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Comparison of the pregnancy rates and costs per calf born after fixed time AI or AI following estrus detection in *Bos indicus* heifers

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Abstract

This study compared pregnancy rates (PR) and cost per calf born after fixed-time AI (FTAI) or AI following estrus detection (EDAI), before and after a single PGF$_{2\alpha}$ treatment in Bos indicus (Brahman-cross) heifers. On Day 0, the bodyweight (BW), body condition score (BCS) and presence of a CL (46% of heifers) was determined. The heifers were then alternately allocated to one of two FTAI groups (FTAI-1, n = 139) and (FTAI-2, n = 141) and an estrus detection and AI group (EDAI; n = 273). Heifers in the FTAI groups received an intravaginal progesterone (P$_4$) releasing device (IPRD; 0.78 g P$_4$) and 1 mg of estradiol benzoate (EB) im on Day 0. Eight days later the IPRD were removed and heifers received 500 µg PGF$_{2\alpha}$ and 300 IU eCG im; 24 hrs later they received 1 mg EB im and were FTAI 30 to 34 h later (54 and 58 h after IPRD removal). Heifers in the FTAI-2 group were started on treatment 8 days after those in the FTAI-1 group. Heifers in the EDAI group were inseminated approximately 12 h after detection of estrus between Days 4 and 9 at which time heifers not detected in estrus received 500 µg PGF$_{2\alpha}$ im and estrus detection and AI continued until Day 13. Heifers in the FTAI groups had a higher overall PR (proportion pregnant as per the entire group) than the EDAI group (34.6% vs. 23.2%; P = 0.003), however conception rate (PR of heifers submitted for AI; PRAI) tended to favor the estrus detection group (34.6% vs. 44.1%; P = 0.059). The cost per AI calf born was estimated to be $267.67 and $291.37 for the FTAI and EDAI groups, respectively. It was concluded that in Brahman heifers typical of those annually mated in northern Australia that FTAI compared to EDAI increases the number of heifers pregnant and reduces the cost per calf born.

Keywords
1. Introduction

The success of an AI program is often judged on the number of females pregnant after one insemination and the cost per calf produced. The north Australian beef industry is mostly comprised of Brahman (*Bos indicus*) or Brahman-infused genotypes [1]. Cattle are generally extensively managed on large properties and are often only mustered twice a year to conduct various husbandry procedures. Consequently the dissemination of improved genetics through the use of AI poses challenges for cattle producers in this region. It is important to identify the most efficacious and cost effective means of using AI in these herds to increase genetic improvement in the north Australian beef industry.
Bos indicus cattle have a unique reproductive physiology as compared to their Bos taurus counterparts which needs to be considered when recommending the most appropriate ovulation synchronization protocol. For example, Brahman cattle commonly attain puberty at a later age than Bos taurus genotypes [2, 3]. As a result the proportion of two year old Brahman heifers in northern Australia that have a CL at commencement of breeding has been reported to be 43% as compared to 63% in tropical composites (50% Bos taurus, 50% Bos indicus) [3]. This implies that a considerable proportion of Brahman females in northern Australia are pre-pubertal at the commencement of the breeding period. The use of PGF$_2$α alone for estrus synchronization is ineffective in pre-pubertal heifers as a prerequisite for its use is the presence of a responsive CL [4]. Treatment of heifers with progesterone (P$_4$) has been shown to advance the onset of puberty [5], while typical ovulation synchronization protocols that enable fixed-time AI (FTAI), utilize intravaginal P$_4$ releasing devices (IPRD) and estradiol benzoate (EB) [6, 7]. Therefore, prepubertal heifers may benefit from FTAI protocols as they can be induced to ovulate, form a CL, and have the opportunity to become pregnant after FTAI.

