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Use of GnRH agonist implants for long-term suppression of fertility in extensively managed heifers and cows

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Abstract

The ability of gonadotrophin releasing hormone (GnRH) agonist implants to suppress ovarian activity and prevent pregnancies, long-term, was examined in heifers and cows maintained under extensive management. At three cattle stations, heifers (2-year-old) and older cows (3- to 16-year-old) were assigned to a control group that received no treatment, or were treated with high-dose (12 mg, Station A) or low-dose (8 mg, Station B and Station C) GnRH agonist implants. The respective numbers of control and GnRH agonist-treated animals (heifers + cows) at each station were: Station A, 20 and 99; Station B, 19 and 89; Station C, 20 and 76. Animals were maintained with 4% bulls and monitored for pregnancy at 2-monthly intervals for approximately 12 months. Pregnancy rates for control heifers and control cows ranged from 60–90% and 80–100%, respectively, depending on the study site. The respective number of animals (heifers + cows) treated with GnRH agonist that conceived, and days to first conception, were: Station A, 9 (9%) and 336 ± 3 days; Station B, 8 (10%) and 244 ± 13 days; Station C, 20 (26%) and 231 ± 3 days. Treatment with high-dose GnRH agonist prevented pregnancies for longer (∼300 days) than treatment with low-dose GnRH agonist (∼200 days). In the majority of heifers and cows treated with GnRH agonist, ovarian

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follicular growth was restricted to early antral follicles (2–4 mm). The findings indicate that GnRH agonist implants have considerable potential as a practical technology to suppress ovarian activity and control reproduction in female cattle maintained in extensive rangelands environments. The technology also has broader applications in diverse cattle production systems.

Keywords: GnRH agonist; Ovarian function; Fertility; Heifers; Cows

1. Introduction

Gonadotrophin releasing hormone (GnRH) is a neuropeptide that stimulates gonadotroph cells in the anterior hypophysis to secrete LH and FSH (D'Occhio, 1993, 1994). At gonadotroph cells, GnRH binds to membrane-bound surface receptors that undergo micro-aggregation and internalisation (Hazum and Conn, 1988). This activates second messenger signal transduction pathways and culminates in release of stored LH and FSH and de novo synthesis of both gonadotrophins (Huckle and Conn, 1988; Hawes et al., 1992). Internalisation of GnRH receptors after binding by GnRH induces a transient insensitivity in gonadotroph cells to stimulation by GnRH. Under normal circumstances, GnRH receptors are replenished within several hours and this restores the responsiveness of gonadotroph cells to GnRH.

Numerous agonists of GnRH have been synthesised that characteristically have a longer half-life in circulation relative to natural sequence GnRH, and increased binding affinity for the GnRH receptor (Karten and Rivier, 1986). The response of cattle to long-term treatment with GnRH agonist involves an acute phase and a chronic phase (D'Occhio and Aspden, 1999). During the acute phase, which can last for several days, the secretion of LH and FSH are increased (Gong et al., 1995, 1996; Maclellan et al., 1997). The chronic phase is characterised by a reduction in GnRH receptors on gonadotroph cells (Melson et al., 1986), uncoupling of signal transduction mechanisms (Hawes et al., 1992), insensitivity to GnRH (D'Occhio and Aspden, 1996), depletion in pituitary content of LH and FSH (Aspden et al., 1996, 1997), lack of pulsatile secretion of LH and FSH (Gong et al., 1995, 1996; Jimenez-Severiano et al., 1999) and the absence of preovulatory surge releases of LH (D'Occhio et al., 1997). Suppression of the anterior pituitary can be maintained long-term provided that GnRH agonist is present in circulation at a threshold concentration (D'Occhio and Aspden, 1996). A lack of pulsatile secretion of LH and FSH in heifers during long-term treatment with GnRH agonist results in the arrest of follicular growth at the gonadotrophin-dependent stage (Gong et al., 1996; D'Occhio et al., 2000) and failure to ovulate (D'Occhio et al., 1997).

Agonists of GnRH have potential applications for the control of reproductive function in heifers and cows maintained under extensive management in rangelands environments, where the absolute control of bulls is not possible. One application is for the prevention of unwanted pregnancies in heifers and older cows that are surplus to breeding requirements. This group of female cattle have traditionally been sterilised using surgical procedures (Jeffery et al., 1997). A second potential application of GnRH agonists is for reversible suppression of fertility in breeding heifers and cows to ensure that mating occurs at an

appropriate time relative to seasonal cycles of rainfall and pasture availability (D'Occhio et al., 1996). Agonists also have other general applications for the control of reproduction in diverse cattle production systems. In the present study, GnRH agonist implants were evaluated for their ability to prevent conception, long-term, in heifers and cows maintained in extensive beef cattle management systems in the dry tropics of northern Australia.

