Final report

Evaluation of a GnRH agonist hioimplant for oestrous suppression and pregnancy prevention in heifers

Project code: NAP3.110

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PROJECT DETAILS

Project Number

NAP3.110

Project Title

Evaluation of a GnRH agonist bioimplant for oestrous suppression and pregnancy prevention in heifers.

Project Description

The project involved further evaluation of the potential of a GnRH agonist bioimplant to achieve a long term contraceptive effect in heifers. In MRC Project NAP3.105, prototype GnRH agonist bioimplants suppressed ovarian activity and prevented pregnanices in heifers and cows for varying periods of time. In the latter project the return to normal ovarian function in heifers and cows treated with GnRH agonist appeared to coincide with increased pasture availability and associated greater live weight gain. This raised the question of whether the GnRH agonist was capable of suppressing ovarian function in heifers undergoing a relatively fast rate of live weight gain during the wet summer months, typical of northern Australia. Also of interest was the dose of GnRH agonist required to achieve long term suppression of ovarian function in heifers. Heifers were treated with graded doses of GnRH agonist (deslorelin) using a bioimplant placed in the ear, and monitored over 12 months for ovarian activity and pregnancies.

Project Objectives

Establish that the GnRH agonist bioimplant suppresses oestrous behaviour and prevents conception in pubertal (2-year-old) heifers that are undergoing a relatively fast rate of live weight gain during the wet season in northern Australia.

Identify the cost/benefit of using the technology to prevent pregnancies in heifers.

Project Duration

1 January 1998 to 31 December 1998

Project Funding

Total Funding $30,000

Abstract

The principal aim in this project was to determine whether a GnRH agonist bioimplant would suppress ovarian function and prevent conception in heifers undergoing a relatively fast rate of live weight gain. A second objective was to identify a dose of GnRH agonist (deslorelin) that would induce a contraceptive effect in heifers for 12 months, or longer. A group of 198 sexually mature heifers (26-month-old) were divided into 4 groups and, in January 1998, received one of the following treatments:

Control	(n=50)	Not implanted
GnRH low dose	(n=50)	Implanted with 3 mg deslorelin
GnRH medium dose	(n=50)	Implanted with 6 mg deslorelin
GnRH high dose	(n=48)	Implanted with 12 mg deslorelin

Heifers were maintained on natural pastures together with 4 herd bulls. At the start of the trial, heifers had a live weight of 308 ± 3 kg (mean± SEM) and they gained 153 ± 3 kg during the 12 months of the trial. Heifers gained 42 ± 1 kg from O and 4 months, 36 ± 1 kg from 4 and 8 months, and 76 ± 2 kg from 8 and 12 months. Hence, the greatest rate of live weight gain (0.63 ± 0.02 kg/day) occurred during the latter part of the trial, which allowed testing of the relationship between rate of live weight gain and capacity of the GnRH agonist to suppress ovarian function.

Only 10 heifers from the control group were initially included in the trial, and control heifers were progressively replaced as they conceived. This ensured that non-pregnant cyclic heifers, which could conceive if mated, were present in the herd throughout the trial. Ovarian activity and pregnancies were monitored at monthly intervals using trans- rectal ultrasonography.

Throughout the trial, control heifers typically conceived within 4 to 6 weeks after introduction into the herd, confirming that there was no seasonal effect on the capacity of heifers to conceive, provided they were showing normal ovarian function.

The GnRH low dose treatment suppressed ovarian function for approximately 3 months, after which time there was a progressive increase in the number of heifers in this group that conceived, as follows: 4 months, 2/50 (4%); 8 months, 28/49 (57%); 12 months, 36/48 (75%). The majority of heifers treated with the GnRH medium dose showed suppressed ovarian function for around 5 months and cummulative conceptions for this group were: 4 months, 1/49 (2%); 8 months, 15/49 (36%); 12 months, 26/48 (54%). Around 90% of heifers treated with the GnRH high dose continued to show only minimal ovarian activity up to 12 months and numbers of heifers pregnant were: 4 months, 0/48;
8 months, 0/48; 12 months, 3/45 (6%).

The results of this trial have established the contraceptive efficacy of a GnRH agonist bioimplant in heifers. The GnRH high dose implant was effective in suppressing ovarian function in heifers undergoing a fast rate of live weight gain, relative to conditions typical of northern Australia. The GnRH agonist bioimplant represents the next generation technology for the reproductive management of female cattle in extensive beef herds.

Executive summary

Industry Issue

The control of pregnancies in heifers that are surplus to breeding requirements is an important component of cattle management in the extensive beef production systems of northern Australia. Particularly significant is the prevention of pregnancies in turn-off heifers and cast-for-age cows. Traditionally, fertility control has been achieved by surgical procedures that include paralumbar (flank) and vaginal (passage) spaying. More recently, the Willis Drop-Ovary Technique has been introduced as an alternate surgical procedure. The latter would appear to be the preferred surgical approach, but requires a relatively high level of technical proficiency. A challenge for the beef industry is to identify a contraceptive technology for heifers and cows that is non-invasive, practical, can be readily applied, and satisfies animal production imperatives whilst recognising the importance of consumer and community awareness of animal welfare.