The objectives of this study were to compare the most suitable AI program for a group of high grade Brahman heifers that were typical of those mated in northern Australia. The PR and costs per calf born after ovulation synchronization and FTAI were compared to that following estrus detection and AI (EDAI), before and after a single PGF$_{2\alpha}$ treatment. The program with the most heifers pregnant and the lowest cost per calf born would be considered the most suitable for use in northern Australia.
2. Materials and methods

2.1. Field study

2.1.1 Heifer selection and management

The study was performed on a commercial beef cattle property in central Queensland, Australia (25°01′44.42″S, 150°26′06.21″E) during late spring – early summer (November – December). Ethical approval was granted by The University of Queensland’s Animal Ethic Committee – approval number: SVS/210/11/MLA. A group of rising 2 year old, high-grade Brahman heifers (> 75% Brahman content; n = 589), were used in the study. The heifers were representative of Brahman and Brahman-cross heifers mated annually in this region. All heifers were managed in a 2806.36 ha paddock which contained a variety of blue grass (Dichanthium sericeum), spear grass (Austrostipa spp.) and buffel grass (Cenchrus ciliaris) pasture before and after the trial. Prior to and during the trial all heifers had ad libitum access to a dry season supplement (10% urea, 2% phosphate and 52 to 60% protein) fed in troughs. At the start of the study (Day 0), all heifers were weighed and body condition scored (BCS; 1 = poor to 5 = fat [8, 9]) and underwent a transrectal ultrasonographic reproductive exam 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 or absence of a CL (diagnosed by the presence of the echogenic appearance of CL [10, 11]), pregnancy and any reproductive tract abnormality were recorded. Heifers which had a bodyweight (BW) ≤
280 kg (n = 28), a BCS < 2 (n = 2), were pregnant (n = 3), had an immature reproductive tract (reproductive tract score 1; [12, 13]) or other abnormalities (n = 3) were rejected from the study. The selected heifers (n = 553) had a mean (±SEM) BW of 327.4 ± 1.1 kg (range 280 to 418 kg) and BCS of 2.5 ± 0.0 (range 2 to 3). At pregnancy diagnosis, approximately six to eight weeks after AI the BW and BCS were again recorded. All heifers were vaccinated for clostridial diseases and leptosporosis prior to the trial.

2.1.2 Experimental design and treatment allocation

On Day 0, heifers were allocated to a treatment group as they presented in the squeeze-chute. Heifers were allocated alternately to a FTAI group (n = 280) or an EDAI group (n = 273). The FTAI heifers were further subdivided into two similar sized groups FTAI-1 (n = 139) and FTAI-2 (n = 141) to ensure all heifers in this group were inseminated between 54 and 58 h after removal of the IPRD; heifers in the FTAI-2 group were put on treatment 8 days after those in the FTAI-1 group. The allocation procedure was retrospectively analyzed for evidence of bias between the allocation of heifers in FTAI-1, FTAI-2 and EDAI groups with regards to BW, BCS and CL presence at Day 0 (Table 1). Heifers in treatment groups FTAI-1 were managed in a 261 ha paddock from Day 0 to 8 and a 50 ha paddock from Day 8 to 10. Heifers from FTAI-2 were managed in a 409 ha paddock from Days 8 to 16 and a 50 ha paddock from Days 16 to 18. Heifers in the EDAI were managed in a 154 ha paddock nearby the handling facility from Day 4 to 13. All
aforementioned paddocks were similar with respect pasture species and quality and quantity of available pasture.

Treatment to synchronize ovulation commenced on Day 0 for FTAI-1 and Day 8 for FTAI-2. Each heifer in the FTAI groups had a half-dose IPRD (Cue-Mate®; 0.78 g P₄; Bioniche Animal Health, Aust/Asia, Sydney, NSW, Australia) inserted intravaginally. The half-dose IPRD was prepared as previously described [6, 7]. At the time of IPRD insertion all heifers received 1 mg EB (Bomerol™, Bayer Australia, Sydney, NSW, Australia) im. Eight days later, the IPRD were removed and all heifers received 500 µg cloprostenol (PGF₂α; Ovuprost™; Bayer Australia, Sydney, NSW, Australia) im, and 300 IU of eCG (Pregnecol™; Bioniche Animal Health, Aust/Asia) im and were FTAI 30 to 34 later. Approximately 24 h after IPRD removal all heifers in the FTAI groups received 1 mg EB im. All heifers in the EDAI group initially received no treatment, but were observed twice daily (morning and afternoon for a duration of 2.5 to 3 h) from Day 4 to 9 for signs of estrus (standing to be mounted, riding) and were inseminated 12 h later. No estrus detection aids were used. On Day 9 all heifers that had not been detected in estrus (n = 217) were treated with 500 µg PGF₂α (Ovuprost™) im. and subsequently observed for signs of estrus and inseminated until Day 13. All heifer treatments are outlined in Fig. 1.

