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How much progesterone do you really need? An investigation for fixed-time artificial insemination of Angus yearling heifers.

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Abstract

The study compared the use of an intravaginal progesterone (P_4) releasing device (IPRD) that contained half the standard dose of P_4 to an IPRD that contained the standard dose of P_4. The IPRDs were compared in an ovulation synchronisation protocol for fixed-time artificial insemination (FTAI) of yearling Angus heifers (n = 176). Heifers originating from two herds were allocated to one of two treatment groups: 1) 1-Pod (0.78 g P_4; 1-Pod Cue-Mate®) and; 2) 2-Pod (1.56 g P_4; Cue-Mate®). Heifers were allocated randomly according to herd, bodyweight and presence of a corpus luteum (CL) that was recorded five days prior to commencement of synchronisation treatments (Day -5). Post analysis of allocation procedures confirmed heifers allocated to the 1-Pod and 2-Pod treatment groups to have an average bodyweight of 309.1 ± 2.4 kg and 307.4 ± 2.4 kg, respectively, and 31.4% and 32.6% with a CL present, respectively. On Day 0, every heifer received an IPRD (Cue-Mate®) either 1-Pod or 2-Pod depending on allocated treatment group, and 1 ml oestradiol benzoate (ODB; Bomerol®) i.m. On Day 8, the IPRD was removed from all heifers and every heifer received 2 ml cloprostenol ($PGF_{2\alpha}$; Ovuprost™), i.m. and 1.5 ml equine chorionic gonadotrophin (eCG; Pregnecol®). On Day 10, at 54 h after IPRD removal, all heifers received 1 ml gonadotrophin releasing hormone (GnRH; Ovurelin®) and were FTAI. Foetal ageing was performed on Day 96. Heifers in the 1-Pod group resulted in a pregnancy rate to FTAI of 54.1% vs. 47.1% (p = 0.357) for the 2-Pod group. The overall pregnancy rate, after being exposed to bulls post FTAI was 89.4% vs. 83.5% (p = 0.262), for the 1-Pod and 2-Pod group, respectively. The results suggest that a new cost effective approach to synchronisation of yearling Angus heifers should be considered.

Introduction

Artificial insemination (AI) in the Australian beef industry has traditionally been used in stud herds, particularly in southern regions where management systems tend to be less extensive as compared to the north. As new genetic technologies become commercially available to the industry, both stud and commercial beef producers, are considering use of AI to introduce new genetics and rapidly improve the genetic merit of their herds. Fixed-time AI (FTAI) is an assisted reproductive technology that empowers beef producers to introduce new genetics into their herds in a cost effective and practical manner as it requires no detection of oestrus and activities are pre-organised to a day and time.

Heifers, both *Bos indicus* and *Bos taurus* genotypes, consistently yield lower than expected pregnancy rates than multiparous cows in FTAI programmes[1]. The physiological reason for these reduced outcomes, to the authors' knowledge, has not been determined. Yet, heifers tend to be group of choice for assisted reproduction programmes as they represent the newest generation of genetics and pose less challenges with management of the cow and calf unit. The *Bos indicus* heifer has been the focus of FTAI research in both the northern Australian and South American beef industries. Advances in this research resulted in best practice ovulation synchronisation protocols being developed that utilise intravaginal progesterone (P_4) releasing devices (IPRD) that contain half the standard dose of P_4 (0.78g; 1-Pod Cue-Mate®)[2-5]. The use of these devices has resulted in marginally better pregnancy rates to FTAI, but most importantly, has resulted in consistent and reliable outcomes, where a high degree of variability used to exist.

Several anecdotal reports from commercially executed FTAI programs, have indicated that use of IPRDs that contain a low-dose of P_4 (<1 ng/ml) for ovulation synchronisation for FTAI in *Bos taurus* heifers, have yielded similar or improved pregnancy rates than standard dose IPRDs. A South American-based study[6] compared the use of two different IPRDs, a CIDR (1.38 g P_4) and a once-used DIB (1 g P_4). Poll Hereford heifers (2 yo) were synchronised and submitted to an oestrus detection + FTAI programme. Conception rates to oestrus detected AI were significantly higher in the once-used DIB treated heifers compared to the CIDR treated heifers (83.7 vs. 33.3; $p<0.01$), but had lower conception rate to FTAI (46.0 vs. 57.0; $p < 0.01$). Overall the pregnancy rate from the programme was not significantly different (71.9 vs. 59.0; $p>0.01$). This indicates that fertility of *Bos taurus* heifers is similar, or improved when a low dose IPRD is used for ovulation synchronisation. The low conception rate to FTAI was explained by the altered time to ovulation in the once-used DIB group.