2. Materials and methods

2.1. Animals and management

The heifers (2-year-old) and cows (3- to 16-year-old) used in this study were of Zebu (Brahman, *Bos indicus*) genotype. Details of the location of experimental sites and management of the animals are provided in a companion publication (Fordyce et al., 2001). In brief, groups of heifers and cows were treated with GnRH agonist at three beef cattle stations located in the dry tropics of northern Australia. Animals were maintained under extensive rangelands management during the study.

2.2. GnRH agonist bioimplants

The GnRH agonist implants contained the agonist deslorelin (D-Trp6-Pro9-des-Gly10-GnRH ethylamide) (Karten and Rivier, 1986). The implants comprised an extruded lipid mix containing soluble pore forming agents and are biocompatible. They were formulated to contain 8 and 12 mg deslorelin/implant for the low-dose and high-dose, respectively. The release rates of deslorelin from the implants in vitro were approximately 10 μg deslorelin/24 h (8 mg implant) and 20 μg deslorelin/24 h (12 mg implant) (J. Walsh, unpublished data, Peptech Animal Health Pty Limited, Sydney, Australia). The release rates in vivo are not known. The implants are placed subcutaneously on the dorsal surface of the ear using aseptic procedures and a commercial implanting device.

2.3. Experimental design

The study was conducted at three beef cattle stations in the dry tropics of northern Australia. At each station, groups of heifers and cows were confirmed non-pregnant (Fordyce et al., 2001) and were then allocated by stratified randomisation to either a control group that received no treatment, or a treatment group that were implanted with a GnRH agonist implant as summarised in Table 1. After treatment, heifers and cows at the respective stations were maintained as a single group under standard management, together with 4% herd bulls (4 bulls per 100 females). At 2-monthly intervals heifers and cows were weighed and examined for pregnancy by rectal palpation. Heifers and cows were slaughtered after approximately 12 months of treatment and the status of the ovaries and reproductive tracts recorded. The latter were compared to the reproductive tracts of contemporary ovariectomised heifers. The study commenced around the beginning of the dry season (April–June;

Table 1
Treatment allocation of heifers and cows at the three study sites

	Dose of GnRH agonist	Animals treated			
		Control		GnRH agonist	
		Heifers	Cows	Heifers	Cows
Station A	High	10	10	51	48
Station B	Low	8	11	41	48
Station C	Low	10	10	39	37
Total		28	31	131	133

Control heifers and cows that conceived were replaced by additional non-pregnant control animals during the study.

autumn) in the dry tropics of northern Australia (Station A, May; Station B, June; Station C, April).

2.4. Statistical analyses

Data at single time points were analysed by ANOVA using the General Linear Models (GLM) procedure of SAS/STAT (SAS, 1990). Data analyses for live weight over time were undertaken by repeated measures analysis using SAS/STAT procedure mixed with REML estimation (autoregressive-1) and the model being y = treatment, time, treatment × time, with animal as the repeated measure (SAS, 1992). The CONTRAST statements of SAS/STAT GLM and MIXED were used for comparisons between treatment group means. χ^2 analysis was used for comparisons of proportions of conceptions at single time points. Analysis of proportions of conceptions over time was undertaken using procedure LIFETEST of SAS/STAT (SAS, 1990). Data are reported as arithmetic means ± S.E.M.

3. Results

3.1. Live weight

Heifers and cows at Station A and Station B showed seasonal fluctuations in live weight that were typical of cattle in the dry tropics of northern Australia (Tables 2 and 3). Live weight remained relatively stable or declined during the dry winter and spring and then underwent a dramatic increase in late-spring and early-summer with the onset of seasonal summer rainfall. At Station C, the live weight of heifers and cows did not decline during winter; rather, both heifers and cows at Station C showed a gradual and progressive increase in live weight during the study (Tables 2 and 3). There were no differences in live weight between control animals and animals treated with GnRH agonist, except for cows at Station C at 12 months. The latter was due to the introduction of a new group of control cows (Table 3). At all three stations heifers and cows were heavier at the end of the study compared with commencement weights.