(Insert Fig. 1 near here)

2.1.3 Sire allocation and artificial insemination
Sires used in the experiment (n = 34) were part of a large scale genetic evaluation project. Heifers were allocated to sire upon presentation for AI. Sires were used in numerical order from 1 to 34 across all groups until semen stores were exhausted. The sire allocation procedure was retrospectively analyzed for evidence of bias among groups with regards to BW, BCS and CL presence at Day 0.

A straw from each batch of semen used was evaluated by the Queensland Government’s Beef Breeding Services Laboratory, Rockhampton, Australia or Just Genes Artificial Breeding services, Everton Park, Australia. All semen used had acceptable post-thaw quality (concentration > $1 \times 10^8$ sperm/mL; > 35% live and > 35% progressively motile). Straws of semen were thawed at 35°C for 15 to 30 sec in a water bath prior to insemination. Heifers from group FTAI-1 and FTAI-2 were FTAI on Day 10 and 18, respectively. Artificial inseminations for heifers in the FTAI groups were performed between 54 and 58 h after IPRD removal by experienced AI technicians. Two squeeze chutes were operated simultaneously as Technicians 1 and 2 inseminated the majority of the heifers and Technician 3 acted as a relief inseminator if either Technician 1 or 2 became fatigued. This enabled all heifers to be inseminated within the scheduled time period. Inseminations for heifers in the EDAI groups were performed using the AM-PM rule after the detection of estrus using only one squeeze-chute and alternating AI technicians. From Day 19, the heifers in the FTAI-1 and FTAI-2 groups were managed together.

2.1.4 Pregnancy diagnosis
Pregnancy diagnosis was performed by transrectal ultrasonography on Day 60 for all heifers (Day 0 = commencement of synchronization treatments in the FTAI-1 group). This corresponded to 42 and 50 d post-FTAI for heifers in the FTAI-2 and FTAI-1 groups, respectively, and between 47 to 56 d post-AI for heifers in the EDAI group. Fetal ageing was used to record heifers as either pregnant to AI or not detectably pregnant to AI (NDP). The overall pregnancy rate (PR) was defined as the proportion of heifers in each group diagnosed pregnant. The PR to AI (PRAI) was defined as the proportion of heifers submitted to AI that were diagnosed pregnant. During the course of the study no bulls were ever observed with the study heifers.

2.1.5 Statistical analysis

Statistical analyses were performed using GenStat 14th edition [14]. A P-value of < 0.05 in all statistical analyses was considered to be significant. To ensure that BW, BCS and presence of a CL did not differ group allocations on Day 0 several analyses were performed. The BW and BCS were analyzed using residual maximum likelihood (REML) methods with FTAI-1 and FTAI-2 groups or FTAI or EDAI treatments and sire and technician as fixed model terms, respectively. The proportion of heifers with a CL present on Day 0 was analyzed using a generalized linear model with a binomial distribution and logit link with FTAI-1 and FTAI-2 groups or FTAI or EDAI treatments, sire and technician as model terms respectively. Predicted means from the analyses were back-transformed for presentation. Results of the allocation procedures for FTAI or EDAI treatments, FTAI-1 and FTAI-2 groups and AI technician are reported in Table 1.
To determine whether the PR or PRAI differed between the FTAI-1 and FTAI-2 groups, a two-sample binomial test comparing the two proportions was performed. Data from the FTAI-1 and FTAI-2 groups were combined as there was no significant difference (see section 3.1). To determine whether the PR or PRAI differed between the FTAI or EDAI treatments a two-sample binomial test comparing the two proportions was performed. To determine the effect of CL presence, BW and BCS at Day 0 and BW gain, BCS change from Day 0 to pregnancy diagnosis and the interaction between treatment and CL on PR, the data was analyzed using a generalized linear model (GLM) with a binomial distribution and logit link. Model terms for the PR analysis included FTAI and EDAI treatments, CL presence, BW and BCS at Day 0, BW gain and BCS change from Day 0 to 60 and the interaction between FTAI and EDAI treatments, and CL. The model terms for the PRAI analysis were the same as the PR analysis but also included sire and AI technician. All two and three way interactions were initially included but omitted from the final model when found to be non-significant. The significance of each term was assessed by approximate Chi-square statistic, given all other terms were present in the model. Predicted means from the analysis were back-transformed for presentation.
2.2 Cost benefit analysis