The objective of this study is to compare the use of an IPRD containing a standard-dose of P_4 (Cue-Mate®; two P_4 impregnated pods containing 0.78 g P_4 each, 1.56 g P_4) and the use of an IPRD containing half the standard dose of P_4 (1-Pod Cue-Mate®, one P_4 impregnated pod 0.78 g P_4) as part of an ovulation synchronisation protocol for FTAI in yearling Angus heifers. The aim is to determine whether using an IPRD with half the standard dose of P_4 as compared to an IPRD with the standard dose P_4 will result in similar or improved pregnancy rates to FTAI.

Methodology

Heifer selection and management

The study was conducted during mid-spring on a station located in the northern NSW tablelands. Ethical approval was granted by The University of New England Animal Ethics Committee (AEC16-078). All animals used in the study were representative of replacement Angus heifers typically mated in the northern NSW tablelands. The heifers formed part of the joining group for Angus Australia's, Angus Sire Benchmarking Project, Cohort 7 (ASBP). Heifers were sourced from two co-located herds at the trial site and were run as a single group for the duration of the study under the same management and nutritional regime. All trial observations and data analysis was performed by an independent research group (Invetus, Armidale, NSW, Australia).

Heifer allocation procedure

Five days prior to the commencement of the study (Day -5) yearling Angus heifers (n = 177) originating from two related herds (Herd 1 and Herd 2), were weighed and body condition scored (BCS; 1 to 5[7]). At the same time all heifers underwent a reproductive exam by transrectal ultrasonography using a SonoSite M-Turbo ultrasound machine equipped with a L52X/10-5 mHz linear array transrectal transducer (SonoSite Inc., Bothel, WA, USA). The presence of absence of a corpus luteum (CL)[8], diameter of the largest follicle (LF), and any abnormalities of the reproductive tract were recorded. Heifers were rejected from the trial if they had an abnormal reproductive tract (n = 1).

Remaining heifers were allocated to two treatment protocols: i) half dose IPRD containing 0.78 g P_4; 1-Pod (n = 86), and ii) standard dose IPRD containing 1.56 g P_4; 2-Pod (n = 86). To allocate the heifers to each group, they were sequentially blocked into pairs and randomly allocated to treatments within blocks using a coin toss. Allocation procedures allowed for herd of origin such that equal numbers (n = 35 and n = 51 head, respectively) from each herd were included in each treatment group. Group mean bodyweights, LF diameters and the proportion of animals with a CL were statistically compared to confirm groups were not significantly different for these parameters at $p < 0.05$.

Ovulation synchronisation protocols

The synchronisation treatments used in the 1-Pod and 2-Pod treatment groups are represented in Figure 1. On Day 0, every heifer in the 1-Pod treatment group had a half-dose IPRD (Cue-Mate®; 0.78 g P_4; Vetoquinol Australia, Brisbane Airport, QLD, Australia) inserted intravaginally. The half-dose Cue-Mate® was prepared according to Butler et al.[5]. Every heifer in the 2-Pod treatment group had a standard IPRD (Cue-Mate®; 1.56 P_4) inserted intravaginally. As the IPRD was inserted, every heifer received 1 ml oestradiol benzoate (ODB;

Bomerol®, 1 mg/ml, Bayer Australia, Sydney, NSW, Australia) i.m. On Day 8, the IPRD was removed from all heifers and every heifer received 2 ml cloprostenol ($PGF_{2\alpha}$; Ovuprost™, 250 µg/ml, Bayer Australia) i.m. and 1.5 ml equine chorionic gonadotrophin (eCG; Pregnecol®, 200 IU/ml; Vetoquinol Australia). On Day 10, at 54 h after IPRD removal, all heifers received 1 ml gonadotrophin releasing hormone (GnRH; Ovurelin®, 100 µg/ml; Bayer Australia) and were FTAI.