Project Strategy

A potential non-surgical technology for contraception in female cattle is the use of a gonadotrophin releasing hormone (GnRH) agonist bioimplant. The reproductive hormone cascade in cattle is initiated by the release of endogenous GnRH from the base of the brain. GnRH acts at the anterior pituitary gland to stimulate a pulsatile pattern of release of luteinising hormone (LH) and follicle stimulating (FSH), which is necessary for normal ovarian function. Agonists of GnRH prevent the pulsatile release of LH and FSH and this leads to suppressed ovarian follicular growth and failure to ovulate. Recently, a GnRH agonist bioimplant was developed which releases microgram quantities of GnRH agonist (deslorelin) in a controlled, sustained manner (Peptech Animal Health Pty Limited). During preliminary testing in Project NAP3.105, prototype GnRH agonist bioimplants induced contraceptive responses in heifers and cows for periods ranging from 200 to 350 days. In the latter studies, a return to normal ovarian function and pregnancies in treated animals coincided with increased pasture availability and accelerated rate of live weight gain. This observation raised the question of whether a GnRH agonist bioimplant can maintain suppressed ovarian function in heifers and cows that are undergoing a relatively fast rate of live weight gain. The present project was therefore specifically designed to address this question. A second objective was to identify a dose of GnRH agonist (deslorelin) that would induce a contraceptive effect in heifers for 12 months, or longer.

Sexually mature heifers (26-month-old) were randomly divided into 4 groups and, in January 1998, received one of the following treatments: Group 1 (n = 50), control, no treatment; Group 2 (n = 50), GnRH agonist Low Dose (3 mg bioimplant); Group 3 (n = 50), GnRH agonist Medium Dose (6 mg bioimplant); Group 4 (n = 48), GnRH agonist High Dose (12 mg bioimplant). Heifers were maintained on pasture together with 4 herd
bulls, and were monitored for appropriate rates of live weight gain. Ovarian activity and pregnancy status were monitored at monthly intervals using ultrasonography.

Project Outcomes

Heifers had a live weight of 308 ± 3 kg (mean± SEM) at the start of the trial and they gained 153 ± 3 kg during the 12 months of the study. Progressive live weight gain during the trial was: 0 to 4 months, 42 ± 1 kg (0.35 ± 0.01 kg/day); 5 to 8 months, 36 ± 1 kg
(0.28 ± 0.01 kg/day); 9 to 12 months, 76 ± 2 kg (0.63 ± 0.02 kg/day). The greatest rate of live weight gain occurred during the latter part of the trial, which allowed testing of the primary objective concerning the relationship between rate of live weight gain and capacity of the GnRH agonist bioimplant to suppress ovarian function. Control heifers conceived throughout the trial

demonstrating that there were no environmental limitations to pregnancy in heifers showing regular ovarian cycles. The GnRH agonist Low Dose treatment suppressed conception for approximately 3 months while the Medium Dose treatment prevented pregnancy for around 6 months. Pregnancies at 12 months were: Low Dose, 36/48 (75%); Medium Dose, 26/48 (54%). Approximately 90% of heifers in the GnRH agonist High Dose treatment continued to have suppressed ovarian activity at 12 months of treatment, and only 3/45 (6%) had conceived at this time.

The results of this trial have established the contraceptive efficacy of a GnRH agonist bioimplant in heifers. Furthermore, the GnRH agonist High Dose suppressed ovarian function in heifers undergoing a relatively fast rate of live weight gain.

Project Conclusion

A world-first GnRH agonist bioimplant for contraception in heifers and cows has been demonstrated in this project. The GnRH agonist bioimplant represents the next generation, non-surgical, technology for the reproductive management of heifers and cows in extensive beef herds in· northern Australia. The technology has application internationally in beef cattle production systems.

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1. Project methodology and detailed results

1.1 Methodology

MLA is committed to investing in top quality scientific research, performed by suitably qualified, experienced and registered researchers and organisations. In experiments that involve livestock, MLA acknowledges that such research will first need to be assessed, and if deemed relevant, approved by a recognised Animal Care and Ethics Committee (AEC). The responsibility for obtaining AEC approval lies with the researcher. MLA has in the past not specifically asked for evidence that such AEC approval had indeed been obtained

The GnRH agonist used in this project was deslorelin (D-Trp^{6}-Pro^{9}-des-Gly^{10}-GnRH ethylamide). The agonist was formulated into controlled-release bioimplants that were placed subcutaneously in the dorsal surface of the ear under aseptic conditions. When incubated *in vitro,* implants released approximately 20 µg deslorelin/24h (J. Walsh, Peptech Animal Health Pty Limited).

Sexually mature heifers (26-month-old) were randomly divided into 4 groups based on live weight and, in January 1998, received one of the following treatments:

Group 1	n=50	Control	no treatment
Group 2	n=50	GnRH agonist Low Dose	3 mg bioimplant
Group 3	n=50	GnRH agonist Medium Dose	6 mg bioimplant
Group 4	n=50	GnRH agonist High Dose	12 mg bioimplant

Heifers were maintained on pasture together with 4 sexually mature bulls. For control heifers, 10 animals were initially included at the start of the trial, and control heifers were then progressively replaced during the trial as they conceived. Ovarian follicular growth and pregnancies were monitored at monthly intervals, from Oto 12 months, by trans-rectal ultrasonography using an Aloka 210 real-time linear array scanner and 7.5 MHz transducer. Live weights were also recorded monthly.

Data at single time points were analysed by ANOVA using the General Linear Models (GLM) procedure of SAS/STAT. Data analyses over time were undertaken by repeated measures analysis using SAS/STAT procedure MIXED with REML estimation, and of type autoregressive (1). The CONTRAST statement of SAS/STAT procedure of GLM was used for comparisons of group means. Chi-square analysis was used where appropriate.

