 11 (161)	51 heifers 46 cows	116 ± 2[b]	0.72 ± 0.02[b]	92 ± 4[b]	0.57 ± 0.03[b]
0 to 11 (350)	51 heifers 46 cows	84 ± 2	0.24 ± 0.01	26 ± 5	0.08 ± 0.01
			STATION 2		
0 to 6 (181)	41 heifers 43 cows	-51 ± 2[a]	-0.29 ± 0.01[a]	-52 ± 3[a]	-0.29 ± 0.02[a]
7 to 12 (190)	40 heifers 43 cows	121 ± 2[b]	0.63 ± 0.01[b]	127 ± 5[b]	0.67 ± 0.03[b]
0 to 12 (371)	40 heifers 43 cows	69 ± 2	0.19 ± 0.01	71 ± 4	0.19 ± 0.01
			STATION 3		
0 to 6 (176)	39 heifers 37 cows	31 ± 2[a]	0.18 ± 0.01[a]	17 ± 2[a]	0.10 ± 0.01[a]
7 to 11 (142)	38 heifers 37 cows	51 ± 3[b]	0.36 ± 0.02[b]	44 ± 3[b]	0.31± 0.02[b]
0 to 11 (318)	39 heifers 37 cows	79 ± 4	0.25 ± 0.01	61 ± 4	0.19 ± 0.01

[a,b] Mean live weight gains within columns, and within station, without a common superscript differ ($P<0.05$; paired sample t-test)

3.3.2 Ovarian function

At Station 1, approximately 30% of heifers were showing ovarian cycles (plasma progesterone ≥ 2 ng/ml) at the commencement of the study. Oestrous cyclicity increased in control heifers to 100% during the study (Figure 3.13; Table 3.14). Heifers treated with a deslorelin implant showed only random occurrences of elevated progesterone, and no consistent pattern of an increase in numbers of heifers expressing oestrous cycles. A majority of cows at Station 1 were determined to be cycling at the start of the experiment, and control cows continued to have oestrous cycles (60 to 100%) during the study. Ovarian cyclic activity was suppressed for the duration of the study in cows treated with deslorelin (Figure 3.13; Table 3.14).

Heifers at Station 2 showed a relatively large reduction in body weight during the first 6 months of the experiment, which was the likely cause for the low proportion of control heifers (~20%) that showed ovarian activity during this period (Figure 3.14; Table 3.15). Heifers treated with a deslorelin implant had suppressed plasma concentrations of progesterone throughout the study (Figure 3.14; Table 3.15). A majority of cows at Station 2 showed cyclic ovarian activity at the beginning of the study and about 70% of control cows had relatively greater progesterone at 2 months of the study (Figure 3.14). Occurrences of elevated plasma concentrations of progesterone were observed in 10 to 20% of cows treated with a deslorelin implant.

At Station 3, most control heifers showed cyclic ovarian activity during the study (Figure 3.15). For heifers treated with a deslorelin implant, 20% had elevated plasma concentrations of progesterone at 4 months of the study which increased to 40% at 8 months. All control cows at Station 3 showed elevated plasma concentrations of progesterone during the study. Amongst cows treated with a

deslorelin implant 15% showed elevated progesterone at 4 months of the study which increased to 40% at 8 months (Table 3.16).

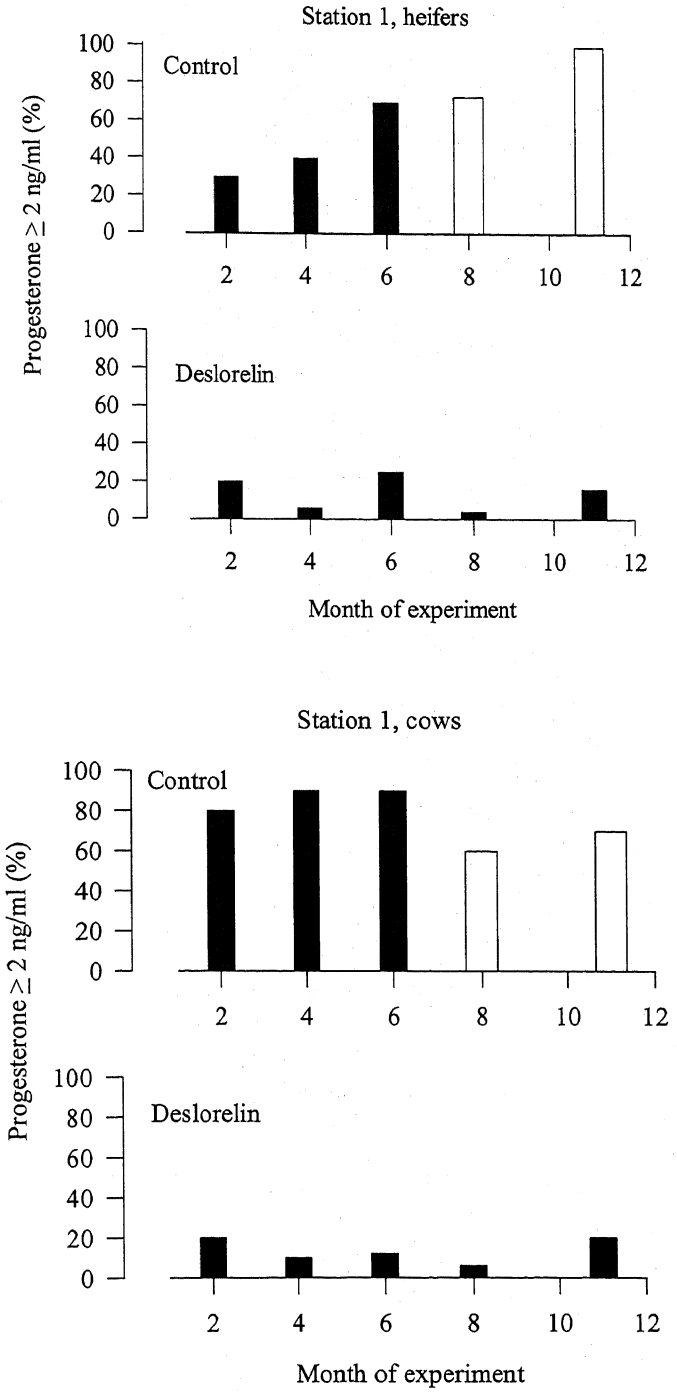


Figure 3.13. Proportion (%) of heifers (upper profiles) and cows (lower profiles) with plasma progesterone ≥ 2 ng/ml on Station 1. The white open bars at 8 and 11 months for controls represent a replacement untreated group of control heifers and cows.

Table 3.14. Occurrence (%) of elevated plasma concentrations of progesterone ($\geq$ 2 ng/ml) in heifers and cows on Station 1.

	Heifers			**Cows**		
	Months					
Treatment	4	8	11	4	8*	11
Control	40[a]	73[a]	100[a]	90[a]	60[a]	70[a]
Deslorelin	6[b]	4[b]	16[b]	10[b]	6[b]	20[b]

[a,b] Percentage in columns without a common superscript differ ($P<0.05$; chi square on actual numbers of cattle with progesterone $\geq$ 2 ng/ml, versus number with progesterone < 2.0 ng/ml, comparison between treatments)
* new group of control cows introduced at 6 months