A partial budget was created to enable comparison of costs per calf [15] of FTAI and EDAI. A list of fixed costs was constructed based on expected expenses in $AUD 2014 (Table 2). The PR data from the study were used. This assumes that no losses from pregnancy diagnosis to parturition occurred, and if losses were to occur, then they would be similar for FTAI and EDAI heifers. The fixed costs were utilized in the model to create a cost per calf born by AI.

(Insert Table 2 near here)

3. Results

3.1 Field Study

The PR of heifers in the FTAI-1 and FTAI-2 groups did not differ significantly (34.5% vs. 34.8%; P = 0.969), so results from the FTAI-1 and FTAI-2 groups were combined. The overall submission rate of heifers in the EDAI group was 143/273 (52.4%). Of the heifers submitted for AI in the EDAI group, 55 (39%) were submitted to AI prior to PGF$_2$α treatment (Day 9), and 88 were submitted between Day 9 and 13.
The PR for the FTAI and EDAI treatments differed significantly (Table 3) FTAI resulted in a significantly higher PR (P < 0.003). However, the PRAI tended to be higher in the EDAI treatment group (P < 0.059; Table 3).

There was no significant differences between heifers allocated to sires or AI technicians with respect to BW (P = 0.372 and P = 0.824), BCS (P = 0.978 and P = 0.519) or proportion of heifers with a CL (P = 0.171 and P = 0.839). Neither AI technician nor sire had an effect on PRAI (P = 0.270 and P = 0.565, respectively). Mean (± SEM) BW (328.5 ± 2.1 vs. 326.7 ± 1.3 kg; P = 0.583) and BCS (2.52 ± 0.03 vs. 2.48 ± 0.01; P = 0.216) at Day 0 did not differ between heifers that were diagnosed pregnant to AI as compared to heifers that were NDP. Neither did the change in BW (47.4 ± 1.6 vs. 51.4 ± 0.9; P = 0.216) and BCS (0.78 ± 0.03 vs. 0.78 ± 0.02; P = 0.817) between Day 0 and pregnancy diagnosis (Day 60) differ between pregnant and NDP heifers.

A CL by treatment interaction was observed for the PR and PRAI (Table 4). As expected, heifers in the EDAI group which did not have a CL at Day 0 had a significantly lower PRAI than those that did have a CL, while PRAI in the FTAI heifers did not differ between this that did and did not have a CL.
3.2. Economic Comparison

Synchronization treatments comprised 14.9% and 2.4% of the expense of the FTAI and EDAI programs, respectively. Semen comprised 43.1% and 31.0% of the expense of the FTAI and EDAI programs, respectively and labor comprised 42.0% and 67.1% of the expense of the FTAI and EDAI programs, respectively. The cost per calf produced by AI was calculated to be $267.67 for the FTAI program and $291.37 for the EDAI program (Table 5), a difference of $23.70.

(Insert Table 5 near here)

4. Discussion

In Brahman heifers typical of those mated annually in northern Australia, treatment to synchronize ovulation and FTAI resulted in a higher overall PR to AI and a lower cost per calf born than EDAI before and after treatment with PGF$_{2\alpha}$. This was primarily due to differences in response to the synchronization treatments in heifers which were cycling as compared to those that were not. Using the proportion of heifers submitted to AI in the EDAI group and the proportion of all heifers with a CL at Day 0, it was estimated that approximately 50% of the heifers were cycling at the commencement of the study. It is common for only about 50% of heifers to be cycling at the time of first mating at approximately two years of age in northern Australia. This is considered to be due to low to
moderate annual growth rates (approximately 150 kg/year) [16] and the current Brahman
genotype used in northern Australia Johnston et al. [3] demonstrated that on average,
Brahman heifers attained puberty at a later age (750.6 days) than tropical composites (50%
tropically adapted Bos taurus, 50% Brahman; 650.8 days) and reported the proportion of
Brahman heifers that had a CL at the time of onset of breeding was 43% as compared to 63%
in the tropical composites. It is apparent that the application of ovulation synchronization
protocols that can assist in the AI of anovulatory heifers is advantageous in north Australian
herds. Treatment of heifers with a combination of P₄ and EB can advance the onset of
puberty [5]. This was demonstrated previously by Butler et al. [6] where a higher proportion
of Brahman heifers (31% estimated to be cycling) ovulated and subsequently had a normal
luteal phase after treatment with an IPRD and EB (60.9%) than heifers treated with two
injections of PGF₂₀ administered 10 days apart (22.2%).