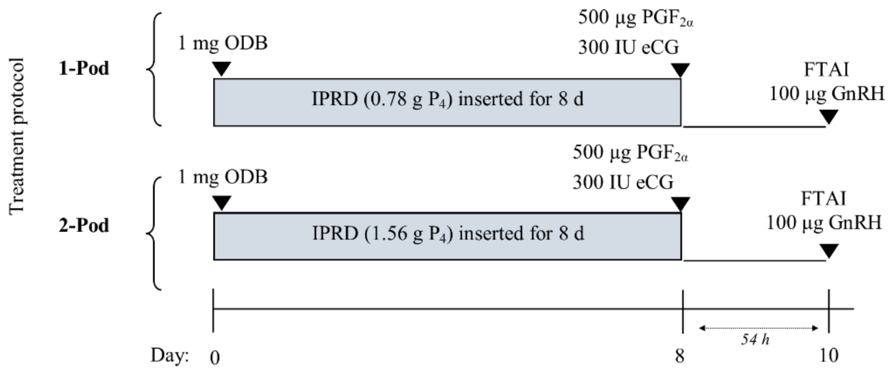

Figure 1 - Protocols used to treat Angus heifers in the 1-Pod (n = 86) and 2-Pod (n = 86) treatment groups.

Artificial insemination and pregnancy diagnosis

All heifers were inseminated, by a single technician, using frozen semen sourced from bulls being evaluated in the ASBP. Sires (n = 24) were allocated on a rotational basis across all heifers, by inseminating two heifers per sire as they randomly presented to the crush. This process was repeated until all heifers had been inseminated. Applied straws were recorded and reconciled against treatment groups. Sires were similarly distributed across both treatments, with the exceptions of Sires #14 and #38 which were subsequently found to have been applied to heifers in the 1-Pod group only.

Ten days after FTAI, sires (n = 4; 2.3%) were introduced in to the entire group of heifers, remaining with the group for 42 days. Each sire had passed a BBSE prior to mating.

Foetal aging was performed on all heifers 12 weeks (Day 96) after the day of FTAI using transrectal ultrasonography (BoviScan Curve sector scanner, with a 4.0 MHz convex rectal probe). The heifers were diagnosed as either pregnant to FTAI, 1st return to oestrus, 2nd return to oestrus, or not detectably pregnant.

Statistical analysis

Bodyweights and LF diameters were compared between treatment groups using a fixed effects linear model and TIBCO Spotfire S+ 8.2 (2010). Comparisons were made using the models:- (*Bodyweight.Pre ~ Treatment + Herd), (LF ~ Treatment + Herd + Block)* and (*Bodyweight.Post ~ Treatment + Herd + Block + Pregnancy)*. Model suitability was confirmed via residual plots. In all instances models appeared appropriate. Means by treatment were compared via Analysis of Variance and Tukeys All Pairwise Comparison Test (at $p<0.05$).

Two heifers, one from each treatment group and both originating from Herd 2, were found deceased between the day of FTAI (Day 10) and the day of pregnancy diagnosis (Day 96), so were removed from the data set prior to analysing the pregnancy outcomes.

The proportions of heifers pregnant to FTAI and pregnant overall on Day 96 along with the proportion of heifers observed to have a CL present on Day -5 were compared using Statistix 10.0, Analytical Software 2013. Point estimates of the difference in proportions between groups were calculated in Microsoft EXCEL, as were 95% Confidence Intervals for the differences using the methodology outlined in Petrie A. and Watson P[9].

Results

Bodyweight and large follicle diameter

There were no significant differences observed between treatment groups in bodyweight prior to treatment (Day -5), bodyweight post treatment (Day 96) and LF diameter prior to treatment (Day -5) at $p < 0.05$ (Table 1**Table *1***). The term *Herd* was significant for both bodyweight assessments, as was Block for bodyweight on Day 96. Pregnancy on Day 96 was not significant (Table 2).

Table 1: Analysis of allocation procedure to confirm no bias between Angus heifers, originating from two different herds, allocated to the 1-Pod or 2-Pod treatment groups. Mean bodyweight ± SEM (kg), mean body condition score (BCS) ± SEM, mean large follicle (LF) diameter ± SEM, and proportion with a CL present.