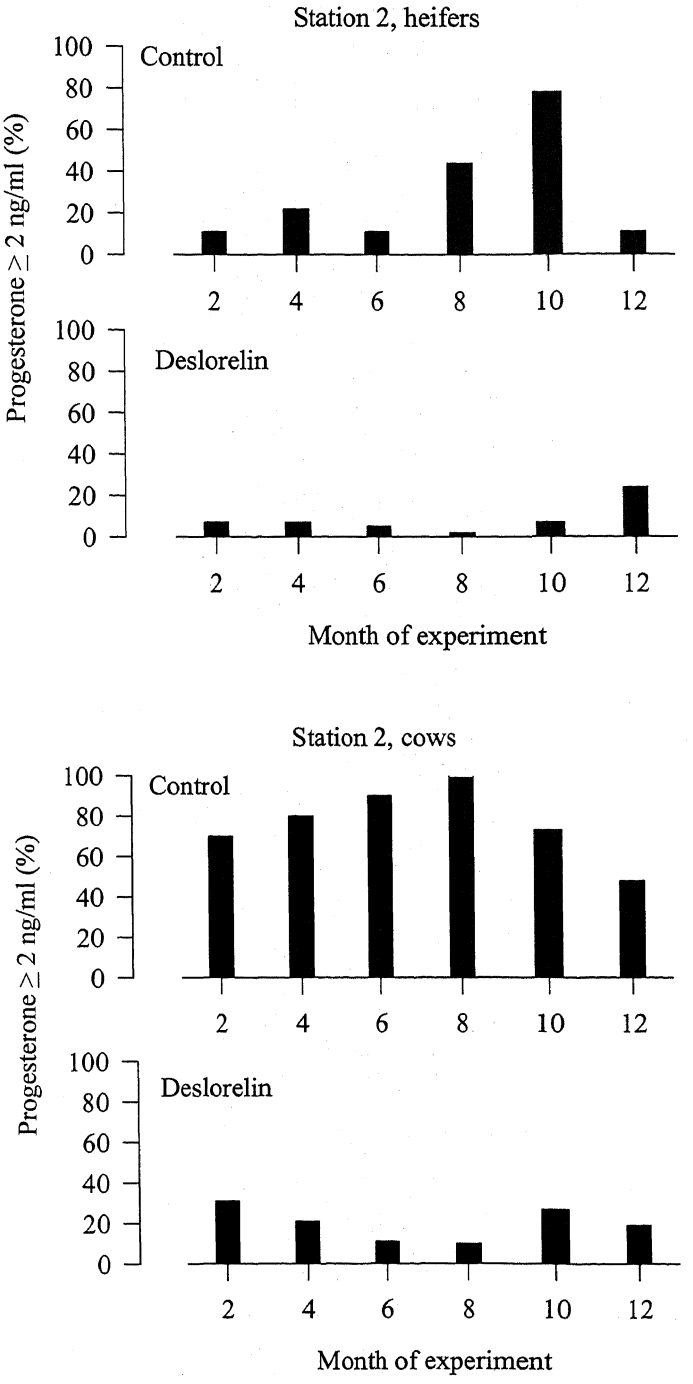


Figure 3.14. Proportion (%) of heifers (upper profiles) and cows (lower profiles) with plasma progesterone ≥ 2 ng/ml on Station 2.

Table 3.15. Occurrence (%) of elevated plasma concentrations of progesterone ($\geq$ 2 ng/ml) in heifers and cows on Station 2.

	Heifers			**Cows**		
	Months					
Treatment	4	8	12	4	8	12
Control	22[a]	44[a]	11[a]	80[a]	100[a]	48[a]
Deslorelin	7[a]	2[b]	24[a]	21[b]	10[b]	19[b]

[a,b] Percentages in columns without a common superscript differ ($P<0.05$; chi square on actual numbers of cattle with progesterone $\geq$ 2 ng/ml, versus number with progesterone < 2.0 ng/ml, comparison between treatments)

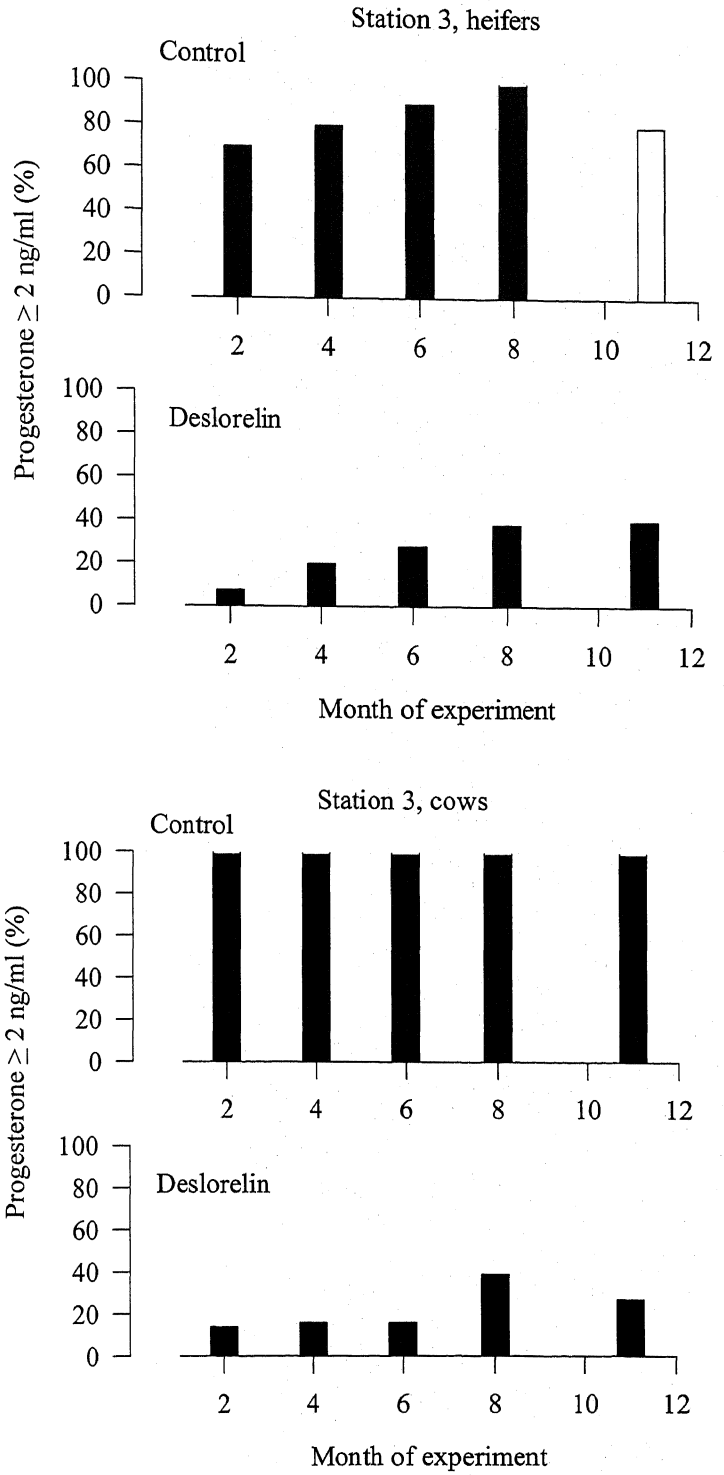


Figure 3.15. Proportion (%) of heifers (upper profiles) and cows (lower profiles) with plasma progesterone ≥ 2 ng/ml on Station 3. The white open bar at 11 months for control heifers represents a replacement group of control heifers introduced.

Table 3.16. Occurrence (%) of elevated plasma concentrations of progesterone ($\geq$ 2 ng/ml) in heifers and cows on Station 3.

	Heifers			**Cows**		
	Months					
Treatment	4	8	11*	4	8	11
Control	80[a]	100[a]	60[a]	100[a]	100[a]	100[a]
Deslorelin	20[b]	38[b]	40[b]	16[b]	39[b]	27[b]

[a,b] Percentages in columns without a common superscript differ ($P<0.05$; chi square on actual numbers of cattle with progesterone $\geq$ 2 ng/ml, versus number with progesterone < 2.0 ng/ml, comparison between treatments)
* new group of replacement control heifers introduced at 9 months

3.3.3 Cumulative pregnancies

At Station 1, untreated control heifers showed a progressive increase in cumulative pregnancies from about 5 months to the end of the study (Figure 3.16; Table 3.17). For heifers treated with a deslorelin implant, 5 conceived between 11 and 12 months of the study. Of the first group of control cows introduced, 80% conceived within the first 5 months of the study. A new group of control cows was introduced at 5 months and 80% of these conceived between 6 and 12 months of the study. Three of the cows treated with deslorelin conceived at around month 12 of the study.

Control heifers at Station 2 showed an increase in cumulative pregnancies after 6 months of the study (Figure 3.17; Table 3.18). This coincided with the period of increase in rate of body weight gain from December to June. By month 12 of the study 62% of control heifers had conceived. Only 10% of heifers implanted with deslorelin conceived between 9 and 12 months of the study. Control cows showed a progressive increase in cumulative pregnancies from 2 to 8 months of the study and by month 12 90% had conceived. Three cows implanted with deslorelin conceived between 3 and 9 months of the study, and a further 3 conceived from 9 to 12 months.

At Station 3, about 80% of the initial group of control heifers had conceived by 6 months of the study and 60% of the second group of control heifers had conceived by the end of the study (Figure 3.18; Table 3.19). The relatively greater conception rates for control heifers at Station 3 were most likely related to greater body weight at the start of the study and continued body growth during the study. Of the heifers treated with deslorelin, four had conceived by 6 months of the study and 12 had conceived by 12 months. Control cows had all conceived by 6 months of the study. Twenty-four percent of the cows treated with deslorelin conceived between 6 and 12 months of the study.