1.2 Results

1.2.1 Liveweight

Results for live weight (LW) gain for control heifers are not reported since heifers in this group were progressively replaced during the trial as they conceived. Heifers treated with GnRH agonist had an increase in LW during the study (Figure 1, Table 1). The rate of LW gain showed seasonal changes during the study, and overall was relatively high for heifers maintained on pasture in a sub-tropical environment (Figure 1, Table 2). The fastest rate of LW gain (0.63 ± 0.02 kg/day) occurred from 9 to 12 months of the study (September to December) (Table 2). Hence, the relationship between rate of live weight gain and capacity of GnRH agonist treatment to suppress ovarian function could be

evaluated in the project. There were no differences in absolute LW, or rate ofLW gain, between groups of heifers treated with different doses of GnRH agonist.

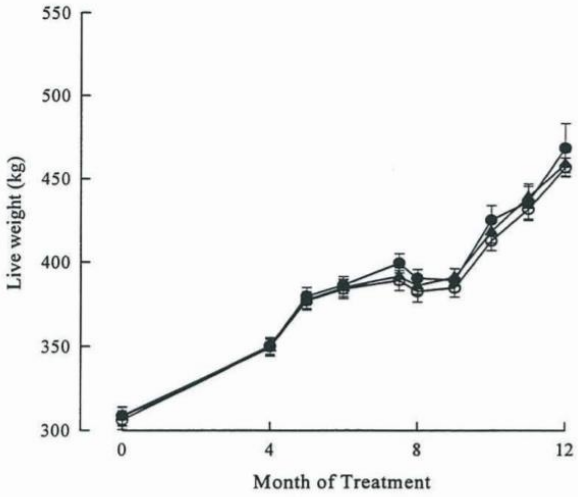


Figure 1. Live weight gain in heifers treated with GnRH agonist at Low Dose (●), Medium Dose (▲) and High Dose (○). Results are means ± SEM.

Table 1: Live weight (mean± SEM) of heifers treated with GnRH agonist.

	Month of treatment			
GnRH agonist dose	**0**	**4**	**8**	**12**
Low	309 ± 6 [a] (50)	350 ± 5 [b] (50)	390 ± 5 [c] (39)	468 ± 15 [d] (12)
Medium	308 ± 6 [a] (49)	349 ± 5 [b] (49)	386 ± 5 [c] (45)	459 ± 7 [d] (26)
High	306 ± 5 [a] (48)	350 ± 5 [b] (48)	378 ± 5 [c] (48)	457 ± 6 [d] (45)
Pooled data	308 ± 3 (147)	350 ± 3 (147)	384 ± 3 (132)	459 ± 4 (83)

[a, b, c, d] Means within rows without a common superscript differ ($P < 0.05$).

Numbers in parentheses are number of heifers; this declined for heifers treated with the Low Dose and Medium Dose of GnRH agonist since heifers in these groups were progressively removed from the study as they conceived and were confirmed pregnant.

Table 2: Rate of live weight gain (mean ± SEM) for heifers treated with GnRH agonist. Results are pooled for heifers treated with Low, Medium and High doses of GnRH agonist.

Months (days)	Number of heifers	Live weight gain (kg)	Daily live weight gain (kg)
0 to 4 (121)	147	42 ± 1^{a}	0.35 ± 0.01^{a}
5 to 8 (132)	132	36 ± 1^{b}	0.28 ± 0.01^{b}
9 to 12 (120)	83	76 ± 2^{c}	0.63 ± 0.02^{c}
0 to 12 (373)	83	153 ± 3	0.41 ± 0.01

[a, b, c] Means within columns without a common superscript differ ($P < 0.01$).

1.2.2 Ovarian follicular activity

Results for ovarian follicular activity for heifers treated with GnRH agonist are shown in Figure 2 to Figure 4 and are summarised in Table 3 to Table 5 (see also Appendix A). Detailed information on follicular activity was not recorded for control heifers, as these heifers were included in the project primarily to demonstrate that conception could occur throughout the trial in heifers undergoing normal ovarian function (Appendix A, IO.I).

1.2.2.1. GnRH agonist Low Dose (3 mg bioimplant)

Heifers treated with Low Dose GnRH agonist had predominantly small follicles (<5 mm diameter) until around 3 to 4 months, after which time follicular activity increased (Figure 2, Table 3). The latter was associated with a progressive increase in the number of heifers that showed cyclic ovarian activity and conceived (Figure 2, Table 3) (Appendix A, 10.2).

The corpus luteum recorded in a proportion of heifers at I month was present at the start of treatment and did not reflect ovulation between O and 1 month. This also applied to heifers treated with Medium Dose and High Dose GnRH agonist.

1.2.2.2. GnRH agonist Medium Dose (6 mg bioimplant)

Ovarian follicular growth in heifers receiving Medium Dose GnRH agonist was restricted to small follicles until around 5 to 6 months, at which time these heifers started to show a return to normal ovarian function (Figure 3, Table 4) (Appendix A, 10.3).

1.2.2.3. GnRH agonist High Dose (12 mg bioimplant)

Treatment with High Dose GnRH agonist restricted follicular growth to small follicles in the majority of heifers for the duration of the study (Figure 4, Table 5). Two pregnancies were recorded at 11 months and a third pregnancy at 12 months (Appendix A, 10.4).