The effect of the presence of a CL at the time of commencement of ovulation
synchronization treatments on PR outcomes to FTAI varies in the literature. In a north
Australian study [7] of FTAI in Brahman heifers the presence of a CL did not affect the PR
to FTAI following treatment with an IPRD and EB to synchronize ovulation. Similarly, in
studies involving Bos taurus genotypes, Leitman et al. [17, 18] demonstrated that PR did not
differ significantly between pre-pubertal and pubertal Angus and Angus-cross heifers
following treatment with an IPRD and GnRH to synchronize ovulation. The first and second
ovulation immediately following onset of puberty in heifers has been reported to be less
fertile than the third ovulation [19]. This is because a shortened luteal phase is often observed
between the first and second ovulation [20], which reduces the P₄ priming that is considered
important for normal fertility [19]. However the mechanisms associated with P₄ priming are
still poorly understood. The insertion of an IPRD for eight days may provide sufficient P₄
priming to enable development and ovulation of a normal ovum and the development of a functionally normal CL. The results of the current study demonstrated that the presence of a CL at the start of treatment to synchronize ovulation affected the likelihood of Brahman heifers becoming pregnant to EDAI but not FTAI. In this study, only one ovarian exam was performed and so only a crude estimate of heifers that are pubertal and pre-pubertal can be made [7, 11]. An assumption can be made that heifers that did not present with a CL at the commencement of this study were mostly comprised of pre-pubertal heifers. As FTAI heifers without a CL were exposed to P₄ and EB treatments prior to the induction of ovulation, this may have increased the likelihood of conception following FTAI. A small proportion of heifers in the EDAI group with no CL may have ovulated in response to the PGF₂α treatment via a luteolysis independent mechanism, but not to the degree of heifers treated with P₄ and EB [21].

In this study a partial budget analysis comparing cost per calf produced by FTAI versus EDAI was conducted. The total cost of ovulation synchronization drugs per animal for FTAI protocols are invariably higher than the costs for EDAI protocols that only utilize PGF₂α [22], hence FTAI programs are often perceived by beef cattle producers to be less economical. However, the main costs of both the FTAI and EDAI programs were semen and labour. In an FTAI program, 100% of females are submitted to AI and receive a dose of semen. Therefore more straws of semen are used than with an EDAI program. Unfortunately, the cost per straw of semen in northern Australia is based primarily on perceived value of the sire and not on the predicted genetic merit of his offspring. Also, because semen is often collected from bulls custom-processed on farm, rather than being produced in a commercial artificial breeding center, the quality of semen may vary considerably. However, there was no evidence of this in the present study. The other cost
factor is labor. Although three AI technicians were used in this study, EDAI protocols typically only require one technician as relatively small numbers of females are inseminated each day. Currently, on most properties in northern Australia the staff are not sufficiently trained in AI to conduct a large AI program, and therefore an AI technician would need to be present during an entire EDAI program. However, in a FTAI program, all females are inseminated on one day, requiring the AI technicians to be present on only one day per FTAI management group. As adoption of AI increases in northern Australia, training of property staff in AI may become more common which will reduce the insemination costs. The time required for estrus detection also contributes to the cost of implementing EDAI programs and has been factored into this analysis.

The significantly higher PR achieved with FTAI in this study offset the higher costs of semen and synchronization treatments resulting in a lower overall cost per calf produced by AI. Not factored into this study is the unrealized benefit of production of more genetically superior calves through FTAI programs as compared to EDAI programs. Production of more genetically superior calves adds genetic value to beef cattle herd contributing to higher profits in the breeding program [23]. It can be concluded that dissemination of genetics through the use of AI in northern tropical beef herds in Australia, is most efficacious and cost effective when FTAI is utilized.