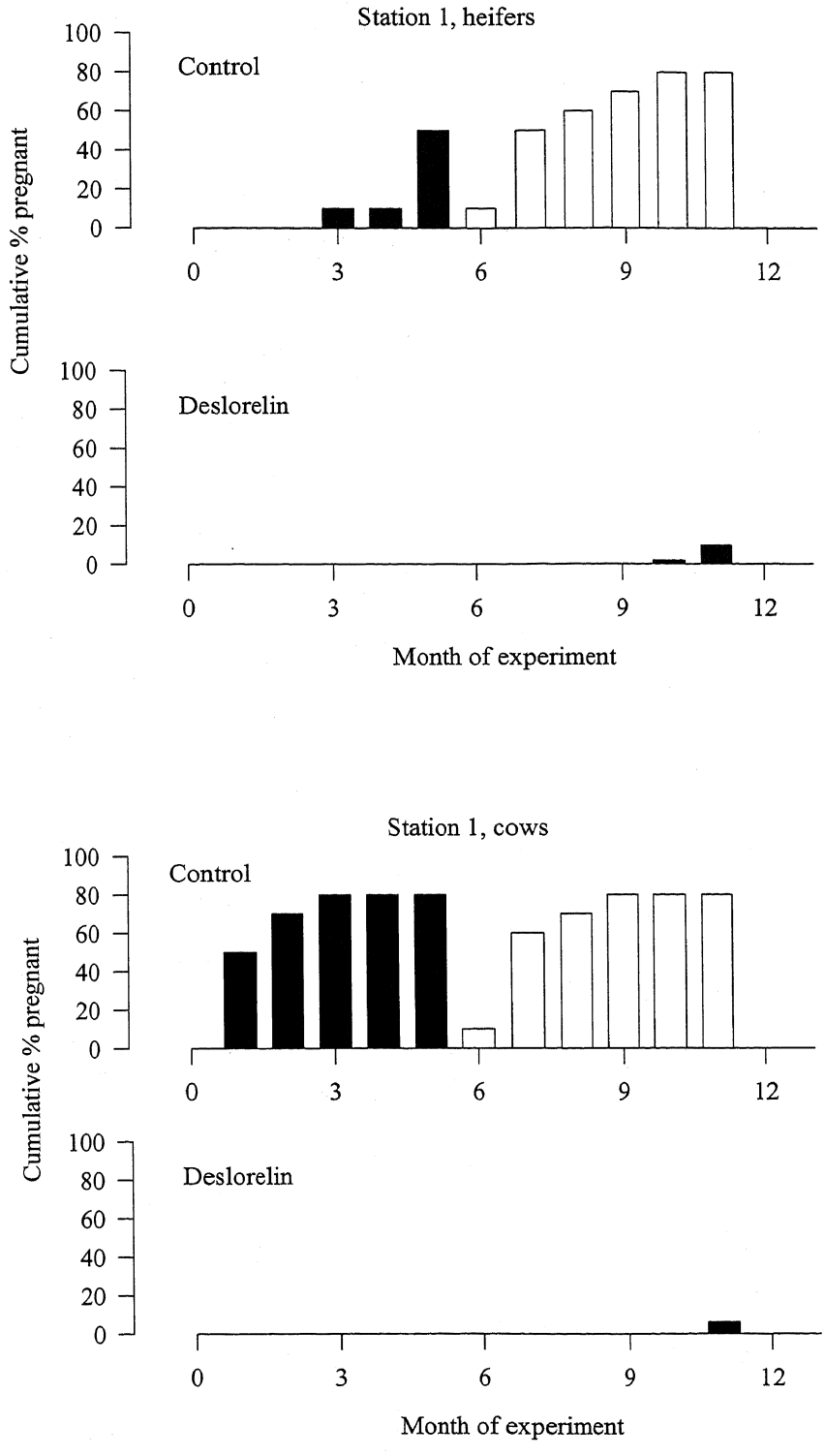


Figure 3.16. Cumulative percent pregnant of heifers (upper profiles) and cows (lower profiles) on Station 1. White open bars depict introduction of replacement groups of control cows and heifers at 6 months. Day of conception was determined from estimated foetal size.

Table 3.17. Cumulative percentage pregnant heifers and cows on Station 1.

	Months				
Treatment	1	3	6	9	11
			HEIFERS*		
Control	0^{a}	10^{a}	50^{a}	70^{a}	80^{a}
Deslorelin	0^{a}	0^{b}	0^{b}	0^{b}	10^{b}
			COWS*		
Control	50^{a}	80^{a}	60^{a}	80^{a}	80^{a}
Deslorelin	0^{b}	0^{b}	0^{b}	0^{b}	6^{b}

[a,b] Percentages in column 11 months without a common superscript differ ($P<0.05$; chi square on actual numbers of cattle pregnant, versus number non-pregnant, comparison between treatments separately for heifers and cows; [a,b] Where there were no pregnancies in treated animals differences were noted by inspection.)
* new group of control heifers and cows introduced at 6 months.

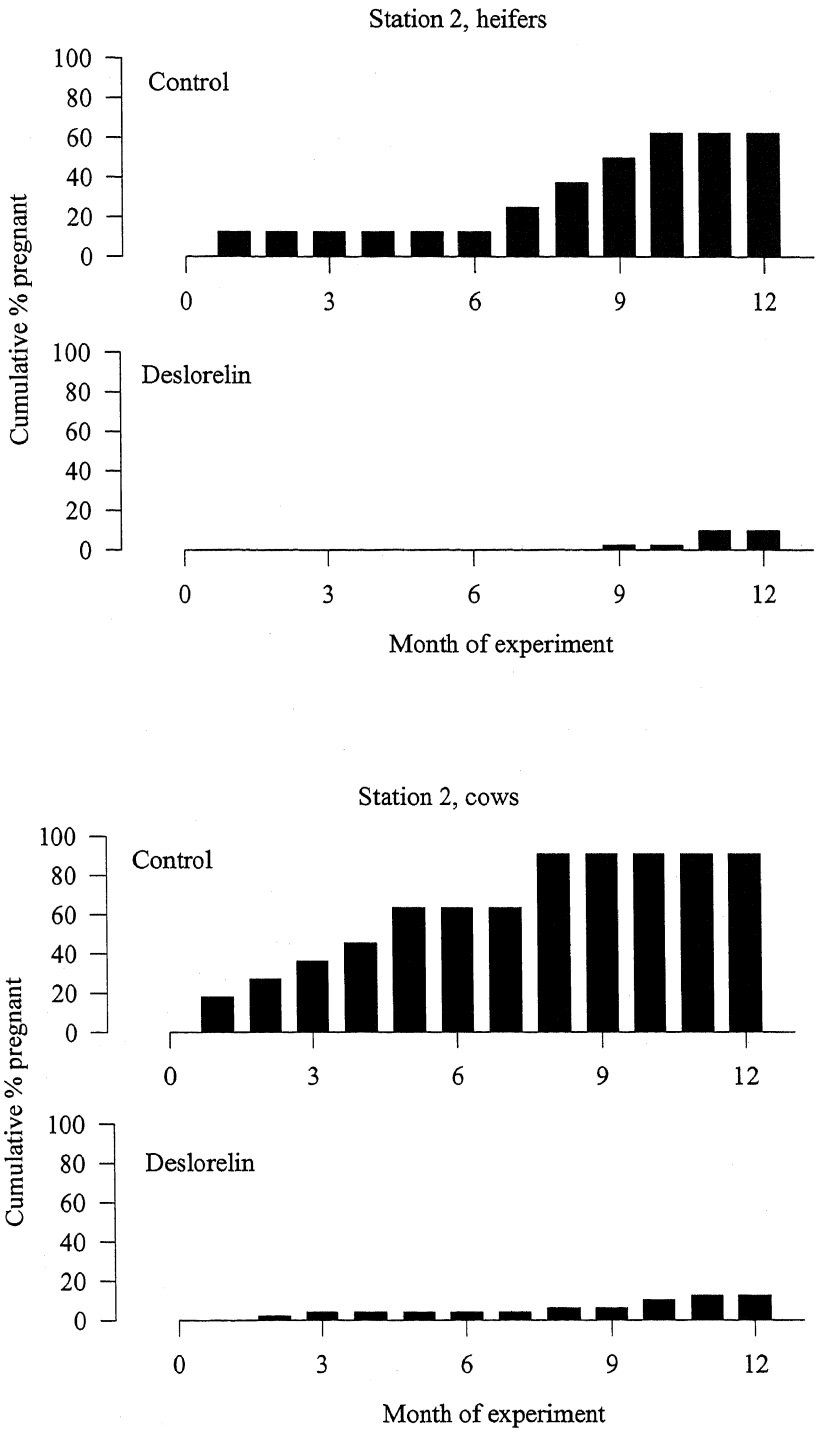


Figure 3.17. Cumulative percentage pregnant of heifers (upper profiles) and cows (lower profiles) on Station 2.

Table 3.18. Cumulative percentage pregnant heifers and cows on Station 2.

	Months				
Treatment	1	3	6	9	12
			Heifers		
Control	12[a]	12[a]	12[a]	50[a]	62[a]
Deslorelin	0[b]	0[b]	0[b]	2[b]	10b
			Cows		
Control	18[a]	36[a]	64[a]	91[a]	91[a]
Deslorelin	0[b]	4[b]	4[b]	6[b]	12[b]

[a,b] Percentages in columns, containing non-zero results, without a common superscript differ ($P<0.05$; chi square on actual numbers of cattle pregnant, versus number non-pregnant, comparison between treatments, separately for heifers and cows; [a,b] Where there were no pregnancies in treated animals differences were noted by inspection.)