Figure 2. Changes in ovarian follicular status over time for heifers treated with Low Dose GnRH agonist. Follicle categories are small (≤ 5 mm), medium (6-9 mm) and large (≥10 mm). A decrease

in small follicles after around 3 months was associated with an increase in large follicles, ovulation, development of a corpus luteum and pregnancies.

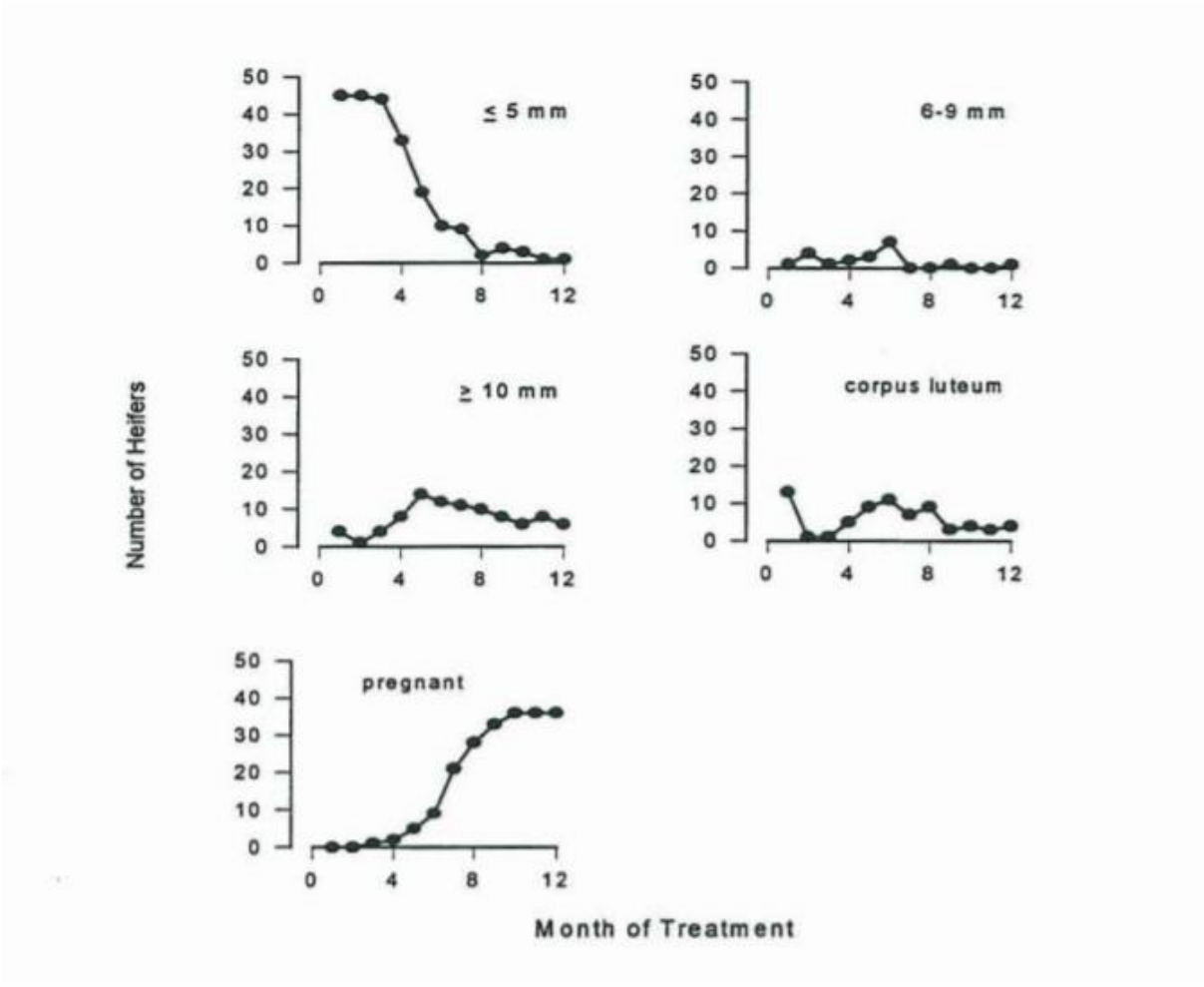


Table 3. Ovarian follicular activity in heifers treated with GnRH agonist, Low Dose. Data are presented as the proportion of heifers for the different categories of reproductive status.

Month	Follicle size (mm)			Corpus luteum	Pregnant
	small (≤ 5 mm)	medium (6-9 mm)	large (≥ 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 within columns without a common superscript differ ($P < 0.05$).

Figure 3. Changes in ovarian follicular status over time for heifers treated with Medium Dose GnRH agonist. Follicle categories are small (≤5 mm), medium (6-9 mm) and large (≥10 mm). A decrease in small follicles after around 5 months was associated with an increase in large follicles, ovulation, development of a corpus luteum and pregnancies