Treatment	**1-Pod**			**2-Pod**		
Herd	*1*	*2*	**Total**	*1*	*2*	**Total**
n	35	51*	86	35	51*	86
Median BCS (Day -5)	2.6 ± 0.0	2.6 ± 0.0	2.6 ± 0.0[a]	2.6 ± 0.0	2.7 ± 0.0	2.7 ± 0.0[a]
Bodyweight (kg)						
Day -5	301.3 ± 3.4	314.7 ± 3.2	309.2 ± 2.5 [a]	301.0 ± 3.8	310.9 ± 3.0	306.8 ± 2.4 [a]
Day 96	414.2 ± 4.3	427.5 ± 4.8**	422.0 ± 3.4	413.9 ± 4.3	424.9 ± 4.0**	420.4 ± 3.0
Total Gain	112.9 ± 3.3	112.8 ± 2.7	112.8 ± 2.1	112.9 ± 2.6	114.0 ± 3.3	113.6 ± 2.2
Average Daily Gain	1.1	1.1	1.1	1.1	1.1	1.1
Ovarian scan (Day -5)						
LF diameter (mm)	10.5 ± 0.5	9.8 ± 0.4	10.1 ± 0.3 [a]	10.3 ± 0.5	9.3 ± 0.4	9.7 ± 0.3 [a]
Proportion CL (%)	17.1	41.2	31.4[a]	17.1	43.1	32.6 [a]

[a] Means/proportions with the SAME superscript are NOT significantly different at p<0.05.
* Two heifers, one each from the 1-Pod and 2-Pod groups both originating from Herd 2, were found deceased between Day 10 and 96.
** Data includes (n = 50) due to incidence in*

Table 2: Results of terms other than Treatment: bodyweight at Day -5 and Day 96 and large follicle (LF) diameter at Day -5, between, herd, block and pregnancy.

	Bodyweight	**LF Diameter**	**Bodyweight**
Day	-5	-5	96
F-Statistic	0.51	0.73	0.181
p-value	0.478	0.395	0.671
Means Comparison (*Treatment*)	(nsd at p<0.05)	(nsd at p<0.05)	(nsd at p<0.05)
P-value (other terms in model)			
Herd	0.0008	0.07	0.004
Block	-	0.95	0
Pregnancy	-	-	0.32

nsd = no significant difference between groups

Pregnancy rates

No significant differences between treatment groups were observed for the proportion of animals in each group with a CL present prior to treatment, for the proportion of animals pregnant to FTAI nor pregnant overall at p<0.05. Confidence intervals for the difference between the 1-Pod and 2-Pod treatments spanned 0 in all three instances, confirming the finding of no significant differences between treatments (Table 3 and Table 4). The presence or absence of a CL at Day -5 had no effect on the outcome to pregnancy to FTAI or pregnancy overall (Table 1).

Table 3: Results of pregnancy diagnosis performed at Day 96, reported after foetal ageing to either pregnant to fixed-time artificial insemination (FTAI), the bulls on the 1st or 2nd return to oestrus, or not detectably pregnant (NDP).

	Treatment Group							
	1-Pod				**2-Pod**			
Group	*Herd 1*	*Herd 2*	**Total**		*Herd 1*	*Herd 2*	**Total**	
n	35	50*	85		35	50*	85	
FTAI	20	26	46	**54.1%[a]**	15	25	40	**47.1%[a]**
Bull 1st cycle	9	13	22	25.9%	4	12	16	18.8%
Bull 2nd cycle	3	5	8	9.4%	9	6	15	17.6%
Total Pregnant	32	44	76	**89.4%[a]**	28	43	71	**83.5%[a]**
NDP	3	6	9	**10.6%**	7	7	14	**16.5%**

[a] proportions with the SAME superscript are NOT significantly different at $p<0.05$.
* Two heifers, one each from the 1-Pod and 2-Pod groups both originating from Herd 2, were found deceased between Day 10 and 96.

Table 4: Comparison of proportions and the 95% confidence interval (CI) of Angus heifers that were treated with either a 1-Pod or 2-Pod Cue-Mate® for ovulation synchronisation for FTAI. Results report pregnant to fixed time artificial insemination (FTAI), and overall pregnant after FTAI and being exposed to bulls.