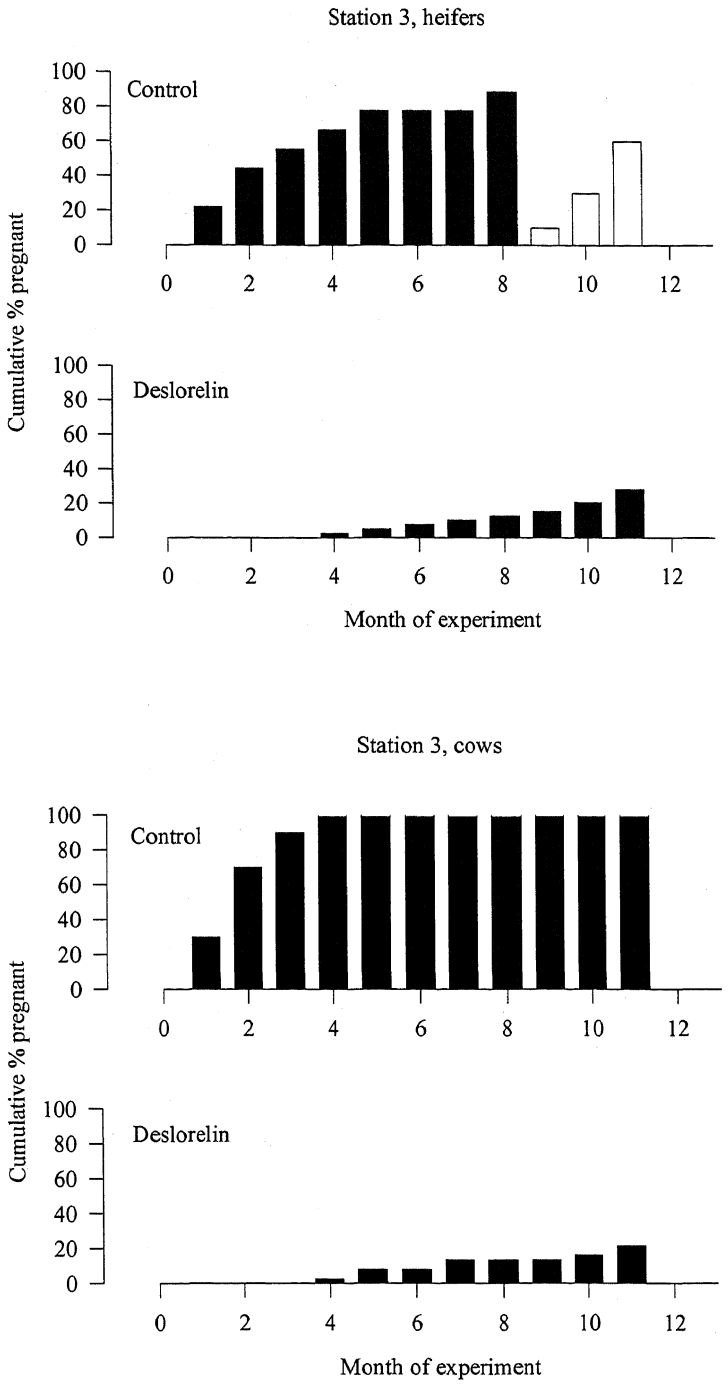


Figure 3.18. Cumulative percentage pregnant of heifers (upper profiles) and cows (lower profiles) on Station 3. White open bars in upper graph indicate a new group of replacement control heifers.

Table 3.19. Cumulative percentage pregnant heifers and cows on Station 3.

	Months				
Treatment	1	3	6	8	11*
			Heifers		
Control	22[a]	56[a]	78[a]	89[a]	60[a]
Deslorelin	0[b]	0[b]	8[b]	13[b]	28[a]
			Cows		
Control	30[a]	90[a]	100[a]	100[a]	100[a]
Deslorelin	0[b]	0[b]	8[b]	13[b]	24[b]

[a,b] Percentages in columns, containing non-zero results, without a common superscript differ ($P<0.05$; chi square on actual numbers of cattle pregnant, versus number non-pregnant, comparison between treatments, separately for heifers and cows; [a,b] Where there were no pregnancies in treated animals differences were noted by inspection.)
* new group of control heifers introduced at 9 months.

3.3.4 Pregnancy data at slaughter for heifers and cows treated with GnRH agonist

Summary pregnancy data for heifers and cows treated with deslorelin are shown in Table 3.20. At both Station 2 and 3, 10% of heifers and cows were pregnant at the time of slaughter. At Station 3, the first pregnancies were estimated to have occurred at about 340 days while the first pregnancies at Station 2 were estimated to be at about 240 days. Twenty-six percent of heifers and cows at Station 1 conceived during the study, with the first conceptions being at about 230 days.

Table 3.20. Final pregnancy data at slaughter for heifers and cows (combined data) treated with GnRH agonist.

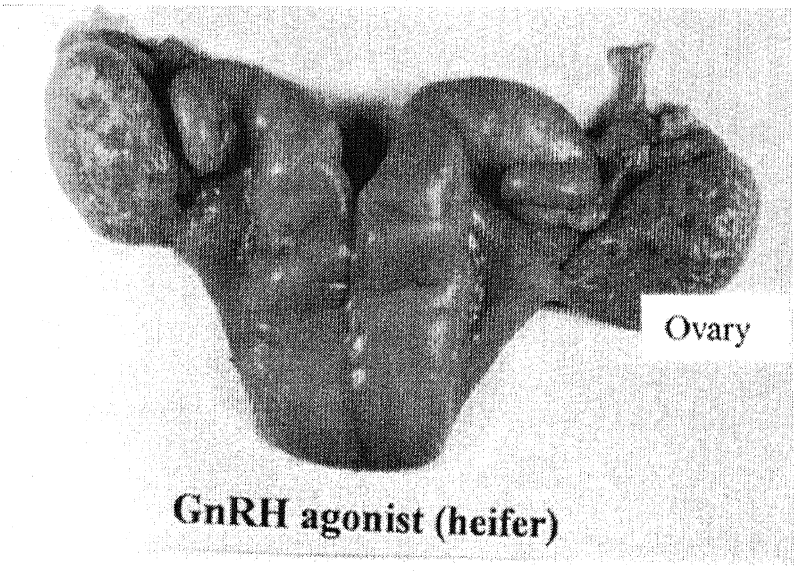
Experiment	Number Heifers+Cows	Estimated* Duration (Days)	Number Pregnant	Mean (±SEM)days to first conception**
Station 1	76	387	20 (26%)	231 ± 19
Station 2	84	376	8 (10%)	244 ± 13
Station 3	99	394	9 (9%)	336 ± 3

** Day of conception was determined from estimated foetal size

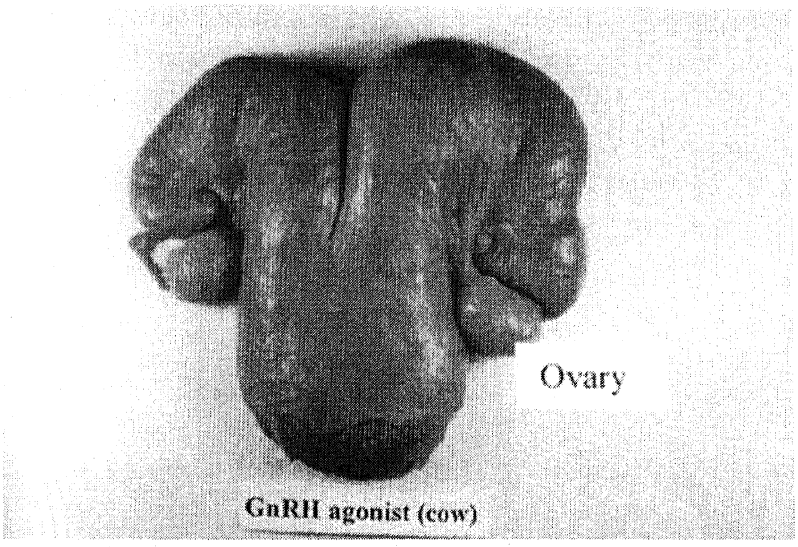
* These are number of days only for heifers and cows that conceived. All other heifers and cows in the respective groups did not show a return to normal ovarian function.

3.3.5 Ovaries and reproductive tracts of heifers and cows treated with GnRH agonist

Representative reproductive tracts from a heifer and cow treated with GnRH agonist are shown in Figure 3.19, A and B. Ovarian follicle size data were not recorded, however, observations indicated that treatment of heifers (A) and cows (B) with GnRH agonist restricted ovarian follicular growth in the majority of animals to early antral follicles (1-2 mm). Under these conditions of follicle growth, heifers and cows treated with GnRH agonist did not show oestrus.



A. Reproductive tract and ovaries of a heifer treated with GnRH agonist for 394 days. Ovarian follicular growth was restricted to small antral follicles (1-2 mm).



B. Reproductive tract and ovaries of a cow treated with GnRH agonist for 394 days. Ovarian follicular growth was restricted to a few small antral follicles (1-2 mm).

Figure 3.19. Representative reproductive tracts and ovaries of a heifer (A) and cow (B) treated with GnRH agonist.