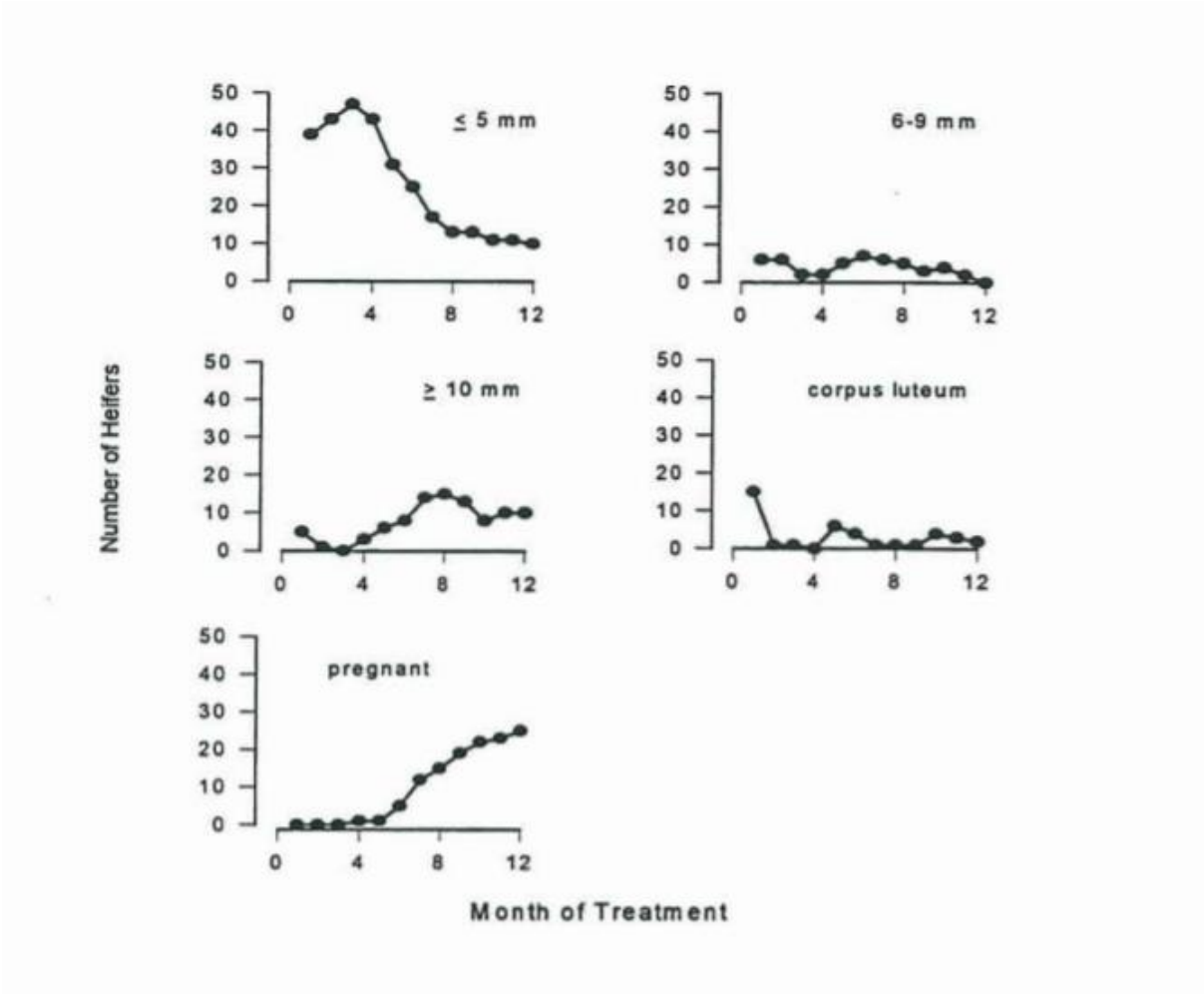


Table 4. Ovarian follicular activity in heifers treated with GnRH agonist, Medium Dose. Data are presented as the proportion of heifers for the different categories of reproductive status.

Month	Follicle size (mm)			Corpus luteum	Pregnant
	small (≤ 5 mm)	medium (6-9 mm)	large (≥ 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).

Figure 4. Changes in ovarian follicular status over time for heifers treated with High Dose GnRH agonist. Follicle categories are small (≤5mm), medium (6-9 mm) and large (≥10 mm). Heifers showed predominantly low follicular growth and only 3 heifers were pregnant at 12 months.