Table 2
Longitudinal changes in live weight for control heifers and heifers treated with a GnRH agonist implant

	Month of treatment[a]			
	0	4	8	12
Station A				
Control	307 ± 4 (10)a	309 ± 7 (9)a	316 ± 6 (16)a	398 ± 8 (11)b
GnRH agonist	303 ± 2 (51)a	303 ± 4 (27)a	314 ± 2 (51)b	388 ± 3 (51)c
Station B				
Control	294 ± 8 (8)a,b	270 ± 7 (8)b	307 ± 8 (7)a	360 ± 12 (8)c
GnRH agonist	307 ± 5 (41)a	283 ± 5 (39)b	306 ± 5 (41)a	373 ± 5 (40)c
Station C				
Control	316 ± 9 (10)a	345 ± 11 (10)a,b	368 ± 5 (3)b,c	396 ± 11 (13)c
GnRH agonist	307 ± 4 (39)a	337 ± 3 (39)b	354 ± 5 (35)c	387 ± 6 (39)d

Means within rows without a common letter (a–d)differ ($P < 0.05$).
[a] There were no differences in live weight between control heifers and heifers treated with GnRH agonist.

3.2. Pregnancies in heifers

Analyses of pregnancies over time showed large differences ($P < 0.001$) in proportions of conceptions between control heifers and heifers treated with GnRH agonist, at all three stations (Fig. 1). Analyses of proportions of pregnancies in heifers at individual time points during the experiments are shown in Table 4. For control heifers at Station A, 50% conceived between 2 and 6 months and 90% were pregnant at 12 months. The first pregnancies for heifers treated with GnRH agonist at Station A occurred between 11 and 12 months (10%). At Station B, control heifers showed a cumulative increase in pregnancies between 6 months (12%) and 12 months (62%). Among heifers treated with GnRH agonist at Station B,

Table 3
Longitudinal changes in live weight for control cows and cows treated with a GnRH agonist implant

	Month of treatment[a]			
	0	4	8	12
Station A				
Control	459 ± 15 (10)a	418 ± 44 (3)a,b	393 ± 15 (12)b	460 ± 20 (10)a
GnRH agonist	445 ± 8 (48)a	415 ± 11 (23)b	414 ± 8 (48)b	471 ± 9 (46)c
Station B				
Control	308 ± 8 (11)a,b	285 ± 6 (11)a	317 ± 7 (9)b	382 ± 15 (10)c
GnRH agonist	317 ± 5 (48)a	298 ± 5 (47)b	330 ± 6 (47)a	388 ± 5 (48)c
Station C				
Control	407 ± 12 (10)a	439 ± 11 (10)a	416 ± 6 (3)a	502 ± 15 (6)b
GnRH agonist	401 ± 8 (37)a	427 ± 7 (37)b	425 ± 7 (37)b	462 ± 8 (37)c

Means within rows without a common letter (a–c) differ ($P < 0.05$).
[a] There were no differences in live weight between control cows and cows treated with GnRH agonist, except for Station C at 12 months; the latter was due to the introduction of a new group of control cows.