3.4 Discussion

Treatment of heifers and cows with a controlled-release deslorelin implant blocked ovarian cyclic activity in the majority of the animals. This barrier to conception was most likely related to restriction of the growth of ovarian follicles in deslorelin-treated cattle to early antral follicles of approximately 1-2 mm, as was observed on the ovaries of deslorelin-treated cattle at slaughter. Recruitment of follicles from the gonadotrophin-sensitive antral pool (McNatty *et al.*, 1999; Webb *et al.*, 1999) and commencement of a wave of ovarian follicular development (Adams, 1999) is reliant upon a transient increase in FSH secretion from the anterior pituitary gland. Heifers and cows receiving GnRH agonist do not exhibit typical fluctuation of secretion of FSH and follicular growth is subsequently arrested at the early antral stage (Gong *et. al.*, 1995, 1996). In the current study, restricted follicular growth was maintained for greater than 12 months in the majority of heifers and cows treated with a deslorelin implant. For animals that conceived, the mean time to conception was about 200 days for heifers and cows treated with the 8 mg implant and approximately 300 days for heifers and cows treated with the 12 mg implant. The 8 mg and 12 mg implants suppressed contraception in both heifers and cows even though body weights differed by 100 to 150 kg at the start of the study. These findings demonstrated the potential of a GnRH agonist implant to achieve long-term infertility and suppress pregnancies in heifers and cows, apparently independent of body weight.

A small proportion (10%) of heifers and cows treated with the 8 mg implant at Station 2 showed cyclic ovarian activity and became pregnant during the study, compared with 25% of heifers and cows at Station 3 that also received the 8 mg implant. The reason for the difference between the two stations is not understood but may have been related to different patterns of changes in body weight. Cattle at

Station 2 showed a relatively large decline in body weight during the first 6 months of the study while at Station 3 cattle showed a steady and progressive gain in body weight. Although relative changes in body weight was the only apparent difference between the two stations, this explanation could be considered inconsistent with the earlier statement that a GnRH agonist implant suppressed ovarian activity in heifers and cows independent of body weight. However, the timing of changes in body weight may influence the response to treatment with GnRH agonist rather than absolute body weight.

Previous studies demonstrated that slow release GnRH agonist implants suppressed ovarian activity in heifers (Herschler and Vickery, 1981; D'Occhio *et al.*, 1996). In these previous experiments, ovarian follicular function was suppressed for relatively short periods of time. The outstanding observation in the present study, therefore, was the prevention of conception for about 400 days in 90% of heifers and cows treated with a 12 mg deslorelin implant.

Heifers and cows in the tropics and subtropics typical of northern Australia often require 12 months to attain a body weight and conformation appropriate for marketing. This applies to both older cows that have recently weaned a calf and young heifers surplus to breeding herd requirements. Pregnancy prevention in these categories of female cattle prior to marketing has been traditionally achieved by surgical sterilisation. The 12 mg deslorelin implant would appear to be a potential alternative to surgical intervention for preventing unwanted pregnancies in heifers and cows destined for sale in northern Australia.

At all three stations, the occurrence of elevated plasma progesterone in heifers and cows treated with deslorelin was relatively greater between 0 and 6 months of treatment, whereas conceptions in these animals tended to occur after the first 6

months. These findings suggested that elevated plasma progesterone in animals treated with GnRH agonist did not necessarily reflect cyclic ovarian activity or the presence of a CL. Heifers and cows treated with a GnRH agonist would not be expected to ovulate (Gong *et al.*, 1996; D'Occhio *et al.*, 1997) so it is possible that occurrences of elevated progesterone may have been the result of follicular luteinisation. The adrenal gland in cattle has also been shown to produce progesterone (Watson and Munro, 1984). Therefore, a possible contribution to elevated plasma progesterone from the adrenal glands cannot be excluded in some control animals and animals treated with deslorelin.

It is not understood why a relatively small proportion of heifers and cows treated with the 8 mg and 12 mg implants displayed ovarian activity and conceived relatively early in the study (4 to 6 months), while most of the treated animals showed long-term suppression of ovarian activity. It is possible that conceptions in the former group of animals may have represented variation amongst animals in insensitivity to treatment with GnRH agonist. Another possibility is that a proportion of 8 mg and 12 mg GnRH agonist implants did not release the predicted respective amounts of agonist.

3.5 Conclusions

The present study has demonstrated the efficacy of a controlled release deslorelin implant to achieve long-term suppression of ovarian activity and prevent pregnancies in heifers and cows under extensive management conditions. Also, the GnRH agonist treatment does not appear to adversely affect body weight gain. Use of a GnRH agonist implant to manage reproduction in heifers and cows provides an alternative to surgical interventions and other treatments such as the use of steroidal compounds.

Chapter 4: Evaluation of GnRH agonist implants for contraception in heifers undergoing continuous body weight gain

4.1 Introduction

In Chapter 3, results indicated that GnRH agonist implants achieved long-term suppression of pregnancies in heifers and cows. As reported in Chapter 3, one group of heifers and cows treated with GnRH agonist had continuous body weight gain throughout the study, and this group also had the earliest pregnancies amongst treated animals. These observations raised the possibility of an interaction between changes in body weight and effectiveness of a GnRH agonist implant to suppress ovarian activity in heifers and cows. One aim of the study in the present chapter was to examine the efficacy of GnRH agonist implants to prevent pregnancies in heifers undergoing continuous body weight gain, rather than fluctuations between live weight gain and weight loss that is typical of tropical and subtropical environments.

It is important for future commercialisation of GnRH agonist implants that the minimum dose required to prevent pregnancies for certain durations be defined. A second component of the study in the present chapter examined the period of pregnancy prevention achieved with GnRH agonist implants that contained 3 mg, 6 mg or 12 mg of agonist.

4.2 Materials and Methods

4.2.1 General materials and methods

The deslorelin implant and method of use (Section 2.2), and statistical analyses methods (Section 2.5) are described in Chapter 2.

4.2.2 Experimental design

Zebu crossbred heifers (Droughtmaster, 26-month-old; 308±3 kg) were block randomised on body weight into four groups; Group 1 (n=50), control, no treatment; Group 2 (n=50), 3 mg GnRH agonist deslorelin implant (low dose); Group 3 (n=50), 6 mg deslorelin implant (medium dose); Group 4 (n=48), 12 mg deslorelin implant (high dose). Heifers, and four sexually mature bulls, were maintained on pasture in a typical subtropical environment at Brigalow Research Station. Ten control heifers were included at the beginning of the study, and control heifers were progressively replaced during the study as they became pregnant. Heifers not treated with deslorelin (control animals) were managed within the experimental herds during the study. These control animals were used to evaluate fertility of the bulls and the inability of deslorein-treated heifers to conceive was due to the deslorelin implant and not to factors other than the implant.

Ovarian follicular activity and pregnancies were monitored at monthly intervals from 0 to 12 months by trans-rectal ultrasonography as subsequently described. Body weights were also recorded monthly.

4.2.3 Experimental site

The site of the experimental work described in this chapter was the Queensland Department of Primary Industries, Brigalow Research Station (latitude 24°50's; longitude 149°47'E) which is located 48 km north of Theodore in the subtropics of central Queensland.

4.2.4 Ultrasonography

The ovaries and uteri of heifers were monitored using ultrasonography. The machine used was an Aloka Echo Camera SSD – 210DX ultrasonographic scanner equipped with a 7.5 MHz linear array transrectal probe (Veterinary Medical and Surgical Supplies, Newcastle, NSW, Australia). Ovarian structures recorded were follicles and CL. Ultrasonography allowed early detection of pregnancies in control heifers and heifers treated with deslorelin. Pregnancies were confirmed about 30 days after first detection and pregnant heifers were removed from the study.

4.3 Results

4.3.1 Body weight

Results for body weight gain for control heifers are not reported as these heifers were removed from the trial as they became pregnant and replaced with new groups of control heifers. Heifers treated with deslorelin showed a progressive increase in body weight during the study, except between 7 and 9 months (Figure 4.1, Table 4.1). Body weight gains did not differ between heifers receiving the different doses of deslorelin (Table 4.2; Figure 4.1), therefore, the data were pooled for analysis (Table 4.3). The most rapid rate of body weight gain (0.63 ± 0.02 kg/day) was from 9 to 12 months of the study (Table 4.3).