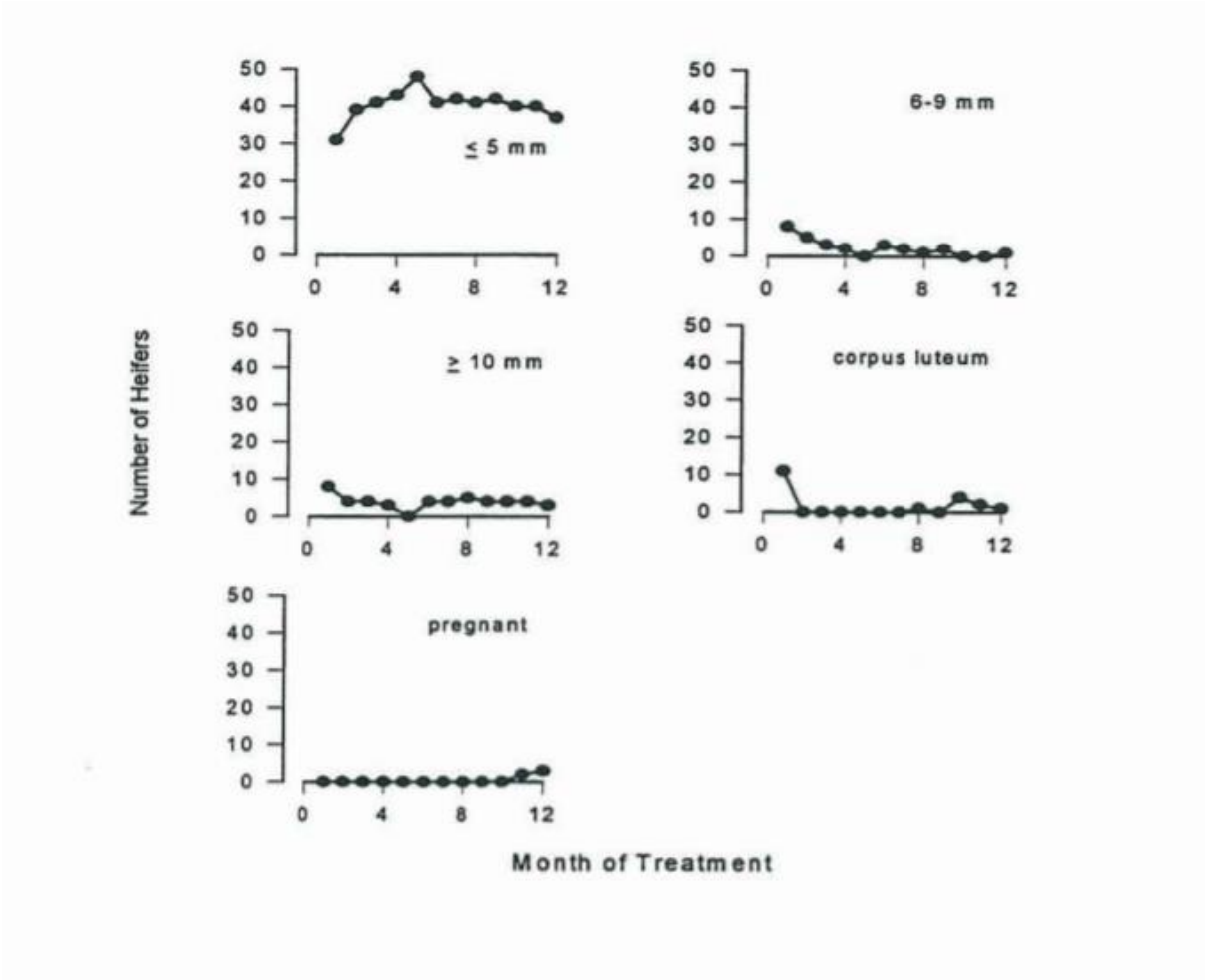


Table 5. Ovarian follicular activity in heifers treated with GnRH agonist, High Dose. Data are presented as the proportion of heifers for the different categories of reproductive status.

Month	Follicle size (mm)			Corpus luteum	Pregnant
	small (≤ 5 mm)	medium (6-9 mm)	large (≥ 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, c] Proportions within columns without a common superscript differ (P < 0.05).

1.2.3 Pregnancies

Results for pregnancies are summarised in Table 6. Control heifers conceived throughout the study, confirming that there were no environmental limitations to pregnancy in heifers that showed normal ovarian function. Heifers treated with Low Dose GnRH agonist showed a progressive increase in the proportion pregnant from approximately 4 months onwards. A similar increase in pregnancies occurred from about 6 months in heifers treated with Medium Dose GnRH agonist. For heifers treated with High Dose GnRH agonist, 2 heifers were observed pregnant at 11 months and a third heifer at 12 months.

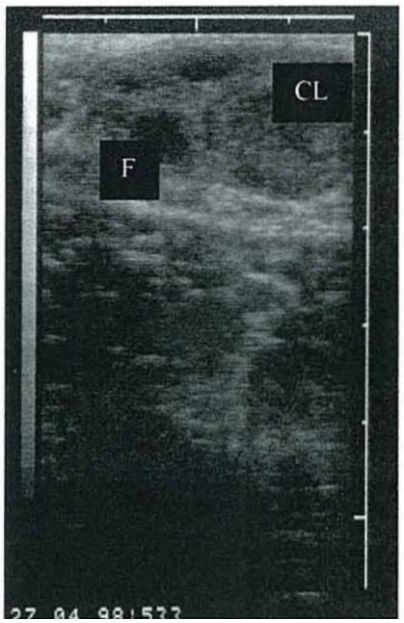
Table 6. Cumulative pregnancies for control heifers and heifers treated with GnRH agonist

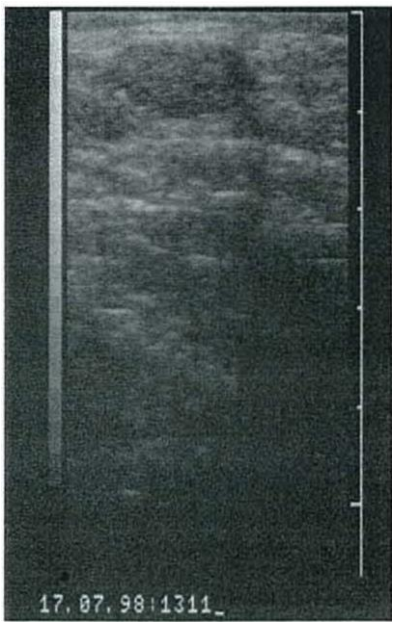
	Month of treatment			
GnRH agonist dose	1	4	8	12
Control	0/11 [a, x]	12/19 (63%) [a, y]	27/34 (79%) [a, y, z]	44/47 (93%) [a, z]
Low	0/50 [a, x]	2/50 (4%) [b, x]	28/49 (57%) [b, y]	36/48 (75%) [b, y]
Medium	0/50 [a, x]	1/49 (2%) [b, x]	15/49 (30%) [c, y]	25/48 (52%) [c, z]
High	0/48 [a, x]	0/48 [b, x]	0/48 [d, x]	3/45 (6%) [d, x]