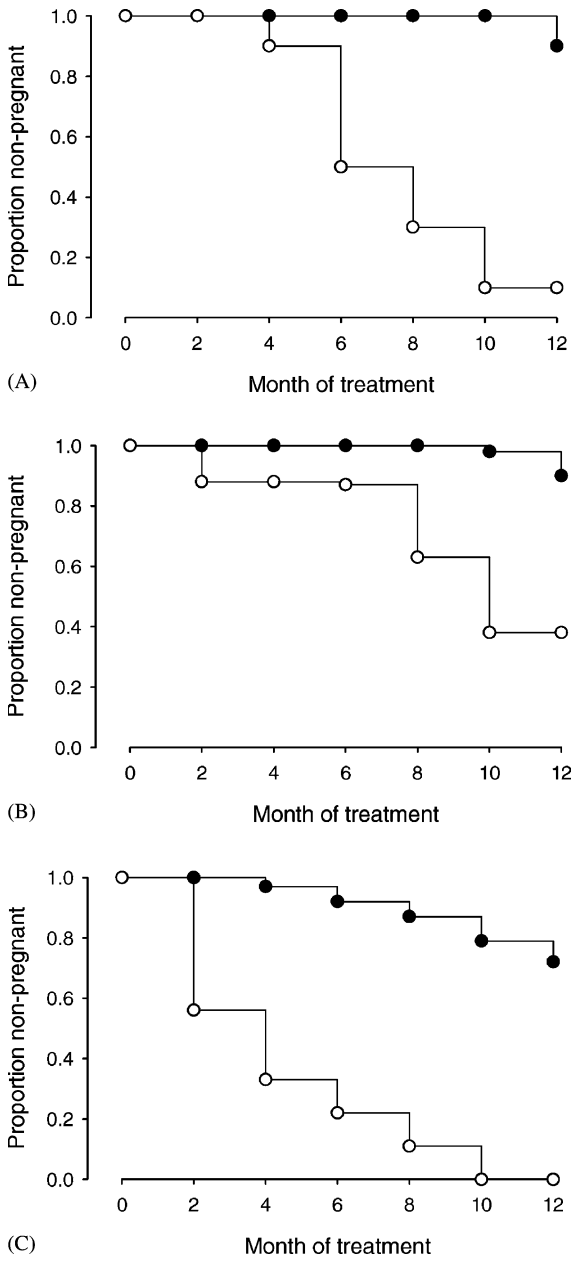

Fig. 1. Proportion of control heifers (open circles) and heifers treated with a GnRH agonist (closed circles) that conceived during the study at Station A (upper plot), Station B (middle plot) and Station C (lower plot). Profiles are based on output from survival analysis procedure LIFETEST of SAS/STAT, which showed large differences ($P < 0.001$) in conceptions over time between control heifers and GnRH agonist-treated heifers at all three study sites.

Table 4
Proportion of control heifers and heifers treated with a GnRH agonist that conceived during the study

	Proportion of heifers pregnant					
	Month of treatment					
	2	4	6	8	10	12
Station A						
Control	0/10a (0%)	1/10a (10%)	5/10a (50%)	7/10a (70%)	9/10a (90%)	9/10a (90%)
GnRH agonist	0/51a (0%)	0/51a (0%)	0/51b (0%)	0/51b (0%)	0/51b (0%)	5/51b (10%)
Station B						
Control	1/8a (12%)	1/8a (12%)	1/8a (12%)	3/8a (37%)	5/8a (62%)	5/8a (62%)
GnRH agonist	0/41a (0%)	0/41a (0%)	0/41a (0%)	0/41b (0%)	141b (2%)	4/41b (10%)
Station C						
Control	4/9a (44%)	6/9a (67%)	7/9a (78%)	8/9a (89%)	3/10a[a] (30%)	6/10a (60%)
GnRH agonist	0/39b (0%)	1/39b (2%)	3/39b (8%)	5/39b (13%)	8/39a (20%)	11/39a (28%)

Proportions within property without a common letter (a, b) differ ($P < 0.05$).
[a] A new group of control heifers was introduced between 8 and 10 months.

pregnancies occurred between 9 months (2%) and 12 months (10% cumulative) (Table 4). Control heifers at Station C showed a progressive increase in pregnancies between 1 and 8 months, at which time 89% were pregnant. The first pregnancies in heifers treated with GnRH agonist at Station C occurred between 2 and 4 months (2%) and this was followed by a steady increase in pregnancies to 12 months (28% cumulative) (Table 4).

3.3. Pregnancies in cows

Analyses of pregnancies over time showed large differences ($P < 0.001$) in proportions of conceptions between control cows and cows treated with GnRH agonist, on all three stations (Fig. 2). Analyses of proportions of pregnancies in cows at individual time points during the experiments are shown in Table 5. Control cows at Station A and Station C conceived early in the study and respective cumulative percentages pregnant at 6 months were 80 and 100%. At Station B, pregnancies in control cows were delayed between 0 and 6 months (64% cumulative), likely due to a decline in live weight during this time, and a dramatic increase in pregnancies occurred from 6 to 9 months (91% cumulative) commensurate with an increase in live weight. The first pregnancies in cows treated with GnRH agonist at Station A were recorded at 12 months (6%). At Station B, the first pregnancy in cows implanted with GnRH agonist was recorded at 8 months (2%) and the cumulative pregnancies at 12 months was 8% (Table 5). At Station C, the first pregnancy in cows treated with GnRH agonist was recorded at 4 months (3%) and the cumulative pregnancies at 12 months was 24%.