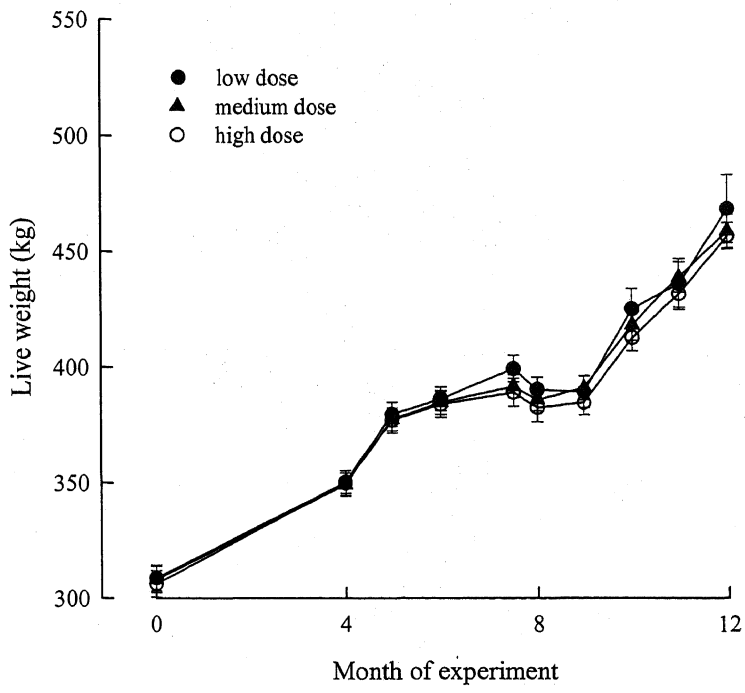


Figure 4.1. Body weight gain in heifers treated with deslorelin implants that contained 3 mg agonist (low dose, ●), 6 mg agonist (medium dose ▲) or 12 mg agonist (high dose, ○). Results are means ± SEM.

Table 4.1. Body weight (kg, mean ± SEM) in heifers treated with deslorelin.

	Month of treatment			
Deslorelin dose	0	4	8	12
3 mg	309 ± 6[a] (50)*	350 ± 5[b] (50)	390 ± 5[c] (39)	468 ± 15[d] (12)
6 mg	308 ± 6[a] (49)	349 ± 5[b] (49)	386 ± 5[c] (45)	459 ± 7[d] (26)
12 mg	306 ± 5[a] (48)	350 ± 5[b] (48)	378 ± 5[c] (45)	457 ± 6[d] (45)
Pooled data	308 ± 3 (147)	350 ± 3 (147)	384 ± 3 (129)	459 ± 4 (83)

[a,b,c,d] means within rows without a common superscript differ ($P<0.05$; comparisons with repeated measures ANOVA).
* numbers in parentheses indicate number of heifers; this declined for heifers receiving the 3 mg and 6 mg dose of deslorelin as heifers in these groups were progressively removed from the study as they were confirmed pregnant.

Table 4.2. Body weight gains (mean ± SEM) for heifers treated with deslorelin.

	Live weight gains (kg)		
	Deslorelin		
Months (days)	3 mg (n)	6 mg (n)	12 mg (n)
0-4 (121)	41 ± 2 (50) [a]	41 ± 2 (49) [a]	44 ± 2 (48) [a]
4-8 (132)	38 ± 2 (39) [a]	38 ± 21 (45) [a]	33 ± 2 (45) [a]
8-12 (120)	78 ± 4 (12) [a]	75 ± 5 (26) [a]	75 ± 2 (45) [a]
0-12 (373)	158 ± 6 (12) [a]	154 ± 6 (26) [a]	151 ± 4 (45) [a]

[a] Means in rows do not differ ($P>0.05$; ANOVA)

Table 4.3. Rate of body weight gain (mean ± SEM) for heifers treated with deslorelin. Results are pooled for heifers treated with the 3, 6 and 12 mg doses of deslorelin.

Months (days)	n	Body weight gains (kg)	Body weight daily gains (kg/day)
0-4 (121)	147	42 ±1[a]	0.35 ± 0.01[a]
4-8 (132)	129	36 ±1[b]	0.28 ± 0.01[b]
8-12 (120)	83	76 ± 2[c]	0.63 ± 0.02[c]
0-12 (373)	83	153 ± 3	0.41 ± 0.01

[a,b,c] Means in columns without a common superscript differ ($P<0.05$; ANOVA/Contrast)

4.3.2 Ovarian follicular activity

Data for ovarian follicular activity in heifers treated with GnRH agonist are depicted in Figures 4.2, 4.3 and 4.4 and summarised in Tables 4.4, 4.5 and 4.6. Detailed data on follicular activity were not recorded for control heifers because these heifers were included in the study principally to determine if conception could occur under the conditions of the study in heifers with normal ovarian function. Representative ultrasonographic micrographs of the ovaries of heifers treated with deslorelin are depicted in Figure 4.5.

Heifers treated with the 3 mg deslorelin implant had relatively small follicles (≤ 5 mm diameter) until around 3 to 4 months of the experiment, when follicular activity increased (Figure 4.2, Table 4.4). This increase in follicular activity was associated with a progressive increase in numbers of heifers demonstrating cyclic ovarian activity and conceptions (Figure 4.2, Table 4.4). The CL observed in a proportion of heifers at 1 month of the experiment was present from the start of treatment and was not indicative of ovulation between 0 and 1 months. This also applied to heifers treated with the 6 and 12 mg deslorelin implants.

Ovarian follicular growth in heifers treated with the 6 mg deslorelin implant was restricted to small follicles (≤ 5 mm diameter) until around 5 to 6 months of the study, at which time these heifers began to resume normal cyclic ovarian activity (Figure 4.3, Table 4.5).

Treatment with the 12 mg deslorelin implant restricted follicular growth to small follicles (≤ 5 mm diameter) in most heifers for the 12 months of the study, when these heifers began to resume normal ovarian activity (Figure 4.4, Table 4.6).

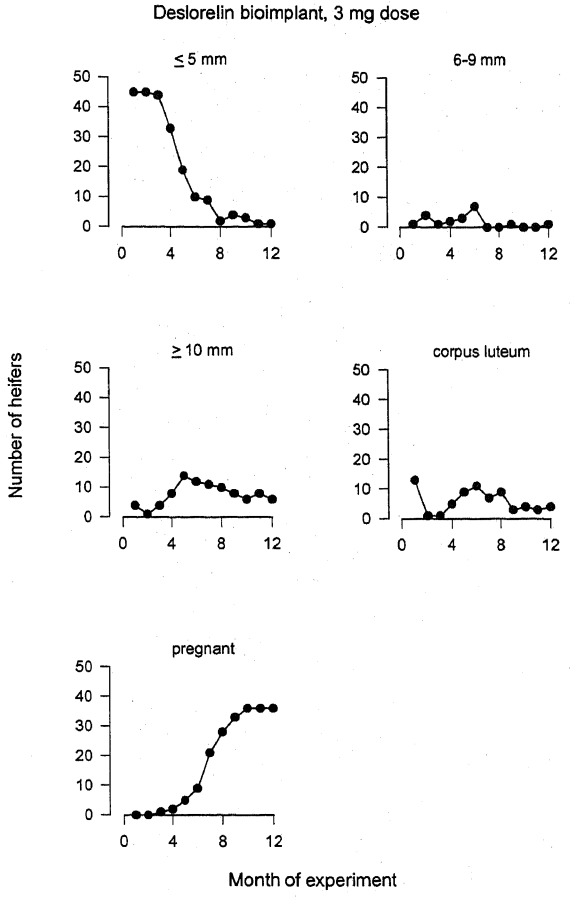


Figure 4.2. Changes in ovarian follicular status and cumulative pregnancies over time for heifers receiving a 3 mg deslorelin slow-release implant. Follicle categories are small (≤ 5 mm diameter), medium (6-9 mm) and large (≥10 mm). A decrease in numbers of small follicles from about 3 months was associated with an increase in large follicles, ovulation, development of a corpus luteum and conceptions.

Table 4.4. Ovarian follicular activity and cumulative pregnancies in heifers receiving the 3 mg dose of deslorelin. Data indicates the proportion of heifers for the different categories of reproductive variables.

Month	Follicle size			Corpus luteum	Pregnant
	≤ 5 mm	6-9 mm	≥10 mm		
1	45/50[a]	1/50[a]	4/50[a]	13/50[a]	0/50[a]
4	33/50[b]	2/50[a]	8/50[a]	5/50[b]	2/50[a]
8	2/49[c]	0/49[a]	10/49[a]	9/49[a,b]	28/49[b]
12	1/48[c]	1/48[a]	6/48[a]	4/48[b]	36/48[b]

[a,b,c] Proportions in columns without a common superscript differ (P<0.05; Chi Square; [a,b] Where there were zero data items differences were noted by inspection.)