	1-Pod vs. 2-Pod	
	Pregnant to FTAI	**Pregnant Overall**
Lower 95% CI for difference (%)	-7.9	-4.4
Point estimate of difference (%)	7.1	5.9
Upper 95% CI for difference (%)	22.6	15.5
Chi-squared statistic	0.85	1.26
p-value	0.357	0.262

Table 5: The effect of the presence or absence of a CL at Day – 5 on the outcome of pregnancy to FTAI or Pregnancy overall (FTAI + exposure to bulls).

Status	*CL presence at Day -5*		Chi-Squared statistic	p-value
	CL present	**No CL**		
Pregnant to FTAI				
	1-Pod			
Yes	20.0%	34.1%	1.25	0.264
No	11.8%	34.1%		
	2-Pod			
Yes	15.7%	31.3%	0.01	0.942
No	18.1%	34.9%		
*Pregnant overall**				
	1-Pod			
Yes	25.9%	63.5%	2.63	0.105
No	5.9%	4.7%		
	2-Pod			
Yes	27.7%	55.4%	0.03	0.864
No	6.0 %	10.8 %		

*includes heifers pregnant to FTAI and bulls for 42 days after FTAI.

Discussion and conclusions

The use of IPRDs that contain a lower dose of P_4 (0.78 g) have formed the basis of best practice ovulation synchronisation protocols for FTAI in *Bos indicus* heifers. Very little research has been conducted comparing doses of P_4 in IPRD devices designed for ovulation synchronisation and FTAI in *Bos taurus* heifers. This study has confirmed that in typical yearling Angus heifers it is not necessary to use IPRDs that contain a higher dose of P_4.

Carvalho et al.[10] was one of the first published reports that highlighted the relationship between circulating P_4 concentrations and the effects on follicular wave emergence, follicular diameter and ovulation rates. This report compared, *Bos indicus*, *Bos indicus* x *Bos taurus* and *Bos taurus* heifers and proposed that the *Bos indicus* heifer required a lower dose of P_4 in IPRD for ovulation synchronisation due to their unique ovarian function as compared to the other genotypes. The results from this study did not suggest that a lower dose IPRD would not be suitable for *Bos taurus* heifers. In fact, due to the emphasis of the *Bos indicus* heifer in this study, the discussion did not argue the dose requirements for IPRDs to be used in *Bos taurus* heifers. Anecdotal evidence from field FTAI programmes suggested that a study investigating the use of IPRDs that contain half the standard dose of P_4 (0.78 g) was warranted.

Treating heifers to ensure the optimal circulating P_4 concentrations result during ovulation synchronisation protocols is desired. If heifers are treated with a P_4 dose that is above the optimal concentration, it is expected that a reduction in luteinising hormone (LH) pulsatility will occur[11] thereby inhibiting the growth of the dominant follicle[10, 12]. The larger the diameter of the ovulatory follicle has been related to the likelihood of conception[13].

Suggesting that measures to improve the growth of the dominant follicle are preferred to improve fertility in FTAI programs. The diameter of the ovulatory follicle was not measured in this study. As there is limited information on *Bos taurus* heifers treated with varying doses of P_4 in IPRDs, a study that measured the ovarian function in response to ovulation synchronisation protocols would be warranted and provide further information to develop best practice protocols in these types of heifers.

The Cue-Mate® IPRD used in this study is comprised of two P_4 impregnated replaceable silicon pods that are mounted on a firm wishbone. Due to its design, a single P_4 impregnated pod, can be replaced with a silicon 'blank' pod to effectively halve the dose of P_4 without altering the physical attributes of the IPRD[5]. This device offers a commercially available product that enables optimal P_4 dosing of heifers.

Interestingly, in this study 31.4% and 32.6% had a CL present five days prior to synchronisation treatment (although this is not a true indication of cycling status as heifers that were in the oestrus, or proestrus at the time of the ovarian exam may not have had luteal tissue visible). Nevertheless, the overall result to FTAI was comparable to other industry reports in Angus yearling heifers[1]. The presence or absence of the CL did not affect the pregnancy rate to FTAI or overall pregnancy rate after being exposed to bulls. There are two possible reasons for this observation, the IPRD, regardless of P_4 concentration was sufficient to stimulate heifers to cycle that did not have a CL present[14], and/or the rising plane of nutrition assisted in stimulating cyclic activity.