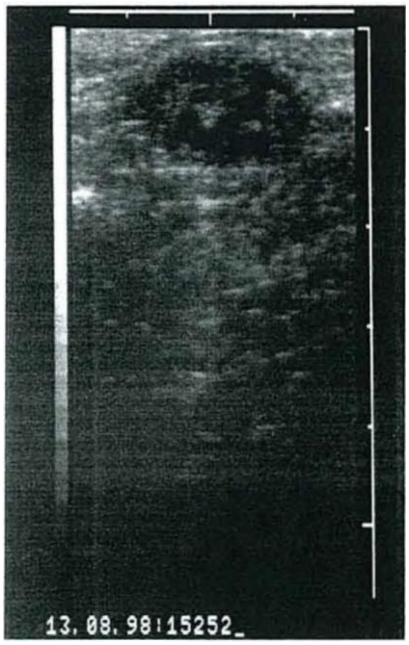
[a, b, c, d] Proportions within columns without a common superscript differ (P < 0.05).
[x, y, z] Proportions within rows without a common superscript differ (P < 0.05).

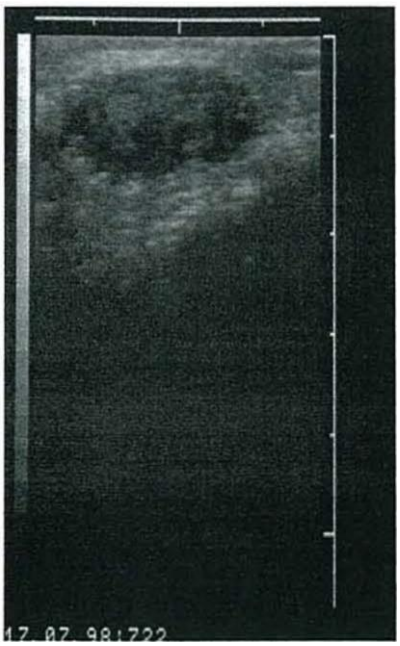
1.2.4 Representative ultrasound micrographs of the ovaries of heifers treated with GnRH agonist

Representative ultrasound micrographs of the ovaries of heifers treated with GnRH agonist are shown below. The micrograph at top left .shows the ovary of a control heifer (#533) and illustrates typical ovarian structures including a corpus luteum (CL) and medium sized follicle (F). In contrast, the ovaries of heifers treated with GnRH agonist are almost devoid of follicles (#1311) or can have follicles which are restricted to 1-2 mm in size (#15252; #722).









2. Discussion of results

The present project has established, for the first time internationally, the efficacy of a GnRH agonist bioimplant to suppress ovarian function and prevent conception in heifers. A dose-response relationship was observed, with suppression of ovarian activity in the majority of heifers in the respective groups achieved for approximately 3 months with Low Dose GnRH agonist, 6 months with Medium Dose GnRH agonist, and 12 months with High Dose GnRH agonist. In the latter group, only 3/45 (6%) heifers were pregnant at 12 months. For heifers treated with Low Dose and Medium Dose GnRH agonist, there was variation among heifers in the duration of suppressed ovarian function. It is not known whether this variation was due to different rates of release of agonist between implants, differences between heifers in sensitivity to GnRH agonist, or differences in the recovery of anterior pituitary gland function among heifers subsequent to exposure to GnRH agonist. These outstanding questions could be addressed, in part, by removing implants after different duration of treatment and observing heifers and cows for a return to normal ovarian activity.

A major objective in this project was to determine whether a GnRH agonist bioimplant would suppress ovarian function and prevent conception in heifers undergoing a relatively fast rate of live weight gain. It was found that the High Dose GnRH agonist treatment suppressed ovarian function in heifers that were undergoing a relatively fast rate of live weight gain, particularly from 9 to 12 months. Hence, it can be concluded that the response of heifers to treatment with GnRH agonist is not related to live weight gain. The response to GnRH agonist treatment can therefore be expected to remain consistent throughout the year, irrespective of prevailing environmental conditions. Also, a threshold dose of GnRH agonist should be effective across a range of live weights for heifers and cows.

A second objective in this project was to identify a dose of GnRH agonist (deslorelin) that would induce a contraceptive effect in heifers for 12 months, or longer. This was achieved with the High Dose (12 mg) GnRH agonist bioimplant.

3. Industry implications

The GnRH agonist bioimplant evaluated in this project represents the next generation, non-surgical, technology for the control of fertility in heifers and cows maintained under extensive management in northern Australia. The agonist bioimplant will be a future preferred technology as it is practical and non-invasive. With regard to the latter, the beef industry in northern Australia will increasingly implement husbandry practices which recognise the importance of consumer and community awareness of animal welfare.

A further outstanding advantage of the GnRH agonist bioimplant, relative to all surgical procedures, is the reversibility of GnRH agonist treatment. The GnRH agonist bioimplant technology should therefore be considered within a whole-enterprise framework of reproductive management of both breeder and non-breeder heifers and cows. This allows for a paradigm shift in the conceptual framework for managing reproduction in extensive beef herds. In future, conceptions patterns in extensive herds can be determined by selective treatment of heifers and cows with GnRH agonist, with a decreased concern over the control of bulls.

4. Cost/benefit of GnRH agonist bioimplant technology

An accurate calculation of the cost/benefit of GnRH agonist bioimplant technology requires information on the cost of a unit implant. Unfortunately, this information is not available at the

present time. Discussions are however proceeding between Meat and Livestock Australia and Peptech Animal Health Pty Limited.