3.4. Summary pregnancy data at slaughter

Summary pregnancy data at slaughter for heifers and cows treated with GnRH agonist are shown in Table 6. For Station A and Station B, 10% of females were pregnant

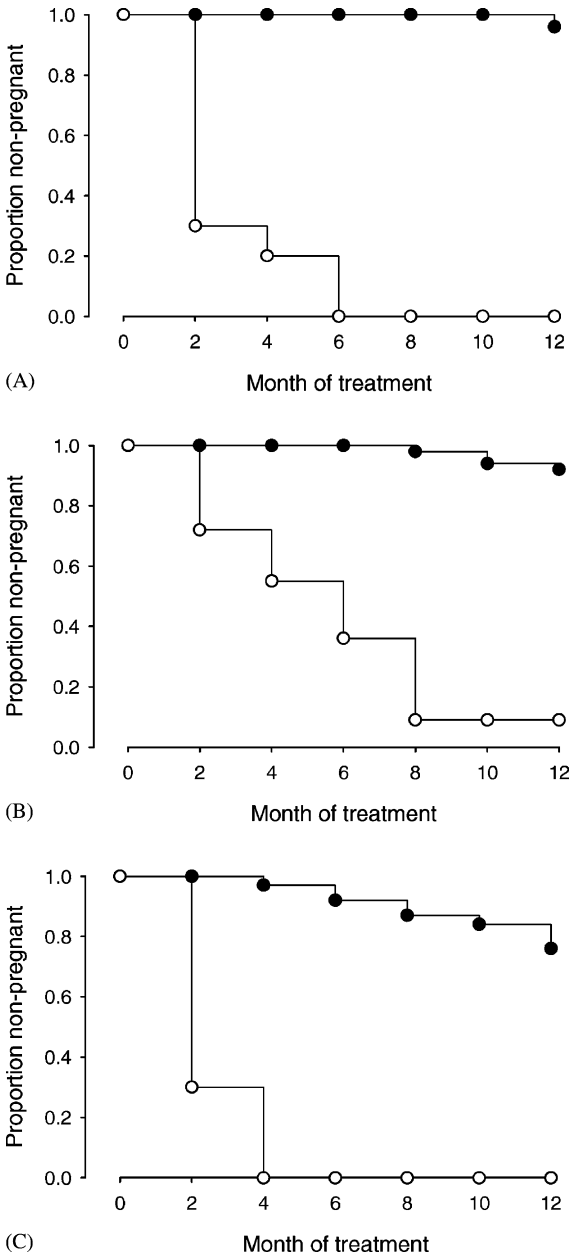

Fig. 2. Proportion of control cows (open circles) and cows treated with a GnRH agonist (closed circles) that conceived during the study at Station A (upper plot), Station B (middle plot) and Station C (lower plot). Profiles are based on output from survival analysis procedure LIFETEST of SAS/STAT, which showed large differences ($P < 0.001$) in conceptions over time between control heifers and GnRH agonist-treated heifers at all three study sites.

Table 5
Proportion of control cows and cows treated with a GnRH agonist that conceived during the study

	Proportion of cows pregnant					
	Month of treatment					
	2	4	6	8	10	12
Station A						
Control	7/10a (70%)	8/10a (80%)	1/10a[a] (10%)	7/10a (70%)	8/10a (80%)	8/10a (80%)
GnRH agonist	0/48b (0%)	0/48b (0%)	0/48b (0%)	0/48b (0%)	0/48b (0%)	3/48b (6%)
Station B						
Control	3/11a (27%)	5/11a (45%)	7/11a (64%)	10/11a (91%)	10/11a (91%)	10/11a (91%)
GnRH agonist	0/48b (0%)	0/48b (0%)	0/48b (0%)	1/48b (2%)	3/48b (6%)	4/48b (8%)
Station C						
Control	7/10a (70%)	10/10a (100%)	10/10a (100%)	10/10a (100%)	10/10a (100%)	10/10 a (100%)
GnRH agonist	0/37b (0%)	1/37b (3%)	3/37b (8%)	5/37b (13%)	6/37b (16%)	9/37b (24%)

Proportions within property without a common letter (a, b) differ ($P < 0.05$).
[a] A new group of control cows was introduced between 4 and 6 months.

at slaughter and for Station C 26% were pregnant. The number of days to first pregnancies were: Station A, 336 ± 3 days; Station B, 244 ± 13 days; Station C, 231 ± 19 days. The high-dose GnRH agonist implant used at Station A prevented pregnancies for a longer period that the low-dose GnRH agonist implant used at Station B and Station C.