This study has presented new options for ovulation synchronisation of typical yearling heifers mated annually in the southern Australian beef industry. The study confirms that the pregnancy rates after FTAI and subsequent exposure to bulls are similar between the use of a half-dose IPRD (0.78 g P_4) or a standard-dose IPRD (1.56 g P_4). The results of the study will benefit veterinarians and their beef producer clients that are engaging in genetic improvement programmes by providing practical and cost effective methods to engage in FTAI.

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References

1. Bo GA, Dent R. Pregnancy rates in Australian Angus Cattle treated with progesterone releasing devices, eCG and GnRH or oestradiol and inseminated at a fixed-time. In: Beggs DS, editor. Australian Cattle Veterinarians, Cairns, QLD, Australia, 2014.

2. Edwards SAA, Atkinson PC, Satake N, Boe-Hansen GB, McGowan MR. Ovarian dynamics in response to two modified intravaginal progesterone releasing device and oestradiol benzoate based ovulation synchronisation protocols designed for use in Brahman heifers. *Anim Reprod Sci* 2014;148:18-25.

3. Edwards SAA, Boe-Hansen GB, Satake N, Chandra K, McGowan MR. A field investigation of a modified intravaginal progesterone releasing device and oestradiol benzoate based ovulation synchronisation protocol designed for fixed-time artificial insemination of Brahman heifers. *Anim Reprod Sci* 2015;160:105-111.

4. Butler SAA. Ovarian responses in *Bos indicus* heifers treated to synchronise ovulation. *School of Veterinary Science*. The Unversity of Queensland, Gatton, Queensland, Australia, 2011.

5. Butler SAA, Atkinson PC, Boe-Hansen G et al. Pregnancy rates after fixed-time artificial insemination of Brahman heifers treated to synchronize ovulation with low-dose intravaginal progesterone releasing devices with or without eCG. *Theriogenology* 2011;76:1416 - 1423.

6. Martinez MF, de Nava G, Demmers KJ et al. Intravaginal Progesterone Devices in Synchronization Protocols for Artificial Insemination in Beef Heifers. *Reprod Domest Anim* 2012;47:230-237.

7. Jephcott S, Norman S. Interpreting results of pregnancy diagnosis in extensive beef herds. *Pregnancy diagnosis in cattle*. Australian Association of Cattle Veterinarians, Eight Mile Plains, Queensland, Australia, 2004:65-74.

8. Kastelic JP, Pierson RA, Ginther OJ. Ultrasonic morphology of corpora lutea and central luteal cavities during the estrous cycle and early pregnancy in heifers. *Theriogenology* 1990;34:487-498.

9. Petrie A, Watson P. *Statistics for veterinary and animal science, 2nd Ed.* Blackwell Publishing Ltd, Oxford, UK, 2006.

10. Carvalho JBP, Carvalho NAT, Reis EL et al. Effect of early luteolysis in progesterone-based timed AI protocols in *Bos indicus*, *Bos indicus* x *Bos taurus*, and *Bos taurus* heifers. *Theriogenology* 2008;69:167-175.

11. Ireland JJ, Roche JF. Effect of progesterone on basal LH and episodic LH and FSH secretion in heifers. *J Reprod Fertil* 1982;64:295-302.

12. Adams GP, Matteri RL, Ginther OJ. Effect of progesterone on ovarian follicles, emergence of follicular waves and circulating follicle-stimulating hormone in heifers. *J Reprod Fertil* 1992;96:627-640.

13. Sá Filho MF, Crespilho AM, Santos JEP, Perry GA, Baruselli PS. Ovarian follicle diameter at timed insemination and estrous response influence likelihood of ovulation and pregnancy after estrous synchronization with progesterone or progestin-based protocols in suckled *Bos indicus* cows. *Anim Reprod Sci* 2010;120:23-30.

14. Anderson LH, McDowell CM, Day ML. Progestin-induced puberty and secretion of luteinizing hormone in heifers. *Biol Reprod* 1996;54:1025-1031.

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