A major advantage of the bioimplant technology is that it provides a non-surgical approach to fertility management in heifers and cows. This eliminates issues of mortality and morbidity associated with all surgical procedures. The bioimplant utilises standard implanting techniques commonly used in the beef industry. The latter provides important producer independence in managing reproduction, compared with reliance on technical expertise for surgical procedures.

5. Publications

D'Occhio, M.J. and Aspden, W.J. (1999) Endocrine and reproductive responses of male and female cattle to agonists of gonadotrophin releasing hormone. Invited Review. Journal of Reproduction and Fertility, Supplement 54 (in press).

D'Occhio, M.J. (1999) GnRH agonist bioimplants for control of reproduction in heifers and cows. The North Australia Program, 1998 Review of Reproduction and Genetics (Editor S. Blakely), NAP Occassional Publication No. 8, 87-91.

D'Occhio, M.J., Whyte, T.R, Barnes, A., Hawke, T. and Ebbem, K. (1999) Evaluation ofa new GnRH agonist bioimplant for pregnancy prevention in heifers. In: Brigalow Research Station Technical Report 1998-1999 (in press).

6. Acknowledgements

This project involved a partnership between Central Queensland University (Professor Michael D'Occhio; Dr William Aspden; Mr Timothy Whyte), Peptech Animal Health Pty Limited (Dr Timothy Trigg; Dr Paul Schober; Mr John Walsh) and Meat and Livestock Australia (Dr David Skerman; Mr Shane Blakely; Mr Peter Loneragan). All three organisations contributed funding for the project. The Queensland Department of Primary Industries (Queensland Beef Industry Institute) also made an important contribution to the project. Heifers used in the project were obtained from Swans Lagoon Beef Cattle Research Station and we are grateful for the assistance provided by Dr Geoffry Fordyce. The project was conducted at Brigalow Research Station and excellent management and technical expertise was provided by Mr Anthony Barnes, Mr Tony Hawke and Mr Karl Ebbern.

7. Appendix

7.1 Appendix A: Detailed reproductive data

7.1.1 Control heifers

	Follicles			Corpus luteum	Pregnant
	Small (≤ 5 mm)	Medium (6-9 mm)	Large (≥ 10 mm)		
1 month				11/11	0/11
2 months				10/11	4/11
3 months				14/15	9/15
4 months				18/19	12/19
5 months				20/21	16/21
6 months				25/26	17/26
7 months				27/27	23/27
8 months				33/34	27/34
9 months				39/39	32/39
10 months				42/42	35/42
11 months				47/47	40/47
12 months				47/47	44/47

7.1.2 GnRH agonist - Low Dose (3 mg)

	Follicles			Corpus luteum	Pregnant
	Small (≤ 5 mm)	Medium (6-9 mm)	Large (≥ 10 mm)		
1 month	45/50	1/50	4/50	13/50	0/50
2 months	45/50	4/50	1/50	1/50	0/50
3 months	44/50	1/50	4/50	0 /50	1/50
4 months	33/50	2/50	8/50	5/50	2/50
5 months	19/50	3/50	14/50	9/50	5/50
6 months	10/50	7/50	12/50	11/50	10/50
7 months	9/50	0/50	11/50	9/50	21/50
8 months	2/49	0/49	10/49	9/49	28/49
9 months	4/49	1/49	8/49	3/49	33/49
10 months	3/49	0/49	6/49	4/49	36/49
11 months	1/49	0/49	8/49	3/49	36/49
12 months	1/48	1/48	6/48	4/48	36/48

7.1.3 GnRH agonist - Medium Dose (6 mg)

	Follicles			Corpus luteum	Pregnant
	Small (≤ 5 mm)	Medium (6-9 mm)	Large (≥ 10 mm)		
1 month	39/50	6/50	5/50	15/50	0/50
2 months	43/50	6/50	1/50	1/50	0/50
3 months	47/49	2/49	0/49	1/50	0/50
4 months	43/49	2/49	3/49	0/49	1/49
5 months	31/49	5/49	6/49	6/49	1/49
6 months	25/49	7/49	8/49	4/49	5/49
7 months	17/49	6/49	14/49	1/49	12/49
8 months	13/49	5/49	15/49	1/49	15/49
9 months	13/49	3/49	13/49	1/49	19/49
10 months	11/49	4/49	8/49	4/49	22/49
11 months	11/49	2/49	10/49	3/49	23/49
12 months	10/48	0/48	10/48	2/48	25/48

7.1.4 GnRH agonist - High Dose (12 mg)

	Follicles			Corpus luteum	Pregnant
	Small (≤ 5 mm)	Medium (6-9 mm)	Large (≥ 10 mm)		
1 month	31/48	8/48	8/48	11/48	0/48
2 months	39/48	5/48	4/48	0/48	0/48
3 months	41/48	3/48	4/48	0/48	0/48
4 months	43/48	2/48	3/48	0/48	0/48
5 months	48/48	0/48	0/48	0/48	0/48
6 months	41/48	3/48	4/48	0/48	0/48
7 months	42/48	2/48	4/48	0/48	0/48
8 months	41/48	1/48	5/48	1/48	0/48
9 months	42/48	2/48	4/48	0/48	0/48
10 months	40/48	0/48	4/48	4/48	0/48
11 months	40/48	0/48	4/48	2/48	2/48
12 months	37/45	1/45	3/45	1/45	3/45