Acknowledgements
The authors would like to thank Bioniche Animal Health Aust/Asia for the supply of Cue-Mate® devices and Pregnecol®, and Bayer Australia for supply of the Ovuprost® and Bomerol®, and Meat and Livestock Australia (MLA) for funding support. The authors gratefully acknowledge Mark and Belinda Wilson for use of their heifers, facilities and staff to conduct the trial and the Australian Brahman Breeders Association for supply of the semen. The authors thank Professor Reuben Mapletoft for his time and efforts in review of this manuscript.

Role of the Funding Source

Bioniche Animal Health Aust/Asia, Bayer Australia and MLA had no input to study design, data collection, interpretation of data or paper preparation and submission.

Conflict of interest

At the time this trial was conducted there were no perceived conflicts of interest from any of the authors.

Author contributions
Dr Edwards was responsible for conducting the experiment, analysis, interpretation of the data and was responsible for the submission and writing of the paper. Professor Bo and Professor McGowan led the trial design and also had a substantial intellectual contribution to the interpretation of the data, whilst Mrs Chandra conducted all the statistical analysis. Mr Atkinson provided intellectual contribution and was involved with collection of data.
References


synchronize ovulation with low-dose intravaginal progesterone releasing devices with or without eCG. Theriogenology. 2011;76:1416 - 23.


Table 1 – Predicted mean bodyweight (BW), body condition score (BCS) ± SEM and proportion of heifers with a CL present on Day 0 by treatment group and AI technician. Superscripts (A and B) represent significantly different proportions (P < 0.05).

<table>
<thead>
<tr>
<th>FTAI Group</th>
<th>Synchronization Treatment</th>
<th>AI Technician</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>FTAI Group Sync Treat AI Tech</td>
</tr>
<tr>
<td></td>
<td>FTAI-1</td>
<td>FTAI-2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>139</td>
<td>141</td>
<td>280</td>
</tr>
<tr>
<td>BW (kg)</td>
<td>325.0 ± 2.2</td>
<td>331.2 ± 2.1</td>
<td>328.2 ± 1.5</td>
</tr>
<tr>
<td>BCSa (1 to 5)</td>
<td>2.47 ± 0.03</td>
<td>2.52 ± 0.02</td>
<td>2.50 ± 0.02</td>
</tr>
<tr>
<td>CL (%)b</td>
<td>46.76</td>
<td>48.97</td>
<td>47.89</td>
</tr>
</tbody>
</table>

- Body condition score measured on a 1 to 5 scale (1 = thin and 5 = fat).
- Proportion of heifers with a CL present as determined by transrectal ultrasonography.
- Data from FTAI-1 and FTAI-2 heifers combined.
Table 2 – Costs associated with the fixed-time AI (FTAI) and estrus detection and AI (EDAI) programs (prices are based on $AUD in 2014).

<table>
<thead>
<tr>
<th>Item</th>
<th>Details</th>
<th>Cost ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synchronization treatments for FTAI</td>
<td>Intravaginal progesterone releasing device(^a) = $7.00&lt;br&gt; Estradiol benzoate = $2.00&lt;br&gt; Prostaglandin F(_{2\alpha}) = $2.00&lt;br&gt; eCG = $2.80</td>
<td>$13.80</td>
</tr>
<tr>
<td>PGF(_{2\alpha}) treatment used in EDAI</td>
<td>Per heifer: cloprostenol= $2.00</td>
<td>$2.00</td>
</tr>
<tr>
<td>Station labor unit</td>
<td>per person, per day</td>
<td>$200.00</td>
</tr>
<tr>
<td>AI Technician unit</td>
<td>per person, per day</td>
<td>$750.00</td>
</tr>
<tr>
<td>Frozen semen</td>
<td>per straw</td>
<td>$40.00</td>
</tr>
</tbody>
</table>

\(^a\) assumes the use of a half-dose intravaginal progesterone releasing device, therefore half cost of a full device = $14.00/2 = $7.00
Table 3 – Pregnancy rate (PR; proportion of heifers pregnant per number of heifers allocated on Day 0) and PR per AI (PRAI; proportion of heifers pregnant per the number of heifers submitted for AI) of Brahman heifers treated to synchronize ovulation to enable fixed-time AI (FTAI) or AI following estrus detection before and after cloprostenol (PGF2α) treatment (EDAI). Superscripts (A and B) represent significantly different proportions (P < 0.05).