3.5. Ovaries and reproductive tracts at slaughter

Ovarian follicular growth in heifers and cows treated with GnRH agonist for around 400 days (Station A) was restricted to early antral follicles (2–4 mm). The reproductive tracts of heifers treated with GnRH agonist were similar in size to the tracts of contemporary heifers that had been ovariectomised at the start of the study and maintained together with the experimental heifers (data not shown).

Table 6
Pregnancy data at slaughter for heifers and cows treated with a GnRH agonist implant

	Number of heifers + cows[a]	Duration of treatment (days)	Number pregnant (%)	Days to first conception[b]
Station A	99	394	9 (9)	336 ± 3
Station B	84	376	8 (10)	244 ± 13
Station C	76	387	20 (26)	231 ± 3

The results are for heifers and cows combined.
[a] Some animals treated with GnRH agonist were removed from the study and were not slaughtered; however, the data for these animals are included in the Table.
[b] Only animals that conceived were included in the calculation of days to first conception.

4. Discussion

Treatment of heifers and cows with a GnRH agonist implant restricted ovarian follicular growth in the majority of animals to early antral follicles (2–4 mm). Recruitment of follicles from the antral pool (McNatty et al., 1999; Webb et al., 1999), and the initiation of an ovarian follicular wave (Adams, 1999), are dependent on the occurrence of transient increases in circulating concentration of FSH. Heifers and cows treated with GnRH agonist do not have pulsatile secretion of gonadotrophins and hence follicular growth is arrested at the early antral, or gonadotrophin-dependent stage (Gong et al., 1995, 1996). In the present study, suppressed follicular growth was maintained long-term (>12 months) by GnRH agonist implants. For heifers and cows that conceived, the average time to conception was around 200 days for animals that received the low-dose GnRH agonist implant, and approximately 300 days for animals that received the high-dose GnRH agonist implant. The implants induced an apparent similar response in heifers and cows, even though live weight differed by 100–150 kg at the time of implantation. The above findings demonstrated the potential of GnRH agonist implants as a practical technology to suppress ovarian activity in heifers and cows, and prevent pregnancies. Furthermore, it would also appear that an appropriate dose of GnRH agonist is effective over a broad range of live weights.

A relatively small proportion (∼10%) of heifers and cows treated with low-dose GnRH agonist at Station B showed ovarian activity and conceived during the study, compared with the proportion (∼25%) at Station C that were also treated with low-dose GnRH agonist. This apparent difference in response to treatment with low-dose GnRH agonist between heifers and cows at Station B compared with Station C might be explained by environmental factors. At Station B, animals experienced a relatively large decline in live weight during the first 6 months of the study, whereas at Station C animals underwent a steady but progressive increase in live weight. If it is assumed that there was no environmental constraint to reproductive function at Station C, then the cumulative proportion of heifers and cows that showed ovarian activity and conceived reflected the capacity of the low-dose GnRH agonist implant to suppress fertility. The observations at Station B highlighted the central role of the environment in determining the reproductive function of cattle in the dry tropics.

Implants containing GnRH agonists were previously shown to suppress ovarian activity in heifers (Herschler and Vickery, 1981; D'Occhio et al., 1996). In these earlier studies, ovarian activity was suppressed for relatively short periods of time. The outstanding observation in the present study was the prevention of pregnancy for approximately 400 days in 90% of both heifers and cows treated with the high-dose GnRH agonist implant. Cattle managed in the dry tropics often require this length of time to attain the live weight and body conformation necessary for marketing. This applies to relatively young heifers that are surplus to breeding requirements and older cows that have recently weaned a calf. The prevention of pregnancies in these females before marketing has traditionally been achieved by surgical sterilisation (Jeffery et al., 1997). The high-dose GnRH agonist implant represents a feasible alternative technology to control reproduction in market heifers and cows in extensively managed production systems.

It is not clear why a small proportion of heifers and cows that received either a low-dose or high-dose GnRH agonist implant conceived relatively early in the study (4–6 months), whilst the majority of animals remained suppressed long-term. The former group of animals may

have represented a population with a relative insensitivity to treatment with GnRH agonist. This is considered unlikely and it is possible that a small proportion of the GnRH agonist implants failed to release agonist adequately.

5. Conclusions

The present study has demonstrated the efficacy of GnRH agonist implants to achieve long-term suppression of ovarian activity and prevent pregnancies in heifers and cows maintained under extensive management. Treatment with a GnRH agonist implant to control reproduction in female cattle offers a practical alternative to surgical procedures and other treatments that include steroidal preparations.

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