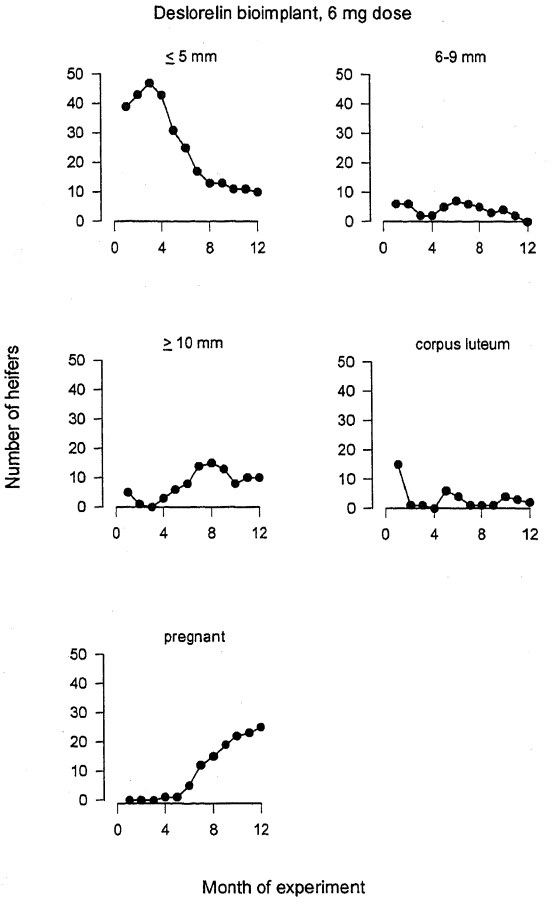


Figure 4.3. Changes in ovarian follicular status and cumulative pregnancies over time for heifers receiving a 6 mg deslorelin slow-release implant. Follicle categories are small (≤ 5 mm diameter), medium (6-9 mm) and large (≥10 mm). A decrease in numbers of small follicles from about 5 months was associated with an increase in large follicles, ovulation, development of a corpus luteum and conceptions.

Table 4.5. Ovarian follicular activity and cumulative pregnancies in heifers receiving the 6 mg dose of deslorelin. Data indicate the proportion of heifers for the different categories of reproductive variables.

Month	Follicle size			Corpus luteum	Pregnant
	≤ 5 mm	6-9 mm	≥10 mm		
1	$39/50^{a}$	$6/50^{a}$	$5/50^{a,b}$	$15/50^{a}$	$0/50^{a}$
4	$43/49^{a}$	$2/49^{a,b}$	$3/49^{a}$	$0/49^{b}$	$1/49^{a}$
8	$13/49^{b}$	$5/49^{a}$	$15/49^{c}$	$1/49^{b}$	$15/49^{b}$
12	$10/48^{b}$	$0/48^{b}$	$10/48^{b,c}$	$2/48^{b}$	$25/48^{c}$

^{a,b,c} Proportions within columns without a common superscript differ ($P<0.05$; Chi Square; ^{a,b} Where there were zero data items differences were noted by inspection.)

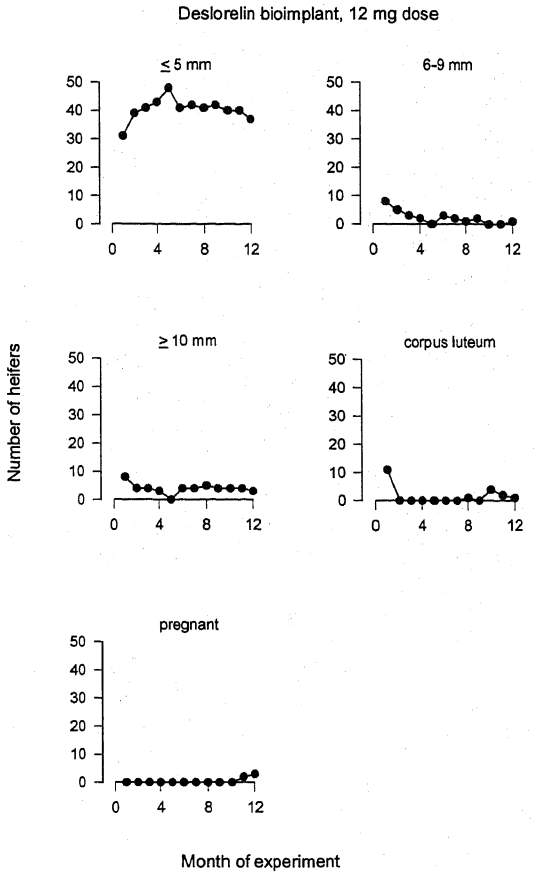


Figure 4.4. Changes in ovarian follicular status and cumulative pregnancies over time for heifers receiving a 12 mg deslorelin slow-release implant. Follicle categories are small (≤ 5 mm diameter), medium (6-9 mm) and large (≥10 mm). Heifers displayed predominantly low follicular growth and only 3 heifers were pregnant at 12 months.

Table 4.6. Ovarian follicular activity and cumulative pregnancies in heifers receiving the 12 mg dose of deslorelin. Data indicate the proportion of heifers for the different categories of reproductive variables.

Month	Follicle size			Corpus luteum	Pregnant
	≤ 5 mm	6-9 mm	≥10 mm		
1	31/48[a]	8/48[a]	8/48[a]	11/48[a]	0/48[a]
4	43/48[b]	2/48[b]	3/48[a]	0/48[b]	0/48[a]
8	41/48[b]	1/48[b]	5/48[a]	1/48[b]	0/48[a]
12	37/45[a,b]	1/45[b]	3/45[a]	1/45[b]	3/45[a]

[a,b] Proportions in columns without a common superscript differ (P<0.05; Chi Square;
[a,b] Where there were zero data items differences were noted by inspection.

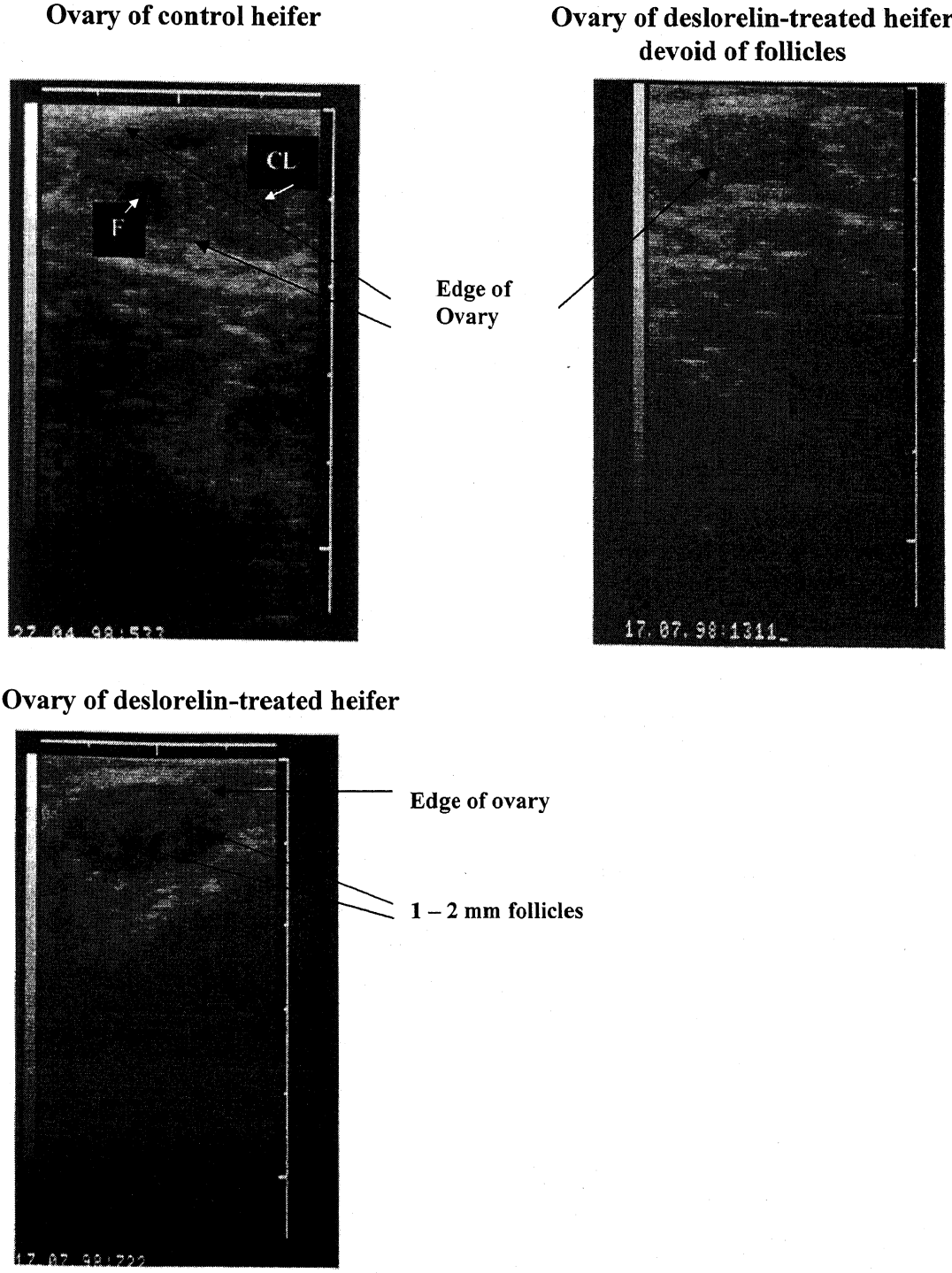


Figure 4.5. Representative ultrasonographic micrographs of the ovaries of a control heifer and heifers treated with deslorelin. The micrograph at top left shows the ovary of a control heifer (#533, top left) and illustrates typical ovarian structures including a corpus luteum (CL) and medium sized follicle (F). In contrast, the ovaries of heifers treated with deslorelin (smaller, darker elipse-like shapes near top of micrographs) are almost devoid of follicles (#1311, top right) or can have follicles which are restricted to around 1-2 mm in size (#722, bottom).