<table>
<thead>
<tr>
<th>Synchronization Treatment</th>
<th>PR</th>
<th>PRAI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FTAI</td>
<td>EDAI</td>
<td>Treatment</td>
</tr>
<tr>
<td>PR</td>
<td>97/280&lt;sup&gt;A&lt;/sup&gt; (34.6)</td>
<td>63/273&lt;sup&gt;B&lt;/sup&gt; (23.1)</td>
<td>0.003</td>
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<tr>
<td>PRAI</td>
<td>97/280 (34.6)</td>
<td>63/143 (44.1)</td>
<td>0.059</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data from FTAI-1 and FTAI-2 heifers combined.
Table 4. The interaction between the presence or absence of a CL at Day 0 and treatment of Brahman-cross heifers to synchronize ovulation with estradiol benzoate, IPRDs containing 0.78g progesterone and fixed-time AI (FTAI) or estrus detection and AI (EDAI) before or after a single prostaglandin F2α treatment. Effect on the pregnancy rate and the pregnancy rate per AI. Superscripts (A, B, and C) represent significantly different proportions (P < 0.05).

<table>
<thead>
<tr>
<th></th>
<th>Pregnancy Rate&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Pregnancy Rate per AI&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(P &lt; 0.001)</td>
<td>(P = 0.007)</td>
</tr>
<tr>
<td></td>
<td>FTAI&lt;sup&gt;a&lt;/sup&gt;</td>
<td>EDAI</td>
</tr>
<tr>
<td>Present</td>
<td>43/134&lt;sup&gt;A&lt;/sup&gt;</td>
<td>51/123&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(32.1)</td>
<td>(41.5)</td>
</tr>
<tr>
<td>Absent</td>
<td>53/146&lt;sup&gt;A,B&lt;/sup&gt;</td>
<td>9/150&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(36.3)</td>
<td>(6.0)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Proportion of heifers pregnant per number of heifers allocated on Day 0.

<sup>b</sup> Proportion of heifers pregnant per the number of heifers submitted to AI.
Table 5 – Comparison of the cost per calf produced by fixed-time AI (FTAI) of Brahman heifers treated to synchronize ovulation or by estrus detection and AI (all costs are expressed in $AUD in 2014).

<table>
<thead>
<tr>
<th>AI protocol</th>
<th>FTAI</th>
<th>EDAI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heifers enrolled in AI program (n)</td>
<td>280</td>
<td>273</td>
</tr>
<tr>
<td>Heifers submitted to AI (n)</td>
<td>280</td>
<td>143</td>
</tr>
<tr>
<td>Heifers treated with PGF2α (n)</td>
<td>-</td>
<td>217</td>
</tr>
<tr>
<td>Cost of synchronization drugs per heifer</td>
<td>$13.80</td>
<td>$2.00</td>
</tr>
</tbody>
</table>

\[ \text{Total cost of synchronization} = (A) \]

\[ \begin{align*}
\text{Total cost of synchronization} &= 3,864.00 \\
\text{Total cost of semen} &= 11,200.00 \\
\text{Total cost of labor} &= 10,900.00 \\
\end{align*} \]

\[ \begin{align*}
\text{Total cost of labor} &= 12,300.00 \\
\end{align*} \]

<table>
<thead>
<tr>
<th>Pregnancy rate to AI (%)</th>
<th>34%</th>
<th>23%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calves produced from AI (n)</td>
<td>97</td>
<td>63</td>
</tr>
</tbody>
</table>

\[ \text{Total cost of AI program} = (A+B+C) = (E) \]

\[ \begin{align*}
\text{Total cost of AI program} &= 25,964.00 \\
\text{Cost of producing AI calf} &= (E/D) \]

Cost of producing AI calf = 267.67 $
Fig. 1. Schedule for treatments to synchronize ovulation in Brahman-cross heifers to enable fixed-time AI (FTAI-1; n = 139 and FTAI-2; n = 141)), and schedule for AI following estrus detection before and after cloprostenol (PGF$_{2\alpha}$) treatment (EDAI; n = 273).