4.3.3 Pregnancies

Data for results for pregnancies are summarised in Table 4.7. Control heifers became pregnant throughout the study demonstrating that conception could occur in heifers having normal ovarian activity. Heifers treated with the 3 mg deslorelin implant showed a progressive increase in the percentage pregnant after approximately 4 months of the study. A similar increase in pregnancies was observed from approximately 6 months in heifers treated with the 6 mg deslorelin implant. In heifers treated with the 12 mg deslorelin implant, two heifers were pregnant at 11 months of the study and a third heifer was pregnant at 12 months.

Table 4.7. Cumulative pregnancies for control heifers and heifers treated with deslorelin.

	Month			
Deslorelin dose	1	4	8	12
Control	$0/11^{a, x}$	$12/19^{a, y}$	$27/34^{a, y,z}$	$44/47^{a, z}$
3 mg	$0/50^{a, x}$	$2/50^{b, x}$	$28/49^{b, y}$	$36/48^{b, y}$
6 mg	$0/50^{a, x}$	$1/49^{b, x}$	$15/49^{c, y}$	$25/48^{c, z}$
12 mg	$0/48^{a, x}$	$0/48^{b, x}$	$0/48^{d, x}$	$3/45^{d, x}$

^{a,b,c,d} Proportions within columns without a common superscript differ ($P<0.05$; Chi Square); comparisons with 0 proportion by inspection only.
^{x,y,z} Proportions within rows without a common superscript differ ($P<0.05$; Chi Square)

4.4 Discussion

Data from the present study indicate, for the first time, that deslorelin implants suppress ovarian activity and prevent pregnancies in heifers in a dose-dependent manner. Previous studies have only examined suppression of ovarian activity in cattle treated with GnRH agonists for relatively short periods (D'Occhio *et al.*, 1996; *Gong et al.*, 1995, 1996). Suppression of ovarian activity in the present study was for approximately 3 months in heifers treated with 3 mg deslorelin, 6 months in heifers treated with 6 mg deslorelin, and 12 months in heifers treated with 12 mg deslorelin. In heifers treated with 12 mg deslorelin only three of 45 (6%) were recorded pregnant at 12 months. For heifers treated with 3 and 6 mg deslorelin there was variation amongst the respective groups of heifers in the duration of suppression of ovarian activity. It is not known if these differences amongst heifers were due to different release rates of GnRH agonist between implants, individual heifer differences in sensitivity to agonist treatment, or differences among heifers in the rate of recovery of anterior pituitary gonadotroph cell function subsequent to treatment with deslorelin. These various possibilities could be examined, in part, by removing implants after different durations of treatment and observing heifers for a return to normal ovarian activity.

Based on observations in Chapter 3, the question arose as to whether there existed relationships between changes in body weight and ability of deslorelin treatment to suppress ovarian activity and prevent pregnancies. It appeared from the findings reported in Chapter 3 that the duration of suppression of fertility at a particular dose of deslorelin might be reduced in heifers and cows that are undergoing a relatively rapid rate of body weight gain. The study reported in the present chapter was designed to address the latter issue and accordingly heifers were maintained on

pastures expected to result in a progressive increase in body weight. It was found that treatment with a 12 mg deslorelin implant suppressed ovarian activity and prevented pregnancies in 42/45 (93%) of heifers for 12 months. It was concluded that at an appropriate dose, treatment with GnRH agonist can suppress fertility long-term in heifers undergoing a relatively rapid rate of body weight gain.

A second aim in the present study was to identify the dose of deslorelin required to achieve long-term (12 month) suppression of fertility in heifers. As noted above, the 12 mg deslorelin implant prevented pregnancies for 12 months in 42/45 (93%) heifers. The 3 and 6 mg deslorelin implants may have applications in management systems where suppression of fertility is required for shorter durations. As the cost of GnRH agonist is a major factor in GnRH agonist implants, the amount of agonist required for a particular application is an important consideration. Also, in the present study, the 6 mg deslorelin implant prevented pregnancies in 48/49 (98%) heifers for 6 months. It should be noted that heifers treated with 6 mg deslorelin which conceived at approximately 6 months would not deliver a calf if sold at about 12 months after treatment. This is an important consideration if the primary objective in a management system is to prevent calving during the time when heifers and cows are being prepared for sale. Heifers and cows that are 6 months pregnant or at an earlier state of pregnancy at slaughter would not be expected to incur any disadvantages from a carcass quality or value perspective. The 6 mg deslorelin implant could, therefore, have application to prevent calving in heifers and cows undergoing a 12 month preparation for slaughter in situations where the separation of bulls from females is difficult.

It is important also to note that as the 3 and 6 mg implants were becoming ineffective in suppressing ovarian activity, at approximately 3 and 6 months

respectively, with greater numbers of cattle becoming pregnant during the remainder of the study. This provided preliminary evidence for reversibility of the treatment after relatively long-term GnRH agonist treatment. This reversibility would provide an advantage over the current surgical methods of preventing pregnancies in cattle in terms of possible applications. For example, an application of GnRH agonist implants could be used to delay puberty in maiden heifers to ensure that their first conception occurred at an appropriate time relative to whole herd management.

4.5 Conclusions

Treatment with deslorelin implants was effective for preventing pregnancies in heifers undergoing a relatively rapid rate of body weight gain. The period of contraception achieved using the implants was dependent upon the amount of deslorelin contained in the implant, with the 12 mg implant providing contraception for approximately 12 months.

Chapter 5: General discussion and directions for future research

5.1 General discussion

The findings reported in this thesis have established the efficacy of deslorelin implants to achieve long-term suppression of ovarian activity and prevent pregnancies in heifers and cows. An outstanding observation was that treatment with a 12 mg deslorelin implant prevented pregnancies for 12 months, a period that is normally sufficient to prepare heifers and cows for slaughter in tropical and subtropical environments typical of northern Australia. The 12 mg deslorelin implant, therefore, offers an alternative to traditional surgical intervention and treatment with steroidal compounds to prevent pregnancies in heifers and cows surplus to breeding herd requirements.

It was also found that the ability of the 12 mg deslorelin implant to prevent pregnancies was not related to the rate of body weight gain in heifers, nor did it influence amount of body weight gain. The implant is, therefore, broadly applicable across diverse management systems in both northern and southern Australia where heifers can have different rates of body weight gain.

The 6 mg deslorelin implant prevented pregnancies for about 6 months in heifers. The importance of this observation was that heifers that conceive at 6 months would not deliver a calf within a 12 month period when they are being prepared for slaughter. These heifers would be at 6 months of gestation and would not be expected to incur any disadvantage from a marketing or carcus quality perspective at slaughter. This is an important consideration for practical application of GnRH agonist implant technology, as a 6 mg deslorelin implant would cost significantly less than a 12 mg deslorelin implant.

A feature of treatment with GnRH agonist is that heifers and cows return to normal fertility after recovering from suppression of reproductive function with the agonist. This reversibility of fertility could have application for controlled, reversible suppression of fertility in breeding heifers and cows. An example of where it might be desirable to temporarily suppress fertility in breeding animals is the delay of puberty in maiden heifers to ensure that they conceive at an appropriate time relative to whole herd management. Hence, the GnRH agonist implant technology should be viewed as a technology that provides new opportunities for reproductive management, rather than simply as another anti-fertility treatment.

5.2 Future directions

- An important aspect for further consideration is the minimal amount of GnRH agonist required for a particular application, as the cost of agonist is a major component of the cost of GnRH agonist implants. Different GnRH agonists also vary in potency and the most cost-effective in terms of potency could be investigated.
- Further research on the controlled-release nature of the implant matrix will help to optimise cost-effectiveness of the implant. At present, there is residual agonist in implants that is not released, apparently irrespective of duration of treatment.
- Research to characterise the reversibility of GnRH agonist treatment is important in order to apply GnRH agonist implant technology for both anti-fertility and controlled fertility objectives. Using a GnRH agonist implant to prevent out-of-season pregnancies would allow for the more optimal management of genetic improvement and subsequent productivity in a breeding herd program. The proper management of reproduction in replacement heifers is of exacting importance in

extensive beef production systems in tropical and subtropical environments. These environments are characterised by significant variations in seasonal availability of pasture. Therefore, breeding must be controlled so that replacement heifers have their first calf either shortly before or during the annual cycle of best pasture availability. The ability to decide the time of breeding by the use of a reversible GnRH agonist implant to manage ovarian cyclic activity in replacement heifers, would represent an advantageous paradigm shift in the method of reproductive management of extensive beef herd systems.

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